RESEARCH NOTE

Abnormal pollen mitoses (PM I and PM II) in an interspecific hybrid of *Brachiaria ruziziensis* and *Brachiaria decumbens* (Gramineae)

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Introduction

Microgametogenesis was analysed in an interspecific hybrid resulting from a cross between an artificially tetraploidized sexual accession of *Brachiaria ruziziensis* ($2n = 4x = 36$) and the apomictic cultivar (cv. Basilisk) of *B. decumbens* ($2n = 4x = 36$). Although each microspore initiated its differentiation by pollen mitosis, polarization of the nucleus was not observed in 28.85% of the microspores, and the typical hemispherical cell plate was not detected as well. The usual asymmetry was not seen at first pollen mitosis (PM I), and microspores therefore lacked differentiation between the vegetative and the generative cell. After telophase, each cell entered PM II, but this was not always followed by cytokinesis. Tripolar spindle and restitutional nuclei were observed among microspores that lacked the first cytokinesis. Pollen from such abnormal divisions were sterile and larger than those resulting from the normal ones.

Male gametogenesis in flowering plants depends on a determinative asymmetry in the cell division at pollen mitosis I (PM I), which gives rise to a larger vegetative cell and a smaller generative cell. To achieve this asymmetry, the microspore undergoes several unique cellular events, including the establishment of cell polarity through nuclear migration, development of an asymmetric mitotic spindle, and an expected process of cytokinesis to form a hemispherical cell plate. Control of gametophytic cytokinesis is, therefore, a critical process in pollen cell fate determination which results in the asymmetric distribution of cellular components that presumably include cell fate determinants (Twell *et al*. 1998; Park and Twell 2001). During cell division in somatic cells, a pre-prophase band of microtubules marks the future division plane and the exact site of cytokinesis, and the cell plate arises from the phragmoplast and grows toward the cell wall (Sylvester 2000). On the other hand, in gametophytic cytokinesis at PM I the pre-prophase band is absent and a unique hemispherical cell plate is formed and surrounds the generative nucleus (Terasaka and Niitsu 1990). Asymmetric cytoplasm cleavage is ensured by a curved profile of phragmoplast microtubules that appear to guide the centrifugal growth of the cell plate from its margin (Brown and Lemmon 1991).

Mutations altering cell division asymmetry during pollen mitosis have been reported in *Arabidopsis thaliana* (Park *et al*. 1998) and *B. decumbens* (Junqueira Filho *et al*. 2003). This paper reports a new occurrence of symmetry in pollen mitoses (PM I and PM II) in a hybrid involving *B. decumbens* as the male genitor. Absence of cytokinesis following PM I and PM II was also observed.

Materials and methods

The hybrid under analysis was synthetized in the *Brachiaria* breeding programme carried out by Embrapa Beef Cattle (Campo Grande, state of Mato Grosso do Sul, Brazil) in 1988, and has displayed promising performance in agronomical evaluation. Inflorescences in the ideal stage for microsporogenesis and microgametogenesis studies were collected in the field, fixed in a mixture of 95% ethanol, chloroform and propionic acid (6 : 3 : 2 v/v) for 24 h, and stored under refrigeration until use. Microsporocytes and pollen grains were prepared by squashing and stained with 0.5% propionic carmine. All meiotic phases and stages of pollen development were evaluated. More than 6000 microspores and pollen grains were carefully analysed.

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Results

Cytological characterization of the hybrid revealed many different kinds of meiotic abnormalities, but most of them were related to its polyploid and interspecific hybrid condition. Meiotic products were characterized by tetrads with various micronuclei in some microspores. After callose dissolution, microspores were released into the anther locule and appeared normal. In due time, each microspore entered PM I. In 71.15% of the microspores analysed, pollen mitosis (PM I) developed normally showing the characteristic polarization of the nucleus (figure 1a) resulting in two unequal cells. However, in 28.85% of the microspores, PM I was characterized by symmetry,

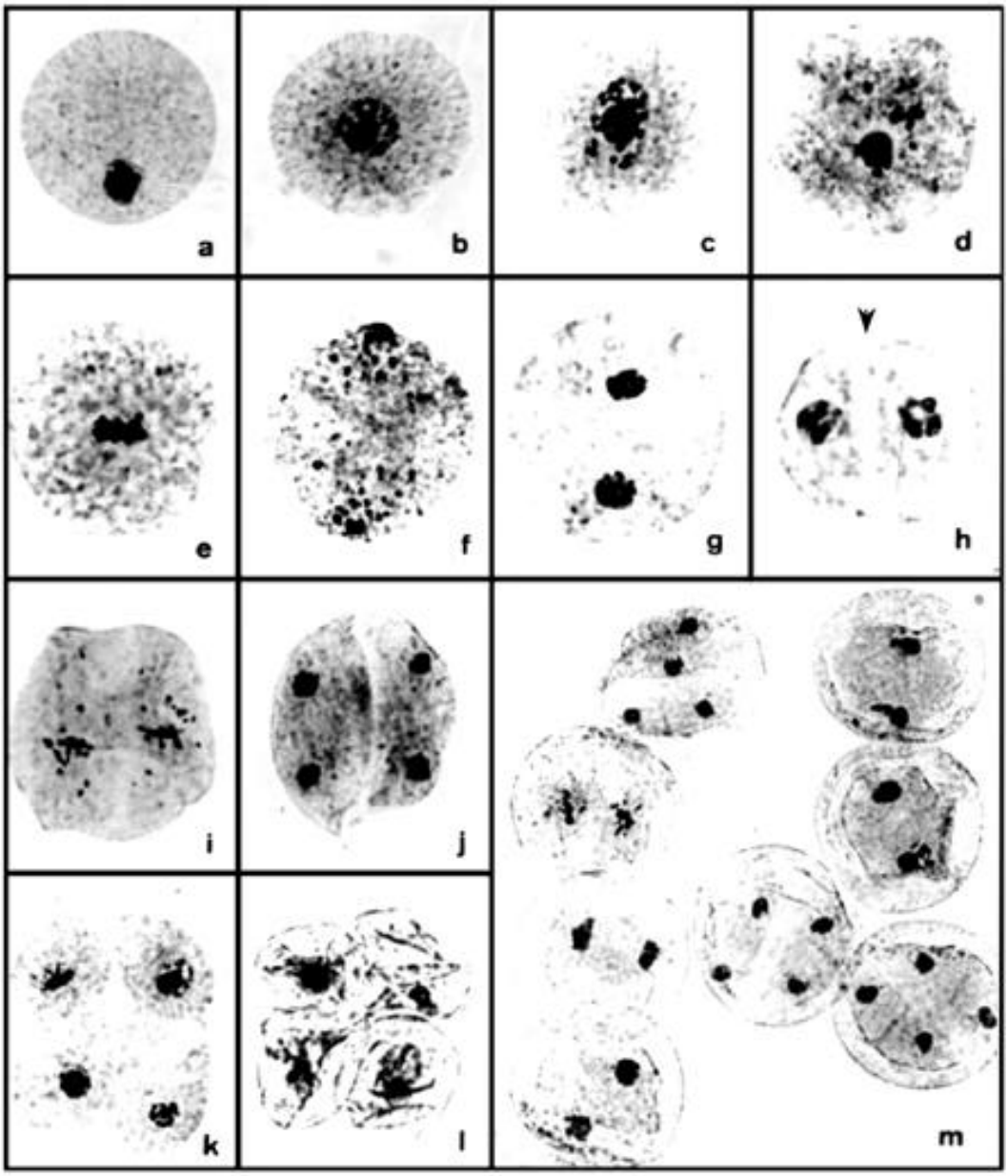

Figure 1. Symmetric pollen mitoses (PM I and PM II) followed by cytokinesis after both divisions. (a) Interphase microspore showing normal nucleus polarization. (b) Interphase microspore with absence of nucleus polarization. (c to g) Stages of PM I in microspores without nucleus polarization: prophase (c), prometaphase (d), metaphase (e), anaphase (f), telophase (g). (h) Cytokinesis (arrowhead) dividing the microspore into two equal-sized cells. Observe the equal size and chromatin condensation in both nuclei. (i to k) Stages of PM II: metaphase (i), telophase (j), four equal-sized cells resulting from the second cytokinesis (k). (l) Tetrad of uninucleate pollen grains with well-developed pollen wall. (m) Microspores in different stages of pollen mitoses (PM I and PM II). (400×).

i.e. the nucleus was not displaced from the cell centre to one pole of the cell (figure 1b). In these microspores, all PM I stages occurred without polarization (figure 1c–g), and the typical hemispherical cell plate was not observed. After cytokinesis, two equal-sized cells resulted from this symmetric division (figure 1h). Both cells also showed equal chromatin condensation, and the nuclei had the same spherical shape and size (figure 1g,h); consequently, the generative and vegetative nuclei could not be differentiated. After telophase, each cell entered PM II, but this was not always followed by cytokinesis. In 7.62% of microspores, PM II occurred in a common cytoplasm and both nuclei underwent a new symmetric mitosis (figure 2a–e). In the remainder, after cytokinesis both nuclei also divided by mitosis. In the latter, after a second cytokinesis (figure 1i–k), a tetrad of four uninucleate pollen grains of

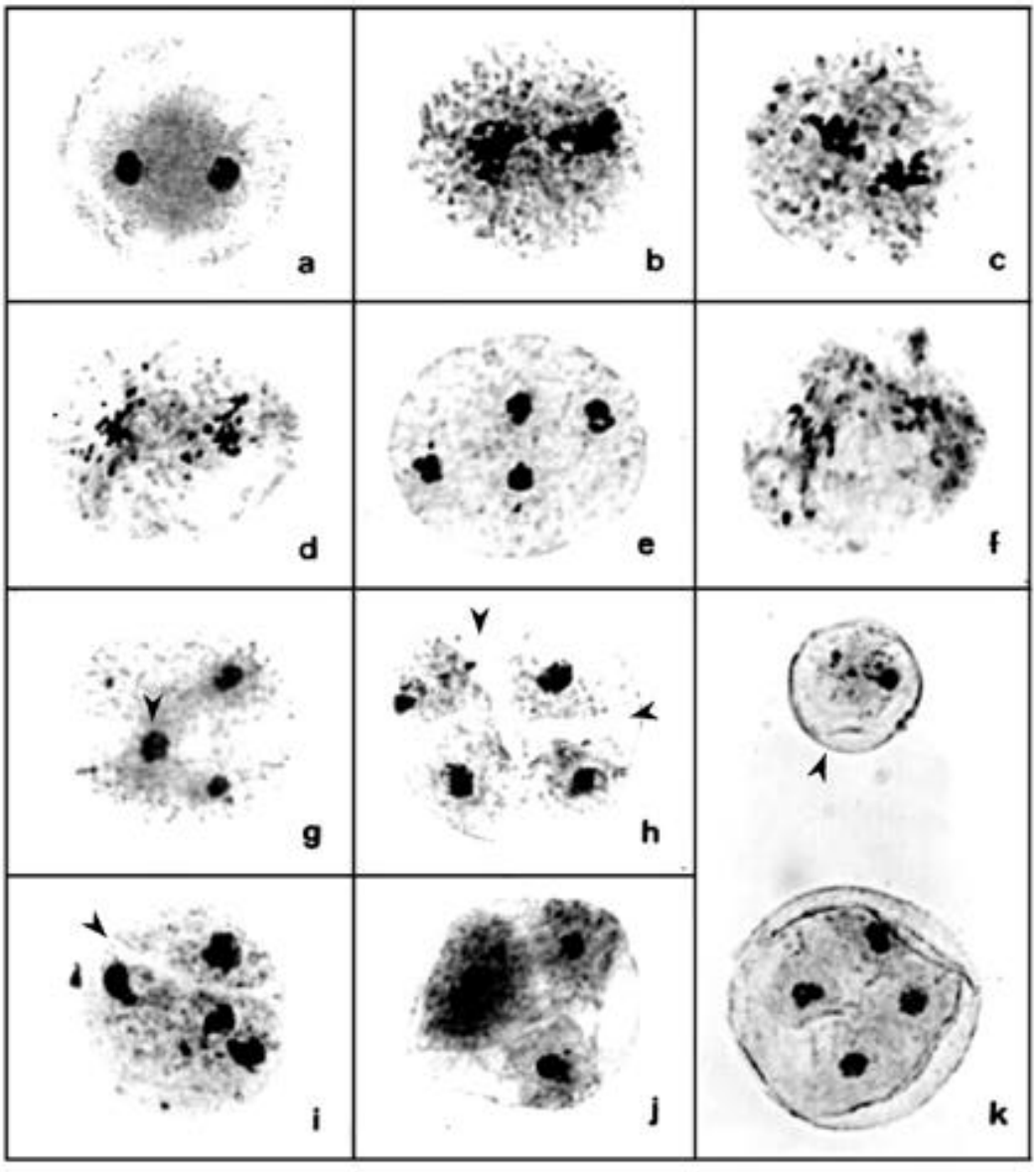

Figure 2. Symmetric pollen mitosis II (PM II) in the absence of first cytokinesis. (a) Binucleate interphase microspore. (b to e) Stages of PM II: prophase (b), metaphase (c), anaphase (d), telophase (e). (f) Anaphase with tripolar spindle. (g) Restitutional nuclei in telophase (arrowhead) after tripolar spindle formation. (h) Abnormal cytokinesis after telophase. Observe that only one cell underwent cytokinesis (arrowhead). (i) Abnormal cytokinesis after telophase resulting in uninucleate and trinucleate cells (arrowhead). (j) Triad of uninucleate pollen grains. The big pollen resulted from nucleus restitution. (k) Normal pollen grain (arrowhead) and tetranucleate pollen grain resulting from symmetric pollen mitoses without cytokinesis. (400×).

equal size was formed (figure 1l). In the former, on the other hand, the outcome depended on whether one or both cytokineses failed. When both cytokineses did not occur, tetranucleate pollen grains (figure 2k) were formed. However, in some cases, the first cytokinesis occurred and the second failed in one cell, resulting in a triad, in which nucleus restitution was scarcely observed (figure 2h). Restitutional nuclei were also found to result from tripolar spindle in PM II in those pollen grains in which the first cytokinesis failed to occur (figure 2f,g). As a result of such diversity in abnormalities during pollen mitoses, different types of pollen grains were observed (table 1). Pollen resulting from abnormal cell divisions were sterile and larger than those resulting from the normal divisions (figure 2k).

Discussion

Although many mutations affecting pollen development have been described in some plants, mutations specifically altering cell division asymmetry and cell fate have been reported only in *A. thaliana* (Park *et al*. 1998) and *B. decumbens* (Junqueira Filho *et al*. 2003). In the former, the division planes of mutant *gemini pollen1* (*gem1*) were shown to be aligned with the polar axis, resulting in symmetric division. In the latter, in a considerable proportion of microspores, polarization of the nucleus was not observed, and the typical hemispherical cell plate was not detected. Division was characterized by symmetry and pollen lacked differentiation between the vegetative and the generative cell. Both nuclei were of equal size, presented equal chromatin condensation, and had a spherical shape. After PM I and cytokinesis, each cell underwent a new symmetric mitosis, without polarization of the nucleus. At the end of PM II, four equal nuclei were observed in each pollen. After cytokinesis, these gave rise to four equal-sized uninucleate and sterile pollen grains, with similar tetrad configurations.

The interspecific hybrid under analysis exhibited behaviour of pollen mitoses very similar to that reported in one accession of *B. decumbens* by Junqueira Filho *et al*. (2003). However, in this hybrid, after PM I some cells did not undergo cytokinesis and both nuclei entered PM II sharing the same cytoplasm. After PM II, cytokinesis was also lacking in some cases. In *A. thaliana*, *gem1* was also found to affect cytokinesis during pollen mitosis, resulting in altered cell division asymmetry and cell fate (Park *et al*. 1998). Binucleate spores with ectopic dividing walls in *gem1* suggested that cytokinesis may be spatially uncoupled from nuclear division at PM I.

Another characteristic of the present mutant similar to the *gem1* mutation of *A. thaliana* (Park *et al*. 1998), and the mutation reported in *B. decumbens* (Junqueira Filho *et al*. 2003), is the incomplete penetrance of the character. In *gem1*, abnormal division was recorded in 10% of the spores, in the *B. decumbens* accession 43.24% of the spores were abnormal, and in the present hybrid 28.85% of spores were symmetrical at PM I. Differences in the size of mature pollen grains found among those that resulted from symmetric and asymmetric cell division were also reported to occur in the *gem1* of *A. thaliana* (Park and Twell 2001). The importance of division asymmetry at PM I in controlling differential cell fate has long been recognized (La Cour 1949). Early observations showed that the differentiation of vegetative and generative cells was clearly associated with a qualitative difference in the cytoplasm surrounding nuclei following asymmetric division at PM I.

In the *Brachiaria* mutants affecting division symmetry at PM I, i.e. in the present hybrid and in the mutant described by Junqueira Filho *et al*. (2003), spores developed into sterile pollen grains. Mutations that cause male sterility in plants are considered to be of great interest in breeding programmes. The *Brachiaria* breeding programme aims at producing hybrids by intraspecific or interspecific crosses using sexual accessions as mother plants and apomictic accessions as pollen parents. Because the sexual progenitors are allogamous, and manual emasculation is difficult to accomplish, a male-sterile mutant might improve the efficiency of the programme by avoiding

Table 1. Behaviour of pollen development in the interspecific hybrid of *B. ruziziensis* × *B. decumbens*.

Pollen mitoses	Characteristics	Occurrence of cytokinesis	No. of scored cells (%) per division
PM I	Asymmetric division	–	905 (71.15)
	Symmetric division	–	367 (28.85)
PM II	Symmetric division	Present	449 (92.38)
		Absent	37 (7.62)
Pollen (from symmetric division)	Uninucleate		510 (11.38)
	Trinucleate		96 (2.14)
	Tetranucleate		374 (8.35)
	Normal		3500 (78.13)

self-pollination of sexual genitors. If, however, the mutant is causing serious sterility it should be reconsidered in the selection of potential genitors. Another point to take into consideration is that apomictic accessions of *Brachiaria* are also pseudogamous. This means that the central-cell nucleus needs to be fertilized for endosperm and healthy seed development, even though the egg cell is not fertilized in an aposporous embryo sac. The present mutation affecting pollen development, added to many other abnormalities detected during microsporogenesis due to polyploidy, and also by the hybrid condition, compromised pollen fertility. Pollen grains in the hybrid were completely sterile. The *Brachiaria* breeding programme aims at producing great quantities of seeds to meet the demand of cattle breeders to cover million of hectares of pastures in the Brazilian savanna. Thus, hybrids like the present one, displaying severe meiotic abnormalities compromising pollen fertility, are unsuitable as cultivars.

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