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An original meiotic mutation in *Paspalum regnellii*

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Abstract Cytogenetic studies carried out over a period of 2 consecutive years on a native Brazilian accession of *Paspalum regnellii* (2n=40) revealed a meiotic mutation that has not been previously reported for any other species. Among 13 inflorescences investigated during the first collection year, three presented anomalous meiotic behavior starting from metaphase I. At the beginning of this phase, the chromosomes occupied the entire equatorial plate in a membrane-to-membrane arrangement, and the spindle fibers, which were clearly visible, did not converge towards the poles. Degeneration of spindle fibers occurred at the end of metaphase I. Chromosome segregation did not occur and the bivalents were left scattered at random in the cytoplasm. Remnants of chromosome fibers could be seen close to the centromere during this stage. The bivalents gave origin to micronuclei in telophase I, with extremely wide variations in number and size among cells. With the absence of spindle formation during meiosis II, metaphase and anaphase II were not observed. Second cytokinesis occurred in prophase II cells after the occurrence of first cytokinesis. The final product of meiosis was completely abnormal, with a predominance of polyads with microspores of different sizes that resulted in abortive pollen grains. In the affected inflorescences, all microsporocytes presented this anomaly, which caused total sterility.

Key words *Paspalum regnellii* · Meiosis · Mutant · Pollen sterility

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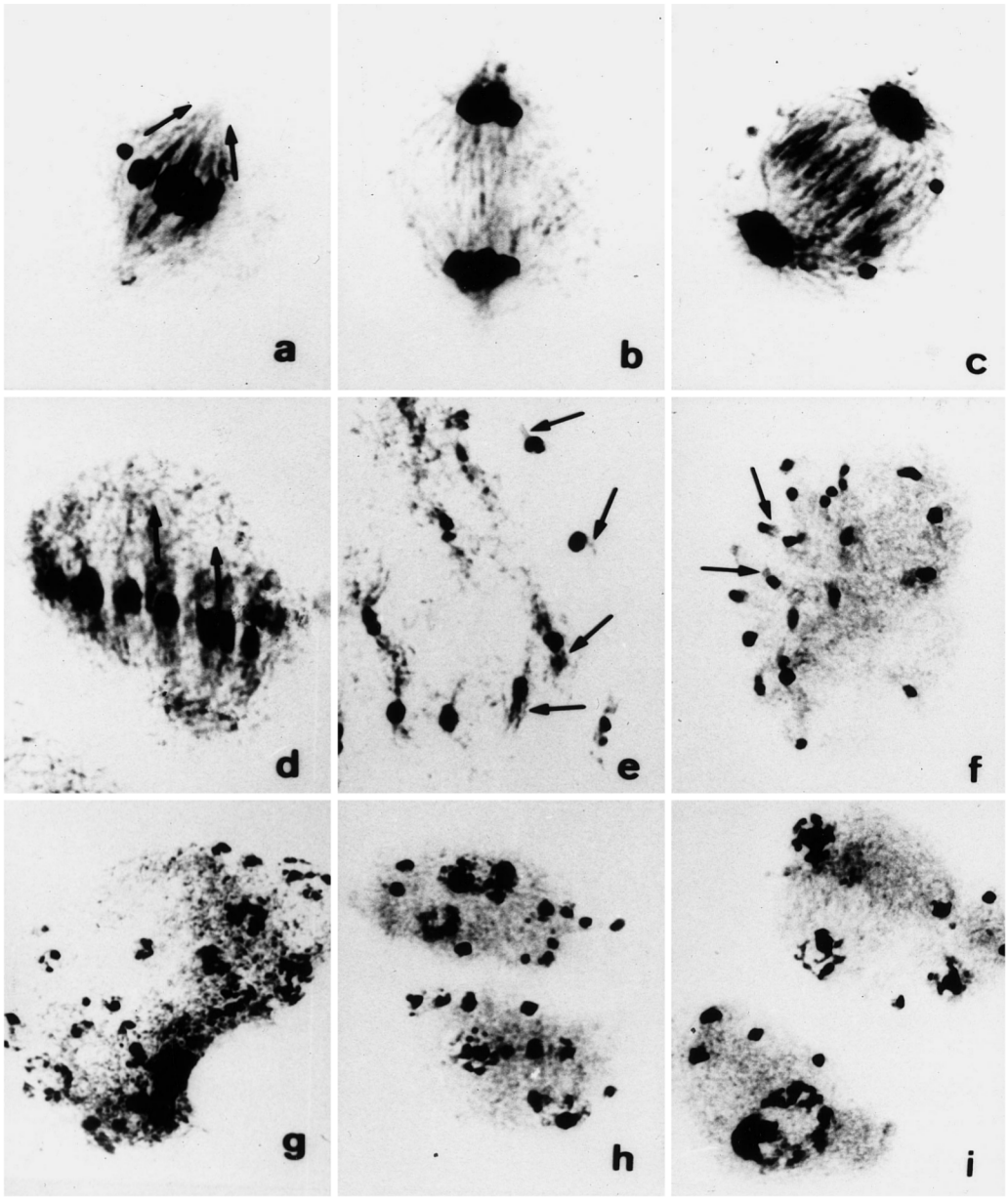
Introduction

Micro- and megasporogenesis comprise three sequential stages that culminate with the production of gametes. These stages, pre-meiosis, meiosis and post-meiosis, are controlled and coordinated by a diversity of genes (Mascarenhas 1992). Meiosis, in addition to being the stage of longest duration, is also the stage that consumes the most cell energy. It is controlled by a larger number of genes. Although consisting of individual components, suggesting independent hierarchical gene control at each step, meiosis is a highly coherent integrated process. These steps may be changed, however, by the presence of mutant genes, resulting in abnormal meiotic products that prevent gamete formation and impair plant fertility. Similarly, pre- and post-meiosis also may be affected by the action of mutant genes. These events, in contrast to meiosis, are controlled by a relatively small number of genes. However, the major mutants causing male sterility are post-meiotic (Kaul and Nirmala 1989).

Meiotic mutants have been extensively reported in higher plants (Baker et al. 1976; Koduru and Rao 1981; Kaul and Murthy 1985). More than 20 genes that affect different steps of meiosis have been reported for maize alone (Golubovskaya 1989). In *Arabidopsis thaliana*, male-sterile mutants have been described extensively in the past 5 years (Chaudhury et al. 1992, 1994; Aarts et al. 1993; Dawson et al. 1993; Preuss et al. 1993, 1994; Xu et al. 1995; He et al. 1996; Peirson et al. 1996) and some of them have been studied at the molecular level (Peirson et al. 1996). In the genus *Paspalum*, however, few meiotic mutants have been reported, despite the fact that microsporogenesis was evaluated in a considerable number of species (Snyder 1961; Fang and Li 1966; Pi and Chao 1974; Burson 1975; Mehra and Chaudhary 1981).

During studies directed at the cytogenetic characterization of *P. regnellii*, we detected a mutation that has not been previously reported for any other species. This report describes the characteristics and behavior of this new meiotic mutant.



Materials and methods

We analyzed a *P. regnellii* accession (BRA-019186) belonging to the germ plasm collection of the Centro de Pesquisa de Pecuária do Sudeste (CPPSE/EMBRAPA) located in São Carlos, São Paulo, where it is kept in pots. The accession was collected in the municipality of Rio Claro, São Paulo, where it occurred as a native species.

Inflorescences in the ideal stage for meiotic studies were collected on two occasions, in the spring/summer of 1994 and the same time in 1995. Thirteen inflorescences were collected on the first occasion and 84 on the second. The material was fixed in Carnoy for 24 h, transferred to 70% alcohol and stored under refrigeration until use. Microsporocytes were prepared by squashing and staining with 1% propionic carmine. All phases of meiosis were rigorously evaluated, starting from diakinesis.

Fig. 1a–j Aspects of meiotic behavior in plants affected by the mutation. **a** Normal metaphase I with spindle fibers converging towards the poles (*arrows*). **b, c** Normal anaphase I and telophase I demonstrating the convergence of spindle fibers. Two micronuclei are also visible in **c**. **d** Metaphase I in an affected plant showing membrane-to-membrane bivalent arrangement and parallel spindle fibers (*arrows*). **e, f** Bivalents dispersed through the cytoplasm after metaphase I. The *arrows* point to some bivalents, residues of chromosome fibers close to the centromere. **g** Telophase I with countless micronuclei of different sizes. **h, i** Prophase II with countless micronucleoli

Results

During the cytogenetic characterization of accession BRA-019186 of *P. regnellii* carried out to determine

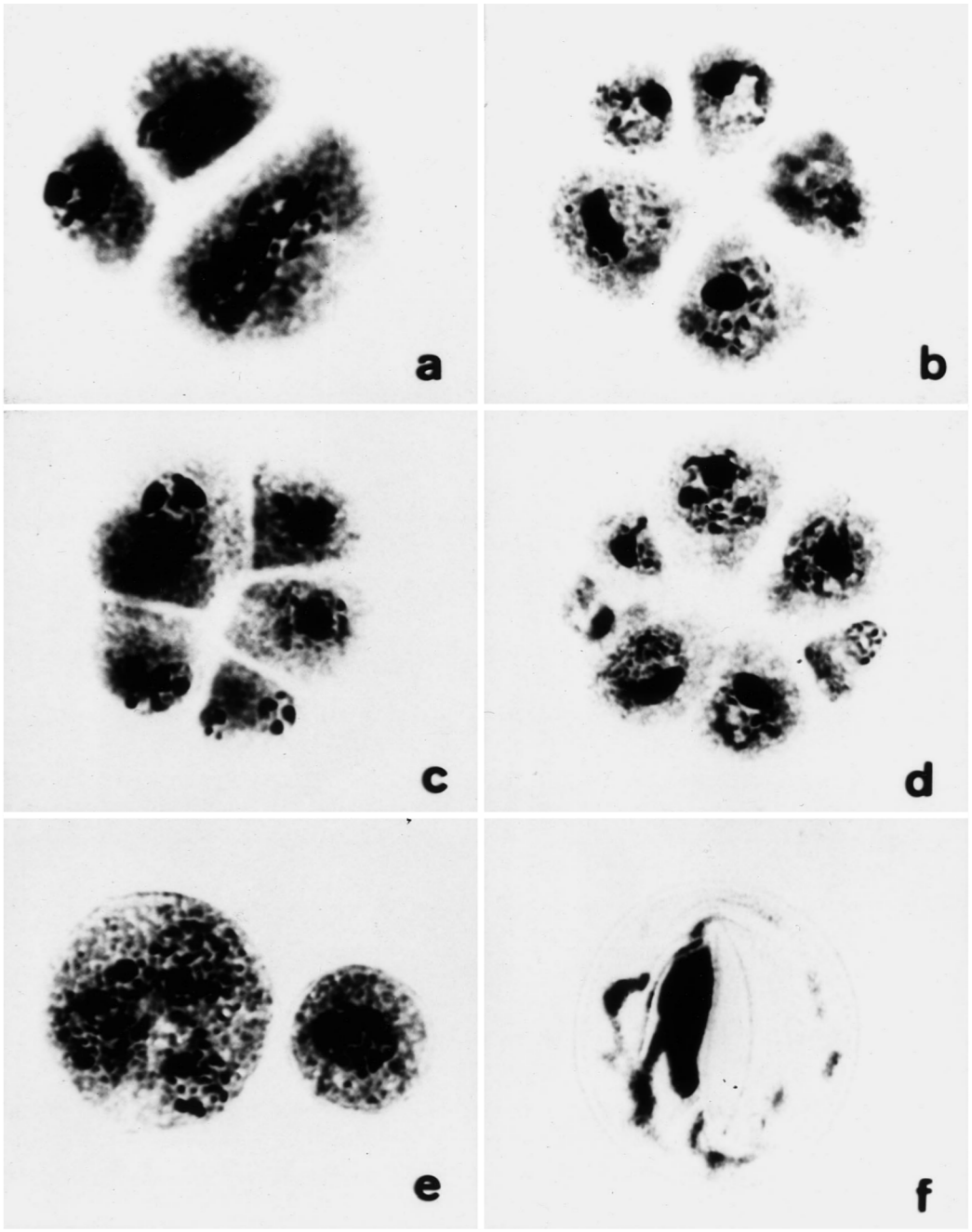


Fig. 2a–f Final product of meiosis in plants affected by the mutation. **a** Microspore triad. **b, c** Pentads with microspores of different sizes. **d** Heptad with microspores of different sizes. **e** Two abnormal microspores, the larger one being trinucleate. **g** Degenerating pollen grain

chromosome number and to evaluate meiotic behavior, we detected, in 3 of the 13 inflorescences from the first collection, a mutation that fully affected the development of meiosis, starting from metaphase I. Up to this stage, meiosis progressed normally and 20 regularly distributed bivalents were clearly observed at diakinesis. Univalent chromosomes were rarely observed and multivalent associations never occurred. Some irregularities started to become visible at metaphase I. Whereas in normal metaphase I bivalents clustered in the center of the cell and spindle fibers converged to the poles (Fig. 1a), in affected plants bivalents occupied the entire equatorial plate in a membrane-to-membrane arrangement and spindle fibers, in addition to being short and deeply stained, did not converge to the poles (Fig. 1d). Figure 1b shows anaphase I and Fig. 1c shows telophase I in unaffected cells,

where spindle fibers can be seen in a normal arrangement. In contrast, in affected plants no conventional anaphase I or telophase I was observed. Soon after metaphase I, without chromosome segregation, the bivalents left the equatorial plate and became irregularly distributed throughout the cytoplasm (Fig. 1e, f). In this stage spindle fibers were clearly almost completely degenerated and were limited to a small region near the centromere, as seen in Fig. 1e, f. Figure 1g shows a typical telophase I of abnormal cells, with countless micronuclei. The number of micronuclei varied as a function of the distance between bivalents. When some bivalents were close to each other they organized a single nucleus, and when they were separated by a distance they formed micronuclei whose maximum number was equal to that of the bivalents, i.e., 20. After telophase I the cells underwent first cytokinesis, giving origin to two cells with many micronuclei (Fig. 1h, i). The phases of meiosis II that depend on the spindle fibers (metaphase and anaphase) were not observed. Thus, soon after first cytokinesis, with the nuclei and micronuclei still organized, second cytokinesis began. Whereas first cytokinesis was normal, leading to the formation of only two cells, second cytokinesis led to the formation of aberrant meiotic products ranging from microspore triads to polyads. Figure 2a–d shows some meiotic products in which microspores of different sizes are observed. Figure 2e shows two microspores already isolated, the larger one trinucleate. Although different sizes, at first the pollen grains formed stained with the same intensity. However, during their development chromatin started to disintegrate (Fig. 2f) and the pollen grains eventually became completely inviable.

Although only inflorescences from the first collection presented this meiotic behavior, all of their cells were affected by the irregularity.

Discussion

The genus *Paspalum* comprises more than 400 tropical and subtropical species, most of them native to South America. In Brazil, the genus has approximately 220 species and occurs in practically all herbaceous communities in the various ecosystems of the country, where many species form natural pastures (Valls 1987).

Although various studies are available that describe the meiotic behavior of the genus *Paspalum*, some of them conducted on species native to Brazil, meiotic mutants have been reported only for *P. secans* (Snyder 1961), *P. conjugatum* (Fang and Li 1966; Mehra and Chaudhary 1981), *P. longifolium* (Pi and Chao 1974; Mehra and Chaudhary 1981), *P. commersoni* (Pi and Chao 1974), *P. proliferum* (Burson 1975), *P. dilatatum* and *P. orbiculare* (Mehra and Chaudhary 1981). With the exception of *P. proliferum* (Burson 1975), in which the absence of first cytokinesis was observed, the mutations in the remaining species were related to the synapsis of the chromosomes.

Meiotic mutations affecting different steps of meiosis have been extensively reported in higher plants (for reviews, see Baker et al. 1976; ; Golubovskaya 1979, 1989; Koduru and Rao 1981; Kaul and Murthy 1985). However, mutations that specifically affect the dividing spindle were described almost exclusively for maize (Clark 1940; Golubovskaya and Sitinikova 1980; Golubovskaya and Mashnenkov 1981; Golubovskaya and Distanova 1986; Taschetto and Pagliarini 1993). The particular mutation observed in the accession BRA-019186 of *P. regnelli* has never before been reported for any other species.

In metaphase I, this mutation was very similar to the divergent spindle mutant (*dv*) described in maize by Clark (1940), Golubovskaya and Mashnenkov (1981) and Staiger and Cande (1991). In this mutant, the spindle fibers are parallel or diverge instead of converging to the poles. However, disjunction is normal and each chromosome group forms a nucleus, usually elongated, that produces its own spindle in meiosis II. The behavior of the three abnormal plants of *P. regnellii*, in principle, was identical to that of the *dv* gene, as demonstrated in Fig. 1d. However, whereas in the *dv* mutant the fibers remain intact until the end of anaphase I, carrying out their function of pulling the chromosomes to the poles, in *P. regnellii* the spindle fibers undergo early disintegration, preventing chromosome segregation. The bivalents with random orientation start to occupy the entire cytoplasm (Fig. 1e, f). In maize, other genes that affect chromosome segregation by interfering with spindle fiber orientation have been reported (Golubovskaya and Sitinikova 1980; Golubovskaya and Distanova 1986).

The meiotic behavior of affected *P. regnellii* plants was similar to that of a maize mutant described by Taschetto and Pagliarini (1993), except for chromosome organization in metaphase I and cytokinesis. In this mutant, meiosis progressed normally until diakinesis, but then went directly from this phase to telophase. All phases of first and second division that depend on the spindle fibers were absent. Since the bivalents became dispersed in the cytoplasm, the number of nuclei varied among cells. The final product of meiosis was a variety of polyad forms. In the plants analyzed here, the chromosomes reached metaphase I, but, due to the early disorganization of the spindle fibers, anaphase did not occur. The bivalents that were scattered at random in the cytoplasm formed micronuclei that varied in number and size from cell to cell (Fig. 1g). Whereas in the maize mutant described by Taschetto and Pagliarini (1993) first cytokinesis did not occur, in *P. regnellii* this phase occurred, leading to the formation of prophase II with a varying number of micronuclei (Fig. 1h, i). Since fiber reorganization did not occur, the phases of second division that depend on the spindle did not occur and second cytokinesis occurred in cells in prophase II, forming structures ranging from triads to a variety of polyads (Fig. 2a–d). The basic difference between the maize and *P. regnellii* mutants is the formation of spindle fibers observed in *P. regnellii* and their early disintegration.

Abnormal chromosome segregation related to late depolymerization of the spindle fibers was reported for a maize mutant (Golubovskaya and Sitinikova 1980), whereas abnormalities related to microtubule disorganization were reported for a mutant of *Aspergillus nidulans* (Oakley and Morris 1981). In this fungus, a mutation in the beta-tubulin molecule prevented chromosome movement to the poles. In the mutant plants of *P. regnellii* there was indeed early disorganization of the spindle fibers, but the cause of this phenomenon is unknown.

Some meiotic mutants present characteristics that may be exploited successfully in breeding programs. Among them are those that cause male sterility. The mutation detected in these plants, by fully affecting meiosis, causes sterility, as demonstrated by the inviability of pollen grains (Fig. 2f). However, since only microsporogenesis was investigated, we do not know if megasporogenesis was also affected, causing female sterility as well. However, it should be considered that most of the tetraploid species (2n=4x=40) of *Paspalum*, including *P. regnellii*, are apomictic and therefore do not utilize their gametes for reproduction.

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