Meiotic instability in invader plants of signal grass *Brachiaria decumbens* Stapf (Gramineae)

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ABSTRACT. *Brachiaria decumbens* is a tropical grass, native of the African savannas, with a narrow natural distribution. On the other hand, *B. decumbens* cv. *Basilisk* is a perennial forage grass widely used for pasture in the tropics. This cultivar was introduced in Australia in 1930 and thence to Brazil in the 60s. Although it covers millions of hectares in the country, it is considered a weed outside the pasture environment. Invader plants of *B. decumbens,* probably of the cultivar *Basilisk,* were collected on the grounds of the State University of Maringá, PR, and cytologically analyzed. Meiotic abnormalities were recorded with great frequency, some of which had been previously reported neither for *B. decumbens* nor for other *Brachiaria* species. Among the irregularities, abnormal chromosome segregation, desynapsis, chromosome stickiness and fusional syncytes were reported. The influence of the meiotic instability in causing pollen sterility is discussed.

Key words: *Brachiaria decumbens,* meiotic instability, chromosome stickiness, fusional syncytes, pollen viability, seed production.

RESUMO. Instabilidade meiótica em plantas invasoras de *Brachiaria decumbens Stapf* (Gramineae). *Brachiaria decumbens* é uma gramínea tropical, nativa das savanas africanas, com ampla distribuição natural. Por outro lado, *B. decumbens* cv. *Basilisk* é uma gramínea perene amplamente utilizada nos trópicos como pastagem. Esta cultivar foi introduzida na Austrália em 1930 e daí, em 1960, foi trazida para o Brasil. Embora cubra milhões de hectares de pastagens do Brasil, é considerada uma erva daninha quando ocorre fora destes ambientes. Plantas invasoras de *B. decumbens,* provavelmente da cultivar *Basilisk,* foram coletadas no campus da Universidade Estadual de Maringá e citologicamente avaliadas. Alta instabilidade meiótica foi encontrada entre as plantas analisadas, sendo que algumas anormalidades nunca haviam sido descritas para *Brachiaria decumbens* ou qualquer outra espécie de *Brachiaria.* Entre as anormalidades encontradas, descreve-se a ocorrência de segregação cromossômica irregular, dessinapse, aderência cromossômica e sincícios fusionais. É discutida a influência da instabilidade meiótica sobre a esterilidade do pólen.

Palavras-chave: *Brachiaria decumbens,* instabilidade meiótica, aderência cromossômica, sincícios fusionais, viabilidade de pólen, produção de sementes.

Animal production in the tropics is largely dependent on either native or introduced pastures. To solve the problems in animal nutrition, especially during the dry season, a few *Brachiaria* accessions were introduced in Brazil as early as 1952. However, in the 60s *Brachiaria* seeds, mainly *B. decumbens* cv. *Basilisk,* were imported from Australia to form thousands of hectares of pastures in the savanna regions of Brazil (Valle and Glienke, 1991).

B. decumbens cv. *Basilisk* is a perennial tropical forage grass, native of East African savannas, introduced in Australia from seeds given by the Uganda Department of Agriculture in 1930. It was approved for commercial release in Australia in 1966 and registered in 1973 (Oram, 1990). Well adapted to infertile acid soils, this cultivar forms an aggressive, high-yielding sward that withstands heavy grazing and trampling (Keller-Grein *et al*., 1996). Although it is a palatable grass, it produces forage of low nutritive value, especially during the dry winter months (Valle *et al*., 1989). It is also susceptible to spittlebugs, a characteristic that reduces its value as a pasture plant in areas where this pest is a major constraint, such as in the neotropical savannas (Lapointe, 1993).

B. decumbens has a narrow natural distribution. The species is very common in Kenya, Rwanda, Burundi and Uganda and it is found in deciduous bushland, grasslands and at forest edges (Keller-Grein *et al*., 1996). Despite the good adaptation to savanna regions of Brazil and the high economic importance of *B. decumbens* for animal production in many tropical countries, few cytogenetical data about the species are available. Studies have mainly been devoted to chromosome counts (Valle and Glienke, 1991). In this contribution we are reporting the occurrence of meiotic instability recorded in some invader plants of this species.

Material and methods

Plants analyzed were collected in a sward of *B. decumbens* at the State University of Maringá, Maringá, state of Paraná, Brazil, where it occurs as a weed. There are strong reasons to believe that these plants belong to the *Basilisk* cultivar because there is hardly any natural occurrence of *Brachiaria* grasses in Brazil. This genus is essentially African and the invader plants may have grown from plant seeds cultivated as forage in nearby pastures.

Inflorescences in the ideal stage for meiotic study were collected and fixed in ethanol:acetic (3:1) for 24 hours and stored under refrigeration at 4ºC until use. Microsporocytes (PMCs) were prepared by squashing and staining with 0.5% propionic carmine. Chromosome number was determined at diakinesis or at metaphase I. All meiotic phases were evaluated and abnormalities recorded. Almost 2000 PMCs were scored from invader plants on the grounds of the university campus. Pollen fertility was evaluated using the same stain employed in meiotic analysis. Photomicrographs were made with a Wild Leitz microscope using Kodak Imagelink - HG, ISO 25 black and white film.

Results and discussion

Interest in analyzing these plants centered around the fact that a previous analysis had shown a high frequency of meiotic abnormalities never described in *B. decumbens;* rather, some of them have never been described in any other *Brachiaria* species. Table 1 presents the percentage of abnormal cells and that of each abnormality per phase. Many different types of abnormalities were found among the PMCs.

The chromosome number scored at diakinesis was found to be 2n=36, characterizing the plants as tetraploid (2n=4x=36). Whereas diploidy (2n=18) and tetraploidy (2n=36) have been reported for different accessions of *B. decumbens* (Valle and Glienke, 1991), *B. decumbens* cv. *Basilisk* is tetraploid (Valle *et al*., 1989). Chromosome associations at diakinesis also confirmed the polyploid condition. Univalent, bivalent and quadrivalent chromosomes were recorded at this phase, with a prevalence of bivalent configurations. The presence of few multivalent associations in tetraploid grasses such as *Brachiaria* (Mendes-Bonatto, 2000) and *Paspalum* (Freitas *et al*., 1997; Takayama *et al*., 1998) has been interpreted as evidence of segmental allopolyploidy, i.e., where the parental genomes are partially homologous.

Table 1. Total percentage of abnormal PMCs, and percentage of each abnormality per phase

Phase	No. of PMCs	% of abnormal PMCs	Abnormalities/ phases	% of each abnormality
Metaphase I	348	52.3	Precocious migration to poles	100.00
Anaphase I	227	79.74	Desynapsis	29.83
			Laggards	33.16
			Additional plate	7.73
			Stickiness	29.28
Telophase I	279	37.99	Micronuclei	100.00
Prophase II	119	32.77	Micronuclei	100.00
Metaphase II	178	71.91	Precocious migration to poles	92.97
			Stickiness	7.03
Anaphase II	129	64.34	Desynapsis	31.32
			Laggards	54.22
			Additional plate	14.46
Telophase II	167	44.91	Micronuclei	100.00
Tetrad	455	69.45	Micronuclei	72.79
			Triads	2.22
			Pentads	16.77
			Hexads	7.91
			Heptads	0.31

The most frequent abnormalities in the two meiotic divisions were those related to chromosome segregation, such as precocious migration to the poles during metaphase (Figure 1a) and laggards at anaphase (Figure 1b, 1d) that led to the formation of micronuclei at telophase (Figure 1c, 1e). These abnormalities are characteristically found in polyploids. With regard to laggards, an interesting behavior was detected. Besides the typical laggards spread between the poles, during the anaphase of both divisions, a group of chromosomes originated an additional plate (Figure 1b) found in several PMCs. A large number of chromosomes segregating independently of the genome set also suggests allopolyploidy, since the two parental genomes did not present the same rhythm of meiosis. The

combination of two distinct genomes in an interspecies hybrid frequently results in aberrant mitotic and meiotic divisions because the genomes show asynchrony in cell cycle. A well-explained case of asynchrony in cell cycle leading to chromosome elimination was reported by Adamowski *et al.* (1998) in an allotetraploid accession of *Paspalum subciliatum.*

Another common segregational abnormality frequently reported in these plants has been the presence of numerous univalent chromosomes in both divisions. The behavior of these chromosomes was characteristic of synaptic mutants (Figure 2 a, b) in which the chromosomes are widespread between the poles. Asynapsis and desynapsis are widely reported in higher plants (Gottschalk and Kaul, 1980 a, b; Koduru and Rao, 1981), but were never described in the genus *Brachiaria*. 'Asynaptic mutants' partially or totally impaired chromosome association during zygotene-pachytene. Since homologous chromosomes do not associate and chiasmata are not formed, chromosome disjunction is aberrant. On the other hand, in the presence of 'desynaptic mutants' chromosome pairing is normal until pachytene, and abnormalities are cytologically recorded at diplotene-diakinesis when univalents and bivalents can be seen in the cell. According to Koduru and Rao (1981), since a distinction is not always possible, as some species are not amenable to analysis at pachytene, 'synaptic mutants' is a better term to describe the lack of prophase I chromosome pairing.

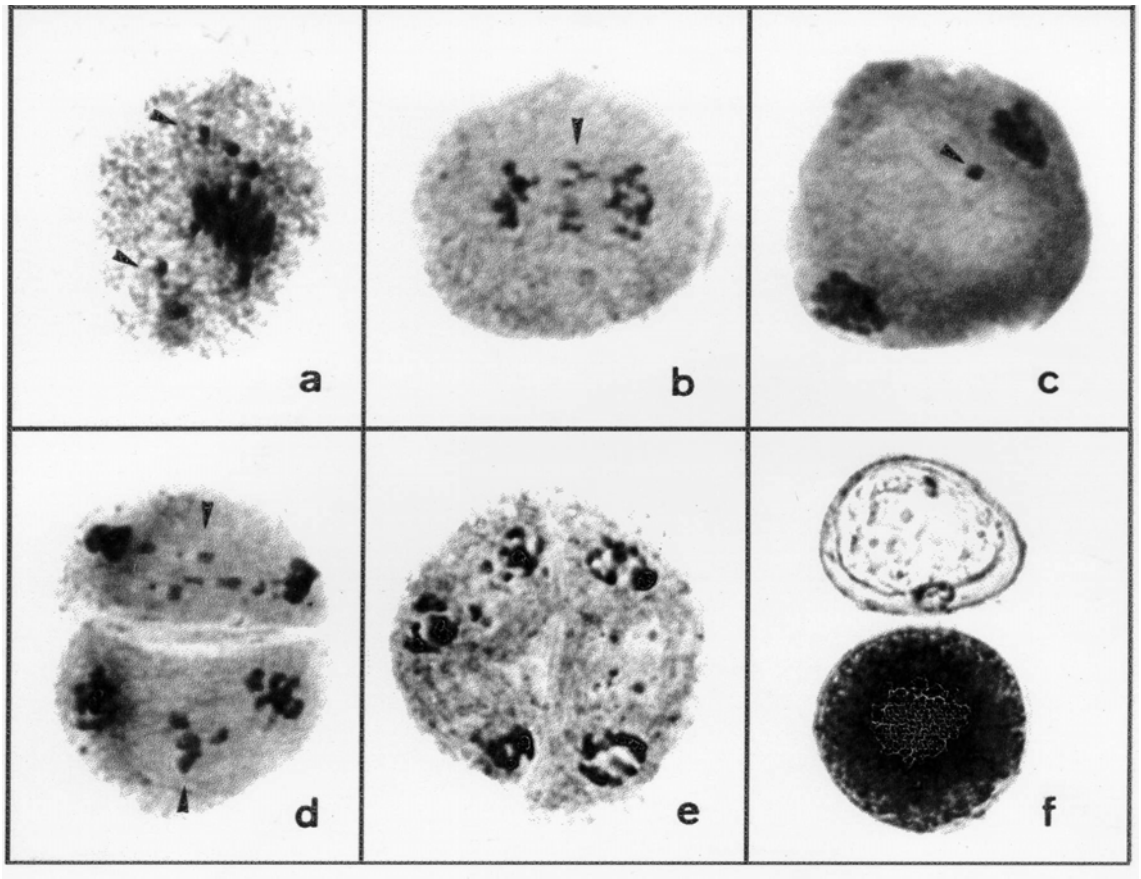

Figure 1. Abnormalities related to chromosome segregation. a) Metaphase I showing precocious chromosome migration at both poles. b) Anaphase I with a group of laggards in a third plate. c) Telophase I with one micronucleus. d) Late anaphase II with many laggards. e) Telophase II showing five non-equally sized nuclei. f) Fertile pollen grain (dark) and sterile one (empty)

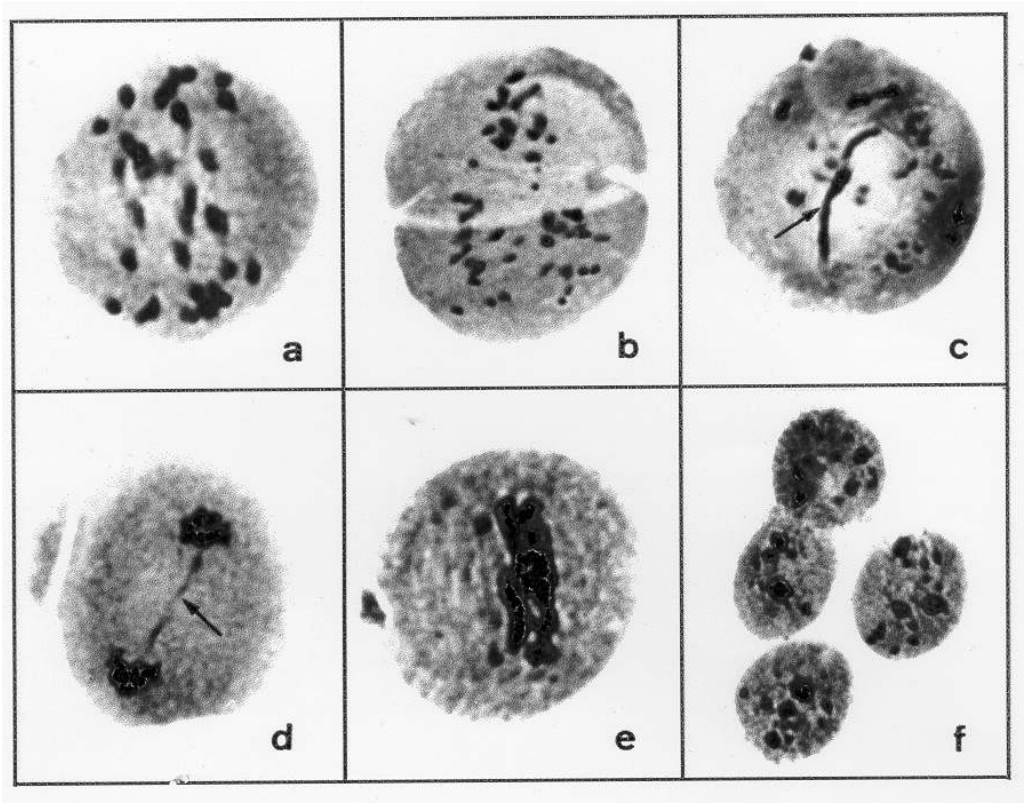

Figure 2. Abnormalities related to chromosome pairing and chromosome stickiness. a) Metaphase I/anaphase I showing chromosome spreading between the poles. b) Metaphase II/anaphase II also showing chromosome spreading between the poles. c) Diakinesis with some bivalents presenting chromosome stickiness (arrow). d) Telophase I showing a thin bridge as a result of chromosome stickiness (arrow). e) Anaphase I showing the chromosomes clumped by severe stickiness. f) Microspores presenting numerous micronuclei as a result of stickiness

Another abnormality also recorded with great frequency in the first and second division, mainly during anaphase I or II, was the occurrence of chromosome stickiness (Figure 2 c - f). Chromosome stickiness is characterized by intense clustering during any phase of the cell cycle. Phenotypic manifestation may be highly variable, ranging from a mild phenomenon involving only a few chromosomes of the genome, to a wide phenomenon involving the entire chromosome complement. In severe cases of stickiness, the impossibility of chromosome separation leads to the formation of single or varying numbers of pycnotic nuclei that culminate in full chromatin degeneration. In the plants analyzed the phenotypic manifestation of the phenomenon ranged from mild, involving a few chromosomes (Figure 2 c, d), to severe stickiness, involving all chromosomes (Figure 2 e). Thin (Figure 2 d) to thick (Figure 2 e) bridges were recorded at anaphase, and sometimes persisting until telophase, impairing chromosome segregation. The intensity of the phenomenon could also be evaluated by the presence of numerous pycnotic micronuclei in microspores (Figure 2 f). A similar case of chromosome stickiness was found in one tetraploid accession of *B. brizantha* analyzed by Mendes-Bonato (2000) and consists of the only report of the phenomenon for the genus *Brachiaria.* Chromosome stickiness may be caused by genetic or environmental factors (Consolaro and Pagliarini, 1996; Souza and Pagliarini, 1996). Although many studies have reported the occurrence of chromosome stickiness, the primary cause and the biochemical basis of this phenomenon are still unknown. Gaulden (1987) hypothesized that stickiness may be caused by the defective functioning of one or two types of specific non-histone proteins involved in chromosome organization that might be necessary for chromatid separation and segregation. Moreover, variability in the degree of stickiness depends on the number of the target protein molecules affected by its inhibitors. Thus not all the cells are equally affected by the phenomenon.

All these abnormalities lead to micronuclei formation at the end of first and second division what

could affect the final product of meiosis. The fate of micronuclei in higher plants may be diverse. In some species the micronuclei originated during meiosis remain as such in the tetrad stage, whereas they form microcytes in other species. In *B. decumbens* both behaviors were observed and polyads with five to seven microcytes/microspores of different sizes were found (Figure 3). This behavior of micronuclei seems to be somewhat common in the genus *Brachiaria* because it occurred in *B. brizantha* (Mendes-Bonato, 2000) and in interspecific hybrids of *Brachiaria* too (Letteriello *et al*., 1998).

The most unusual abnormality recorded in these plants was the occurrence of cell fusion leading to syncyte formation. The number of cells involved in the process ranged from two to several (Figure 4 a-e), and generally they were in the same phase of meiosis. Another interesting aspect of the syncytes is that many of them showed severe chromosome stickiness (Figure 4 b, 4 e). Cell fusion leading to syncyte formation, a phenomenon widely reported in higher plants (Price, 1956; Kamra 1960 a, b; Nirmala and Rao, 1996; Caetano-Pereira *et al*., 1998, 1999), was reported in an hexaploid accession of *B. brizantha* by Mendes-Bonato (2000). According to Nirmala and Rao (1996), a syncyte is a periplasmodial mass containing more than one nucleus that may occur individually or coalesced, while forming a polyploid nucleus. They may be formed by suppression in the cell wall formation in premeiotic mitosis (archesporial syncytes) or by PMC fusion caused by dissolution of the cell wall during the prophase of meiosis (fusional syncytes). While archesporial syncytes have regular contours, the latter present irregular ones. Thus, in *B. decumbens* analyzed the syncytes with irregular contours may be characterized as 'fusional syncytes'. In the hexaploid accession of *B. brizantha* analyzed by Mendes-Bonato (2000), the syncytes presented regular contours and were characterized as 'archesporial syncytes'. Syncyte formation may be induced by genetic and environmental factors (Nirmala and Rao, 1996; Rao and Koduru, 1978; Rao *et al*., 1991), and generally lead to the formation of abnormal pollen grains.

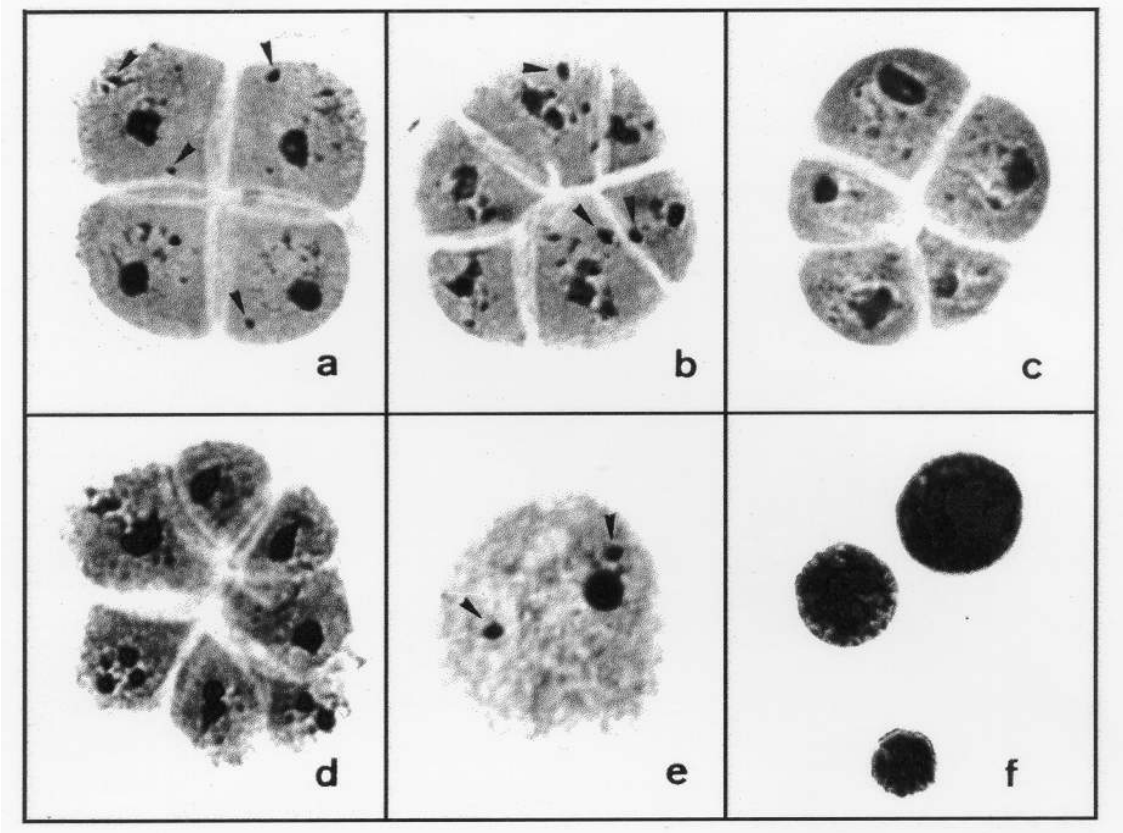

Figure 3. Some aspects on the fate of micronuclei at the end of meiosis. a) Tetrad of microspores with numerous micronuclei (arrowheads). b) Hexad showing unequally sized microspores and micronuclei in some of them (arrowheads). c) Pentad with microspores without micronuclei. d) Heptad with unequally sized microspores. e) Microspore with two micronuclei (arrowheads). f) Variation in size of pollen grains

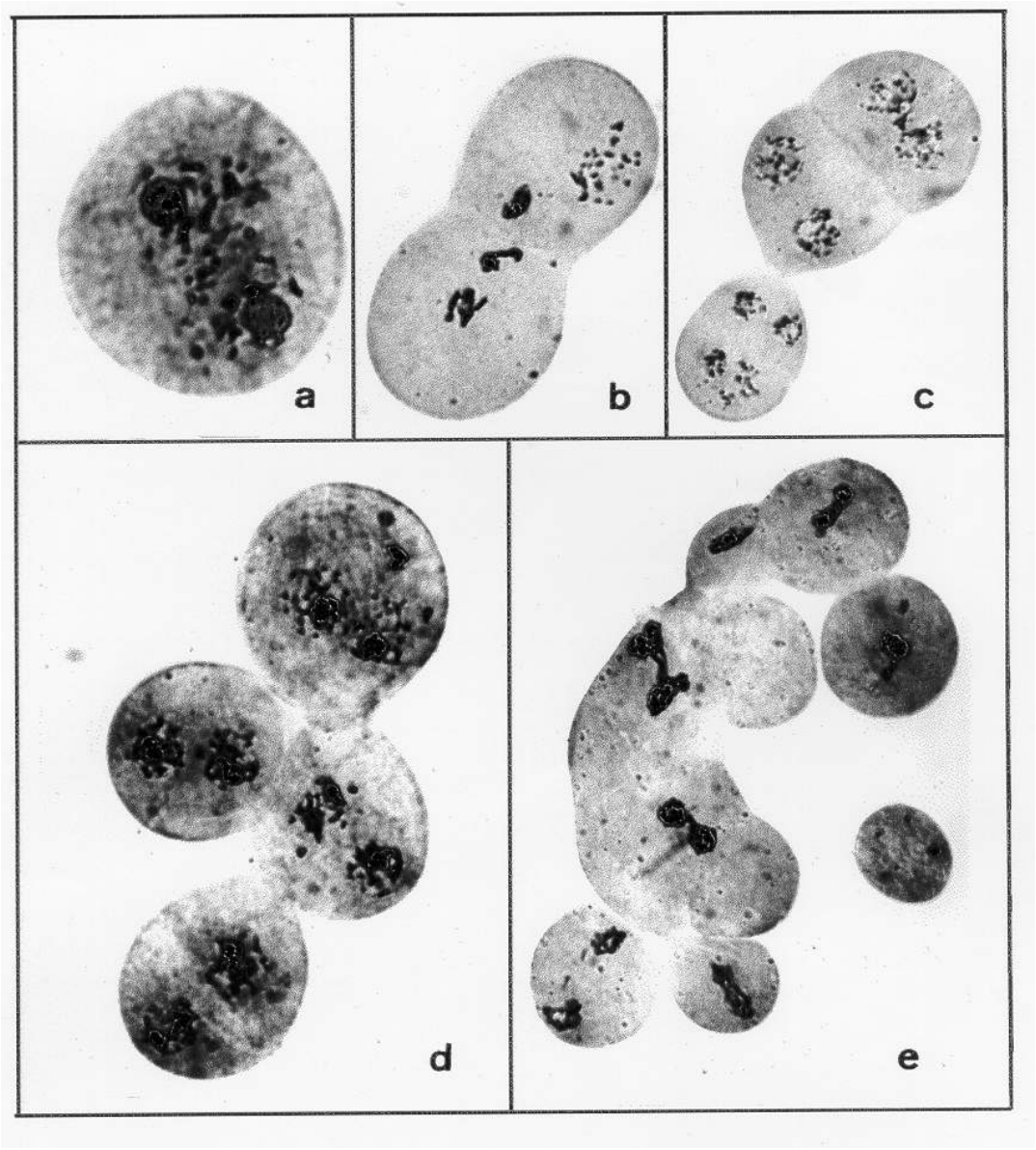

Figure 4. Cell fusion among microsporocytes. a) Fusion between two microsporocytes in prophase I. b) Fusion between microsporocytes in different phases of meiosis. Observe the stickiness clumping the chromosomes into three groups. c) Fusion between two differently sized microsporocytes in telophase I. d) Fusion among four microsporocytes. e) Fusion among microsporocytes. Observe the severity of stickiness clumping the chromosomes.

Such high levels of meiotic abnormalities obviously compromise pollen viability. Pollen sterility was estimated at 52.73%. Microspores (Figure 3 e), pollen grains with micronuclei and pollen grains of different sizes (Figure 3 f) were frequently observed. In spite of their high meiotic instability and pollen sterility, the seed production in these plants was not apparently affected, suggesting that they are apomictic.

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