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A male-sterile mutation in soybean (*Glycine max*) affecting chromosome arrangement in metaphase plate and cytokinesis

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ABSTRACT: A spontaneous male-sterile, female-fertile mutation affecting bivalent arrangement at the metaphase plate and cytokinesis was detected in line BR98-197 of the soybean breeding program developed by Embrapa – National Soybean Research Centre. Untill diakinesis, meiosis was normal with chromosome pairing as bivalents. From this phase, in several meiocytes, bivalents were not able to organize a single metaphase plate and remained scattered in the cytoplasm in a few or several groups. In these meiocytes, chromosomes segregated in both divisions giving rise to several micronuclei. However, the main cause of male sterility was the absence of cytokinesis after telophase II. Instead of the typical tetrads of microspores, four nucleate coenocytic microspores were formed. In the mutant, pollen mitoses did not occur, and after engorgement by starch, pollen underwent a progressive process of degeneration.

Introduction

In autogamous crop species with small flowers, such as soybean, manual cross-pollination to produce large quantities of hybrid seed is difficult and tedious (Fehr, 1987). Insect cross-pollination of male-sterile soybean plants facilitates the production of hybrid seed (Lewers *et al.,* 1996). A number of molecular approaches have been used to study and regulate male sterility on various agricultural crops for F1 hybrid production (Ku *et al*., 2003). On the other hand, there are many plant species that display natural mutants that cause male sterility. Mutant nuclear genes (Mendelian inheritance) affecting male cell and organ development are designated *ms* genes. They usually are recessive and typically expressed in specific sporophytic tissues at different stages (Horner and Palmer, 1995).

Several soybean male-sterile, female-sterile lines (*st* series, from *st2* to *st8*) have been described genetically and cytologically (Palmer and Horner, 2000), and also male-sterile, female-fertile mutations (*ms* series, from *ms1* to *ms9*) have been reported (Palmer, 2000; Palmer *et al.,* 2001). Spontaneous male-sterile, female-fertile mutations have been identified in Brazilian lines of soybean breeding program developed by Embrapa – National Soybean Research Centre. These mutants have been studied in the genetical and cytological context (Bione *et al*., 2002a, b, c, d, 2003, 2004). Some of these mutations are similar to those reported by Palmer and co-workers through some decades in American lines of

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soybean, while others are novel. Additional cytological data about the *ms* mutant series could improve knowledge on their uses in breeding programmes. In this paper, the cytological details of microsporogenesis of a spontaneous male-sterile, female-fertile mutant in soybean (BR98-197) are provided.

Material and Methods

Soybean male-sterile plants (MS) have been recorded in the breeding program carried out by Embrapa Soybean in Londrina PR Brazil. The male-sterile plants could not be distinguished from fertile ones until pod formation, when a low number of pods or their total absence under isolation were observed on green plants that retained their leaves at maturity. Male-fertile plants were selected and evaluated for segregation in the following year. They were designated BR98-197. The heterozygous form BR98-197H is kept at the Soybean Germplasm Collection by Embrapa Soybean, Londrina PR, Brazil.

Plant progeny tests were conducted in a greenhouse so that heterozygous plants segregating for sterility could be identified. About 12 seeds resulting from self-pollination of normal plants were grown in pots. Progenies with fertile and male-sterile plants were considered heterozygotes. Remaining seeds from heterozygotes for the MS character were once more sown in pots. The first flowers were tested for pollen viability and 0.5% propionic carmine was used for staining. Sterile plants were identified, flower buds were collected between 9:00 and 12:00 a.m. in ideal stage for meiotic analysis and fixed in FAA (3 ethanol 95%: 1 acetic acid: 1 formaldehyde) for 24 h; transferred to 70% alcohol and stored at 4ºC until use. Microsporocytes were prepared by squashing and stained with 0.5% acetic carmine. Nine male-sterile plants were cytologically analyzed. The number of microsporocytes analyzed per plant ranged from 110 to 450, comprising the stages from metaphase I to telophase II. One thousand pollen grains stained with 0.5% acetic carmine were randomly scored from each sterile plant to estimatepollen sterility. Starch content in pollen grains was evaluated with lugol.

Results

Light-microscope analysis of meiotic behaviour in nine male-sterile plants of line BR98-197H revealed a typical *ms* mutation affecting microspore development. While fertile plants presented totally regular meiosis and pollen fertility, the male-sterile plants presented considerable amount of meiotic abnormalities from metaphase I through telophase II (Table 1). Untill diakinesis, meiosis was normal with chromosomes pairing as 20 bivalents (Fig. 1 a). However, from this phase, in several meiocytes in each plant, bivalents were not able to organize a single metaphase plate (see Table 1), remaining scattered in the cytoplasm in a few or several groups (Fig. 1 b to f). In these meiocytes, chromosomes segregated in anaphase I (Fig. 1 g) and anaphase

Table 1.

Percentage of normal cells at different phases of meiosis and pollen sterility in the line BR98-197

Plant	Number of cells/plant	Phases of meiosis							Pollen sterility
		Metaphase I	Anaphase I	Telophase I	Metaphase II	Anaphase II	Telophase II	Tetrads*	
1	405	59.3	69.8	58.8	68.0	81.5	69.6	0.00	100.0
2	241	34.3	91.7	72.1	100.0	100.0	87.2	0.00	100.0
3	396	23.8	75.0	48.6	62.5	57.9	63.1	0.00	100.0
4	450	37.6	50.0	72.1	55.6	61.539	80.0	0.00	100.0
5	178	25.0	80.0	60.6	75.0	75.0	74.4	0.00	100.0
6	185	51.9	58.8	63.0	23.5	100.0	70.5	0.00	100.0
7	287	47.8	91.7	84.8	41.8	100.0	77.3	0.00	100.0
8	129	32.2	60.0	73.3	37.5	71.4	83.3	0.00	100.0
9	110	75.0	14.3	88.9	12.0	100.0	90.3	0.00	100.0
Means/phase		43.0	65.6	69.1	51.5	83.0	77.3	0.00	100.0

* Typical tetrads were not formed

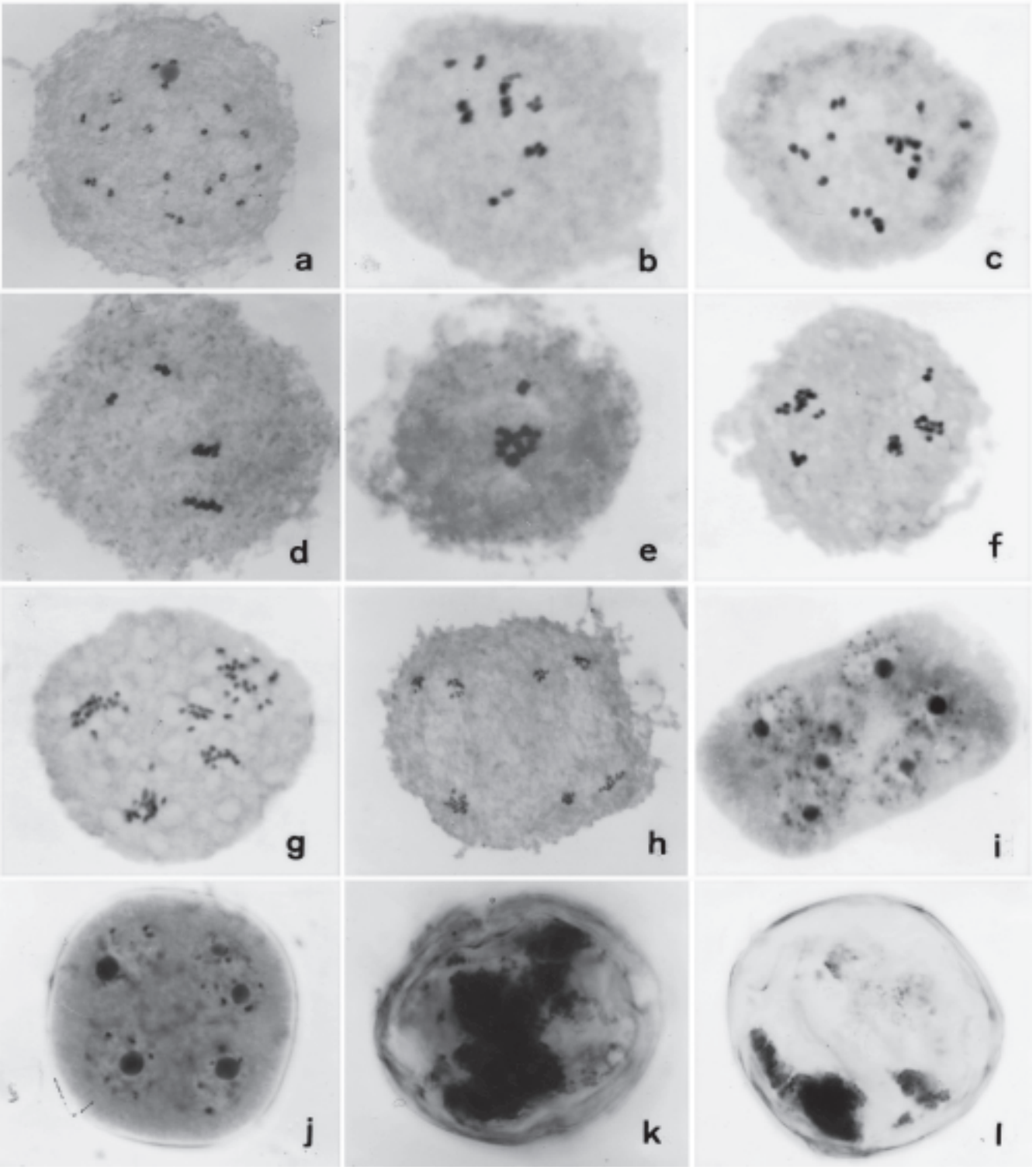

FIGURE 1. Aspects of abnormalities recorded in the two meiotic divisions of the soybean BR98-197 mutant. a) Diakinesis with 20 bivalents. b-f) Bivalents scattered at the cytoplasm forming a few or several groups. Note the absence of a typical metaphase plate. g-h) Chromosome segregation at anaphase I (g) and anaphase II (h). i) Telophase II with various nuclei. j) Four-nucleate coenocytic microspore. k) Pollen grain with starch reserve. l) Degenerative and sterile pollen grain. (X 400)

II (Fig. 1 h) giving rise to several micronuclei in telophase II (Fig. 1 i).

Despite the severity of these abnormalities which involved the meiotic products, their frequency was insufficient to explain complete pollen sterility. The main cause of pollen sterility was attributed to total absence of cytokinesis following telophase II. Typical tetrads of microspores were not detected in the present mutant. Instead of four haploid microspores, the meiotic products were replaced by four-nucleate coenocytic microspores (Fig. 1 j). After pollen wall formation, and without suffering the pollen mitoses, the coenocytic microspores underwent gradual engorgement by starch, and entered into a progressive process of degeneration. Pollen sterility was complete.

A ratio of a 3 male-fertile:1 male-sterile plant was found among self-pollinated progeny in line BR98-197H, where the mutation originally appeared and within segregating families.

Discussion

Absence or defects in cytokinesis following telophase II have been reported in *ms1* (Brim and Young, 1971; Albertsen and Palmer, 1979) and *ms4* (Delannay and Palmer, 1982; Graybosch and Palmer, 1985) male-sterile soybean mutants. Sterility of *ms1* mutants is caused by failure of cytokinesis after telophase II. The four-nucleate microspores develop a pollen-like wall and become engorged with lipid and starch reserves. In fact, coenocytic microspores usually degenerate after engorgement. In *ms4* mutants, Delannay and Palmer (1982) reported total absence of cytokinesis following telophase II also resulting in four-nucleate coenocytic microspores, which soon degenerated or underwent some irregular divisions, and formed various kinds of 1 to 4 cell aggregates that degenerated almost immediately. Some of the aggregates persisted until anthesis and, in some instances, functional pollen grains were released due to a delayed cytokinesis which occurred after pollen-wall deposition. In these cases, the results were tetrad-like clusters of cells resembling pollen grains. While the extent and frequency of cytokinesis varied between and within locules of single anthers, they also were influenced by temperature. According to Delannay and Palmer (1982), cellular aggregates seemed to be a more unique feature of *ms4* male-sterile plants than that of individualised pollen grains. On the other hand, Graybosch and Palmer (1985) reported that in their *ms4* mutant, cytokinesis following telophase II varied between incomplete, totally absent, or disoriented, and resulted in microspores with different number of nuclei.

Despite absence of cytokinesis, the mutant under analysis presented abnormalities after diakinesis affecting a great number of meiocytes. These abnormalities were never reported in any other Brazilian or American male-sterile line of soybean either alone or associated with other abnormalities as found here. A low frequency of irregular chromosome segregation has been reported in both divisions in some Brazilian male-sterile lines by Bione *et al*. (2002 c, 2003), but in meiocytes presenting normal metaphase plate. In the present mutant, the inability of bivalents to converge into a single metaphase plate could be associated to irregular orientation of spindle fibers. Regular chromosome segregation into two daughter cells is possible with a properly ordered bipolar division spindle. In higher plants, the centrosome and spindle polar organisers are not identified as cellular morphological structures. Plant spindles have no asters, however, microtubule (MT) bundles converge with their (-) ends at the poles and interdigitate with their (+) ends at the spindle equator, just as in the astral spindle (Euteneuer *et al*., 1982). The principles of plant spindle formation are still not completely understood, although several hypotheses have been suggested (Mazia, 1987; Smirnova and Bajer, 1998; Chan and Cande, 1998). Shamina *et al*. (2000) considered that it is essential to examine the morphological phenotypes of mutations which disrupt various stages of division spindle formation to address the problem. In this context, mutant plant meiocytes are an important instrumental tool because they are sizeable and sharply synchronized with respect to the stages of division within the anther. Taking into account the bivalent disposition inside the cytoplasm in the present male-sterile line, it is possible that the mutation affected the microtubule organizing centers (MOTCs).

At least three male-sterile mutant lines identified in the Brazilian breeding program and critically analyzed in the cytological context by Bione *et al*. (2002 a, 2003, 2004), presented differential characteristics from those reported by Palmer and co-workers for a specific *ms* mutation (mainly *ms1 , ms2,* and *ms4*). All of them were related to abnormal cytokinesis after telophase II, but always with some additional characteristics. Various independent occurrences of spontaneous male-sterile mutations (*st* and *ms* loci) have been reported in the American soybean breeding programs (Skorupska and Palmer, 1989, 1990; Palmer 2000), and they are currently being identified in Brazilian soybean breeding

programs too (Bione *et al*., 2002 a, b, c, d, 2003, 2004). Data suggest that some loci controlling male fertility in soybean tend more towards spontaneous mutations than others. Skorupska and Palmer (1990) and Palmer (2000) have identified new *ms* mutations in populations that were characterized by chromosomal instability.

Segregation ratio for male-fertile : male-sterile plants in BR98-197H was 3:1. As the ratio remained constant in the progenies of heterozygous fertile plants after selfing, this may suggest that mutation has been caused by a single recessive gene. Allelism tests of the present mutant with *ms* soybean mutant series available at the Soybean Genetic Type Collection (USDA/ARS) are in progress in Embrapa Soybean. These tests will show whether the mutant with some differential cytological characteristics is allele to *ms1* or to *ms4* genes, or whether it is a new *ms* mutant.

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