



*Research Note*

## ***ms17*: a meiotic mutation causing partial male sterility in a corn silage hybrid**

M.S. Pagliarini<sup>1</sup>, V.F. Souza<sup>1</sup>, N. Silva<sup>1</sup>, C.A. Scapim<sup>2</sup>, M. Rodovalho<sup>2</sup>  
and M.V. Faria<sup>3</sup>

<sup>1</sup>Departamento de Biologia Celular e Genética,  
Universidade Estadual de Maringá, Maringá, PR, Brasil

<sup>2</sup>Departamento de Agronomia, Universidade Estadual de Maringá,  
Maringá, PR, Brasil

<sup>3</sup>Departamento de Agronomia,  
Universidade Estadual do Centro-Oeste do Paraná, Guarapuava, PR, Brasil

Corresponding author: M.S. Pagliarini  
E-mail: mspagliarini@uem.br

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**ABSTRACT.** Cytological analysis under light microscopy of the single hybrid P30R50 of silage corn revealed an abnormal pattern of microsporogenesis that affected the meiotic products. Meiosis progressed normally until diakinesis, but before migration to the metaphase plate, bivalents underwent total desynapsis and 20 univalent chromosomes were scattered in the cytoplasm. At this stage, meiocytes also exhibited a number of chromatin-like fragments scattered throughout the cell. Metaphase I was completely abnormal in the affected cells, and univalent chromosomes and fragments were distributed among several curved spindles. Anaphase I did not occur, and each chromosome or group of chromosomes originated a micronucleus. After this phase, an irregular cytokinesis occurred, and secondary meiocytes with several micronuclei were observed. Metaphase II and anaphase II also did not occur, and after the second cytokinesis, the genomes were fractionated into polyads, generating several unbalanced microspores, with various-sized nuclei. About 35% of the tetrads were abnormal in the hybrid.

This spontaneous mutation had been previously reported in a USA maize line called *ms17* and was found to cause male sterility.

**Key words:** *ms17*; Corn silage; Meiotic mutation; Breeding; Male sterility

## INTRODUCTION

In higher plants, male sterility results from many individual developmental events that specify the ontogeny of the male organs. Mutations that impair any step in these processes give rise to defective stamen or pollen development, causing male sterility. Male sterility can be controlled by nuclear or cytoplasmic genes, or both, that affect stamen or pollen development (Kaul, 1988). Male sterility can be the result of gene mutation or environmental factors (Horner and Palmer, 1995). Phenotypes of male sterile mutants range from complete absence of male reproductive organs to the presence of normal stamens, but with functional pollen that fails to dehisce (Dierig and Tomasi, 2001).

Meiosis is genetically the most significant activity of an organism, consisting of highly coordinated physiological, biochemical, and cytogenetic events that lead to gene recombination and chromosome reduction in the gamete. Although these events, which occur during microsporogenesis, are continuous, they are controlled by a large number of genes, the majority of which are dominant and non-allelic to one another (Beadle, 1932; Baker et al., 1976; Gottschalk and Kaul, 1974, 1980a,b; Albertsen and Phillips, 1981; Kaul and Murthy, 1985; Golubovskaya, 1979, 1989; Kaul and Singh, 1991; Peirson et al., 1996; Sosnikhina et al., 2005). Mutations in any of these genes disrupt meiosis and culminate in gametic sterility. A number of male-sterile loci have been identified in several major crops. Corn (*Zea mays* L.) has several non-allelic male-sterile genes that have been characterized (Beadle, 1932; Albertsen and Phillips, 1981; Golubovskaya, 1979, 1989; Palmer et al., 1992).

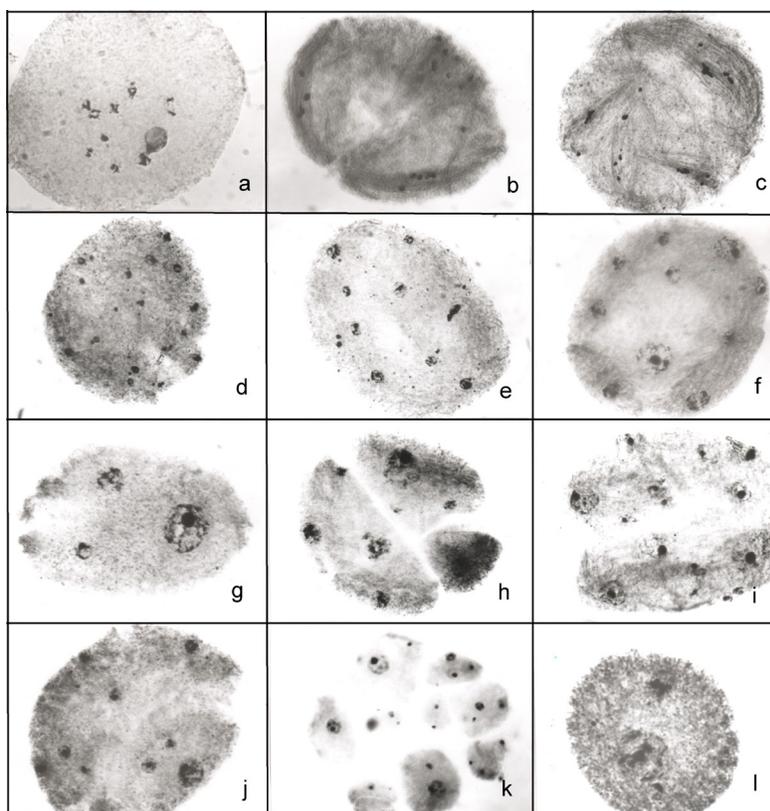
Until recently, it has not been possible to identify and characterize the genes affected in these mutants at the molecular level. However, this has begun to change with the identification of a number of meiotic mutants from *Arabidopsis* and maize (Curtis and Doyle, 1991; Sheridan et al., 1996; Taylor et al., 1998; Sanders et al., 1999). The identification and characterization of plant meiotic genes have enjoyed a tremendous growth in the last decade (Mercier and Grelon, 2008). In this paper, we report the occurrence of a spontaneous mutation affecting microsporogenesis and causing partial male sterility in a commercial corn silage hybrid.

## MATERIAL AND METHODS

The commercial hybrid of corn silage P30R50 (single hybrid; DuPont, Brazil) was cultivated in the Experimental Farm of the State University of Maringá, in the summer of 2008. For meiotic studies, inflorescences were collected and fixed in a mixture of 95% ethanol and acetic acid (3:1) for 24 h, transferred to 70% alcohol, and stored under refrigeration until use. Microsporocytes were prepared by squashing and staining with 1% propionic carmine. Nine plants were analyzed and abnormalities were observed in each phase of microsporogenesis. Photomicrographs were made with a Wild Leitz microscope using Kodak Imagelink - HQ, ISO 25 black and white film.

## RESULTS AND DISCUSSION

In the hybrid P30R50, a special kind of meiotic abnormality was recorded. Meiosis progressed normally until diakinesis, with a few univalents (Figure 1a) and bivalents with terminal chiasma. However, before migration to the metaphase plate, bivalents underwent total desynapsis, and 20 univalent chromosomes were scattered in the cytoplasm (Figure 1b,c). In this stage, meiocytes also exhibited a number of chromatin-like fragments scattered throughout the cell (Figure 1b-e). Metaphase I was completely abnormal in the affected cells and chromosomes were distributed into several abundant curved spindles (Figure 1b,c). Anaphase I did not occur, and each chromosome or group of chromosomes originated a micronucleus (Figure 1d-g). After this phase, the first cytokinesis occurred and secondary meiocytes with several micronuclei were observed in prophase II (Figure 1h-j). Metaphase II and anaphase II also did not occur, and after the second cytokinesis, the genomes were fractionated into polyads (Figure 1k), generating several unbalanced microspores with various-sized nuclei (Figure 1l). Table 1 presents the frequency of affected cells.



**Figure 1.** Aspects of microsporogenesis in the *ms17* corn silage mutant. **a.** Meiocyte in diakinesis showing nine bivalents and a pair of univalents. **b. c.** Metaphase I with abundant curved spindles and several chromatin-like fragments scattered throughout the cell. **d.-g.** Telophase I showing various-sized nuclei. **h.-j.** Prophase II in meiocytes that underwent abnormal cytokinesis. **k.** Polyad of microspores with various-sized nuclei. **l.** Pollen grain with several micronuclei of different sizes.

**Table 1.** Number of cells analyzed, abnormalities recorded, and number of abnormal cells (percentage) per meiotic phase.

Phase	No. of cells analyzed	Abnormality	No. of abnormal cells (%)
Prophase I	1171	Univalents	273 (23.3)
Metaphase I	751	Abnormal spindle	296 (39.4)
Anaphase I	-	Did not occur	-
Telophase I	498	Various-sized nuclei	158 (31.7)
Prophase II	1279	Multinucleated cells	408 (31.9)
Metaphase II	-	Did not occur	-
Anaphase II	-	Did not occur	-
Telophase II	764	Various-sized nuclei	453 (59.3)
Meiotic product	753	Polyads	240 (32.7)
Mean of abnormalities	5216		1828 (35.1)

Besides the polygenes controlling the chiasma frequency (Rees and Thompson, 1956; Lein and Lelley, 1987), two other categories of genes can affect synapsis. The asynaptic genes hinder chromosome pairing (Gottschalk and Kaul, 1980a) while the desynaptic genes promote desynapsis in bivalents before metaphase I (Gottschalk and Kaul, 1980b). In the present hybrid, chromosome pairing was normal, suggesting that a desynaptic gene caused chromosome separation before metaphase I. Desynaptic mutants generally display normal chromosome attachment to the spindle, a phenomenon not observed in this hybrid.

An identical meiotic behavior characterized as meiotic mutation *ms17* was reported in maize by Albertsen and Phillips (1981) and Staiger and Cande (1991). According to these authors, the gene *ms17*, located at chromosome 1, had variable expression that most notably affected spindle formation, as observed in the present corn silage analyzed. In this mutant, an unusual accumulation of microtubules was observed and spindle formation was abnormal, leading to multiple poles and extra spindles. About 35% of tetrads were abnormal in the hybrid. The percentage of abnormal meiotic products was not cited for the *ms17* mutant described by Albertsen and Phillips (1981), but the authors pointed out that it may produce some pollen, since the mechanism for meiotic breakdown is variable.

It is remarkable how meiotic mutations showing the same meiotic behavior can arise independently in different parts of the world. The original mutant *ms17* was selected in segregating progeny of male sterile maize lines by E.B. Paterson, University of Illinois, USA (Albertsen and Phillips, 1981), whereas the present mutant was found in a single commercial corn silage hybrid produced by DuPont, Brazil. In this hybrid, for the mutation to be expressed, both genitors might have contributed to a recessive *ms17* gene. The hybrid P30R50 is one of several commercial hybrids of corn silage of different origins that were selected to initiate the breeding program to obtain inbred lines after selfing. However, considering that the *ms17* mutation affected the meiotic product, we suggest that this hybrid could be discarded from the breeding program as a source of inbred lines to create new silage corn hybrids. Male sterile mutants, however, are considered to be a powerful tool in hybrid breeding programs. Manual cross-pollination for the production of large quantities of hybrid seeds can be difficult and time-consuming. In soybean, for example, genetic male sterility is used to improve efficiency of hybridization by eliminating tedious hand emasculations, to enhance random mating for population development and recurrent selection, and to facilitate the development of testers for inbred line evaluation and development (Cervantes-Martinez et al., 2207).

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