

Microsporogenesis in inbred line of popcorn (*Zea mays* L.)

G.C.L. Ricci¹, N. Silva¹, M.S. Pagliarini¹ and C.A. Scapim²

¹Departamento de Biologia Celular e Genética,
Universidade Estadual de Maringá, Maringá, PR, Brasil

²Departamento de Agronomia, Universidade Estadual de Maringá, Maringá,
PR, Brasil

Corresponding author: M.S. Pagliarini

E-mail: mspagliarini@uem.br

Genet. Mol. Res. 6 (4): 1013-1018 (2007)

Received May 9, 2007

Accepted September 25, 2007

Published November 27, 2007

ABSTRACT. Endogamy places genes for several characteristics in homozygosis, which include those related to meiosis causing abnormalities that may impair gamete viability. An original population (S_0) of popcorn (CMS-43) produced by Embrapa Maize and Sorghum was self-pollinated for seven years, generating inbred lines (S_1 to S_7). Conventional studies of microsporogenesis revealed that meiotic abnormalities did not increase with endogamy. Univalent chromosomes, irregular chromosome segregation, abnormal cell shape, partial asynapsis, cell fusion, absence of cytokinesis, abnormal spindle orientation, and chromosome stickiness were recorded in low frequency in meiocytes. Since the frequency of abnormalities was low, mainly in S_7 , inbred lines from CMS-43 have a high potential for hybridization.

Key words: Endogamy, Inbred lines, Microsporogenesis, Popcorn, Combining ability

INTRODUCTION

The most important breeding objective is to improve yield (Trifunovic et al., 2003), and only lines that possess high breeding value for yield and other traits of interest that have an impact on yield warrant recycling in breeding programs. The main objective of maize breeding programs is to develop new inbred lines with high-combining ability to produce higher grain yields and superior agronomic performance in hybrid combinations. In such breeding programs, the choice of parents is crucial, because it will determine the genetic constitution of the source population, which, in turn, determines the probability of selecting a new superior line (Hallauer and Miranda Filho, 1988).

Meiosis is an event of high evolutionary stability that culminates in the reduction of chromosome number in gametes. Cytological events of meiosis are controlled by a large number of genes acting from pre-meiosis to the post-meiotic mitoses (Baker et al., 1976; Golubovskaya, 1979, 1989). Mutations of these genes may cause anomalies that impair plant fertility (Albertsen and Phillips, 1981; Curtis and Doyle, 1991). When an allogamous plant is submitted to self-pollination, many genes, including those involved in the control of meiosis, experience homozygosis causing inbreeding depression. Irregularities in microsporogenesis due to inbreeding have been reported in several plant species (Lamm, 1936; Myers and Hill, 1943; Morris and Isikan, 1964; Pantulu and Manga, 1972; Karp and Jones, 1982; Defani-Scoarize et al., 1995, 1996; Pagliarini et al., 2002).

The cytological stability of maize inbred lines is an important consideration in view of their extensive use in genetics and plant breeding research (Morris and Isikan, 1964). In alfalfa (Smith and Murphy, 1986) and maize (Morris and Isikan, 1964; Lima et al., 1984; Hallauer and Miranda Filho, 1988; Pagliarini, 1989), seed production was shown to be severely depressed by endogamy. However, little is known about factors directly responsible for this depression. Thus, this study was systematically planned to investigate the effect of endogamy on the meiotic behavior in one population of popcorn and to deduce the possibilities of using the S_7 inbred lines in crosses, taking into account their meiotic stability as one of the selected characters.

MATERIAL AND METHODS

A commercial population of popcorn (S_0), called CMS-43, produced by Embrapa Maize and Sorghum Research Center (CNPMS - Sete Lagoas, MG, Brazil), in 1979, was chosen to determine the effects of endogamy on microsporogenesis. This population was selected in the popcorn germplasm collection of CNPMS due to its resistance to *Puccinia* sp and to *Helminthosporium turcicum*. It originated from crosses among 33 genotypes of white grains (Pacheco et al., 1992). The original population was self-pollinated yearly until the seventh generation (S_0 - S_7) on the Experimental Farm of State University of Maringá (Maringá, Paraná State, Brazil).

For cytological analysis, seeds from S_0 to S_7 generations were cultivated simultaneously in the summer of 2004 in a randomized complete block design with three replications. Three plants per replication were analyzed per generation. Young inflorescences for meiotic studies were collected in the morning and fixed in 3:1 ethyl alcohol:acetic acid

for 24 h and then transferred to 70% alcohol and stored under refrigeration until use. Meiocytes were prepared by squashing and staining with 1% propionic carmine.

More than 550 meiocytes per plant were analyzed in each generation involving cells from pachytene to tetrad stage. All types of meiotic abnormalities were considered. Photomicrographs were made with a Wild Leitz microscope using Kodak Imagelink - HQ, ISO 25 black and white film.

RESULTS AND DISCUSSION

From S_0 to S_7 , 72 plants were evaluated with respect to their meiotic behavior. Different types of meiotic abnormalities were recorded in low frequency in each generation (Table 1). The most common meiotic abnormalities were those related to irregular chromosome segregation (Figure 1a to g) observed in all generation. Precocious chromosome migration to the poles in metaphases (Figure 1d,e) and laggard chromosomes in anaphases led to micronucleus formation in telophases (Figure 1b,c) and microcytes in the tetrads (Figure 1g), or polyads (Figure 1f). These abnormalities are caused by univalent chromosomes that occur in diplotene/diakinesis (Figure 1a). Univalents appear in these phases as a result of the absence of chiasmata in some bivalents. Chiasmata are mechanically important to ensure bipolar alignment and regular segregation of homologs during the first (reductional) meiotic division. Chiasma formation is a character under polygenic control (Rees and Thompson, 1956; Lein and Lelley, 1987). Selfing of allogamous plants leads to gene segregation, so that a different chiasma frequency may appear among lines of the same origin and, as a consequence, different frequencies of univalent chromosomes can occur. The frequency of univalents varied during the selfing cycle; S_3 and S_4 generations were the most affected. Univalent chromosomes, in general, show precocious migration to the poles, leading to micronucleus formation in telophase. Irregular chromosome segregation in both meiotic divisions has been determined to be the main cause of unbalanced gamete formation (Gottschalk and Kaul, 1974; Koduru and Rao, 1981; Pagliarini, 2000). Studies performed in different plant species have shown a negative correlation between univalent chromosomes and fertility (Moraes-Fernandes, 1982; Smith and Murphy, 1986; Pagliarini, 1989, 2000). Negative correlation between univalent chromosomes and combining ability has been recorded in inbred lines of maize (Pagliarini, 1989).

Table 1. Number of abnormal cells and abnormalities recorded in CMS-43 from S_0 to S_7 generations.

Abnormality	Generations								No. of cells affected
	S_0	S_1	S_2	S_3	S_4	S_5	S_6	S_7	
Univalent chromosomes	20	40	18	75	117	57	60	37	424
Precocious migration	32	8	1	21	6	17	7	9	101
Laggard chromosomes	24	16	16	25	9	9	7	3	109
Micronuclei	7	2	0	104	8	14	15	11	161
Cell fusion	0	0	0	2	2	1	0	1	6
Chromosome stickiness	6	4	4	15	4	0	69	0	102
Partial asynapsis	9	13	9	1	1	2	6	0	41
Absence of cytokinesis	3	37	17	11	3	13	1	1	86
Abnormal cell shape	0	12	5	2	2	3	4	3	31
Abnormal spindles	0	0	1	1	0	2	0	0	4
Dyads	0	18	10	14	3	3	4	4	56
Triads	1	4	17	3	2	2	0	0	29
Total	102	154	98	274	175	123	173	69	1250
No. of cells analyzed	3870	3870	3870	3870	3870	5220	5220	5220	35,010
% of abnormal cells	2.6	4.0	2.5	7.1	4.5	2.4	3.3	1.3	

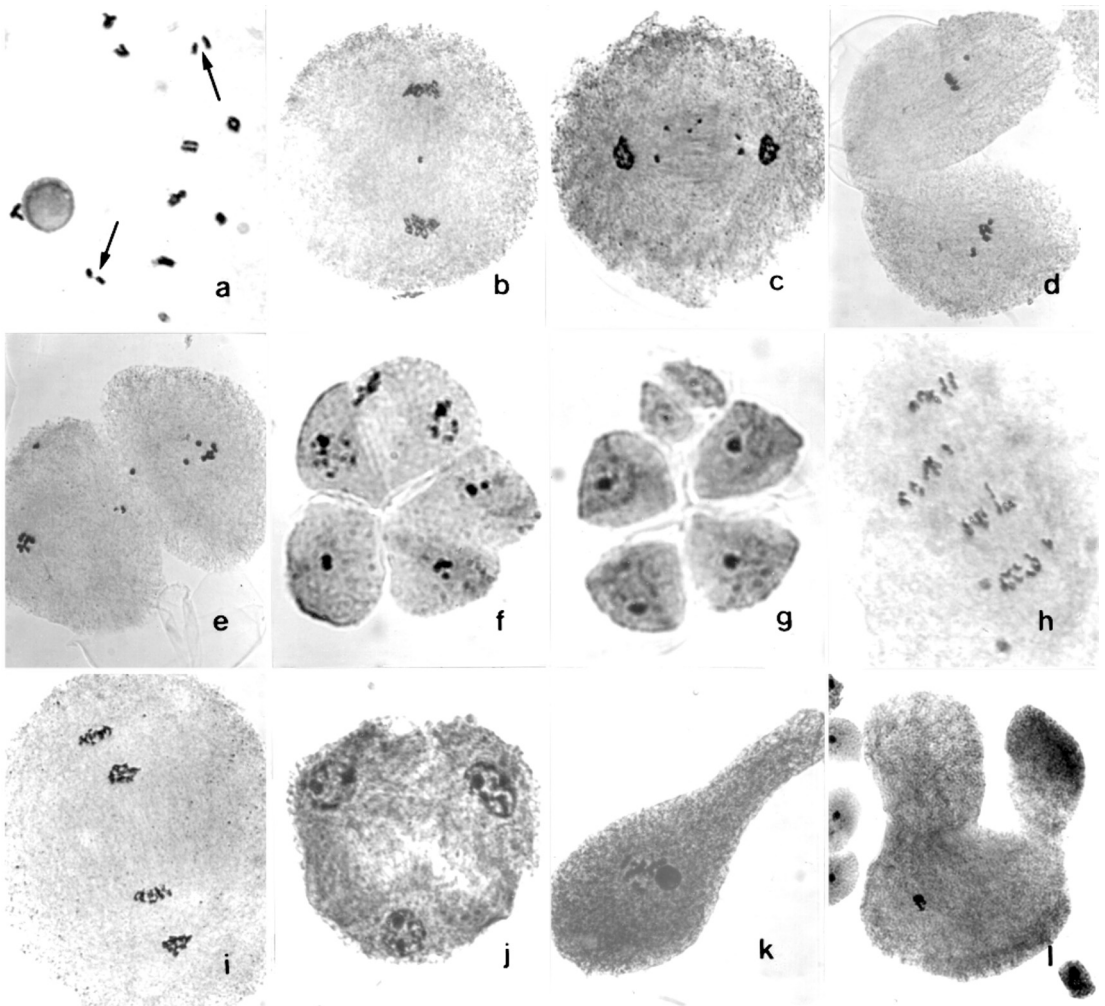


Figure 1. Different types of meiotic abnormalities observed in CMS-43 and its endogamous lines. a) Meicyte in diakinesis showing eight bivalents and two pairs of univalent chromosomes (arrows). b) Early telophases I with one micronucleus. c) Telophase I with several micronuclei. d, e) Metaphases II with precocious chromosome migration and micronuclei in e. f) Pentad of microspores. g) Tetrad with two microcytes. h, i) Anaphase II (h) and telophase II (i) with absence of first cytokinesis and sequential spindle orientation. j) Telophase II with a restitution nucleus. k, l) Meicytes with abnormal cell shapes.

Other types of meiotic abnormalities were recorded from S_0 to S_7 , including partial asynapsis, cell fusion, absence of cytokinesis (Figure 1h and i), abnormal spindle orientation in the second division (Figure 1h and i) leading to restitutional nucleus formation (Figure 1j), abnormal cell shape (Figure 1k and l), and chromosome stickiness. All abnormalities observed in CMS-43 have been reported among inbred maize lines previously analyzed (Pagliarini, 1989; Defani-Sciarize et al., 1995, 1996; Caetano-Pereira and Pagliarini, 1996; Caetano-Pereira et al., 1998; Utsunomiya et al., 2002; Pagliarini et al., 2002). Several lines of evidence obtained for different plant species have demonstrated that each step of meiosis is genetically controlled (Gottschalck and Kaul, 1974; Baker et al., 1976; Koduru and Rao, 1981; Golubovskaya, 1979, 1989). Allogamous species, such as popcorn, have a degree of heterozygosity that ensures

normal meiosis. When this system is broken by inbreeding, some abnormalities may become frequent. The frequency of cells with meiotic abnormalities was very low in the present study, suggesting that CMS-43 is a population with a high frequency of dominant homozygous loci for meiosis control.

Meiotic mutations do not affect vegetative development and do not change the plant phenotype. They can be revealed only during tassel inflorescence and, as a rule, meiotic mutants display complete or partial male and/or female sterility (Golubovskaya, 1989). Although the present lines were not yet tested for combining ability, the meiotic stability ensures that the S₇ lines may have a high potential for the production of new popcorn hybrids.

ACKNOWLEDGMENTS

Research supported by PRONEX/FUNDAÇÃO ARAUCÁRIA - No. 1227-33/04.

REFERENCES

- Albertsen MC and Phillips RL (1981). Developmental cytology of 13 genetic male sterile loci in maize. *Can. J. Genet. Cytol.* 23: 195-208.
- Baker BS, Carpenter AT, Esposito MS, Esposito RE, et al. (1976). The genetic control of meiosis. *Annu. Rev. Genet.* 10: 53-134.
- Caetano-Pereira CM and Pagliarini MS (1996). Unusual shapes of maize microsporocytes. *Nucleus* 39: 107-110.
- Caetano-Pereira CM, Defani-Scoarize MA, Pagliarini MS and Brasil EM (1998). Syncytes, abnormal cytokinesis and spindle irregularities in maize microsporogenesis. *Maydica* 43: 235-242.
- Curtis CA and Doyle GG (1991). Double meiotic mutants of maize: implications for the genetic regulation of meiosis. *J. Hered.* 82: 156-163.
- Defani-Scoarize MA, Pagliarini MS and Aguiar CG (1995). Causes of partial male sterility in an inbred maize line. *Cytologia* 60: 311-318.
- Defani-Scoarize MA, Pagliarini MS and Aguiar CG (1996). Meiotic behavior of inbred lines of maize (*Zea mays* L.). *Nucleus* 39: 10-18.
- Golubovskaya IN (1979). Genetic control of meiosis. *Int. Rev. Cytol.* 58: 247-290.
- Golubovskaya IN (1989). Meiosis in maize: *mei* genes and conception of genetic control of meiosis. *Adv. Genet.* 26: 149-192.
- Gottschalk W and Kaul MLR (1974). The genetic control of microsporogenesis in higher plants. *Nucleus* 17: 133-166.
- Hallauer AR and Miranda Filho JB (1988). Quantitative genetics in maize breeding. State University Press, Ames.
- Karp A and Jones RN (1982). Cytogenetics of *Lolium perenne*. I. Chiasma frequency variation in inbred lines. *Theor. Appl. Genet.* 62: 177-183.
- Koduru PK and Rao MK (1981). Cytogenetics of synaptic mutants and fertility interrelationships in prairie *Bromus inermis* Leyss populations. *Cytologia* 37: 747-757.
- Lamm R (1936). Cytological studies on inbred rye. *Hereditas* 22: 217-240.
- Lein V and Lelley T (1987). A separate control for frequency and within-bivalent distribution of chiasmata in rye (*Secale cereale* L.). *Genome* 29: 419-424.
- Lima M, Miranda Filho JB and Gallo PB (1984). Inbreeding depression in Brazilian populations of maize (*Zea mays* L.). *Maydica* 29: 203-215.
- Moraes-Fernandes MIB (1982). Estudo da instabilidade meiótica em cultivares de trigo. Efeito genotípico, relação com fertilidade e seleção de plantas estáveis. *Pesqui. Agropecu. Bras.* 17: 1177-1191.
- Morris R and Isikan MH (1964). Cytological studies on inbred lines of maize. *Can. J. Genet. Cytol.* 6: 508-515.
- Myers WM and Hill HD (1943). Increased meiotic irregularity accompanying inbreeding in *Dactylis glomerata* L. *Genetics* 28: 383-397.
- Pacheco CAP, Gama EEG, Lopes MA and Santos MX (1992). Formação de compostos de milho-pipoca. Relatório Técnico Anual do Centro de Pesquisa de Milho e Sorgo: 1988/1991, Sete Lagoas.
- Pagliarini MS (1989). Avaliação da frequência de quiasmas em milho (*Zea mays* L.) e suas implicações com a capacidade de combinação para a produção de grãos. PhD thesis, Escola Superior de Agricultura Luiz de Queiroz/USP, Piracicaba.
- Pagliarini MS (2000). Meiotic behavior of economically important plant species: the relationship between fertility and male sterility. *Genet. Mol. Biol.* 23: 997-1002.
- Pagliarini MS, Defani MA, Walter FM and Pereira JE (2002). Recurrence of meiotic abnormalities in maize genotypes from the same origin and their influence on productivity. *Crop Breed. Appl. Biotechnol.* 2: 355-360.
- Pantulu JV and Manga V (1972). Cytology of inbreds and F₁ hybrids of pearl millet. *Theor. Appl. Genet.* 42: 69-74.

- Rees H and Thompson JB (1956). Genotypic control of chromosome behavior in rye. III. Chiasma frequency in homozygotes and heterozygotes. *Heredity* 10: 409-424.
- Smith S and Murphy RP (1986). Relationships between inbreeding, meiotic irregularity and fertility in alfalfa. *Can. J. Genet. Cytol.* 28: 130-137.
- Trifunovic S, Córdova H, Crossa J and Pandey S (2003). Head-to-head and stability analysis of maize (*Zea mays* L.) inbred lines. *Maydica* 48: 263-269.
- Utsunomiya KS, Bione NCP and Pagliarini MS (2002). How many different kinds of meiotic abnormalities could be found in a unique endogamous maize plant? *Cytologia* 67: 169-176.