Short Communication

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

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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 (S₀) of popcorn (CMS-43) produced by Embrapa Maize and Sorghum was self-pollinated for seven years, generating inbred lines (S₁ to S7). 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 S₇, 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 S₇ inbred lines in crosses, taking into account their meiotic stability as one of the selected characters.

MATERIAL AND METHODS

A commercial population of popcorn (S₀), 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 (S₀-S₇) on the Experimental Farm of State University of Maringá (Maringá, Paraná State, Brazil).

For cytological analysis, seeds from S₀ to S₇ 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 S₀ to S₇, 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; S₃ and S₄ 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>
<thead>
<tr>
<th>Abnormality</th>
<th>Generations</th>
<th>No. of cells affected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S₀</td>
<td>S₁</td>
</tr>
<tr>
<td>Univalent chromosomes</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Precocious migration</td>
<td>32</td>
<td>8</td>
</tr>
<tr>
<td>Laggard chromosomes</td>
<td>24</td>
<td>16</td>
</tr>
<tr>
<td>Micronuclei</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Cell fusion</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chromosome stickiness</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Partial asynapsis</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>Absence of cytokinesis</td>
<td>3</td>
<td>37</td>
</tr>
<tr>
<td>Abnormal cell shape</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Abnormal spindles</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dyads</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Triads</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>102</td>
<td>154</td>
</tr>
</tbody>
</table>

| No. of cells analyzed      | 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           |

Table 1. Number of abnormal cells and abnormalities recorded in CMS-43 from S₀ to S₇ generations.
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-Scoarize 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 heterozygosis that ensures

Figure 1. Different types of meiotic abnormalities observed in CMS-43 and its endogamous lines. a) Meiocyte 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) Meiocytes with abnormal cell shapes.
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.

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REFERENCES


