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Effects of endogamy on microsporogenesis in popcorn

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ABSTRACT - *Inbred lines are necessary for the development of popcorn hybrids. Genes for several traits, among them those related to the meiotic process, approach homozygosis during inbreeding. The purpose of this paper was to evaluate the meiotic stability of popcorn endogamic lines developed from a triple hybrid in contribution to hybrid breeding programs. The original population (S_0) and seven annual self-pollinated generations (S_1 to S_7) were analyzed. Cytological results were statistically analyzed by linear regression. The frequency of total and terminal chiasmata decreased with endogamy. Meiotic abnormalities also increased with endogamy although their number was not high. Since the frequency of abnormalities was low and did not affect drastically the meiotic product, inbred lines from the hybrid under analysis may have a high potential for the establishment of new popcorn hybrids.*

Key words: endogamy, inbred lines, microsporogenesis, popcorn, breeding.

INTRODUCTION

An important objective of maize breeding programs is to develop new inbred lines with high combining ability to produce higher grains yields and for 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). The main objective of breeding is to increase yields (Trifunovic et al. 2003), and only lines with high breeding values for yield and yield-related traits should be maintained in breeding programs**.**

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), seed production was severely depressed by endogamy. However, little is know about factors directly responsible for this depression. Irregularities in meiotic behavior could be partially responsible for the inbreeding depression in fertility. Clark (1942) highlighted the practical value of determining and eliminating the causes of sterility in inbred lines to ensure uniform production in hybrid corn. Partial or complete sterility may be manifested in corn by poorly filled ears, aborted pollen and defective seeds. Among several other factors, the author mentioned sterility, caused by aberrant chromosome behavior during microsporogenesis, which could directly affect gamete production. 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

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1982, Defani-Scoarize et al. 1995 1996, Pagliarini et al. 2002). Meiosis is an event of high evolutionary stability that culminates in the reduction of the 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, Golubovskaya 1989). Mutations of these genes may cause abnormalities that impair plant fertility (Albertsen and Phillips 1981, Curtis and Doyle 1991). When an allogamous plant is self-pollinated, many genes, including those involved in the control of meiosis, approach homozygosis, causing inbreeding depression. Thus, this study was conducted to a) investigate the effect of endogamy on the meiotic behavior in one popcorn population and to b) analyze the possibilities of using S_7 inbred lines in crosses, considering their meiotic stability as one of the selected traits.

MATERIAL AND METHODS

A commercial population of popcorn (S_0) named Zelia, was chosen to evaluate the effects of endogamy on microsporogenesis. Zelia is a three-way cross hybrid produced by Pioneer with several desirable agronomic characteristics. The original population was yearly self-pollinated up to the seventh generation (S_0 - S_7) in an experimental farm of Universidade Estadual de Maringá (UEM, state of Paraná, Brazil).

For cytological analysis, seeds from S_0 to S_7 generations (treatments) were cultivated simultaneously in the summer of 2004 in randomized complete blocks 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 refrigerated until use. Meiocytes were prepared by squashing and staining with 1% propionic carmine.

More than 400 meiocytes per plant were analyzed in each generation involving cells from pachytene to tetrad stage. Chiasma frequency was scored in 20 meiocytes in diakinesis per plant, considering the number and distribution along the bivalent. 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 obtained for each cytological study characteristic were statistically analyzed (5% probability) by polynomial regression.

RESULTS AND DISCUSSION

1. Chiasma frequency

The chiasma frequency at diakinesis was divided in the frequency of terminal chiasma (FCt) and the frequency of interstitial chiasma (FCi) per meiocyte. The sum of the two chiasma frequencies represents the total number of chiasmata per meiocyte (FCm). Results for these characters are presented as mean of a generation in Table 1.

1.1. Frequency of chiasma per meiocyte

From S_0 to S_7, chiasma frequency decreased in each generation of inbreeding, beginning with a mean of 18.66 chiasmata per meiocyte in S_0 and reaching 15.57 in S_7. The estimated coefficient of linear regression (linear $b=-0.542$; $P < 0.05$) evidenced a negative correlation between chiasmata frequency and endogamy. Chiasma counting has been regarded as the most straightforward method of scoring the total number of crossing-over events in the genome (Darlington 1929). It is well documented in several plant species that chiasma frequency is a cytological character under polygenic control (Dayal 1977a, Karp and Jones 1982, Lein and Lelley 1987, Rees and Thompson 1958). Thus, in each generation of self-pollination more genes approach homozygosis while chiasma frequency decreases. A reduction of chiasma frequency caused by inbreeding was also reported in several other plant species (Lamm 1936, Parker 1975, Dayal 1977b, Lein and Lelley 1987, Rees and Thompson 1956).

1.2. Frequency of interstitial chiasma

There was a positive correlation between the frequency of interstitial chiasma and endogamy (linear $b=+0.392$; $P < 0.05$), demonstrating a distinct behavior of frequency of chiasma per meiocyte for this trait. Studies performed in rye (Jones 1967, Lein and Lelley 1987) and *Lolium perenne* (Karp and Jones 1983) showed that there is another type of genetic control, also polygenic but totally different, and independent of the chiasma frequency per meiocyte, which distributes the chiasma along the bivalent. Furthermore, the independence of both genetic controls of chiasma formation was clearly

Table 1. Means of chiasma frequency per meiocyte (FCm), frequency of terminal chiasma (FCt), frequency of interstitial chiasma (FCi), univalent chromosomes (UC) and abnormal meiocytes (AM) during endogamy (S_0 to S_7) and the linear regression coefficient for the three-way cross hybrid Zelia

Generations	FCm	FCt	FCi	UC	AM
S_0	18.66	16.30	2.75	2.88	0.96
S_1	17.36	15.02	2.33	1.00	1.69
S_2	18.07	14.50	3.57	1.77	2.73
S_3	18.05	14.86	3.18	2.33	1.32
S_4	16.81	14.65	2.16	3.22	2.49
S_5	16.60	13.86	2.73	4.66	2.46
S_6	16.13	12.30	3.83	4.11	2.91
S_7	15.57	11.78	3.78	4.00	2.36
Mean	17.16	14.13	3.04	3.00	2.12
Linear regression coefficient*	-0.542	-3.04	+0.392	+0.194	+0.398

* significant at 5% level

showed here, where chiasma frequency per meiocyte decreased while the frequency of interstitial chiasma increased with increasing inbreeding levels.

1.3. Frequency of terminal chiasma

Terminal chiasma showed the same behavior of the chiasma frequency per meiocyte, i.e., it decreased during inbreeding (linear b = -3.04; P <0.05). As mentioned earlier, chiasmata represent genetic recombination events. However, according to Zarchi et al. (1972), terminal chiasmata have only a physical function for the maintenance of the bivalent structure to ensure perfect chromosome segregation. In some species, however, terminal chiasma results from the phenomenon of chiasma terminalization, according to Darlington's concept (1929). It was proved that in maize chiasmata terminalize (Torrezan and Pagliarini 1995). In this species, chisama frequency is higher in diplotene than in diakinesis, reinforcing the assumption of chiasma terminalization.

2. Meiotic abnormalities

In the cytological analyses of each generation, some meiotic abnormalities were found among plants.

2.1. Univalent chromosomes

Cytological analyses revealed a gradually increasing tendency of univalent chromosomes (Figure 1a and Table 1) over the generations of inbreeding (linear b = +0.194; P < 0.05). The increased number of univalents was negatively correlated with the chiasma frequency per microsporocyte and the terminal chiasmata frequency, i.e., with the reduction of chiasma frequency per microsporocyte and its terminalization along the bivalent caused by endogamy, homologous chromosomes tend to separate into univalents. Chiasmata are mechanically important to ensure bipolar alignment and regular segregation of homologous chromosomes during the first (reductional) meiotic division. Precocious migration of univalent chromosomes to the poles was observed (Figure 1b), leading to micronuclei formation in telophase (Figure 1c). Irregular chromosome segregation in both meiotic divisions has been considered the main cause of unbalanced gamete formation (Gottschalk and Kaul 1974, Koduru and Rao 1981, Pagliarini 2000).

Studies in different plant species have shown a negative correlation between univalent chromosomes and fertility (Moraes-Fernandes 1982, Pagliarini 1989, Pagliarini 2000, Smith and Murphy 1986). However, the number of univalent chromosomes observed in the present inbred lines, with a mean of 4.66 in S_5 (Table 1), is much lower than those reported in the above studies. This number of univalents, *per se*, would not severely impair pollination because the number of pollen grains available in a popcorn inflorescence would still be high.

2.2. Other types of meiotic abnormalities

Analyses of microsporogenesis showed a slight increase of meiotic abnormalities with endogamy (linear b=+0.398; P<0.05). Different types of meiotic abnormalities, including partial asynapsis (Figure 1 d); anucleate meiocytes (Figure 1e); absence of cytokinesis after the first (Figure 1g) or the second meiotic division (Figure 1h), leading to 2n microspore (Figure 1i) or

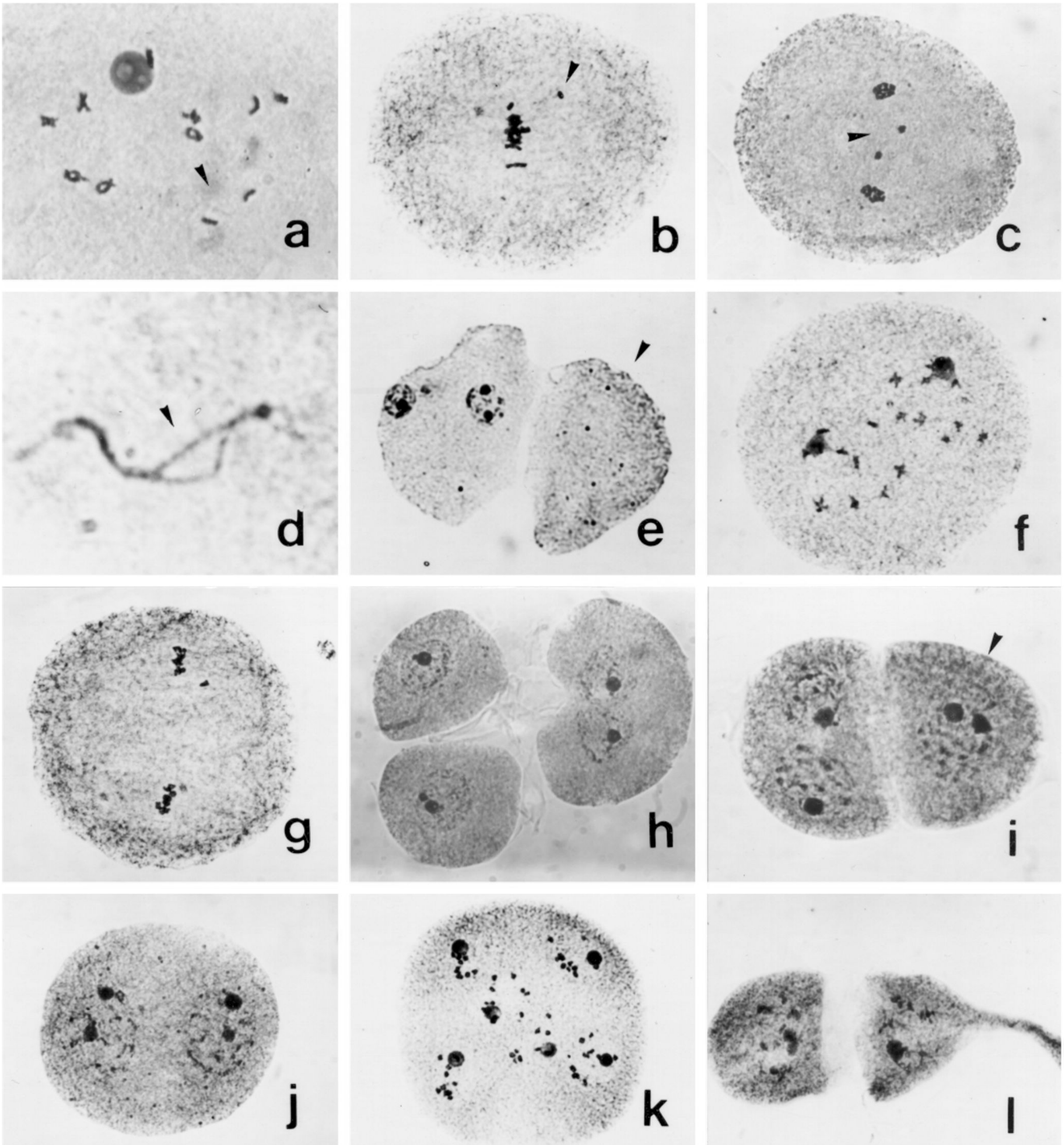

Figure 1. Different types of meiotic abnormalities observed in Zelia and endogamous lines. a) Meiocyte in diakinesis showing nine bivalents and one pair of univalent chromosomes (arrowhead). b) Metaphase I with a univalent migrating precociously to the pole (arrowhead). c) Telophase I with two micronuclei (arrowhead). d) A bivalent in pachytene stage showing partial asynapsis (arrowhead). e) Prophase II with one anucleate cell (arrowhead). f) Prophase II with absence of the first cytokinesis. g) Metaphase II with absence of cytokinesis and precocious chromosome migration to the poles. h) Triad of microspores with absence of the second cytokinesis in one cell. i) Dyad of microspores with a binucleate microspore and one with a restitution nucleus (arrowhead). j) A released binucleate microspore. k) A syncyte resulting from fusion of six meiocytes. l) Prophase II with one irregularly shaped cell (arrowhead)

binucleate microspore (Figure 1j); cell fusions (Figure 1k); and abnormal cell shape (Figure 1l). The mean of abnormal cells encompassing these abnormalities did not exceed 2.91, recorded in S_6.

All these abnormalities have been reported among inbred maize lines previously analyzed by our group (Caetano-Pereira and Pagliarini 1996, Caetano-Pereira et al. 1998, Defani-Scoarize et al. 1995, Defani-Scoarize et al. 1996, Pagliarini et al. 2002, Utsunomiya et al. 2002). Extensive evidences obtained for different plant species have demonstrated that each step of meiosis is genetically controlled (Baker et al. 1976, Golubovskaya 1979, Golubovskaya 1989, Gottschalck and Kaul 1974, Koduru and Rao 1981). Allogamous species, such as popcorn, have a degree of heterozygosis that ensures normal meiosis. When this system is broken by inbreeding, some abnormalities may become frequent. The *mei* mutations do not influence vegetative development and do not change the plant phenotype. They can be revealed only during tassel inflorescence and, as a rule, *mei* mutants display complete or partial male and/or female sterility (Golubovskaya 1989).

The frequency of cells with meiotic abnormalities, including thee cells with univalent chromosomes, was very low in this study. Although the present lines have not yet been tested for combining ability, the meiotic stability ensures, at least partly, a high potential for the production of new popcorn hybrids in the S_7 lines.

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Efeitos da endogamia sobre a microsporogênese de milho-pipoca

RESUMO - *Linhagens endogâmicas são necessárias para a formação de híbridos de milho-pipoca. A endogamia coloca em homozigose genes para diversas características, entre os quais, aqueles relacionados com o processo meiótico. A presente pesquisa teve por objetivo avaliar a estabilidade meiótica de linhagens endogâmicas de milho-pipoca produzidas a partir de um híbrido triplo a fim de fornecer subsídios para o programa de melhoramento. A população original* (S_0) *e sete gerações autofecundadas* (S_1 *a* S_7) *foram analisadas. Os resultados citológicos foram analisados via regressão linear. As freqüências de quiasma total e terminal diminuíram com a endogamia, enquanto as anormalidades meióticas aumentaram, não sendo, porém, elevadas. Considerando que a freqüência de anormalidades foi baixa e não afetou drasticamente o produto final da meiose, as linhagens extraídas do híbrido sob análise podem apresentar um alto potencial para a produção de novos híbridos de pipoca.*

Palavras-chave: milho pipoca, endogamia, microsporogênese, anormalidades meióticas.

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