Cytomixis in Maize Microsporocytes

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Cytomixis has been defined by Gates (1911) as the passage of chromatin material from the nucleus of a pollen mother cell (PMC) to the cytoplasm of another adjacent PMC, a phenomenon detected by him in *Oenothera gigas* and *O. biennis*. The literature on cytomixis reveals that the phenomenon has been observed in PMC, and in meristematic, tapetum, integumental and nuclear cells, as well as in cells of the ovary of various plants (Kaul 1990). The phenomenon has been detected at higher frequency in genetically imbalanced species such as hybrids, apomictic, haploid and polyploid species and intergeneric hybrids (Levan 1941, Yen *et al.* 1993, Consolaro and Pagliarini 1995).

Cytomixis has also been detected in the mitosis of *Vicia faba* roots (Amer and Mikhael 1986), in *Prunus amygdalus* Batsch and *P. persica* Batsch (Soodan and Waffai 1987), *Vigna glaberscens* (Sen and Bhattacharya 1988), *Lindenbergia indica* (Sinha 1988), *Laminaceae* (Kundu and Sharma 1988), *Alopecurus arundinaceus* (Kaul 1990), in a clone of *Saccharum spontaneum* (Singh *et al.* 1990), and in *Pisum sativum* (Nirmala and Kaul 1994), among others. In maize microsporocytes the phenomenon was described by McClintock (1929).

Although cytomixis has been reported for several plant species, its origin is not clear. Among the factors proposed to cause it are the influence of genes (Brown and Bertke 1969, Omara 1976, Bedi 1990, Kaul and Nirmala 1991), abnormal formation of the cell wall during premeiotic divisions (Levan 1941, Kamra 1960), the action of chemical agents such as colchicine and ethyl-methane sulfate (Sinha 1988) and rotenone (Amer and Mikhael 1986), changes in the biochemical processes that involve microsporogenesis modifying the microenvironment of affected anthers (Kaul 1990), and the effect of gamma radiation resulting in an imbalanced and sterile genetic system (Amm *et al.* 1990). According to Nirmala and Kaul (1994), the cytomixis occurring in *Pisum sativum* is due to the presence of a male-sterile mutant gene and its frequency is altered by environmental factors.

In the present study we report the spontaneous occurrence of cytomixis in maize genotypes with different levels of heterozygosis grown on cerrado soils in the Brazilian Central Plateau.

Material and methods

The material studied consisted of 21 inbred lines, 10 single-cross hybrids and 5 double-cross hybrids of maize grown on corrected and acid soil in the Brazilian Central Plateau.

Male inflorescences were fixed in Carnoy's fluid for 24 hr, transferred to 70% alcohol and stored under refrigeration. Slides were prepared by the squashing technique and cells were stained with 1% propionic carmine. All meiotic phases were analyzed for a total of 300 cells per plant. A total 220 and 286 plants of the homozygous and heterozygous genotypes were analyzed, respectively.

Table 1. Incidence of cytomixis (cyt) or cytoplasm connections (conn) among PMC of inbred lines (L) and double-cross hybrids (DCH) of maize grown on corrected and acid soil in the Brazilian Central Plateau

Genotype	Acid soil				Corrected soil			
	No. of plant analysed	No. of plant affected	% affected cells cyt	% affected cells conn	No. of plant analysed	No. of plant affected	% affected cells cyt	% affected cells conn
L1	10	0	0.0	0.0	10	2	0.1	0.2
L2	6	2	0.3	0.7	10	1	0.1	0.2
L3	7	5	0.6	1.0	10	4	0.1	0.4
L4	1	0	0.0	0.0	9	3	0.2	1.4
L5	1	1	0.2	0.1	10	0	0.0	0.0
DCH1	10	0	0.0	0.0	7	1	0.0	0.3
DCH2	10	5	0.2	0.3	4	0	0.0	0.0
DCH3	10	3	0.1	0.4	10	0	0.0	0.0
DCH4	10	1	0.1	0.2	5	2	8.8	25.0
DCH5	10	1	0.0	0.3	10	0	0.0	0.0

Results

Among the 36 genotypes analyzed, 5 inbred lines and the 5 double-cross hybrids presented plants affected by cytomixis in all meiotic stages and on all types of soil. Table 1 shows the number of plants analyzed and affected and the percentage of cells presenting cytomixis or only cytomictic connections between adjacent cells without chromatin passage for each type of soil and for the genotypes under study.

The occurrence of cytomixis or cytomictic connections was observed between cells within the same meiotic stage, or in different meiotic stages and between meiocytes and tapetum cells. Two to several cells were simultaneously involved. We also observed cells in diakinesis with a higher or lower number of bivalents, anucleate cells close to bi- or trinucleate cells, some of them containing remnants of cytoplasm bridges and microspores of the same tetrad joined by cytomictic channels. Abnormal tetrads, meiocyte degeneration, cell fusion and mixoploidy were frequent in affected genotypes. Fig. 1 illustrates some aspects of cytomixis between microsporocytes and between microsporocytes and tapetum cells, and also the occurrence of cytomictic connections between neighboring cells.

Discussion

Chromatin transfer from cell to cell or the connection between cells through cytoplasm channels revealed the occurrence of cytomixis in some of the inbred lines and double-cross maize hybrids analysed in the present study. As reported for *Gossypium* (Sarvella 1958), *Justicia tranquariensis* (Saggoo and Bir 1983) and for forest species (Bedi 1990), cytomictic connections were observed between PMC in different stages of meiosis and even between tapetum cells and meiocytes, as also reported by Cooper (1952).

Whereas Yen *et al.* (1993) reported the occurrence of cytomixis in cells within the division stage and in the early stages of microsporogenesis, in the present material we observed cytoplasmic channels apparently being formed in cells in advanced stages of meiosis. Bahl and Tyang (1988) reported the *de novo* onset of cytoplasmic channels in *Papaver dubium*. These channels probably originated in the form of small projections that, by elongating, would touch another cell. The cell wall may dissolve at the point of contact forming a passage for chromatin or chromosome transfer. According to Soodan and Waffai (1987), the persistence of cytoplasmic channels between adjacent cells prevents their rounding off and the development of a callosis wall. This would create condi-

Fig. 1. Some aspects of cytomixis in maize microsporogenesis. a) Cytomixis between microsporocytes in metaphase I. b) Cytomixis between a microsporocyte and a tapetum cell indicated by the arrow. c) Cytomictic connections between microsporocytes in diplotene.

tions for the movement of chromatin from one cell to another.

The formation of these channels during advanced stages suggests that in the material studied here cytomixis may not have been due to cell wall malformation during premeiotic division resulting from calcium deficiency since the highest frequency of this abnormality was observed in a double-cross hybrid grown on corrected soil. The results suggest a genotypic involvement in the cytomixis occurrence. Among the 36 genotypes evaluated 10 were affected, and for each double-cross hybrid at least one of their parental inbred line were also equally affected. The intergenotypic differ-

ences observed may have been caused by an imbalanced genetic system in response to fluctuations in environmental conditions, as also proposed by Basavaiah and Murthy (1987). According to Nir-mala and Kaul (1994), the photoperiod and/or the temperature alter the frequency of cells affected by cytomixis. According to Soodan and Waffai (1987), the presence of cytomixis in few individuals in a group tested indicates the involvement of specific genes that are expressed only under particular environmental conditions. The PMC of the same microsporangium may undergo variations in their cytotoxic potential or may fail in exposing the specific conditions required to trigger the process. This may explain the low frequency of cells affected by cytomixis in the inbred lines and double-cross hybrids of maize investigated in the present study.

Cytomixis may have serious genetic consequences by leading to the formation of PMC with aberrant chromosome numbers, binucleate PMC, aberrant microspore tetrads and pollen sterility (Omara 1976, Soodan and Waffai 1987, Sinha 1988). Cytomixis is also considered to be the possible cause of the origin of extra centric fragments defined as B chromosomes (Yong-Ren *et al.* 1991) and of an uncommon nuclear behavior including cenocytism, variation in cell size, asynchronous or multipolar divisions, and delayed chromatin condensation (Yen *et al.* 1993). Furthermore, specific heterochromatic chromosomes have been found to be involved in cytotoxic events, suggesting that cytomixis is a process of genetic manipulation for the generation of syncytia (Patra *et al.* 1986). All of these abnormalities interfere negatively with the reproductive potential. Bedi (1990) observed that the reduced fertility of six forest species studied was due to cytomixis. These abnormalities may even modify the reproductive system by imposing total male sterility and promoting cross pollination (Soodan and waffai 1987).

According to Sarvella (1958), the migration of the genomic complement through cytomixis, causing deviations in chromosome number, may be an additional mechanism for the origin of aneuploid or polyploid plants. In maize, a species that presents genetic stability, cytomixis definitely contributed to a reduction of the viability of affected plants by leading to other anomalies such as meiocyte degeneration or deviations in chromosome number and by being also associated with irregularities such as cell fusion and mixoploidy. All of these abnormalities, which were frequent in the materials studied here, may result in partial sterility by suppressing meiosis or by generating imbalanced final products of meiosis.

Summary

A set of inbred lines and single-cross and double-cross maize hybrids grown on acid and corrected soil of the Brazilian Central Plateau were tested for meiotic behavior. Among them, five inbred lines and five double-cross hybrids presented plants exhibiting cells connected by cytoplasmic channels which in several cases showed the passage of chromatin material. All meiotic stages were affected on both soil types. Although the phenomenon occurred at low frequency, it was observed between cells within the same stage, in different stages, or even between meiocytes and tapetum cells. Two to several cells were involved simultaneously. Anucleate cells close to bi- or trinucleate cells were frequently observed as well as cells exhibiting aneuploidy or polyploidy, syncytia, mixoploidy and abnormal tetrads.

Key words : cytomixis, maize, microsporocytes, tapetum.

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