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CYTOMIXIS IN MAIZE HAPLOIDS

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ABSTRACT

Cytomixis was extensively seen in maize haploids treated with colchicine. Genomic imbalance in the form of haploidy is probably responsible for the occurrence of cytomixis. Genotypic differences as well as colchicine treatment influenced the expression of this phenomenon. The highest number of cells in a single cluster with interconnections was 48. Some cells with as high as 27 chromosomes and a few with no chromosome were also seen. There was a kind of relay pattern in the transfer of chromatin material. Chromatin transfer was more pronounced in meta-ana I. A 15–20 fold increase in pollen fertility was observed in the haploids with extensive cytomixis.

Key words: Maize, haploids, cytomixis, genetics, selection.

Cytomixis or the phenomenon of cell to cell migration of nuclear and extranuclear material through cytoplasmic connections was first reported in pollen mother cells of *Crocus sativus* [1]. This phenomenon occurs more commonly in pollen mother cells under abnormal and imbalanced constitutions such as in hybrids, mutants, triploids and apomicts, but is also seen in normal diploids. Not only pollen mother cells but also meristematic tissues, tapetal cells of anthers and epidermis of scales and leaves have been reported to show cytomixis [2].

In maize (*Zea mays* L.), cytomixis was reported in the triploid and its hyperploid progeny [3] and in a trisomic for chromosome 5 [4]. The present investigation records cytomixis in haploids of maize.

MATERIALS AND METHODS

The haploids used for this study were of maternal origin obtained through genetic selection technique [5]. The haploids were derived in a genetic stock showing high frequency (5–6%) of maternal haploids and one breeding material. Colchicine treatment (0.1% aqueous solution for 8 h at 25°C) was given to haploid seeds soaked for 48 h for doubling the

chromosome number. The colchicine treated seeds along with untreated control were sown in the field. Suitable young tassels from the haploids which did not show chromosome doubling after treatment and untreated haploids were fixed in 3 : 1 ethanol : acetic acid mixture with a tinge of ferric chloride. Propiono-carmine squash preparations of PMCs from suitable anthers were made to study meiosis. The photomicrographs showing cytomixis in the present paper were taken from the temporary slides in a Nikon Microphot FX model microscope.

RESULTS AND DISCUSSION

All the haploids, derived either from the genetic stocks or the breeding material, revealed the presence of cytoplasmic connections between and among the PMCs. Per cent cells involved in cytoplasmic connections were higher in the haploids derived from the genetic stock than those derived from the breeding stock. Further, laboratory stock-derived haploids which were not doubled after colchicine treatment showed higher frequency of cytoplasmic connections than the untreated ones and in two of the treated haploids extensive cytomixis was observed in the form of cell to cell chromatin transfer and direct cytoplasmic contact (Figs. 1:1, 2; Table 1). Cytoplasmic connections were of varying breadth (Fig. 1:3) and the number of connections ranged from 1–4. The interconnected PMCs formed clusters with variable number of cells from 2 to as high as 48 cells in a single cluster, and one single cell was found to be connected with a maximum of 5 adjoining cells (Fig. 1:1, part of the 48-cell cluster). Clusterwise distribution of cells in both the plants with extensive cytomixis is presented in Table 2. Clusters having 2–10 cells were more common. Number of chromosomes in different cells of a large cluster varied from 0 to 27 (Fig. 1:1; Table 3). Some giant cells were observed with two groups of ten chromosomes and these cells were also involved in chromatin transfer with nearby cells (Fig. 1:4). In such cases, chromatin transfer was always toward the giant cell but not from the giant cell to others. Not only meiocyte–meiocyte connections but also meiocyte–tapetal cell connection and even transfer of whole nucleus of tapetal cell to meiocyte (Fig. 1:5; 6) were observed. In a few instances, the whole spindle was seen to migrate to the nearby cell (Fig. 1:7). Chromatin transfer was observed mainly in meta–ana I, but in few instances cells in diakinesis had two groups of chromatin along with their respective nucleoli (Fig. 1:8).

Sporadically, one/two fertile pollens were observed in a single anther in the maize haploids. It was most interesting to note a 15–20 fold increase in pollen fertility (on the basis of acetocarmine stainability) in the haploids with extensive cytomixis.

There is no clear understanding about the cause of origin and evolutionary significance of cytomixis. Several suggestions have been made to explain the probable origin of cytomixis. These include the effect of fixation [6], mechanical injury [7], abnormal pathological conditions [8, 9], temperature anomalies [10], physiologically controlled

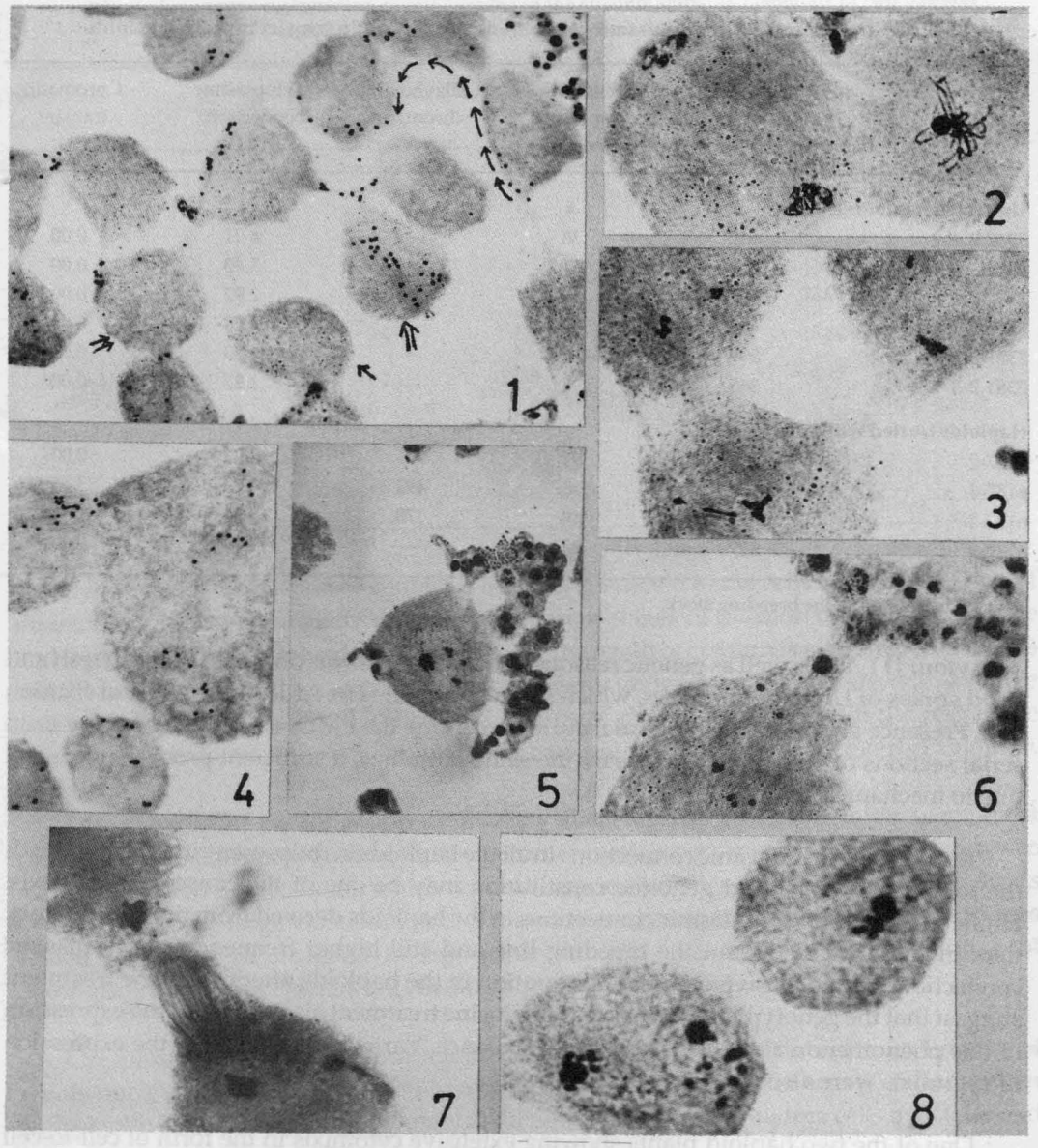

Fig. 1. Different stages of cytomixis in haploid maize.
1) Part of a 48-cell cluster showing cells with no chromosome (→), with 27 chromosomes (⇒) and a kind of relay pattern (→ →) in chromatin transfer. A single cell connected to five nearby cells is also seen (⇉). 2) Cytoplasmic contact between two cells, one at pachytene and the other at late telophase. 3) Cytoplasmic connections of varying breadth. 4) A giant cell with two groups of 10 chromosomes involved in chromatin transfer with nearby cells of normal size. 5) Cytoplasmic connections between meiocyte (in diakinesis) and tapetal cells. 6) Tapetal cell nucleus is migrating to the meiocyte through cytoplasmic bridge. 7) Migration of whole spindle. 8) Cytoplasmic connections between two cells at diakinesis, one of them having two groups of chromatin with the respective nucleolus.

Table 1. Percentages of cytoplasmic connections and chromatin transfer in maize haploids

Plant No.	Total cells observed	Cells with cytoplasmic connections	Cells showing chromatin transfer	Cytoplasmic connections (%)	Chromatin transfer (%)
Untreated haploids:					
5317 B. 6-2	612	38	—	6.21	0.00
5317 F.4-3	916	31	—	3.40	0.00
5317 F.4-9	342	10	—	2.92	0.00
5320.1-3	401	17	—	4.24	0.00
7381 B-2*	599	9	—	1.80	0.00
7381 B-3*	363	5	—	1.37	0.00
Haploids treated with colchicine:					
6187-2	698	91	—	13.03	0.00
6187-4	589	558	492	94.73	83.53
6187-5	331	249	170	75.22	51.35
6187-9	827	92	—	11.12	0.00

*Haploids isolated in the breeding stock.

behaviour [11, 12] as well as genetic reasons [13]. Cytomixis was observed both in fresh and fixed spikes of *Urochloa panicoides*, which suggests that it is not due to the effect of fixation [14]. Presence of normal histological features excepting the PMCs undergoing cytomixis in serial sections of cytomictic anthers by the same authors is a sufficient proof that it is not due to mechanical injury.

Incidence of cytoplasmic connections in all the haploids in the present study agrees with the view that imbalanced genomic constitution may be one of the causes of cytomixis. Higher incidence of cytoplasmic connections in the haploids derived from the genetic stock than in those derived from the breeding line and still higher frequency of cytoplasmic connections and extensive chromatin migration in the haploids after colchicine treatment suggest that the genotypic difference and colchicine treatment also influence the expression of this phenomenon along with genomic imbalance. Varietal differences in the expression of cytomixis were also reported [15] in peach.

One of the two haploid plants showing extensive cytomixis in the form of cell-to-cell chromatin transfer had 7 clusters with more than 20 cells in each cluster. These seven clusters involved about 43% of the total cells analysed in that plant. Presence of cells with more than 20 chromosomes in the large clusters indicates that chromatin flow was toward a particular cell from the neighbouring cells, probably under some attraction force as there was a relay pattern in chromatin transfer (direction of relay pattern is shown in Fig. 1:1 by arrows). This also agrees with the early observations on *Coix* [2].

Table 2. Frequency distribution of cell number in the clusters showing cytomixis in two haploid maize plants (plant No. 1: 6187-4, plant No. 2: 6187-5)

No. of cells in a cluster	No. of clusters		Total No. of cells		Per cent of total cells	
	1	2	1	2	1	2
1	—	—	31	82	5.26	24.77
2	17	16	34	32	5.77	9.66
3	18	17	54	51	9.16	15.40
4-6	16	15	76	71	12.90	21.45
7-10	11	9	92	70	15.62	21.14
11-20	4	2	50	25	8.48	7.5
21-30	3		80		13.58	
> 30	4		172		29.20	
Total			589	331		

Cytoplasmic connection or contact is not only restricted to the cells at the same stage of division but it also occurs between cells at different stages of division (Fig. 1:2, pachytene and telophase). Occurrence of giant cells may be the result of cytomixis in premeiotic mitosis but the real reason for their appearance is not clearly understood. A few cells with average size in diakinesis (as in Fig. 1:8) had two groups of chromatin along with the respective nucleoli. Probably, the entire nuclear material had migrated in pachytene or early diplotene.

The majority of earlier reports indicated that chromatin transfer takes place most frequently at meiotic prophase, but in the present investigation, migration of chromatin was mainly observed in meta-anal, which was also reported earlier in the interspecific hybrids of *Coix* [2]. In the large clusters, some chromosomes remained in the connecting bridges which could not be assigned to any particular cell, as a result, the total number of chromosomes in such clusters is generally less than expected (Table 3).

Cytoplasmic connections between the meiocyte and tapetal cells and even transfer of whole nucleus from the tapetal cell to meiocyte and congregation of tapetal cells together support the view that a group of closely associated and actively dividing cells may take part in cytomixis at any stage of mitosis and/or meiosis [16].

Increase in the frequency of fertile pollen (on the basis of acetocarmine stainability) in the two cytomictic haploid plants is probably due to chromatin transfer resulting in restoration of a balanced chromosome number in the pollen. One of the cytomictic plants

Table 3. Chromosome distribution in cells of three large clusters showing cytomixis in haploid plant 6187-4

Number of chromosomes per cell	Cluster 1		Cluster 2		Cluster 3	
	cells	chromo-somes	cells	chromo-somes	cells	chromo-somes
0	5	0	7	0	9	0
1	1	1	3	3	4	4
2	2	4	4	8	3	6
3	0	0	0	0	5	15
4-6	4	19	4	18	3	18
7-9	11	97	8	69	11	91
10	3	30	4	40	3	30
11-5	6	78	8	105	4	57
16-20	1	16	1	16	2	36
>20	2	48	3	67	4	90
Total	35	293	42	326	48	347

was crossed using pollen from a normal diploid stock and the other was selfed. The crossed cob set six seeds and the selfed one had two, but the normal haploids (those not showing cytomixis to any great extent), when either crossed to normal diploid or selfed, did not set any seed. Seed set in haploid plants would require the formation of fertile female gametes. Whether cytomixis had also taken place during megasporogenesis to increase the frequency of viable female gametes in these two plants could not be ascertained in this study. However, viable seeds obtained in the progeny of haploids would serve as useful material in cytogenetical analysis and breeding.

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