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Research Article

MMS-Induced Cytomixis in Pollen Mother Cells of Broad Bean (*Vicia faba* L.)

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Abstract: Cytomixis was recorded during microsporogenesis in broad bean (*Vicia faba* L.), a member of the family Fabaceae. It is an important pulse crop used as vegetable, silage, forage and stock feed. The phenomenon of cytomixis was observed in various stages of meiosis in methyl methane sulphonate (MMS) treated populations of *Vicia faba* L. Cytomixis was observed to occur through various methods, i.e. by forming cytoplasmic channels, and direct fusion of pollen mother cells (PMCs), the former was more frequent than the latter. Both types were observed at different stages of microsporogenesis. The migration of nuclear content involved all the chromatin/chromosomes or part of it from donor to recipient cell/cells. The occurrence of PMCs with chromosome numbers deviating from the diploid number (2n = 12) through the process of cytomixis may lead to the production of aneuploid and polyploid gametes. Stickiness of chromosomes was observed in all the populations treated with various concentrations of MMS. Increasing the concentration of MMS had a positive effect on the percentage of PMCs showing cytomixis. The level of pollen fertility was found to be affected by cytomixis and chromosome stickiness. It seems possible that genetic factors might have also contributed towards pollen sterility.

Key Words: *Vicia faba*, MMS, microsporogenesis, cytomixis, pollen fertility, stickiness

Introduction

Cytomixis was first observed by Kornicke (1901) in pollen mother cells (PMCs) of *Crocus sativus* L., and then by Gates (1911), who studied the PMCs of *Oenothera gigas* and defined it as a phenomenon of transmigration of chromatin from one cell to an adjoining cell, and coined the term cytomixis. Since that time, cytomixis has been more commonly reported during microsporogenesis in several families of flowering plants (McClintock, 1929; Semyarkhina & Kuptsou, 1974; Soodan & Waffai, 1987; Bahl & Tyagi, 1988; Koul, 1990; Yen et al., 1993; Kumar & Sharma, 2002; Haroun et al., 2004). In addition, such migration of chromatin has also been reported in somatic cells (Bowes, 1973; George & Geethamma, 1985) and in the interphase between somatic and meiotic cells (Cooper, 1952). In Leguminosae, cytomixis has been reported in many genera, e.g., *Lotus* (De Nethan Court & Grant, 1964), *Ononis* L. (Morrisset, 1978), *Vigna* Endl. (Sen & Bhattacharya, 1988), *Lathyrus* L. (Seijo, 1996; Kumar & Sinha, 1991), and *Pisum* L. (Gottschalk, 1970; Nirmala & Kaul, 1994).

Although cytomixis has been reported in several plant species, its origin is not clear. Among the factors proposed to cause cytomixis are: 1. The influence of genes (Kaul & Nirmala, 1991), 2. Abnormal formation of the cell wall during premeiotic divisions (Kamra, 1960), 3. Action of chemical agents such as colchicine and methyl methane sulphonate (Sinha, 1988), and rotenone (Amer & Mikhael, 1986), 4. Changes in the biochemical process that involves microsporogenesis modifying the microenvironment of affected anthers (Koul, 1990), 5. The effect of gamma radiation resulting in an imbalanced and sterile genetic system (Amma et al., 1990), and 6. The presence of a male-sterile mutant gene and its frequency altered by environmental factors (Nirmala & Kaul, 1994), environmental stress, and pollution (Haroun et al., 2004).

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The aim of this study was to investigate cytomixis, its cause, and genetic significance.

Materials and Methods

Seeds of *Vicia faba* L. var. major were obtained from the Genetics Division, Indian Agricultural Research Institute (IARI), New Delhi. Dry (10%-12% moisture content) and healthy seeds of the variety were pre-soaked in distilled water for 12 h and then treated with methyl methane sulphonate (MMS) at 10 different concentrations (0.01%-0.1%) prepared in sodium phosphate buffer with 7.0 pH for 6 h with constant intermittent shaking. The treated seeds were washed in running tap water for 30 min to remove the residual effect of the mutagen sticking to the seed coat. One set of seeds was kept untreated to act as control for comparison. A total of 11 treatments (including control) were sown separately following a complete randomised block design (CRBD) with 3 replications during the Rabi season (2003-2004) at the Agriculture Farm, Aligarh Muslim University, Aligarh. Each treatment consisted of 300 seeds with 100 seeds in each plot of 6 × 6 m. The seed-to-seed and row-to-row distance was maintained at 25 and 40 cm, respectively. For meiotic studies, young flower buds from 30-40 randomly selected plants were fixed in freshly prepared Carnoy's fluid (alcohol:chloroform: acetic acid in a 6:3:1 ratio) for 24 h and washed and preserved in 70% alcohol at 4 °C. Then the anthers were squashed in 2% acetocarmine, dehydrated in NBA Series (50% acetic acid + 50% normal butyl alcohol and then passed to 100% normal butyl alcohol), mounted in Canada balsam, and dried at 45 °C. More than 100 dividing PMCs from each treatment, as well as control populations, were studied and analysed. Cytomixis was observed only in PMCs, not in anther wall cells. It was evident by the rounded shape of PMCs, while the anther wall (which is composed of epidermis, endothecium, middle layers, and tapetum) cells were elongated and flattened. Moreover, these layers were ephemeral and degenerated, providing nourishment to the PMCs. During our cytological investigation, anther wall layers were degenerated and only PMCs were present. Pollen grains were also stained with 2% acetocarmine to study pollen fertility. Photographs were taken from freshly prepared slides using an Olympus X30 research photomicroscope.

Results and Discussion

The experimental results are presented in Table 1, which reveals that the percentage of PMCs showing cytomixis was exponential in relationship to the mutagenic concentration and was altogether nil in the control populations. An increase in the frequency of PMCs showing cytomixis was found with increasing concentrations of MMS (0.01% to 0.1%) (Table 1).

The frequency and intensity of cytomixis depended on the nature of the connection between the adjacent cells. Two types of connections between PMCs were observed: cytoplasmic channels and direct fusion. Cytomixis through cytoplasmic channels was observed at various stages of cell division and PMCs were mostly connected with a single cytoplasmic channel (Figure 1d). Multiple cytoplasmic channels were also found between PMCs, but in low frequency (Figure 1a). The direct fusion of PMCs was observed at various stages of cell division (Figures 1d and e) and the frequency of cells showing cytomixis through this method was greater during the first part of meiotic division (Table 1). Some PMCs were found to have a cytoplasmic channel with one PMC and direct fusion with another (Figure 1c).

Although cytomixis occurred through cytoplasmic channels and direct fusion of cells at different stages of microsporogenesis, the former was more frequent than the latter. Cytomixis through both methods was more common at various stages of meiosis-I than meiosis-II (Table 1). Similar observations have been reported by many researchers (Bahl & Tyagi, 1988; Seijo, 1996 & Haroun et al., 2004).

In the majority of cases, 3-8 meiocytes were involved in cytomixis (Figure 1e), though in some cases, only 2 meiocytes were found to have cytoplasmic connections (Figure 1g). Cytoplasmic channels were of varying breadth to permit the migration of the chromatin/ chromosomes from donor to recipient cell/cells at various stages of meiosis. It was not uncommon to find 2 or 3 cytoplasmic channels emerging from a single PMC and connecting 2 or more cells (Figure 1a). The migration of chromatin materials from one PMC to another was unidirectional, i.e. from a donor to a recipient cell (Figure 1b); however, the chromatin materials migrated from a single meiocyte to more than one adjoining PMCs simultaneously. Wherever more than 2 PMCs were simultaneously involved in cytomixis, the chromatin

Table 1. Effect of increasing concentration of MMS on different stages of meiosis showing an increase in cytomixis and chromosomal stickiness, and reduction in pollen fertility in *Vicia faba* L.

Conc. in %	Total number of PMCs observed	Number of cells with normal (2n=12) chr. Number.	Number of cell with abnormal chr. Number	Number of PMCs showing cytomixis	% age of PMCs showing cytomixis	Types of cytomixis		Number of cells showing cytomixis at various stages of meiosis										PMCs with stickiness (%)	Pollen fertility (%)
						CC	DF	L.Z	P.D	D.	M-I	A-I	T-I	P-II	M-II	A-II	T-II		
Control	110	110 (100%)	–	–	–			–	–	–	–	–	–	–	–	–	–	–	90
0.01	108	108 (100%)	–	4	3.70	2	2	–	–	–	–	–	–	–	–	–	–	2.77	86.43
0.02	115	115 (100%)	–	6	5.21	2	4	–	–	–	–	–	–	–	–	–	–	4.34	84
0.03	110	108 (98.18%)	2 (1.81%)	8	7.27	3	5	3	3	2	–	–	–	–	–	–	–	7.27	88.76
0.04	108	102 (94.44%)	6 (5.55%)	17	15.74	7	10	3	3	3	2	2	2	–	2	–	–	12.03	79.43
0.05	120	110 (91.66%)	10 (8.33%)	29	24.16	12	17	6	5	5	3	3	3	2	2	–	–	15.83	72.43
0.06	118	110 (93.22%)	12 (110.16%)	37	31.35	17	20	8	6	6	4	3	3	2	2	3	–	21.18	68.65
0.07	115	100 (86.95%)	15 (13.04%)	55	47.82	25	30	12	10	8	6	6	4	3	3	3	–	24.34	66.65
0.08	118	103 (86.44%)	15 (12.71%)	72	61.08	32	40	14	12	10	8	8	6	4	4	4	2	29.66	66.43
0.09	120	110 (91.66%)	10 (8.33%)	79	65.83	29	50	16	14	10	10	8	4	5	4	4	4	33.33	64.78
0.1	106	100 (94.33%)	6 (5.66%)	81	76.41	30	51	16	14	12	10	8	4	5	4	4	4	45.28	62.33

L.Z. = Leptotene Zygotene, P.D. = Pachytene Diplotene, D. = Diakinesis, M-I = Metaphase-I, A-I = Anaphase-I, T-I = Telophase-I, P-II = Prophase-II, M-II = Metaphase-II, A-II = Anaphase-II, T-II = Telophase-II, Cyt. = Cytomixis, Conc. = Concentration, C.C. = Cytomictic channel, D.F. = Direct fusion.

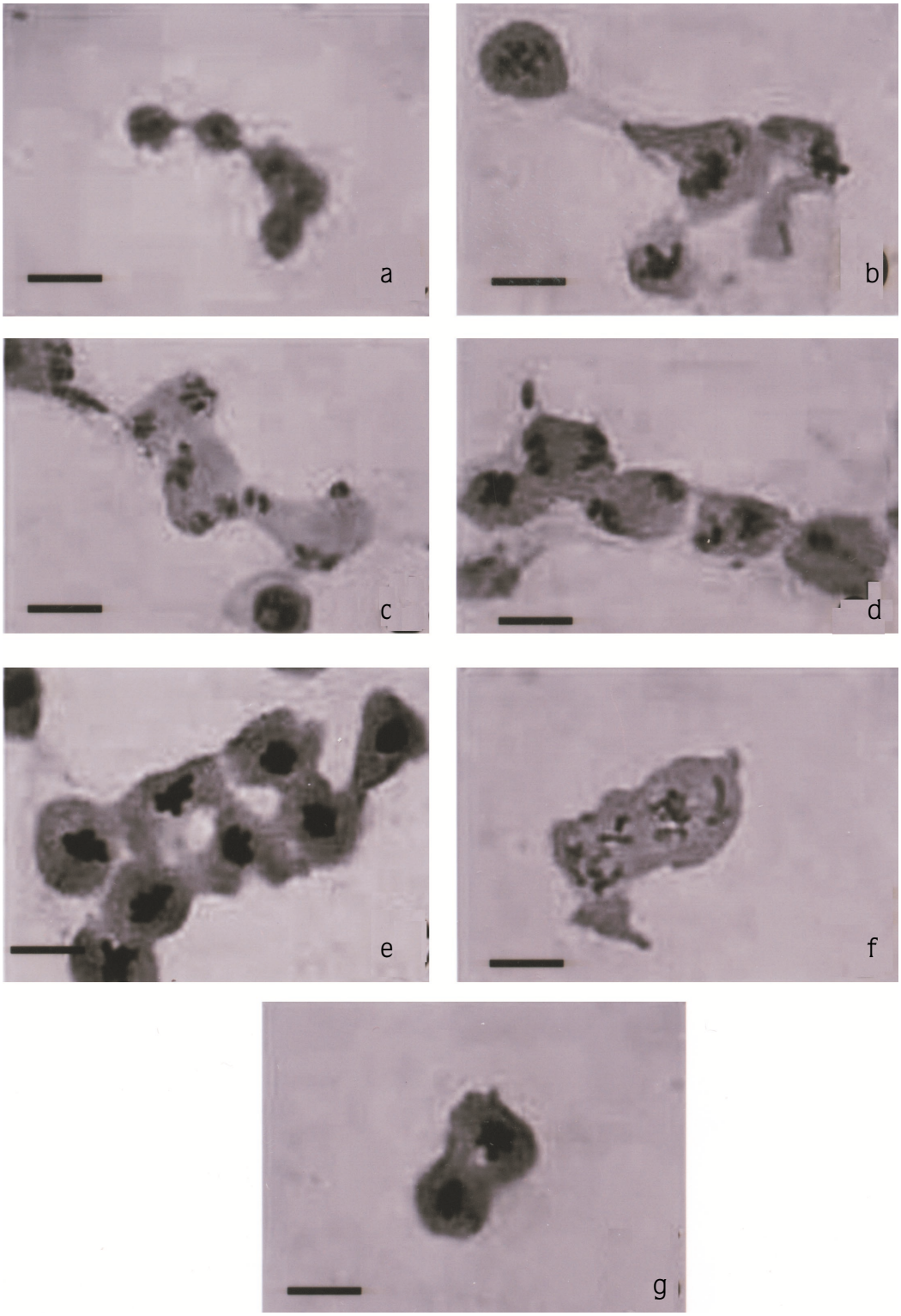

Figure 1. a) PMCs connected through a cytoplasmic channel. b) 2 PMCs showing unidirectional transmigration. c) PMCs showing bivalent transmigration through a cytoplasmic channel. d) PMCs at different stages of meiosis showing cytomixis by direct fusion. e) PMCs showing direct fusion and stickiness of chromosomes. f) A polyploid PMC. g) 2 PMCs showing cytomixis by direct fusion.
Bar scale represents 5 µm

materials passed from the first meiocyte to the second, from second to the third, and so on (Figure 1c). Unidirectional migration of chromatin materials from one PMC to another in a series has been reported by Gottschalk (1970); however, in the present investigation, a single PMC was found donating its chromatin materials simultaneously to 2 PMCs through 2 independent cytoplasmic channels. This type of chromatin migration is different from the pattern described by Gottschalk (1970). In rare cases, all of the chromatin materials of the donor cell migrated to the recipient cells and the donor cell was emptied. The migration of only a part of the chromatin material caused the formation of aneuploid cells.

Sometimes the recipient cells were observed with an additional mass of chromosomes, which did not pair with the normal chromosomes, but did so among themselves and went on further divisions separately. A similar situation was reported in pearl millet by Pantulu and Manga (1972). The fate of such additional masses of chromosomes is not definitely known, but they probably form either micronuclei or micropollen. Loss of chromosomes in meiotic and somatic cells has been observed in *Gossypium* L. by Sarvella (1958). According to Thakur (1978), a loss or gain of one or more chromosomes has 2 obvious possibilities: first, extremely deficient gametes will be lethal and will be eliminated; second, those gametes, which contain a chromosome number other than normal and are able to survive, may be responsible for producing euploids. In cases where migration of all chromatin material takes places, there is the possibility of the development of polyploids (Figure 1f). A noteworthy point of the present investigation is that sometimes chromatin material migrated without the nucleolus or the nucleolus migrated without chromatin materials. The migration of chromosomes in between and among the PMCs ranges from 1 to 6, implying that chromosome migration was broken at different intervals right from the very initiation of the process up to the complete migration of chromatin material.

The frequency of PMCs showing cytomixis was higher during the early stages of meiosis and gradually decreased towards the end of meiosis (Table 1). This observation supports earlier views (Meheshwari, 1950; Levan, 1941) that early stages are more favourable for cytomixis, and is contrary to the belief that all the stages of meiosis are equally susceptible to cytomixis (Verma et al., 1984).

In the phenomenon of cytomixis, 2 or more PMCs in the same phase of division are usually involved, but in the present case the cytoplasmic connections between PMCs at different stages of meiosis, such as anaphase-I and metaphase-II, anaphase-I and anaphase-II, and diakinesis/metaphase and anaphase-I, were observed (Figure 1d). In a condition where anaphase-I chromosomes, which were still undivided, moved to another cell at anaphase-II, the chromosomes were already divided. In such a situation, the anaphase-I chromosomes might be left as laggards or might behave in some other way. Therefore, it is difficult to ascertain the fate of these chromosomes until their behaviour is traced in subsequent stages. During observation, cells larger and smaller than normal, with or without nuclei, were also found. This could have been because of excess chromatin material resulting from their migration and accumulation, followed by their remigration to other cells. That the migration took place at anaphase-II and later stages was also evident from the presence of hexapolar anaphase-II, pentads, and hexads. Pollen fertility was very low and gradually decreased with the increase in frequency of cells showing cytomixis (Table 1). Most of the pollen was shrunken and sterile.

Though triploids are supposed to be sterile, owing to their abnormal chromosome behaviour, in the present case, the extent of sterility was such that it was not attributed to the above irregularities alone. Cytomixis in the present case may also have been responsible for the sterility because: 1. The number of pollen grains was considerably reduced because of degeneration of cells with no or very little genetic material; 2. Among the cells completing all the meiotic stages, most of them were genetically imbalanced because of a higher or lower number of chromosomes than normal cells.

The factors responsible for cytomixis are rather ambiguous. Some possible causes attributed to cytomixis are the effect of fixation (Gottschalk, 1970), mechanical injury (Sarvella, 1958), pathological conditions (Boback & Herich, 1978), temperature anomalies (Basavaiah & Murthy, 1987), polyploid level (Verma, et al., 1984), hybrid condition (Yen et al., 1993), cell response as a consequence of pesticides and antibiotic dosages (Kumar & Sinha, 1991), abnormal genetic behaviour due to treatment with a chemical mutagen (Kumar & Srivastava, 2001; Kumar & Sharma, 2002), crop culture condition (Pierozzi and Benatii Jr., 1998), failure of cell wall

formation during premeiotic mitosis (Kamra, 1960), and genetically controlled behaviour (De Mantu & Sharma, 1983). Cytomixis in the present investigation may have been attributed to abnormal genetic behaviour due to treatment with a chemical mutagen. With the increase in mutagen concentration, the frequency of PMCs that showed chromosomal stickiness and cytomixis increased. According to Kaul (1971), some chemicals, which cause stickiness of chromosomes, may be responsible for cytomixis. Our consistent failure to find chromosomal stickiness and cytomixis in control plants confirms this view. In the present investigation, the reduction in frequency of PMCs showing variation in chromosome number was observed in the population treated with higher concentrations of mutagen (0.08%-0.1% MMS), but cytoplasmic connections between the meiocytes was a common occurrence, thereby suggesting that stickiness prevents the migration of chromosomes from one cell to another.

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