EMS Induced Intercellular Chromatin Transmigration in *Papaver somniferum* L.

Girjesh KUMAR and Sana NASEEM

Plants Genetics Laboratory, Department of Botany, University of Allahabad, Allahabad, India

Abstract

Kumar G., Naseem S. (2013): **EMS induced intercellular chromatin transmigration in** *Papaver somniferum* L. Czech. J. Genet. Plant Breed., **49**: 86–89.

The phenomenon of chromatin migration was observed during microsporogenesis in an ethyl methane sulphonate (EMS) treated population of poppy, which is an important medicinal plant. Cytomixis occurred through a cytoplasmic channel or by direct fusion of pollen mother cells (PMCs); the former was more recurring than the latter. The process was associated with irregular meiosis. PMCs with differing chromosome numbers from the normal diploid number ($2n = 22$) through cytomixis may lead to the production of aneuploid and polyploid gametes. An increase in the concentration of EMS had a positive effect on the percentage of PMCs showing cytomixis. In addition to cytomixis, other chromosomal abnormalities were also found. Cytomixis along with the related chromosomal abnormalities largely affected the post-meiotic products resulting in some pollen sterility.

Keywords: cytomixis; ethyl methane sulphonate; microsporogenesis; pollen sterility; poppy

Cytomixis is the phenomenon of migration of chromatin material through cytoplasmic connections or cytomictic channels. It has been discovered in over a hundred angiosperm species (Heslop-Harrison 1966; Falistocco *et al.* 1995; Ghaffari 2006; Lattoo *et al.* 2006) so far. The phenomenon was at first found in epidermal cells of *Allium nutans* by Miehe (1901) and in pollen mother cells of *Crocus vernus* by Koernicke (1901). Afterwards, Gates (1911) described the phenomenon in male meiocytes of *Oenothera gigas* and named it cytomixis.

Several morphologically and functionally variable connections exist between the cells in plants. Plasmodesmata, an important intercellular connection, are specialized channels spanning the cell wall (McLean *et al.* 1997). However, during microsporogenesis, plant cells can be connected with one another by the channels considerably exceeding plasmodesmata in their size and differing from them in their structure. These cytomictic channels are predominantly the pathways for migration of nuclear material between cells (Lattoo *et al.* 2006). Some researchers associate the formation of cytomictic channels with the need of exchange of certain substances between cells for a synchronous pollen development (Heslop-Harrison 1966). Although views on the significance of cytomixis are mixed and conflicting, most researchers consider cytomixis to be of considerable evolutionary significance (Falistocco *et al.* 1995; Boldrini *et al.* 2006). It is considered a possible cause of aneuploidy and polyploidy (Lattoo *et al.* 2006), and even as one of the modes of origin of B chromosomes (Ghaffari 2006).

The purpose of the present cytological investigation was to conduct a comparative analysis of the

cytomixis frequency in an ethyl methane sulphonate (EMS) treated population of poppy. The study focused on the characterization and consequences of the phenomenon in relation to the meiotic behaviour and reproductive success of the plant. Possible genetic causes, peculiarities at a cytological level, as well as its genetic significance were taken in account.

Seeds of a locally adapted inbred line of *Papaver somniferum* L. viz. Vivek were obtained from CIMAP, Lucknow. Dry and healthy seeds were standardized for approximately 12% moisture content and were pre-soaked in distilled water for 12 h. Seeds were then treated with EMS at 3 different concentrations (0.2, 0.4 and 0.6%) prepared in sodium phosphate buffer with 7.0 pH for 6 h. An untreated set was maintained as control. The treated seeds along with the control were sown in triplicates immediately in the field at the Experimental Research Farm of CIMAP, Lucknow, India, adopting the randomized complete block design (RCBD). For meiotic studies, young floral buds were fixed in a freshly prepared 1:3 acetic alcohol solution (Carnoy's fixative). Slides were prepared using an anther squash technique with 2% acetocarmine.

Cytological investigations of the control population of poppy revealed the species standard chromosome set (n = 11) with 11 bivalents at diakinesis (Figure 1A) and 11:11 chromosomal segregation at anaphase I (Figure 1B). However, cytomixis was observed in the EMS treated population (Figure 1C–K). The data of experimental results are presented in Table 1. The percentage of PMCs showing cytomixis exponentially increased with an increase in the concentration of EMS. The phenomenon was more efficient at early stages of meiosis I (Maheshwari 1950). Owing to cytomixis pollen mother cells (PMCs) with double chromosome complement as well as fewer chromosomes were observed. The chromatin transfer occurred through two types of intercellular connections, i.e. cytoplasmic channels and direct fusion. The former was more frequent than the latter (Bahl & Tyagi 1988; Haroun *et al.* 2004).

Generally, two PMCs were engaged in chromatin transfer but at times more than two and even more PMCs with chromatin masses distributed throughout the cytoplasm were observed. Enucleated PMCs that have completely transferred their nuclear content were found in good numbers. PMCs were mostly found to be connected with a single cytoplasmic channel. Multiple cytoplasmic channels were at a low frequency. PMCs having the cytoplasmic channel with one PMC and direct fusion with another PMC were observed. Cytomixis occurred not only at same stage, but also at different stages of meiosis. Two B-chromosomes were also observed during cytomixis.

Although the phenomenon has been reported in a large array of plant species, the factors responsible for cytomixis are rather ambiguous. Abnormal genetic behaviour due to treatment with a chemical mutagen (Kumar & Sharma 2002)

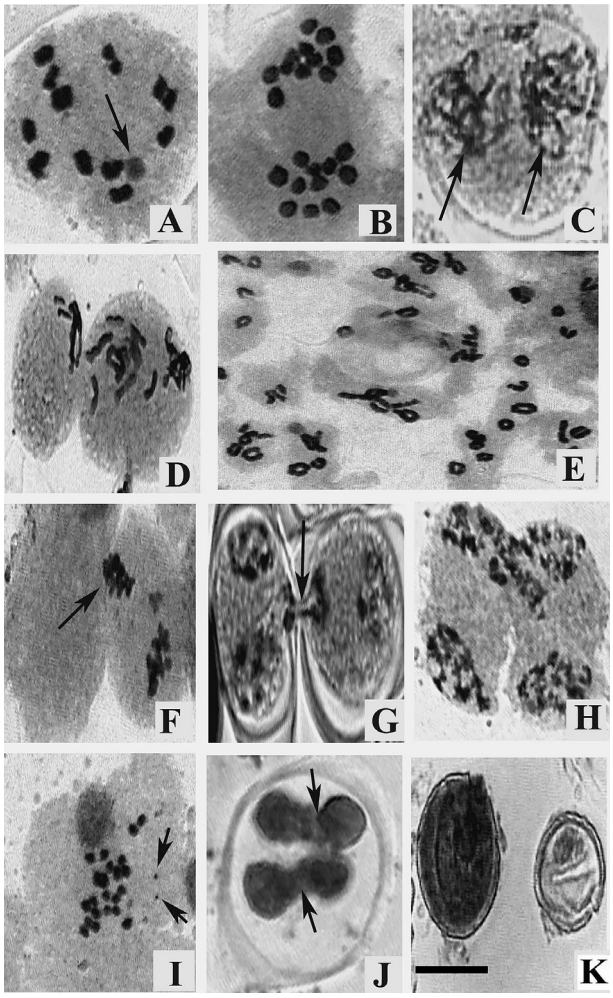

Figure 1. Meiotic stages of desynaptic variants; A – normal diakinesis (11 bivalents; arrow showing nucleolus), B – normal anaphase I (11:11 separation), C – PMC with double chromosome complement, D – chromatin transfer at early prophase I, E – mixing in group, F – complete transfer of sticky chromosomes at metaphase I leaving behind an empty PMC, G – channel formation at telophase I, H – fusion at telophase I, I – 2 B chromosomes (arrow) in PMC involved in mixing, J – mixing between tetrads, K – pollens (sterile unstained); scale bar: 4.2μm

Table1. Effect of increasing the concentration of EMS on the frequency of cytomixis and other chromosomal abnormalities along with pollen viability

EMS conc. (%)	PMCs observed (total No.)	Cytomixis											Other chromosomal abnormalities (%)	Pollen viability (%)
		PMCs involved		type (%)		meiotic stage (No.)								
		(No.)	(%)	CC	DF	P	M I	A I	T I	M II	A II	T II		
Control	235	–	–	–	–	–	–	–	–	–	–	–	–	88.30–94.52 (93.17 ± 0.18)
0.2	214	21	9.81	68.51	31.49	11	5	1	2	–	2	–	32.58	71.43–87.21 (81.15 ± 1.14)
0.4	225	34	15.11	61.54	38.56	13	7	5	4	1	2	2	38.14	58.04–75.38 (62.59 ± 1.43)
0.6	216	46	21.29	55.25	44.75	18	10	6	3	2	3	4	43.02	46.74–60.20 (52.11 ± 0.89)

PMCs – pollen mother cells; CC – cytomictic channel; DF – direct fusion; P – prophase I; M I – metaphase I; A I – anaphase I; T I – telophase I; M II – metaphase II; A II – anaphase II; T II – telophase II

could be one of the possible causes attributed to cytomixis like in the present case. Various chromosomal abnormalities such as laggards, chromatin stickiness, precocious movement, unsynchronization, unorientation, micronuclei, single bridge, forward movement etc. were also recorded. The pollen viability was considerably reduced due to degeneration of cells with no or very little genetic material. Coenocytes were formed at early prophase-I. They may be formed either by the passage of a nucleus from one PMC into another (Price 1956) or through the fusion of PMCs (Mehra & Kalia 1973).

The present cytological investigation clearly reveals that EMS induced cytomixis in poppy may be considered to be a possible source of production of aneuploid and polyploid gametes. Such gametes can be used in breeding programmes to create genetic variability through changed chromosome numbers that may lead to improvement of some novel traits of the plant. Thus, EMS induced cytomixis in poppy should be given more attention in future studies.

Acknowledgements. The authors are extremely thankful to Prof. R. Rajasekharan, Director, CIMAP (Central Institute of Medicinal and Aromatic Plants), Lucknow, for providing the experimental field to grow the crop. Kind help and support of Dr. B. Kumar, Scientist EII at CIMAP, Lucknow, is gratefully acknowledged. Sincere thanks to all the members of Plant Genetics Laboratory.

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Received for publication May 22, 2012
Accepted after corrections January 30, 2013

Corresponding author:

Sana Naseem, PhD., University of Allahabad, Department of Botany, Plant Genetics Laboratory, 211002 Allahabad, India
e-mail: sana.naseem3@gmail.com