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EMS-INDUCED CYTOMICTIC VARIABILITY IN SAFFLOWER (*CARTHAMUS TINCTORIUS* L.)

Seeds of safflower (Carthamus tinctorius L.) were subjected to three treatment durations (3h, 5h and 7h) of 0.5 % Ethyl Methane Sulphonate (EMS). Microsporogenesis was carried out in the control as well as in the treated materials. EMS treated plants showed interesting feature of partial inter-meiocyte chromatin migration through channel formation, beak formation or direct cell fusion. Another interesting feature noticed during the study was the fusion among tetrads due to wall dissolution. The phenomenon of cytomixis was recorded at nearly all the stages of microsporogenesis connecting from a few to several meiocytes. Other abnormalities such as laggards, precocious movement, bridge and non-disjunction of chromosomes were also recorded but in very low frequencies. The phenomenon of cytomixis increased along with the increase in treatment duration of EMS. Cells with these types of cytomictic disturbances may probably result in uneven formation of gametes or zygote, heterogenous sized pollen grains or even loss of fertility in future.

Introduction. Since the first discovery of cytomixis in *Crocus sativus* by Kornicke [1] it has been reported in a number of plant species [2–7], but reports on cytomictic variations in safflower is meager due to pure staining ability, problem of chromosome stickiness and lack of research work on its cytogenetical aspects. The phenomenon of cytomixis is characterized by the migration of chromatin/chromosomes between the surrounding meiocytes through cytoplasmic channels or direct fusion among PMCs.

Although cytomixis has been reported in a number of plant species, its origin is still an enigma. Among the factors proposed to cause cytomixis are: 1) the influence of genes [8]; 2) abnormal formation of the cell wall during premeiotic divisions [9]; 3) action of chemical agents such as colchicine and methyl methane sulphonate [10] rotenone [11]; 4) changes in the biochemical process that involves microsporogenesis modifying the microenvironment of affected anthers [12]; 5) the effect of gamma radiation resulting in an imbalanced and sterile genetic system [13]; 6) the presence of a male-sterile mutant gene and its frequency altered by environmental factors [14], or environmental stress, and pollution [15].

The persistence of cytomictic channels has significant effect on the meiotic process, its end products and fertility of the plant. This process seems to be of evolutionary significance, which is endorsed by many authors [16, 17] and is more frequent in species with unbalanced genomes such as haploids, aneuploids, hybrids [18], mutants [2] triploids [19] and apomicts [20]. It may lead to the production of aneuploid plants with certain morphological characteristics [21] or produce unreduced gametes as reported in several grass species including *Dactylis* [22] and *Aegilops* [23]. Unreduced gamete formation is of evolutionary importance leading to the production of plants with higher ploidy levels. Aneuploid pollen grains produced as a result of cytomixis may also be responsible for reduction in pollen fertility of the *Stipa* species [24].

Material and Methods. Seeds of *C. tinctorius* L. were obtained from the Genetics Division, Indian Agricultural Research Institute (IARI), New Delhi. Healthy seeds of the plant were pre-soaked in distilled water and then treated with ethyl methane sulphonate (EMS) at three treatment durations (3 h, 5 h and 7 h) of 0.5 % EMS. The treated seeds were washed in running tap water 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. For meiotic studies, young flower buds were randomly selected and then fixed in freshly prepared Carnoyrs fluid (alcohol: glacial acetic acid in a 3:1 ratio) for 24h and preserved in 70 % alcohol at 4 °C. The anthers were squashed in 2 % acetocarmine, and photographs were taken from freshly prepared slides using Nikon Image capturing system.

Results. The meiosis in control plants was absolutely normal with 12 bivalents at metaphase-I and 12:12 separation at anaphase-I without any evidence of chromosomal anomalies or cytomictic variations. Cytomixis, the inter PMC (Pollen Mother Cell) transfer of chromatin material is observed only in the populations treated with EMS. Data regarding types of karyological abnormality, cytomictic abnormality, total cytoplasmic abnormality and pollen fertility is provided in the Table. The frequency and intensity of cytomixis depended on the nature of connection between adjacent cells. Maximum amount of cytomixis is contributed to inter-chromatid migration through tube or beak formation (Fig. 2, 3) with a frequency of 1.95 % at 7 h treatment duration followed by cytomixis among PMCs either at same phase of division (Fig. 1, 4, 6) or at different phase (Fig. 5) being 0.85 %, then comes cytomixis through wall dissolution between pollen tetrads being 0.80 % (Fig. 8). Intermeiocyte transfer of genetic material as micronuclei was also noted in considerable amount (Fig. 7). Total frequency of cytoplasmic connections was recorded to be 0.85 ± 0.66 %, 1.62 ± 0.53 % and 3.55 ± 0.72 % at 3, 5, 7 h treatment duration respectively as also depicted in the Fig. 9.

As regard the karyological abnormality percentage, abnormal meiotic course is characterized by the occurrence of chromatin stickiness predom-

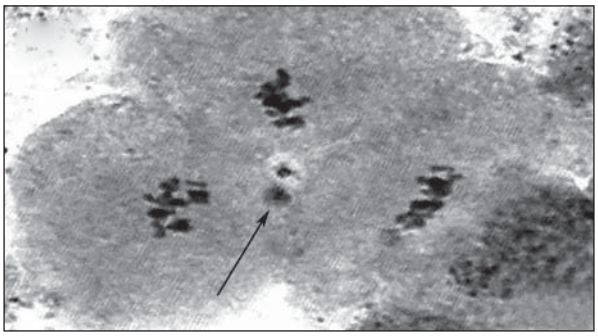

Fig. 1. Migration of chromosome between same phase of three PMCs

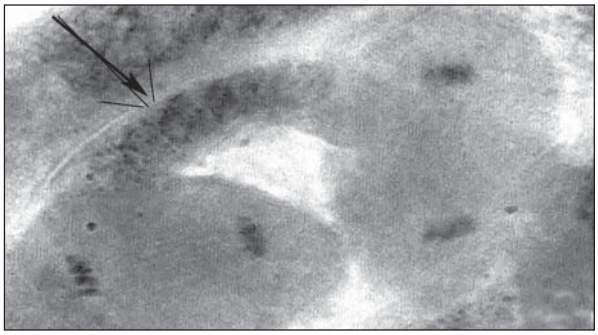

Fig. 2. Cytomixis through Tube formation

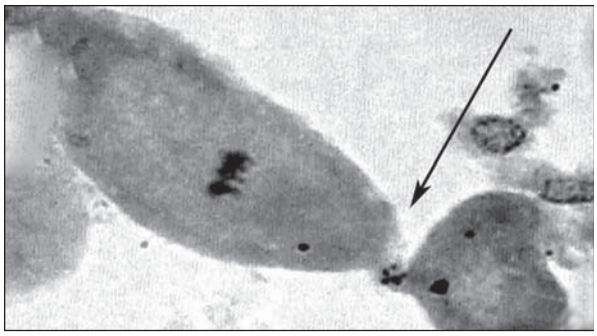

Fig. 3. Cytomixis through Beak formation

EMS induced cytomictic variations in *Carthamus tinctorius* L.

Treatment dose (h)	No. of PMCs Scored	Karyological abnormality, %	Cytoplasmic abnormality, %			Total cytoplasmic abnormality, %	Pollen fertility, %
			cy	icm	cwd		
Control	465	–	–	–	–	–	96.95
3h	451	8.33	0.38	0.25	0.22	0.85 ± 0.66	88.15
5h	463	17.65	0.32	0.95	0.35	1.62 ± 0.53	70.92
7h	492	32.16	0.85	1.95	0.80	3.55 ± 0.72	68.65

Abbreviations: cycytomixis between PMCs, icm– inter chromatid migration, cwd– cytomixis through wall dissolution.

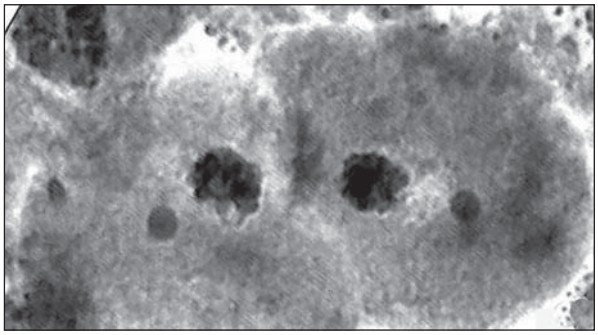

Fig. 4. Cytomixis through cell wall dissolution between similar stages involving movement of extra chromosomal mass

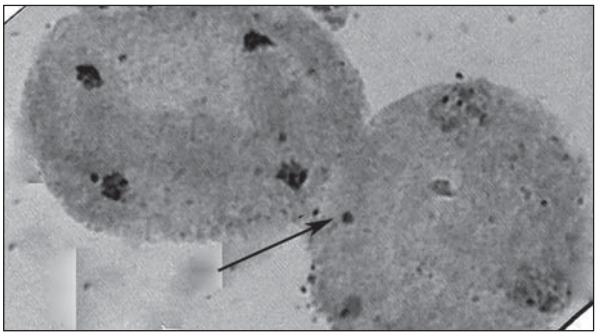

Fig. 5. Cytomixis between more two PMCs of dissimilar stages

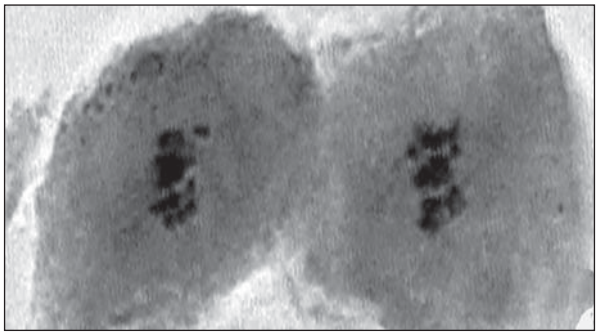

Fig. 6. Cytomixis between two PMCs of similar stages (metaphase-I)

inantly at MI and unequal separation of bivalents/chromosomes at AI/II, bridges and laggards at anaphases were also present in significant amount (Table). Consequent effect of these abnormalities on the microsporogenesis is evident from the occurrence of abnormal tetrads configurations and microsporocytes with micronuclei.

The test for pollen fertility showed fewer amounts of sterile pollen grains in control plants as

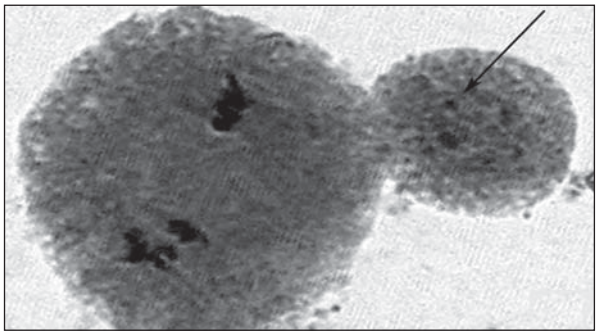

Fig. 7. Cytomixis involving elimination of nucleic material as micronuclei

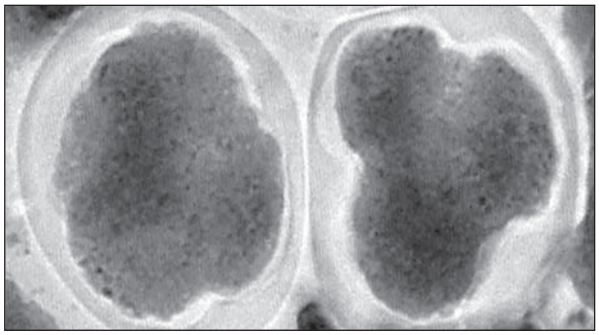

Fig. 8. Direct fusion of tetrads due to cell wall dissolution

compared to treated sets. Pollen fertility recorded in control plants was found to be 96.65 % which decreased to 68.65 % at 7h treatment duration sets. No significant correlation was found between cytomixis and pollen viability.

Discussion. Cytomixis, the migration of chromatin material between and among the meiocytes, meristimatic, tapetal, integumental, nucellar and ovary cells through cytomictic channels or direct adhesion is a well established phenomenon reported in large array of plants [5, 7, 25]. Cytomixis is a potential means to conserve the genetic heterozygosity of gametes [26] and additional means of phylogenetic evolution of karyotypes by reducing or increasing the basic series [27, 28] creation of aneuploids and polyploids [29]. This is a peculiar type of cytological anomaly reported in number of angiosperm species [30, 31]. According to Heslop- Harrison [32] and Risueno et al. [33] the role of cytoplasmic channels is related to transport of nutrients between meiocytes. Although the phenomenon of cytomixis is reported in a number of plant species but the

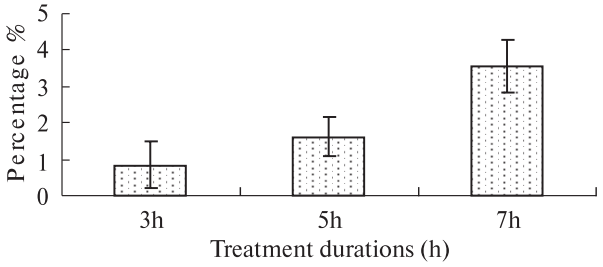

Fig. 9. Percentage of cytomixis at three different time durations of 0.5 % EMS

chromosome transfer in true sense is rare. Kamra [9] working on PMC of *Hordeum* believed that no amount of defective squashing or application of pressure could produce such small protusions so close to one another or to form PMCs with extra fragments or increase in the number of bivalents in them especially at metaphase. In recent years there is accumulating evidence that cytomixis is a normal, genetically controlled phenomenon influenced by physiological and environmental factors [7, 35, 36] rather than being due to fortuitous causes such as fixation, mechanical injuries or pathological anomaly. Cytomixis as a cause of inducing meiotic irregularities and formation of hypo-and hyperploid PMCs has also been reported in several plants [29, 30, 32, 37]. The study revealed that all the stages of meiosis were susceptible to cytomixis as contrary to the general belief that early stages are more favourable [38].

The possible implications of cytomixis are 1. either it may give rise to pollen sterility due to degeneration of cells with no or very little genetic material and 2. most of the cells completing all meiotic stages, would be genetically imbalanced because of less or more number of chromosomes than normal ones [39].

All of the meiotic abnormalities found in the EMS treated set of safflower analyzed revealed that there was no correlation between cytomixis and decrease in pollen fertility percentage but type of meiotic anomalies had direct correlation with pollen fertility. Fertility depends on the efficiency of the meiotic process. Increase in meiotic aberrations caused decrease in pollen fertility percentage which was in concordance with studies conducted by various workers on *Allium, Pilocarpus* [40–43].

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ЦИТОМИКТИЧЕСКАЯ ВАРИАБЕЛЬНОСТЬ У САФЛОРА (*CARTHAMUS TINCTORIUS* L.), ИНДУЦИРОВАННАЯ ЭМС

Семена сафлора (*Carthamus tinctorius* L.) обрабатывали 0.5%-ным этилметансульфонатом (ЭМС) в одном из трех режимов – 3, 5 и 7 ч. Микроспорогенез изучали как в контроле, так и в обработанном материале. Растения, обработанные ЭМС, проявляли интересную особенность частичной интер-мейоцитной миграции хроматина при формировании каналов, образовании клювообразного выступа или прямом слиянии клеток. Другим обнаруженным в исследовании явлением было слияние тетрад из-за растворения стенки. Феномен цитомиксиса отмечался почти на всех стадиях микроспорогенеза и затрагивал от нескольких до многих мейоцитов. Другие аномалии, такие как отставания, преждевременные движения, мосты и неразделения хромосом, отмечались с незначительной частотой. Феномен цитомиксиса возрастал с увеличением длительности обработки ЭМС. Клетки с этими типами цитомиктических нарушений могут, вероятно, приводить к нерегулярному формированию гамет или зигот, гетерогенных по размеру пыльцевых зерен, или даже к потере фертильности в будущем.

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ЦИТОМІКТИЧНА ВАРІАБЕЛЬНІСТЬ У САФЛОРА (*CARTHAMUS TINCTORIUS* L.), ІНДУКОВАНОГО ЕМС

Насіння сафлору (*Carthamus tinctorius* L.) обробляли 0.5%-ним етилметансульфонатом (ЕМС) в одному з трьох режимів – 3, 5 і 7 год. Мікроспорогенез вивчали як у контролі, так і в обробленому матеріалі. Рослини, оброблені ЕМС, виявляли цікаву особливість часткової інтер-мейоцитної міграції хроматину при формуванні каналів, утворенні дзюбоподібного чи прямому злитті клітин. Іншим виявленим в дослідженні явищем було злиття тетрад через розчинення стінки. Феномен цітоміксіса відзначався майже на всіх стадіях мікроспорогенезу і зачіпав від кількох до багатьох мейоцитів. Інші аномалії, такі як відставання, передчасні рухи, мости і нерозділення хромосом, зустрічались з незначною частотою. Феномен цитоміксису зростав із збільшенням тривалості обробки ЕМС, Клітки з цими типами цитоміктичних порушень можуть, ймовірно, приводити до нерегулярного формування гамет або зигот, гетерогенних за розміром пилкових зерен, або навіть до втрати фертильності в майбутньому.

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