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INDUCED CYTOMIXIS IN *CICER ARIETINUM* L. VAR. -50 BY THE TREATMENT OF TRICHLORO ACETIC ACID

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In present investigation, cytomixis was observed during different stages of meiosis in trichloro acetic acid (TCA) treated population of *Cicer arietinum L. Cicer arietinum* L., a member of family Fabaceae, an important pulse crop used as vegetable, silage, forage and stockfed. Cytomixis was observed to occur in forms of cytoplasmic connections and direct fusion of pollen mother cells (PMCs). 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 number deviating from the diploid number (2n= 16) 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 TCA except in 0.01% concentrations. A dose dependent increase in the percentage of PMCs showing cytomixis was found with the increasing concentrations of TCA. The percentage of pollen sterility is clearly affected by cytomixis and stickiness. It seems possible that genetic factors might have also contributed towards the pollen sterility.

Key words : *Cicer arietinum* L., cytomixis, microsporogenesis, TCA.

Cytomixis generally refers to the migration of chromatin from one cell to n adjoining cell through cytoplasmic connections and direct fusion of cytoplasm of the cells. This phenomenon was at first described by Kornick (1901) in pollen mother cells (PMCs) of *Crocus sativus* and the term cytomixis was coined by Gates (1911), who studied the PMCs of *Oenothera gigas*. But now it is believed that it also includes the intercellular transfer of organelles and other cytoplasmic constituents, which has also been reported in spermatogenesis in lower plants (Kwiatkowska *et al.* 2003) The literature reveals that the cytomixis has been observed in a number of plant species (Gottschalk 1970 and Omara 1976) and has mainly been observed in PMCs. In present investigation, cytomixis has been observed by TCA treatment in *Cicer arietinum* during the mutagenesis work. The aim of this is to investigate cytomixis, its cause, nature and genetic significance.

MATERIALS AND METHODS

Dry and healthy seeds of variety K-850 were presoaked in distilled water for 12 h and then treated with trichloro acetic acid with 10 different doses prepared in sodium phosphate buffer at 7.0 pH for 24 h with constant intermittent shaking. One set of seeds was kept untreated to act as control for comparison. For meiotic studies, young flower buds from 25 to 30 randomly selected plants were fixed in freshly prepared Carnoy's fluid (alcohol: chloroform: acetic acid in a 6:3:1ratio) for 2 to 4 h and then washed and preserved in 70% alcohol. The anthers were squashed in 0.5% propionocarmin. More than 100 PMCs from each treatment, as well as control populations, were studied and anaylzed. Cytomixis was observed only in PMCs not in anther wall cells.

Pollen grains were also stained with 1% propionocarmin to study pollen fertility/sterility. The coloured, cytoplasm and nuclei filled

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Table 1 : Effect of different concentrations of TCA on abnormality percentage, types of cytomictic cells, chromosomal stickiness and percentage of sterile pollen grains in Cicer arietinum Var. K-850.

Treatments (%)	Total No. of PMCs observed	No. of cells with abnormal chromosomes No. (%)	PMCs with cytomixis (%)	Types of cytomixis				No. of cells with stickness (%)	Pollen sterility (%)
				M-I		M-II			
				C.C.	D.F.	C.C.	D.F.		
CN	39	-	-	-	-	-	-	-	-
0.01	350	-	3(0.86)	2(0.57)	-	-	1(0.29)	2(0.27)	5.45
0.02	342	2(0.58)	5(1.46)	2(0.58)	2(0.58)	-	1(0.29)	3(0.88)	8.35
0.03	335	3(0.90)	8(2.39)	2(0.60)	4(1.19)	-	2(0.60)	6(1.79)	8.75
0.04	350	7(2.00)	18(5.14)	4(1.14)	8(2.29)	1(0.29)	5(1.43)	12(3.43)	13.42
0.05	348	11(3.16)	31(8.91)	7(2.01)	15(4.31)	3(0.86)	6(1.72)	25(7.18)	18.25
0.06	360	13(3.61)	38(10.56)	8(2.22)	18(5.00)	3(0.83)	9(2.50)	29(8.06)	20.15
0.07	355	11(3.19)	47(13.24)	11(3.13)	22(6.20)	4(1.13)	11(3.13)	35(9.86)	25.05
0.08	352	16(4.51)	55(15.63)	13(3.66)	29(8.24)	4(1.14)	8(2.25)	45(12.78)	29.05
0.09	345	16(4.55)	62(17.97)	13(3.77)	33(9.57)	5(1.45)	11(3.19)	49(14.20)	35.82
0.10	340	17(5.00)	67(19.71)	14(4.12)	35(10.29)	6(1.76)	12(3.53)	53(15.59)	38.12

PMCs: Pollen Mother Cells, M-I: Meiosis-I, M-II: Meiosis-II, C.C.: Cytosplasmic Channel, D.F.: Direct Fusion.

pollen grains having regular shape and size were considered as fertile type while, the colourless, empty pollen grains (without cytoplasm ans nuclei) having irregular shape and size were treated as sterile type. Photomicrographs were taken from freshly prepared as well as permanent slides.

OBSERVATIONS AND DISCUSSION

The observation reveals that the percentage of PMCs showing cytomixis increased with the increasing doses of mutagen (TCA) and was nil in the control (Tabel-1). The frequency and intensity of cytomixis depended on the nature of connection between the adjacent cells. Types, cytoplasmic connections and direct fusion between PMCs were observed. The direct fusion of PMCs was observed at various stages of cell division (figures-2,3,4,5) and the frequency of the cells showing cytomixis through direct fusion was greater during the first phase of meiotic division. Cytomixis through cytoplasmic channels was observed at various stages of cell division and PMCs were mostly connected with a single cytoplasmic channel (Figures-1, 6). Cytomixis through cytoplasmic connections and direct fusions was more common at various stages of meiosis-I than at meiosis-II (Table-1).

Haroun *et al.* (2004) and Bhat *et al.* (2006) also observed that PMCs showing cytomixis increased with the increasing doses of mutagen and was nil in the control as observed in *Cicer arietinum* L. due the mutagenic effect of TCA. Many researchers (Seijo 1996 and Haroun *et al.* 2004) are in support with the finding of present investigation that cytomixis through cytoplasmic channels and direct fusions was more common at various stages of meiosis-I than at meiosis-II. Although it has been reported in several plant species, its origin is not clear. Among the factors proposed to cause cytomixis are: the influence of genes (Kaul and Nirmala 1991); abnormal formation of cell wall during pre meiotic divisions (Karma 1960); action of chemical agents such as

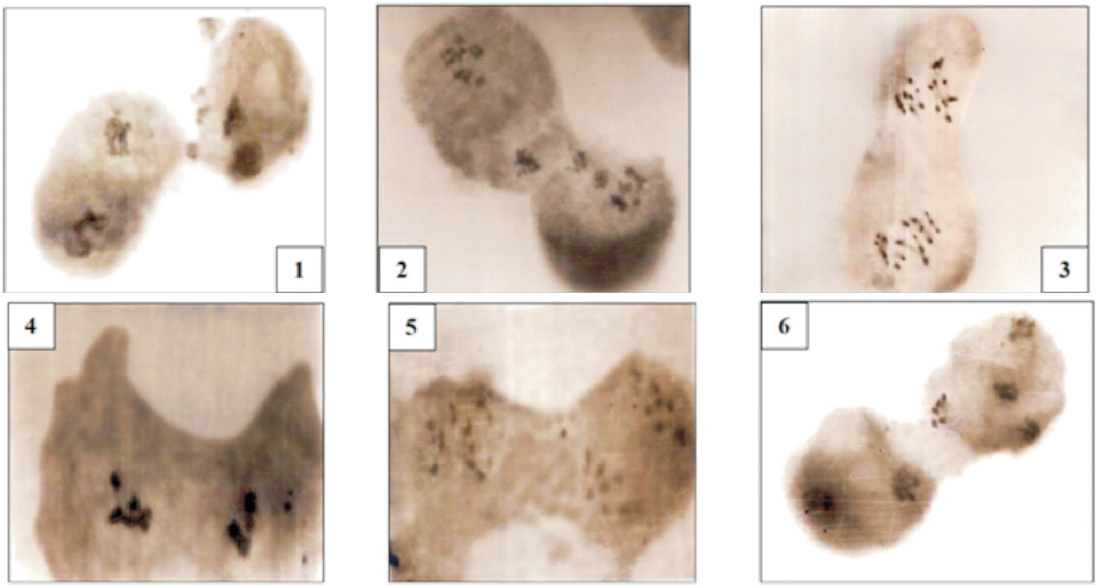

PLATE -1

Plate 1 : Figures 1-6. 1. PMC_s with direct cytoplasmic fusion (1000x). 2. PMC_s showing bivalent transmigration through a cytoplasmic channel (1000x). 3. Cytomixis between microspore mother cells at metaphase I (1000x). 4. PMC_s showing direct fusion and stickiness of chromosomes (1000x). 5 PMC_s at metaphase I and anaphase I showing cytomixis by direct fusion (1000x). 6 Cytoplasmic channel between two PMC_s at telophase I & II stages (1000x).

colchicines and methyl methane sulphonate (Sinha 1988); and rotenone (Amer and Mikhael 1986); the presence of a male sterile mutant gene and its frequency altered by environmental factors (Nirmala and Kaul 1994).

Cytoplasmic channels were of varying breadth or thickness to permit the migration of chromosomes from donor to recipient cell/cells at various stages of meiosis. The migration of chromatin materials from one PMC to another was unidirectional i.e. from a donor to a recipient cell (Figures-2, 6). Unidirectional migration of chromatin materials from one PMC to another in a series has been reported by Gottschalk (1907). In rare cases all of the chromatin material of the donor cell was emptied. The migration of only a part of the chromatin material causes the formation of aneuploid cells. However, cytomixis of intercellular nuclear/chromatin transfer is still an elusive phenomenon, which has been observed in PMCs of many higher plant species (De Souza and Pagliarini 1997) but not in the spermatogenous cells of animals or lower plants.

According to Thakur (1978), a loss or gain of one or more chromosomes has two obvious possibilities: first extremely deficient gametes will be lethal and will be eliminated, second those gametes, which contain a chromosomal number other than normal and are able to survive, may be responsible for producing euploids. In cases where migration of all chromatin material takes place, there is a possibility of development of polyploidy.

In the present study, close relationship is clear between the percentage of cytomixis and the percentage of pollen sterility as previously reported by Haroun (1996), Alice and Maria (1997) and Haround *et al.* (2004). No doubt, cytomixis observed in the late stages of division plays an important role in pollen sterility which consequently decreases seed set and crop productivity. Cytomixis might also have been responsible for the sterility because the number of fertile pollen grains considerably reduced due to degradation

of cells with no or very little genetic material. It may also be due to the fact that most of the cells completing all the meiotic stages, were genetically imbalanced because of a higher or lower number of chromosomes than normal cells (Bhat *et al.* 2006). Cytomixis usually leads to abnormal PMCs which rarely or never show any viability. The cytological aberrations produced at the end of cytomixis are more often non-hereditary and hence are of little significance (Anis and Wani 1997). Therefore, it seems likely that cytomixis alone could be responsible for pollen fertility. An increase in pollen sterility was also reported with an increase in mutagenic treatment by several workers (Anis and Wani 1997 and Kumar *et al.* 2003).

TCA is used as herbicide and also reported as a mutagen. Cytomixis may have been attributed either due to TCA induced abnormal genetic behaviour or disintegration of cell wall and fusion of cytoplasm in between the PMCs of *Cicer arietinum* L., caused by the toxic application of TCA. In present investigation, the Cytoplasmic connections between meiocytes were very common but sometimes stickiness prevented the migration of chromosomes from one cell to another but there were few incidences of aneuploids formation due to migration of only a part of the chromatin material from one cell to another.

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REFERENCES

Alice M S & Maria S P 1997 Cytomixis in *Brassica napus var.* oleifera and *Brassica campestris var.* oleifera (Brassicaceae). *Cytologia* **62** 25-29.

Amer S M & Mikhael E 1986 Cytological effect of pesticides XVI. Effect of the insecticide rotenone on root mitosis of *Vicia faba. Cytologia* **51** 171-176

Anis M & Wani A A 1997 Caffeine induced morpho-cytological variability in Fenugreek, *Trigonella foenumgraecum* L. *Cytologia* **62** 343-349.

Bhat T A, Parveen S & Khan A H 2006 MMS-Induced cytomixis in pollen mother cells of Broad Bean (*Vicia faba L.*). *Turk J Botany* **30** 273-279.

De Souza A M & Pagliarini MS 1997 Cytomixis in *Brassica napus* and *Brassica campestris var.* oleifera (Brassicaceae). *Cytologia* **62** 25-29.

Gates R R 1911 Pollen formation in *Oenothera gigas. Ann Bot* **25** 909-940.

Gottschalk W 1970 Chromosomes and nuclear migration during microsporogenesis of *Pisum ativum. The Nucleus* **131**-9.

Haroun S A 1996 Induced cytomixis and male sterility in pollen mother cells of *Hordeum vulgare* L. *Delta J Sci* **20**172-183.

Haroun S A, A1-Shehri A M & A1-Wadie H M 2004 Cytomixis in the microsporogenesis of *Vicia faba* L. (Fabaceae). *Cytologia* **69** 7-11.

Karma O P 1960 Chromatin extrusion and cytomixis in pollen mother cells of *Hordeum. Hereditas* **46** 592-600.

Kaul M L H & Nirmala C 1991 Male sterile gene action diversity in barley and pea. *Nucleus.* **34** 32-39.

Kornicke M 1901 Uber Ortsveranderung von Zellkarnern. S.B. Niedernhein. Ges. Natur-und Heilkunde Bonn. Pp. 14-25.

Kumar G, Kesarwani S & Sharma V 2003 Clastogenic effect of individual and combined treatment of gamma rays and EMS in *Lens culinaris. J Cytol Genet* **4 (NS)** 149-154.

Kwiatkowska M, Poplonska K & Wojtczak A 2003 *Chara tomentosa* antheridial plasmo-desmata at various stages of spermaogenesis. *Plant Biology* **46** 233-238.

Nirmala C & Kaul M L H 1994 Male Sterility in Pea IV. Gene action duplicacy. *Cytologia* **59** 195-201.

Omara MK 1976 *Cytomixis in Lolium perenne. Chromosoma* **55** 267-271.

Seijo G 1996 Spontaneous cytomixis in the microsporogenesis of sweet pea *(Lathyrus odoratus L.)* (Leguminosae). *Cytologia* **61** 189-195.

Sinha A R P 1988 Morphological and cytological changes induced in *Lindenbergia indica* following treatments. *Genet Polonica* **29** 335-339.

Thakur V 1978 Chromosomal and nuclear transmigration during microsporogenesis in natural populations of some plant species. In: *Recent Trends and Contacts between Cytogenetics, Embryology and Morphology.* Dhyansagar, VR. *et al.* (ed), Today and Tomorrow New Delhi, India, Pp 123-130.