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Induced cytomictic crosstalk behaviour among micro-meiocytes of *Cyamopsis tetragonoloba* (L.) Taub. (cluster bean): Reasons and repercussions

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Abstract. Cytomictic behaviour of chromosomes among pollen mother cells was observed in mutagenic studies in cluster bean (*Cyamopsis tetragonoloba* (L.) Taub.). The study of pollen mother cells (PMC) revealed various chromosomal aberrations among which cytomixis was notified due to its obtrusive peculiarity and is therefore given description in this article. Cytoplasmic and chromatin transmigration were discernible among contiguous or slightly distant PMCs through recreation of passage *via* direct cell-to-cell fusion or channel formation. This cytomictic phenomenon was invariably more pronounced at meiosis I as compared to meiosis II. Plasmodesmatal connections play a paramount role in aiding this behaviour by establishing intercellular crosstalks. The cellular intermingling resulted in syncyte cells which were identified due to their doubled size. Syncyte or unreduced PMC formation leading to unreduced fertile gametes is speculated to act as a possible way out for infraspecific polyploidization of species. Pollen fertility was computed, alongwith this heterosized pollens of varying diameter were segregated. Large sized pollens were 2n pollens; where size difference is a consequence of cytomixis. Cytoplasmic connections among pollens were also observed sporadically. It is opined that syncyte formation and 2n pollen production have evolutionary significance.

Keywords: *Cyamopsis tetragonoloba,* cytomixis, heterosized pollens, infraspecific polypoidy, PMCs, syncyte.

INTRODUCTION

Cytomixis is promiscuous intercellular interaction for exchange of nuclear material, dividing chromosomal bodies and other integral cytoplasmic organelles. The credit for first description of this phenomenon was conferred to Arnoldy (1900) in gymnosperms. Later on, the behaviour was also explored in PMCs of *Crocus sativus* by Koernicke (1901); however, the term 'cytomixis' was christened by Gates (1911) during his findings in *Oenothera gigas* and *Oenothera biennis.* Besides reproductive cells, cytomixis has also been witnessed in root meristematic cells (Jacob 1941), tapetal cells (Cooper 1952), shoot apex

(Guzicka and Wozny 2004) and other diverse somatic cell systems. In angiosperms, it is invariably more frequent in family Poaceae (reported in 82 species) and Fabaceae (reported in 48 species) (Mursalimov *et al.* 2013a)

Occurrence of cytomixis is speculated to be of pathological nature and is frequently documented in species with unbalanced genomes such as haploids, aneuploids, hybrids (de Nettancourt and Grant 1964), mutants (Gottschalk 1970), triploids (Salesses 1970). There are also few instances where cytomixis was more profound among polyploids than their diploid counterparts (Semyarkhina and Kuptsou 1974); where it is perceived to allow elimination of extra DNA in order to stabilize the genome and produce balanced and/or reduced pollen grains (Zhou 2003). The phenomenon has also been documented in PMCs of transgenic tobacco plants (Sidorchuk *et al.* 2007). A unique pattern of B-chromosome pioneered cytomixis was observed in B-carrier plant poppy, where B-chromosome was the first entrants in the recipient cell and A-chromosomes followed them (Patra *et al.* 1988), for which it was argued that heterochroatin blocks of B-chromosomes played a facilitating role for cytomixis.

Cautious contemplation has revealed that cytomixis is a sort of cell selection, which selects and preserves fitting variants but eliminates unbalanced and irreparable PMCs (Kravets 2013). There is a difference of opinion regarding its significance; however general consensus by authors configures an evolutionary trail (Boldrini *et al.* 2006; Li *et al.* 2009). According to Cheng *et al.* (1980), cytomixis acts as an additional facilitator in phylogenetic evolution of karyotypes by reducing or increasing the basic series. However Guan *et al.* (2012) opined contrary views by asserting its deleterious effects on fertility while Veilleux (1985) accredited cytomixis to be a potential means to conserve genetic heterozygosity of gametes.

Plethora of study recruited on cytomictic behaviour suggests that the phenomenon is a resulting event regulated by genetic and environmental factors rather than being due to fortuitous causes such as artifact produced by fixation, mechanical injuries or pathological anomaly (Gottschalk 1970; Song and Li 2009). Factors such as partial or total inhibition of cytokinesis during microsporogenesis (Risuen˜o *et al.* 1969), effect of gamma radiation (Kumar and Yadav 2012; Dwivedi and Kumar 2018), action of chemical agents such as colchicine (Gautam and Kumar 2013), are reported to repercuss into cytomixis. Several environmental constraints such as thermal stress (Sidorchuk *et al.* 2016), cold harsh conditions also intrigue inter-meiocyte fusion and hence syncyte formation (Singhal *et al.* 2011).

Depending upon the intensity and severity, cytomixis is categorized into three main types: weak (local), intensive, and destructive or pathological (Kravchenko 1977). The study is significant because cytomixis is linked to evolution since it may lead to change in ploidy as well as often leads to unreduced gamete. Furthermore, the study is of great relevance in assessing reasons and process of its occurence, and the complex process of microsporgenesis which is substantially affected by cytomixis. Role of plasmodesmatal connections and callose insulation needs more detailed scrutiny. Ionizing radiation i.e. gamma rays was used in the present study for exploiting its mutagenic role for improvement genetic characteristics of the plant system. Role of gamma rays has also been anticipated for its role in inducing polyploids and aneuploids *via* cytomixis in several reports. Gamma ray is ascribed to be most efficient factor that results in imbalanced genetic system (Saraswathy *et al.* 1990).

The plant material cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] is an important legumes, thriving well in semi arid zones of Indian and Pakistan. The plant is highly valued for its guar gum that is extracted form the seed endosperm that add ons its economical value. Besides this, cluster bean occupies a decent position in traditional folklore medicines and is nutraceutically also very important. Cytomictic behaviour in *Cyamopsis tetragonoloba* has been previously described spontaneously (Sarbhoy 1980), but the present article is envisioned to reach new vistas by exploring multitude facets of gamma rays induced cytomixis. Salient features and repercussions entailed in relation to meiotic behaviour and reproductive success will be ambit of this work.

MATERIALS AND METHODS

Plant material

Seeds of Cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] were procured from Central Arid Zone Research Institute (CAZRI) Jodhpur, Rajasthan, India. After preliminary screening, accession number RGC-1038 was selected for cytogenetical work.

Agroclimatic conditions of the experimental site

Present study was conducted in an experimental cage in Roxburgh Botanical Garden, Department of Botany, University of Allahabad, Prayagraj, UP, India during kharif season in July to November. The geographical location is 25°27'43.01''N, 81°51'10.42''E. Prayagraj lies in sub-tropical climatic zone and receives an annual rainfall of 958mm where relative humidity is 59%.

Treatment and Sowing

Fresh seeds of *Cyamopsis tetragonoloba* were arranged into different packets that were irradiated with gamma rays at increasing dose (*viz.* 100 Gy, 200 Gy and 300 Gy) from a Co-62 source radioisotope inside gamma chamber at National Botanical Research Institute (NBRI), Lucknow, India at radiation speed of 2gy per second. These irradiated seeds were sown in respective pots in replicates in complete randomized block design (CRBD) to raise the generation alongwith a control set that was maintained as a standard.

Bud Fixation

Floral buds were fixed in carnoy's fixative (solution constituting 3 parts of 90% alcohol: 1 part glacial acetic acid) for duration of 24 hours. Buds were preserved in 70% alcohol at 4°C in refrigerator for future use.

Meiotic study

Flower buds of appropriate size were teased in a drop of 70% alcohol, followed by staining and mounting in 2% acetocarmine. Squash of the bud was prepared using a tapper. After squash preparation, slides were observed under Olympus light microscope whereas important stages were captured using Nikon Phase Contrast Research photomicroscope (Nikon Eclipse, E200, Japan) at 40X resolution. Pollen fertility was also computed on the basis of glycerine-acetocarmine stainability test using temporary mounts (Marks 1954). Adequately stained, globose, nucleated pollens were marked as fertile whereas sparsely stained, shrivelled and enucleated pollens were regarded as sterile. Variation in pollen diameter was recorded.

Statistical calibration

The data obtained were analysed using statistical software SPSS 16 and means were compared using Duncan's Multiple Range Test (DMRT) ($P \leq 0.05$). All the results were expressed in form of Mean ± Standard Error.

RESULTS

Cytogenetical screening of microsporogenic cells is a reliable test for in-depth view of in Cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.]. Cytogenetical studies revealed that chromosome complement set of the plant is n=7 (Fig. 2B showing metaphase I), confirming the somatic chromosomal configuration to be 2n=14. Meiocytes, in control, were perfectly normal and bivalents morphology was canonical with no considerable indication of aberrations; also there was no sign of cytomictic connections amongst PMCs. However, mutagenic treatment of gamma rays had impacted into a wide range of chromosomal anomalies alongwith cytomictic behaviour

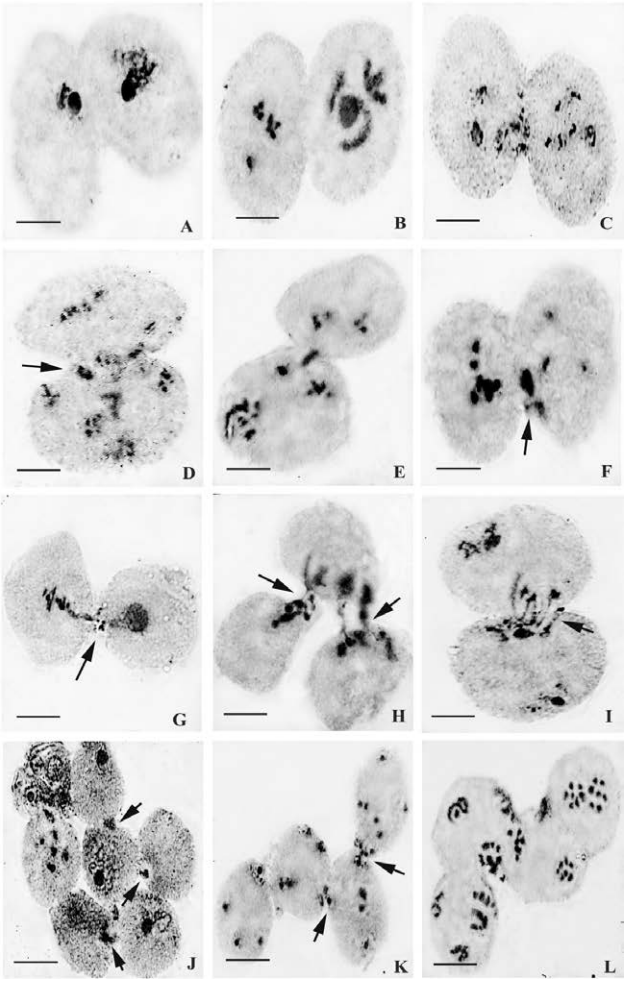

Fig. 1. Cytomixis *via* direct cell fusion (A-F) where A: Direct cell fusion at Diplotene; B: Cell fusion between Prophase 1 and metaphase I; C: Chromatin transfer between two PMCs; D: Horizontal transfer of chromosomes where one PMC is chromatin deficient; E: A chromosomal fragment in the transition phase; F: Migrating chromatin pushed towards periphery as sticky chromatin band. Cytomictic transmigration *via* Channel formation (G–I) where G: Single channel bridging two meiocytes; H: Simultaneous transfer of chromatin from 1 PMC to 2 PMCs; Multiple channel formation. Group formation (J – L) where J and K: Transitory micronuclei pushed at ends of meiocytes; L: Association between cells at Anaphase II stage. Scale bar: 10.45 μm.

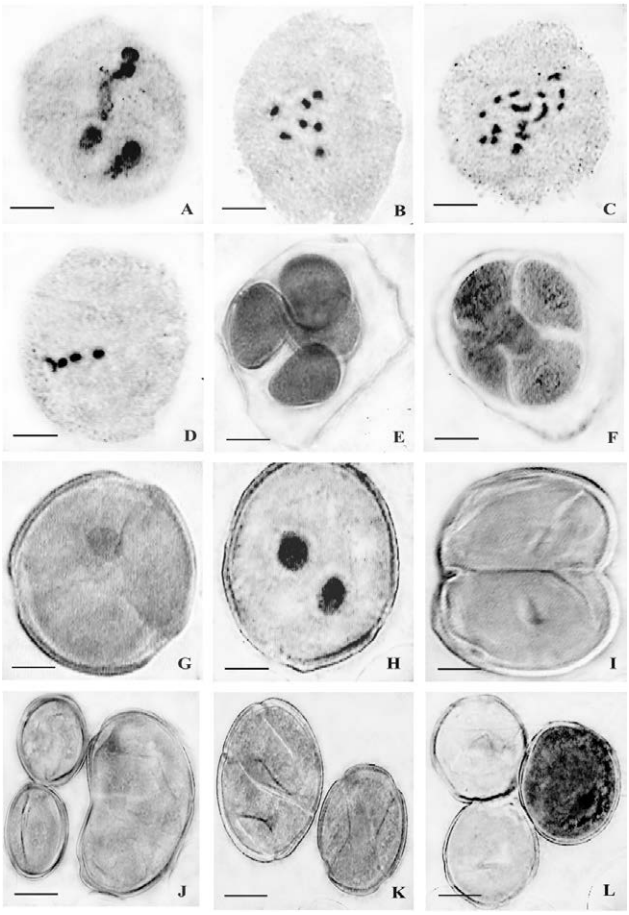

Fig. 2. Consequence of cytomixis on late meiotic phases and pollen morphology. A: Supernumerary nucleoli; B: Normal PMC with seven bivalents; C: Syncyte with 14 bivalents; D: Hypoploid meiocyte; E: Normal tetrad; F: Polyad; G: Normal fertile pollen; H: Two nucleated pollen; I: Two pollens fusing through wall dissolution; J: Heterosized pollens; K: Pollens with differential size and diameter; L: Fertile and sterile pollens. Scale bar: 10.45 μm.

amidst dividing meiocytes.

PMCs exhibiting Cytomictic events

The frequency of cytomixis was computed to be 7.29±0.33 % at 100 Gy dose which increased from this value to 10.84±0.46% at 200 Gy and 14.67±0.60% at 300 Gy. It was witnessed to manifest either through direct cell to cell fusion or *via* cytoplasmic channels; where frequency of direct fusion was higher in comparison at all the three doses. Table 1 represents data on cytomictic frequency at various stages of meiosis. Traversing of cytoplasmic contents, chromatin material, cellular organelles and other vital intrinsic trophic factors between proximate PMCs was witnessed. Onset of transitory events was witnessed by acquisition of cell polarization where nucleus was positioned towards the cell periphery i.e in between the communicating meiocytes unlike the non-cytomictic cells where nucleus was in the central space of PMC. Direct fusion was recorded at diverse stages of division with different degree of cytomictic intensities. For categorising the intensity, three levels of cytomixis were identified according to Kravchenko (1977). Cells at lower doses had loose wall connexion; these formed pairs and led to cause local cytomixis since no indication of chromatin transmigration was observed. Several PMCs deciphered rather intensive cytomictic phenomena where the migrating chromatin and micronuclei were encountered in between the associating PMCs or were pushed towards periphery of the parent PMC. Transferring content was seen to pass *via* cytoplasmic channels as sticky chromatin bands**.** Cytoplasmic channels (CC) were also of distinct morphology. It was, either, in the form of single channels (Fig. 1G) or multiple bridging (Fig. 1I) architectures through which nuclear transaction occurred. Fig.1H shows simultaneous transfer of chromatin from one PMC to two PMCs through channel formation. At some instances, cytomixis occurred *via* group formation where multiple PMCs participated in the confluence (Fig. 1J to L). Distinguishing feature of such grouping was the attainment of chain transfer. Chain transfer was peculiar where one cell donates a nucleus to the recipient cell and this recipient, in turn, transacts its nucleus to the succeeding one and so on.

Besides cytomixis, several other abnormalities were notifiable among which stickiness, univalents, disturbed polarity, unequal separation and laggards were more common alongwith less frequents anomalies such as bridges and micronuclei formation. An increasing trend for other chromosomal anomalies was recorded with respect to gamma irradiation i.e. from 9.80±0.29 at 100 Gy to 16.72±0.40 at 300 Gy gamma dose (Table 1).

Syncyte manifestation

A remunerative phenomenon of syncyte was witnessed at all the three doses of gamma irradiation *viz.* 100 Gy (0.25±0.25%), 200 Gy (0.55±0.28%) and 300 Gy (0.66 ±0.33%). Syncytes are recreated by complete confluence of two PMCs, where whole chromatin material is transferred to the recipient PMC. Therefore the recipient PMC is complemented with doubled chromatin complement. Fig. 2C is a syncytic cell representing 14 bivalents in place of 7 bivalents. Conversely, hypoploid cells were also recorded with lesser number of bivalents (Fig. 2D is a hypoploid cell). Binucleate PMCs with supernumer-

Table 1. Effect of Gamma irradiation on the incidence of Cytomixis and Syncyte formation in Cluster bean [*Cyamopsis tetragonolo* (L.) Taub.].

Treatment	Cytomixis				Syncytes (%)	Frequency of cells showing cytomixis at various stages of meiosis, % (Mean ± SE)									Other abnormalities (Mean ± SE)
	NNo. of PMCs ((Mean)	Frequency of PMCs involved in Cytomixis	Type of cytomixis			Meiosis I						Meiosis II			
			DF	CC		PI			MI	AI	TI	MII	AII	TII	
						Pt	Dp	Dk							
Control	3315	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100 Gy	2297	7.29±0.33	61.66±1.66	38.33±1.66	0.25±0.25	1.91±0.16	1.70±0.27	1.00±0.17	0.77±0.08	0.67±0.04	0.99±0.15	0.45±0.23	-	-	9.80±0.29
200 Gy	3300	10.84±0.46	71.02±2.41	28.96±2.41	0.55±0.28	2.02±0.17	1.65±0.06	1.68±0.14	1.34±0.11	1.24±0.20	0.77±0.07	0.97±0.11	0.68±0.20	0.42±0.25	12.02±0.31
300 Gy	2293	14.67±0.60	75.11±1.78	26.45±1.45	0.66±0.33	2.61±0.18	2.15±0.05	2.04±0.06	1.59±0.15	1.47±0.13	1.59±0.24	1.46±0.07	0.89±0.28	0.79±0.11	16.72±0.40

Abbreviations: PMCs-Pollen Mother Cells, CC-Cytomictic channel, DF-Direct fusion, PI-Prophase I, MI-Metaphase I, AI-Anaphase I, TI-Telophase I, MII-Metaphase II, AII-Anaphase II, TII-Telophase II, Pt-Pachytene, Dp-Diplotene, Dk-Diakinesis.

ary nucleolus were seen that might have been engineered due to nucleolar transfer *via* common meiocytic links.

Abnormal sporads, pollen fertility and size variation

Besides meiocytic fusion, tetrad and pollen grain fusion (Fig. 2I) were also recorded which is a rather uncommon and interesting phenomena to be reported in this study, since cytomictic behaviour is documented to occur more frequently at meiosis I. Abnormal microspores including monads, dyad and polyads (Fig. 2F) were also encountered. Variation in pollen size (diameter) was also recorded which were accounted to be heterosized pollens. In case of control, pollen diameter was not variable but in treated sets, alongwith medium normal sized pollens, differential frequency of small and large sized '2n pollens' were recorded. Fig. 2J and 2K are heterosized pollens. Smaller pollens were generally non-viable whereas larger ones had a differential degree of fertility. Table 2 is a representation of pollen fertility in control and treated sets; it also depicts mean diameter and relative frequency of large, medium and small sized pollen grains. Pollen fertility was also calculated and a sharp reduction was discernible from 97±0.57% at 100Gy to 74.33±0.88% at 300 Gy dose.

DISCUSSION

Meiosis is a beautifully manoeuvred cellular event that has been established to ensure efficient chromosome partitioning, recombination and trait assortment. Furthermore, its proper disposition is essential in restoring gametic viability. Henceforth, it becomes seemingly important to follow and magnify knowledge on the meiotic cell cycle. A typical promiscuous cytomictic behaviour among PMCs of Cluster bean (*Cyamopsis tetragonoloba* (L.) Taub.) was reported in response to mutagenic incidence of gamma rays. Significance for recruiting studies on this phenomenon is vested in the fact that cytomictic events provide a larger view onto the mechanism of intercellular activities and cellular conveyance.

Cytomixis was more profuse at meiosis I than at meiosis II and it appears to be active energy dependent process since KCN solution or decline in temperature checks it (Zhang *et al.* 1985). The perplexity surrounding the mechanism through which chromatin transfer occurs was resolved after identification of the role of cytoplasmic connections (Gates 1911). These connections form an important avenue for cytopasmic crosstalks among proximate PMCs. These are engineered

Table 2. Impact of Gamma rays on Pollen fertility and Relative pollen size frequency in Cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.].

Treatment	Pollen fertility (%)	Diameter(mm)			Relative frequency of different Pollen size (%)		
		Small	Medium	Large	Small	Medium	Large
Control	97.00±0.57	-	19.17±0.61	-	-	100	-
100 Gy	91.33±1.20	15.24±0.36	19.48±0.36	31.72±0.64	7.33±0.33	83.00±1.15	9.66±0.88
200 Gy	82.66±0.66	15.94±0.50	19.98±0.40	32.83±0.57	11.66±0.33	71.00±0.57	17.33±0.88
300 Gy	74.33±0.88	15.48±0.57	19.66±0.70	33.02±0.29	12.66±0.88	64.66±0.66	22.66±0.33

at prophase I of meiosis and are recognised as primary CC. Persisting plasmodesmata expands its extremities, it forms passage of large interconnecting cells, which is termed as cytoplasmic channels. Cell wall dissolution between the adjacent cells may also lead to cytoplasmic connections (Falistocco *et al.* 1995). Hydrolytic enzymes released by endoplasmic reticulum and golgi bodies are involved in CC formation (Yu *et al.* 2004). These primary CC may form via fusion of several plasmodesmata or through enlargement of single plasmodesmata or *de novo* in the region where no plasmodesmata occurs (Wang *et al.* 1998; Mursalimov *et al.* 2013a). However the cellulose-pectin wall is gradually replaced by callose layer at subsequent stages, as explained by Kravets (2013). The callose deposition insulates the cellular crosstalks and ceases the primary CC. It is for this reason that cytomixis is more profusely recorded at mieois I rather than meiosis II. However cytomictic behaviour may still persits by the genesis of secondary CC which is formed by action of enzymes callase that acts on callose wall. Specific organelles-spherosome like vesicles secreate callase and points at which callose catalyzes destruction of callose, secondary CC originates (Mursalimov *et al.* 2013b). These secondary CC remain available for cytomixis at the later stages of meiosis.

Local cytomixis represents association of meiocytes into groups *via* cytomictic channels in the early prophase of meiosis without any participation of migrating chromatin. Severe cytomictic channels such as Fig. 1H was also seen where cytoplasmic content of one PMC emanates in two PMCs. This has also been reported in *Vicia faba* (Bhat *et al.* 2017). Cytopathological symptoms are evident in intensive cytomixis, where transaction of chromatin; migration of the cytoplasmic content, nuclei etc are witnessed whereas destructive cytomixis involves complete destruction of the donor cells and severe pathological signs the filling of the anther cavity with agglutinated chromatin, and the impairment of remaining microsporocytes during meiosis. Actually, destructive cytomixis represents rather the way of the MSC autolysis than the way of communication between microsporogenic cells (Kravets 2013). Actin filaments play a key role in cytomixis since migration of cell contents through cytomictic channels is stopped due to cytochalasin B, a chemical that prevents the growth of actin filaments (Zhang *et al.* 1985).

Several pertinent questions regarding functional state of the transferring chromatin were answered by conducive histone modification experiments using immunostaining technique Mursalimov *et al.* (2015). Migrating chromatin had no signs of selective heterochromatinization and was decrypted to be in transcriptionally active state. Ultrastructural studies indicate that neither nucleus nor chromatin is damaged while traversing through cytomictic channel (Mursalimov and Deineko 2011). These arguments implicits ample evidence that cytomixis is a genetically controlled enigmatic phenomenon occurring due to environmental or physiological factors (Bellucci *et al.* 2003); which has been installed in cells to facilitate inter-cellular transmigration of vital cellular components. Several reports elucidates that cytomictic behaviour is linked to meiotic segregation and aberrant gene functioning at preceding meiotic or mitotic stages subverts to both chromosomal aberration as well as cytomixis. Thus, cytomixis regulation may be controlled by genes responsible for the chromosome segregation such as the *DIF1* gene in *Arabidopsis thaliana* (Bhatt *et al.* 1999).

An intriguing aspect revealed was presence of more than one nucleus in PMCs which displayed coenocytic behaviour. This behaviour is persistently encountered in intergeneric hybrids for example in *Meconopsis aculeate* (Singhal and Kumar 2008). Consequently, one cell gets an extra nucleus, leaving behind the other nucleus deficient cell. Such coenocytes lead in formation of abnormal-sized pollen grains as suggested earlier by Mendes-Bonato *et al.* (2001). Furthermore, fusion of two PMCs led to syncyte formation, also documented in *Chrysanthemum* (Kim *et al.* 2009), *Mertensia echioides* (Malik *et al.* 2014). Frequency of syncytes is quite low but it is easily detectible due to its invariably larger size compared single meiocyte. The product of such meiocytes resulted into the formation of '2n' or large-sized pollen grains. Jones and Reed (2007) approved that presence of

'giant' pollen to be associated with 2n status. Unreduced (diploid) gametes such as 2n pollen are good source for inducing polyploids (Ghaffari 2006; Latoo *et al.* 2006). Syncytes are concluded to oblige with imperative significance since it results into aneuploids which are assets for cytogeneticists. It may have serve as an exemplary model for intergeneric polyploids production. It is witnessed that this additional supernumerary chromatin mass do not pair with main chromatin material of the recipient cell, instead it remains as a separate identity, which may later on from micronuclei or micropollen (Bhat *et al.* 2006). However, its synthesis is of great future prospects since there induction is remunerative of infraspecifc polyploidization. It that may serve novel in the field of genetic variation and crop improvement.

Hypoploid cells are also quite prodigious tool from one viewpoint since they might become deficient in certain intrinsic genetic factors and their scrutiny is thus imperative. Cytoplasmic connection among pollens, although a rare phenomenon, was also witnessed here. Such connections among pollen grains had already been noticed in the intergeneric hybrids of *Roegneria tsukushiensis* x *Psathyrostachys huashanica* (Sun *et al.* 1994) and in *Meconopsis aculeata* (Singhal and Kumar 2008). Heterosized pollens of varying diameter were also recorded. Genesis of heterosized pollens stems from the aneuploid PMCs post cytomixis. Pollen fertility was documented to decline with increasing gamma rays. The descending fertility is apparently an outcome of all the cumulative factors that led to cytogenetical aberrations which eventually affected the reproductive success of microsporogenesis.

This study is succesfull documentation on gamma rays induced cytomixis in *Cyamopsis tetragonoloba* (L.) Taub. It also validates the efficacy of the ionizing radiation for inducing useful cytological variants such as aneuploids and infraspecific polyploids. Gamma rays, plausibly has a substantial role in maintaining genetic heterogeneity (Kravets 2013) or restoring and balancing the unbalanced genomes within the developing male gametophyte, as highlighted by (Falistocco *et al.* 1995; Ghaffari 2006; Song and Li 2009). If cytomixis is a means for synthesising infraspecific polyploids, it is also characterized as genome stabilizing, cell sorting checkpoint. For clearing all the mysteries and to expand our level of knowledge, we hope arrival of more concrete techniques, which might help in furthering our vision.

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