

Induced Cytomictic Variations and Syncyte Formation During Microsporogenesis in *Phaseolus vulgaris* L.¹

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Abstract—The intercellular translocation of chromatin material along with other cytoplasmic contents among the proximate meiocytes lying in close contact with each other commonly referred as cytomixis was reported during microsporogenesis in *Phaseolus vulgaris* L., a member of the family Fabaceae. The phenomenon of cytomixis was observed at three administered doses of gamma rays viz. 100, 200, and 300 Gy respectively in the diploid plants of *Phaseolus vulgaris* L. The gamma rays irradiated plants showed the characteristic feature of inter-meioocyte chromatin/chromosomes transmigration through various means such as channel formation, beak formation or by direct adhesion between the PMC's (Pollen mother cells). The present study also reports the first instance of syncyte formation induced via cytomictic transmigration in *Phaseolus vulgaris* L. Though the frequency of syncyte formation was rather low yet these could play a significant role in plant evolution. It is speculated that syncyte enhances the ploidy level of plants by forming $2n$ gametes and may lead to the production of polyploid plants. The phenomenon of cytomixis shows a gradual inclination along with the increasing treatment doses of gamma rays. The preponderance of cytomixis was more frequent during meiosis I as compared to meiosis II. An interesting feature noticed during the present study was the channel formation among the microspores and fusion among the tetrads due to cell wall dissolution. The impact of this phenomenon is also visible on the development of post-meiotic products. The formation of heterosized pollen grains; a deviation from the normal pollen grains has also been reported. The production of gametes with unbalanced chromosomes is of utmost importance and should be given more attention in future studies as they possess the capability of inducing variations at the genomic level and can be further utilized in the improvement of germplasm.

Keywords: cytomixis, gamma rays, microsporogenesis, syncyte, heterosized pollen grains, *Phaseolus vulgaris* L.

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INTRODUCTION

Cytomixis, a cytological event is characterized by the migration of nuclear material as well as other cell organelles along with cytoplasm between the cells lying in close proximity with each other through the cytoplasmic channels or by direct fusion among the pollen mother cells (PMC's). Arnoldy [1] first reported this phenomenon in the reproductive organs of gymnosperms followed by Koernicke [2] in the pollen mother cells of *Crocus vernus* L. The term cytomixis was given by Gates [3] who reported the phenomenon of intercellular chromatin migration among the pollen mother cells, during the microsporogenesis in *Oenothera gigas* and *O. biennis*. Since then it has been reported in a wide array of flowering plants [4–9]. Though cytomixis has been frequently reported during microsporogenesis however few instances of cytomictic migration have also been reported in the somatic cells such as root meristematic cells [10, 11], in the ovary cells [12], in tapetal cells [13], in the proembryos

of graminaceous plants [14] and in the shoot apex of trees [15]. Cytomixis observed in vegetative tissues displayed a characteristic feature of asynchrony as compared to microsporocytes [16].

The origin, development and the significance of cytomixis are still an enigma. The role of this phenomenon in the evolutionary pathway remains speculative. The process seems to affect the developmental stages of microsporogenesis leading to the production of aneuploid plants as well as polyploid plants [10, 17, 18]. The cytoplasmic connections among the meiocytes acts as an important avenue for the exchange of cytoplasmic content as well as nuclear content. Cytomictic transmigration has been more frequently observed in plants with unbalanced genomic constituent such as aneuploids, haploids, hybrids [19], mutants [4], triploids [20] and apomicts [21]. In some cases the prevalency of cytomixis in tetraploids over their diploid counterparts has also been inferred [22, 23].

Cytomixis is distinguished into three groups on the basis of its intensity—slight (local), intensive and destructive [24]. The slight (local) type of cytomixis

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Table 1. Effect of an increasing dose of gamma rays on the frequency of cytomixis and syncyte formation during meiosis of *Phaseolus vulgaris* L.

Cytomixis			Frequency of cells showing cytomixis at various stages of meiosis, %												
treatment doses	total number of PMC's observed	frequency of PMC's involved, %	type of cytomixis, %		number of syncytes	meiosis I						meiosis II			other chromosomal abnormalities, %
			CC	DF		PI			MI	AI	TI	MII	AII	TII	
						Pt	Dp	Dk							
Control	368	—	—	—	—	—	—	—	—	—	—	—	—	—	—
100 Gy	331	6.94 ± 0.60	30.43	69.56	2	2.11	1.51	0.90	1.20	0.30	0.60	0.30	—	—	10.32 ± 0.22
200 Gy	353	9.81 ± 0.43	32.35	67.64	5	0.84	1.69	1.98	1.41	0.84	1.13	0.84	0.56	0.28	11.84 ± 0.35
300 Gy	317	14.19 ± 0.31	28.88	73.91	6	1.26	1.57	2.20	2.52	1.89	1.26	1.26	1.57	0.94	17.98 ± 0.63

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.

doesn't influence the meiotic course and do not cause destructive effects contrary to intensive and destructive type of cytomixis which affects the meiotic course by causing multiple disturbances considerably [25]. Until now cytomixis has been reported in a number of plant species, but reports on cytomictic transmigration in *Phaseolus vulgaris* L. is meager due to lack of research work done on its cytogenetical aspects. The present cytological investigation is a descriptive analysis of the meiotic behaviour of gamma rays irradiated diploid plants of *Phaseolus vulgaris* L. The aim of the study was to carry out a comparative analysis of cytomixis frequency in gamma rays irradiated sets and to investigate the manifestation and consequences of this phenomenon at chromosomal level in relation to the reproductive success of the plant. The study also reports the formation of syncytes through cytomictic transmigration for the first time in *Phaseolus vulgaris* L.

MATERIALS AND METHODS

Procurement of seeds. Seeds of inbred lines of *Phaseolus vulgaris* L. variety PDR-14 (Uday) were obtained from Indian Institute of Pulses Research (IIPR), Kanpur, India.

Gamma rays irradiation. The packets of seed sample consisting of 200 seeds per dose were prepared for the gamma rays irradiation. The seed samples were administered to gamma rays irradiation from a Co⁶⁰ source at Nuclear Research Laboratory, IARI, New Delhi. The doses given were viz. 100, 200, 300, and 400 Gy respectively. The irradiated seeds were sown immediately in replicates of three in the field adopting a complete randomized block design (CRBD). One

set of untreated seeds were also sown in the field along with treated ones to act as control.

Cytological studies. For meiotic studies, at the time of flowering young floral buds of suitable size were randomly selected and then fixed in freshly prepared Carnoy's fluid (alcohol : glacial acetic acid in a 3 : 1 ratio) for 24 h and preserved in 70% alcohol at 4°C. The slides were prepared using anther squash technique with 2% acetocarmine. Pollen fertility was evaluated by acetocarmine stainability test where the darkly stained pollen grains with prominent nuclei and regular shape were considered as fertile whereas unstained and shrunken pollen grains with diminishing nuclei were considered as sterile. Approximately 20–25 buds from each dose were taken into consideration for cytological study of meiotic course.

RESULTS

The control plants of *Phaseolus vulgaris* L. ($2n = 22$) exhibited normal meiotic division with 11 bivalents at metaphase I and 11 : 11 segregation at anaphase I. The highest dose i.e. 400 Gy was found to be lethal for the plants. Cytomixis as well as chromosomal aberrations were altogether absent in the control plants. However, in the case of gamma rays treated plants cytomictic connections involving the transfer of chromatin material between the proximate meiocytes have been recorded at all the three administered doses viz. 100, 200, and 300 Gy respectively. The data of the type and frequency of cytomixis during various stages of meiosis have been presented in Table 1. The percentage mean and standard deviation of the frequency of PMC's involved in cytomixis are 6.94 ± 0.60 , 9.81 ± 0.43 and

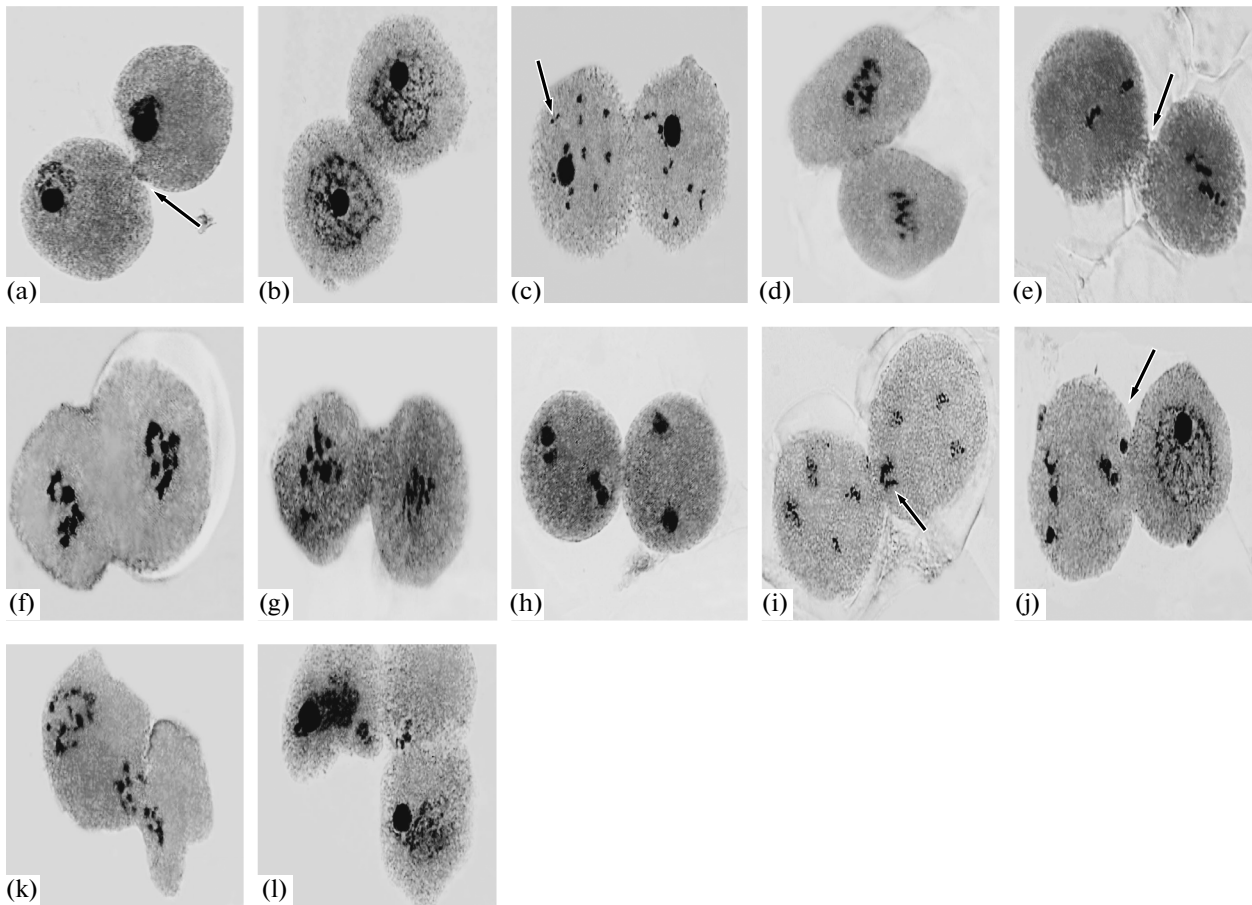


Fig. 1. (a) beak formation between PMC's at pachytene stage; (b) direct fusion of PMC's at diplotene stage; (c) direct fusion of PMC's at diakinesis stage showing an extra bivalent in one PMC's (arrow indicates extra bivalent); (d) fusion at metaphase I; (e) formation of cytoplasmic strands at metaphase II; (f) direct contact by amalgamation of wall between PMC's; (g) chromatin transfer through broad channel formation between PMC's at metaphase I; (h) fusion at telophase I through cell wall dissolution; (i) migration of chromosomes at metaphase II; (j) cytomixis involving elimination of nucleic material in the form of micronuclei between two PMC's at different stages; (k) cytomixis involving transfer of chromosomes into an enucleated PMC; (l) simultaneous transfer of chromatin material from 1 PMC to 2 other PMC's.

14.19 ± 0.31 at 100, 200, and 300 Gy respectively. The maximum percentage of inter PMC (Pollen Mother Cell) transfer of chromatin material is being observed at the 300 Gy dose of gamma rays. The degree of occurrence of this phenomenon concurred with the increase in the dose of gamma rays.

The manifestation of cytomixis starts from early prophase I and persists upto tetrad stage. However the frequency of occurrence of this phenomenon during the later stages of meiosis i.e. meiosis II was rather low. Generally 2 PMC's are involved in chromatin transfer but sometimes chromatin migration is also observed in groups (Fig. 1l). The transfer of chromatin material among group of PMC's occur in a relay fashion either in similar directions or in different directions.

The intercellular chromatin transfer between adjacent PMC's has been exclusively observed in two distinct patterns—it may occur either through cytomictic channel formation or by direct fusion of PMC's. Among the two patterns, direct adhesion between the

PMC's holds the highest share for the efficient transfer of chromatin/chromosomes. The cytomictic channel formation is further categorized into two categories as broad channel and narrow channel. The formation of broad type of channel was relatively more frequent (Fig. 1g) as compared to the latter type. Moreover, the direct fusions of PMC's were encountered with different intensities at similar as well as different stages of cell division (Figs. 1b, 1c, 1d, 1f, 1j). Interestingly, it is noteworthy that channel formation among the microspores (Fig. 2f) and direct fusion among the tetrads due to dissolution of cell wall (Fig. 2g) were also observed during the present study. The intra-microsporal transfer of chromatin through one or more narrow and broad channels or chromatin strands lead to the fusion of nuclei with each other.

Another peculiar finding witnessed during the present investigation was the presence of syncyte (Fig. 2c), though their frequency was relatively low. The syncytes recorded were 2, 5 and 6 in numbers at 100, 200, and

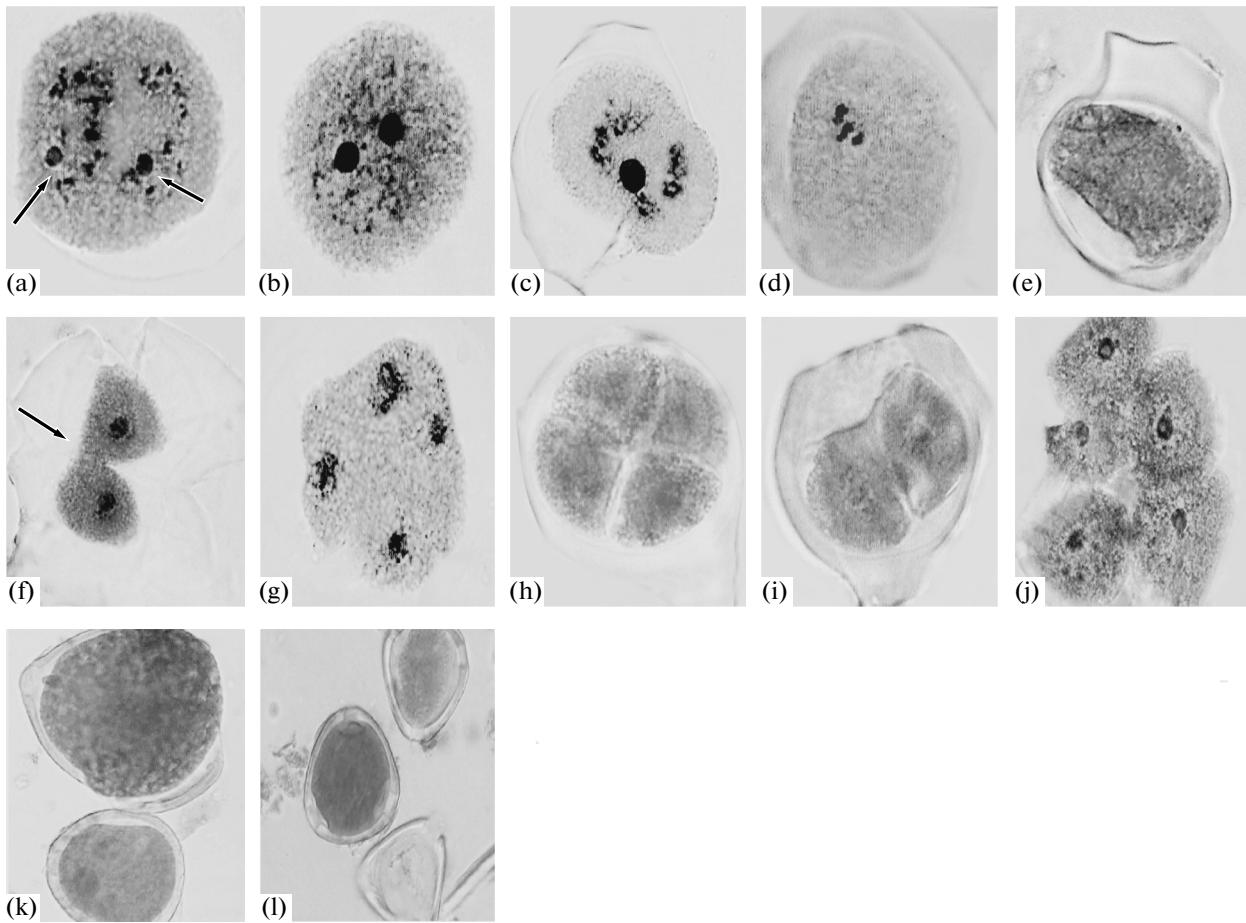


Fig. 2. (a) PMC with supernumerary nucleoli (two nucleoli) in a single cell; (b) a binucleated PMC; (c) syncyte with double chromosome complement; (d) hypoploid PMC; (e) enucleated PMC; (f) tube formation between microspores; (g) direct fusion of tetrad due to cell wall dissolution; (h) normal tetrad; (i) dyad; (j) polyad; (k) heterosized fertile pollen grains; (l) fertile and sterile pollen grains.

300 Gy. Syncyte is formed by the fusion of two PMC's together in which the chromatin material is subsequently transferred to a single cell. After the fusion, the syncyte acts like a single large-sized PMC having the double chromosome complement. The syncyte PMC's can be easily differentiated from the normal ones owing to their large size. The PMC with supernumerary nucleoli (2 nucleoli) was observed during the present finding (Fig. 2a). The migration of chromatin material can either be partial or complete resulting in the formation of binucleated PMC (Fig. 2b), hyperploid, hypoploid (Fig. 2d) and aneuploid PMC (Fig. 2e). As a consequence of cytomixis, aberrant post meiotic products such as dyads (Fig. 2i), polyads (Fig. 2j), heterogeneous sized pollen grains (Fig. 2k), sporads with micronuclei etc. are formed which impairs the meiotic course of a plant. The mean diameter and relative frequency of large, medium and small sized pollen grains have been presented in Table 2. The pollen fertility for control plants was found to be $94.34 \pm 0.23\%$ while it was recorded $89.37 \pm 0.60\%$, $81.99 \pm 1.05\%$, $75.33 \pm 0.91\%$ for 100, 200, and 300 Gy respectively. The phe-

nomenon had an impact on the meiotic course and it might be the reason for the induction of various types of chromosomal aberrations. The percentage mean of chromosomal anomalies increase from 10.32 ± 0.22 to 17.98 ± 0.63 with the increase in the dose of gamma rays.

DISCUSSION

Cytomixis, the phenomenon of intercellular chromatin migration among two or more cells lying in close vicinity with each other is an active energy-dependent process and has been reported in a number of higher plants. It is believed that actin cytoskeleton rather than the tubulin elements are involved in the process [26]. Apart from the higher plants, the occurrence of this phenomenon during spermatogenesis in animals [27] and lower plants [28] have also been reported. It is noteworthy that cytomixis is an efficient mean of cell to cell communication as transportation of nutrient and signaling molecule among the cells occurs through cytomictic channels [29] and it also

Table 2. Effect of an increasing dose of gamma rays on the fertility and relative frequency of large, medium and small sized pollen grains

Treatment doses	Fertility, %	Diameter, μm	Relative frequency of large, medium and small sized pollen grains, %
Control	89.25–95.86 (94.34 \pm 0.23)	42.64 \times 30.16 ^m	100
100 Gy	77.68–92.03 (89.37 \pm 0.60)	41.60 \times 31.51 ^m	100
200 Gy	69.84–83.58 (81.99 \pm 1.05)	63.44 \times 54.08 ^l 40.56 \times 32.24 ^m	16.41 ^l 73.66 ^m
300 Gy	59.81–81.73 (75.33 \pm 0.91)	35.36 \times 30.16 ^s 69.68 \times 57.20 ^l 41.08 \times 34.32 ^m 32.24 \times 29.43 ^s	9.92 ^s 21.46 ^l 67.12 ^m 11.41 ^s

l—large, *m*—medium, *s*—small pollen grains.

accompanies the elimination of excessive and unclaimed cells both in plants and animals [25]. Besides this the histochemical results revealed that the one way transport of nutritious substances and several organelles also occurs through the cytomictic channels into the actively functioning PMC at the prejudice of the weaker ones [30]. The maximum frequency of cytomictic activity has been observed during prophase I in the present investigation and it is in accordance with the earlier findings [5]. The reason cited for this is based on the fact that the cytomictic canals are usually occluded by callose plugs during the later stages [31].

Till date there are conflicting opinions among researchers regarding the origin and cause of cytomixis. According to Heslop-Harrison [32] cytomictic channels are formed de novo. They deny the involvement of plasmodesmata in the formation of cytomictic channels however, during later research by Wang et al. [33], it was found that there might be the possibility of the formation of cytomictic channels due to plasmodesmata fusion. From the physiological perspective, the occurrence of cytomixis in microsporocytes is attributed to the incomplete formation of cell septums and cytoplasmic canals between cells [34, 35]. It is assumed that due to incompletely built cell walls the content of microsporocytes became sensitive to hydrolytic enzyme exposure which further led to the movement of cytoplasmic contents between cells [32, 34].

Regarding the cause of cytomixis, different factors such as effect of gamma radiation [36], due to the influence of genes [37], environmental stress and pollution [6], changes in the biochemical processes that involve microsporogenesis modifying the microenvironment of affected anthers [12], or action of chemical agents such as colchicine [38], methyl methane sulphonate [39], and sodium azide [8] etc. have been assigned as the probable reasons for the induction of

this phenomenon in different plants. While some researchers believed that the phenomenon of cytomixis originates as an artifact of fixation [40] or due to some fortuitous causes such as pathological anomaly [4, 41], mechanical injuries [10] etc. In the present study gamma ray is the causative agent responsible for the induction of cytomixis and production of syncytes and gametes with altered chromosome numbers which can be utilized for improving some peculiar traits of the plant.

Cytomixis have a profound effect on the meiotic course and in the development of post-meiotic products. An array of meiotic abnormalities such as lag-gards, chromosome stickiness, precocious movement of chromosomes, unorientation etc. has been discernible in the treated sets at all the stages of meiosis I and meiosis II. Unequal distribution of chromatin was observed in small percentage of cells at anaphase I. High incidence of chromosomal stickiness was visible in all the phases of meiosis I and meiosis II. The occurrence of the induction of meiotic irregularities as a consequence of cytomixis in plants by different investigators are available as well [18, 42]. The formation of binucleated PMC's, aneuploid cells, hypoploid cells, hyperploid cells, syncytes and aberrant microspores such as monad, dyads, polyads etc. are usually observed as a consequence of cytomixis.

The manifestation of syncytes occurs through cytomictic transmigration among the neighbouring PMC's through cell wall dissolution [17]. The formation of syncytes during microsporogenesis has earlier been reported in *Phleum pretense* [43] *Cyamopsis tetragonoloba* [44], maize [45], *Brachiaria decumbens* [46], *Chrysanthemum* [47], interge-neric hybrids of Triticeae such as *Psathyrostachys huashanica* \times *Secale montanum* [48], and *Roegneria ciliaris* \times *Psathyrostachys huashanica* [49] etc. The syncyte PMC's in the plants are destined to produce heterosized pollen grains or $2n$

pollen grains due to the non-reduction of gametes after meiosis [9, 17, 44, 50]. The $2n$ pollen grains play a significant role in the production of infra-specific polyploids [47]. To our knowledge this is the first documentation on the formation of syncytes and the occurrence of intra-microsporal chromatin transfer within a sporad through channel formation which consequently results in the production of heterosized pollen grains in the species *Phaseolus vulgaris* L. The formation of abnormal sporads has an impact on the pollen fertility also as a negative co-relation was established between the increasing doses of gamma ray and pollen fertility.

Conclusively, from the above study it can be elucidated that the phenomenon of cytomixis induced through gamma irradiation may be considered as an effective mechanism for the production of aneuploid and polyploid gametes which could be further exploited in breeding programmes to generate variability in plants. Syncyte manifestation is another interesting feature on which detailed study should be focused in future as they lend a major role in the production of infra-specific polyploids.

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