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MUTATIONAL STUDIES IN JAYA CULTIVAR OF *Capsicum annuum* Linn.

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Key words : Jaya cultivar, γ-ray, EMS

Mutational studies have been carried out in Jaya cultivar of *Capsicum annuum* Linn. (Chilli) which belongs to the family Solanaceae. Morphological as well as cytological impact on the γ-ray and EMS treated plants have been compared with the control plant. The effect of γ-ray has been observed to be haphazard while that of EMS is systematic.

INTRODUCTION

Induced mutations offer great potentialities in evolving efficient cultivars suitable for different agroclimatic conditions. With this view mutational studies have been carried out on Jaya cultivar of the chilli plant (*Capsicum annuum* Linn.) belonging to the family Solanaceae.Many cultivars of chilli are known and among them Jaya cultivar is the most commonly available cultivar in Gaya town. This cultivar is perennial with the height generally ranging from 0.5 to 1.5 meter. The colour of the flower is white with pentamerous condition. The fruit is typically green when unripe but it may turn orange red or typical red on ripening. The fruit of the cultivar is very bitter in taste.

In the present investigation two most effective mutagens namely Gamma rays (γ-rays) as physical mutagen and Ethyl Methane Sulphonate (EMS) as chemical mutagen have been selected for inducing mutations in Jaya cultivar. The effects of both mutagens have been screened for the morphological and cytological attributes of the studied cultivar.

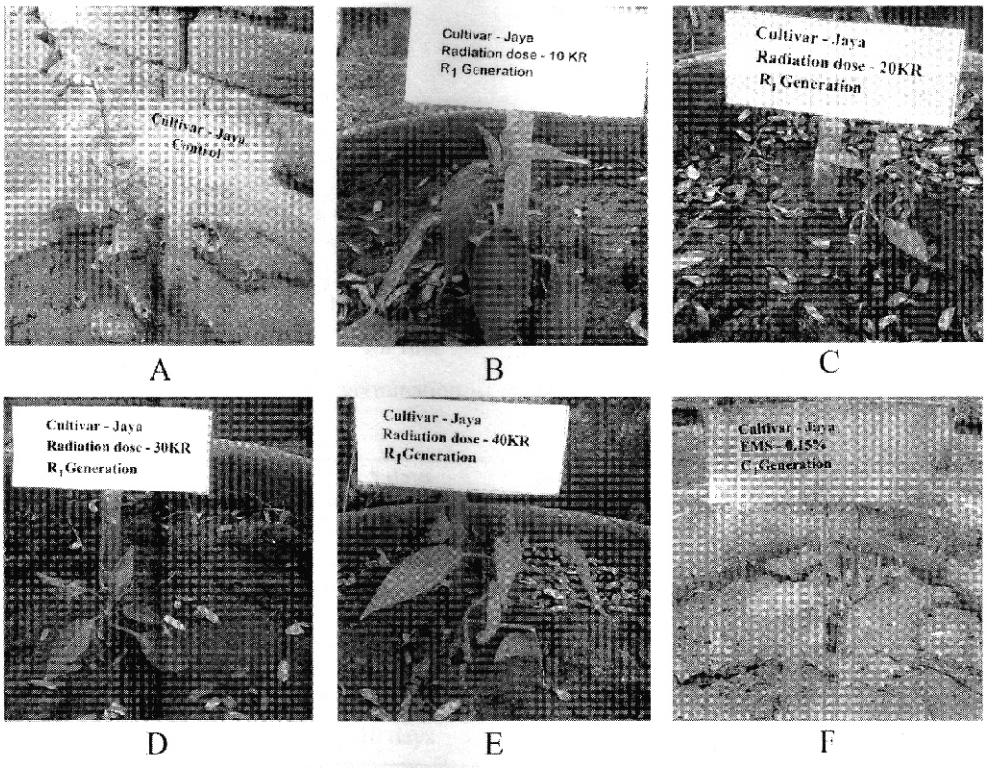
MATERIALS AND METHODS

Seeds of Jaya cultivar were treated with γ-rays at Floriculture Department of Lucknow. Doses of γ-ray given to the seeds are 10 KR, 20 KR, 30 KR &40 KR. Similarly seeds of Jaya were treated with EMS at different concentrations such as 0.10%, 0.15%, 0.20% and 0.30%. Germination percentage of seeds of control plants and the treated plants were noted. After seedling formation detailed morphological changes were observed.

Under cytological studies, effects on mitosis and meiosis of the cultivar were observed. For mitosis, root tips were pretreated in alpha bromo napathalene and fixed in 1 : 3 aceto-alcohol. For meiosis, buds were fixed in 1 : 3 aceto-alcohol at 10 : 45 a.m. Staining was done in 2% acetocarmine and the slides were made permanent by the method adopted by Celariar (1956). Percentage of total form value of somatic chromosome was calculated by the formula given by Huziwara (1962).

OBSERVATIONS

Germination rate of the treated seeds was observed in two generations and its was compared with the germination rate of control plant. Similarly morphological attributes of control and treated plants were also observed and compared (Figs. A, B, C, D, E, F, G & H). Details have been compiled in tables 1 &2.



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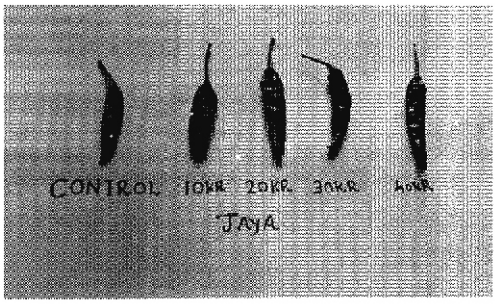

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Fig. A-H : Morphological images of control and treated plants of *Capsicum annuum* (Jaya cultivar).

(A) Control Plants, (B) 10 KR Plants R_1 generation, (C) 20KR plant R_1 generation, (D) 30 KR Plant R_1 generation, (E) 40 KR plants R_1 generation (F) 0.15 per cent C_1 generation, (G) 0.30 per cent C_1 generation (H). Fruits of Control and radiated Plants.

TABLE-1

Germination rate in control and treated plants of "Jaya" cultivar

Control and treated plants	Total no. of seeds taken	No.of days taken by seeds for first germination	Total time taken by seeds in forming seedlings	Total no. of seeds germinating upto seedlings stage	Percentage of germination
Control	100	5 days	28 days	92	92%
Gamma rays doses in KR			**R_1 Generation**		
10 KR	100	7 days	30 days	80	80%
20 KR	100	6 days	29 days	72	72%
30 KR	100	8 days	31 days	65	65%
40 KR	100	10 days	33 days	54	54%
			R_2 Generation		
10 KR	100	6 days	28 days	82	82%
20 KR	100	7 days	29 days	74	74%
30 KR	100	6 days	28 days	67	67%
40 KR	100	8 days	30 days	56	56%
EMS Concentration			**C_1 Generation**		
0.10%	100	7 days	29 days	81	81%
0.15%	100	9 days	31 days	72	72%
0.20%	100	8 days	30 days	67	67%
0.30%	100	11 days	33 days	56	56%
			C_2 Generation		
0.10%	100	6 days	28 days	85	85%
0.15%	100	7 days	29 days	76	76%
0.20%	100	7 days	29 days	70	70%
0.30%	100	9 days	31 days	61	61%

TABLE-2

Morphological attributes of control and treated Plants in "Jaya" Cultivar

Control and treated plants	Plant length in inches	Total no. of branches/ plant	Fruit length (cm)	Extent of bitterness of fruit	Seeds/ fruit average no.	Seeds/ plant average no.	Fruit/ plant average no.	Colour of fruit
Control	26.00	24	5.4cm	Most Bitter	45	2070	46	Dark Green
Gamma rays doses in KR				**R_1 Generation**				
10 KR	22.50	18	5.2cm	Bitter	38	1520	40	Dark Green
20 KR	18.50	12	5.1cm	Less Bitter	43	1290	30	Normal Green
30 KR	19.00	14	5.0cm	Bitter	48	1680	35	Normal Green
40 KR	28.50	20	5.6cm	Less Bitter	50	1900	38	Pale Green
				R_2 Generation				
10 KR	20.50	22	5.3cm	Bitter	38	1216	32	Dark Green
20 KR	19.00	18	5.1cm	Most Bitter	41	1968	48	Normal Green
30 KR	22.00	16	5.2cm	Most Bitter	47	2491	53	Normal Green
40 KR	26.00	12	5.5cm	Less Bitter	49	2499	51	Pale Green
EMS Construction				**C_1 Generation**				
0.10%	14.28	10	4.2cm	Less Bitter	41	1640	40	Dark Green
0.15%	16.00	12	4.7cm	Bitter	45	1980	44	Light Green
0.20%	20.78	8	5.2cm	Most Bitter	49	2254	46	Normal Green
0.30%	23.00	9	5.7cm	Bitter	53	2703	51	Normal Green
				C_2 Generation				
0.10%	16.00	14	4.6cm	Less Bitter	43	1935	45	Dark Green
0.15%	19.50	12	4.2cm	Bitter	45	1800	40	Dark Green
0.20%	22.00	10	4.5cm	Bitter	48	2400	50	Normal Green
0.30%	24.00	8	5.0cm	Bitter	51	2703	53	Normal Green

Under cytological studies, mitosis and meiosis were observed in two generations of the treated plants.

The control plants showed 2n = 24 chromosomes during mitosis (Fig-1). The somatic cells of irradiated plants also showed 2n = 24 chromosomes but here anomalies were very much prominent (Fig.-2). The common anomalies reported during mitosis were stickiness and chromosomal breaks. Here, chromosomal fragments were more in number than stickiness. Percentage of anomalies was more in R_1 generation than in R_2 generation. Similarly in EMS treated root tip cells, abnormalities were recorded but here stickiness of chromosomes was found to be more common than chromosome fragment (Fig.-3). Again in C_2 generation the percentage of anomalies decreased as compared to C_1 generation. Details of mitotic anomalies in treated plants have been summarized in Table-3.

TABLE-3

Mitotic abnormalities in control and treated Plants of "Jaya" cultivar

Control & Treated Plants	Total no. of dividing cells	No. of affected cells	Percentage of affected cells	No. of cells showing breaks	No. of cells showing stickiness
Control	1200	Nil	Nil	Nil	Nil
Gamma rays doses in KR			**R_1 Generation**		
10 KR	1200	120	10.0	80	40
20 KR	1200	260	21.6	160	100
30 KR	1200	380	31.6	260	120
40 KR	1200	470	39.1	340	130
			R_2 Generation		
10 KR	1200	100	8.3	60	40
20 KR	1200	210	17.5	140	70
30 KR	1200	310	25.8	230	80
40 KR	1200	380	31.6	280	100
EMS construction			**C_1 Generation**		
0.10%	1200	60	5.0	20	40
0.15%	1200	190	15.8	60	130
0.20%	1200	310	25.8	100	210
0.30%	1200	386	32.1	96	290
			C_2 Generation		
0.10%	1200	48	4.0	18	30
0.15%	1200	160	13.3	50	110
0.20%	1200	250	20.8	60	190
0.30%	1200	320	26.6	80	240

Detailed meiotic studies were also carried out. The meiotic studies in control plant showed gametic number as n = 12 (Fig-4). The division was non synchronised and some of the pollen mother cells showed typical meiotic anomalies like clumping of chromosomes (Fig.-5), multivalent and univalent formation at metaphase I stage and chromosomal laggards, chromosomal bridges at anaphase I. On the other hand, in irradiated plants, meiotic anomalies were very much pronounced. The meiotic anomalies were recorded at all does of gamma rays, i.e., 10 KR, 20 KR, 30 KR and 40 KR in both R_1 and R_2 generations. Prominent anomalies included multivalent and univalent formations, translocation chains and

rings at metaphase I and unequal separation of chromosomes, chromosomal bridges and chromosomal laggards at anaphase I (Fig-6, 7, 8, 9, 10, 11 & 12). Pollen sterility was also noticeable which showed an increasing tendency with the increase in meiotic anomalies. Similarly meiotic studies were carried out in the EMS treated plants for two generations. In this case also, meiotic anomalies were frequently noticed. C_2 generation showed less frequency of meiotic anomalies (Fig-13,14,15,16,17 &18).

Details of meiotic anomalies and pollen sterility in control as well as in treated plants have been complied in Tables 4, 5, & 6.

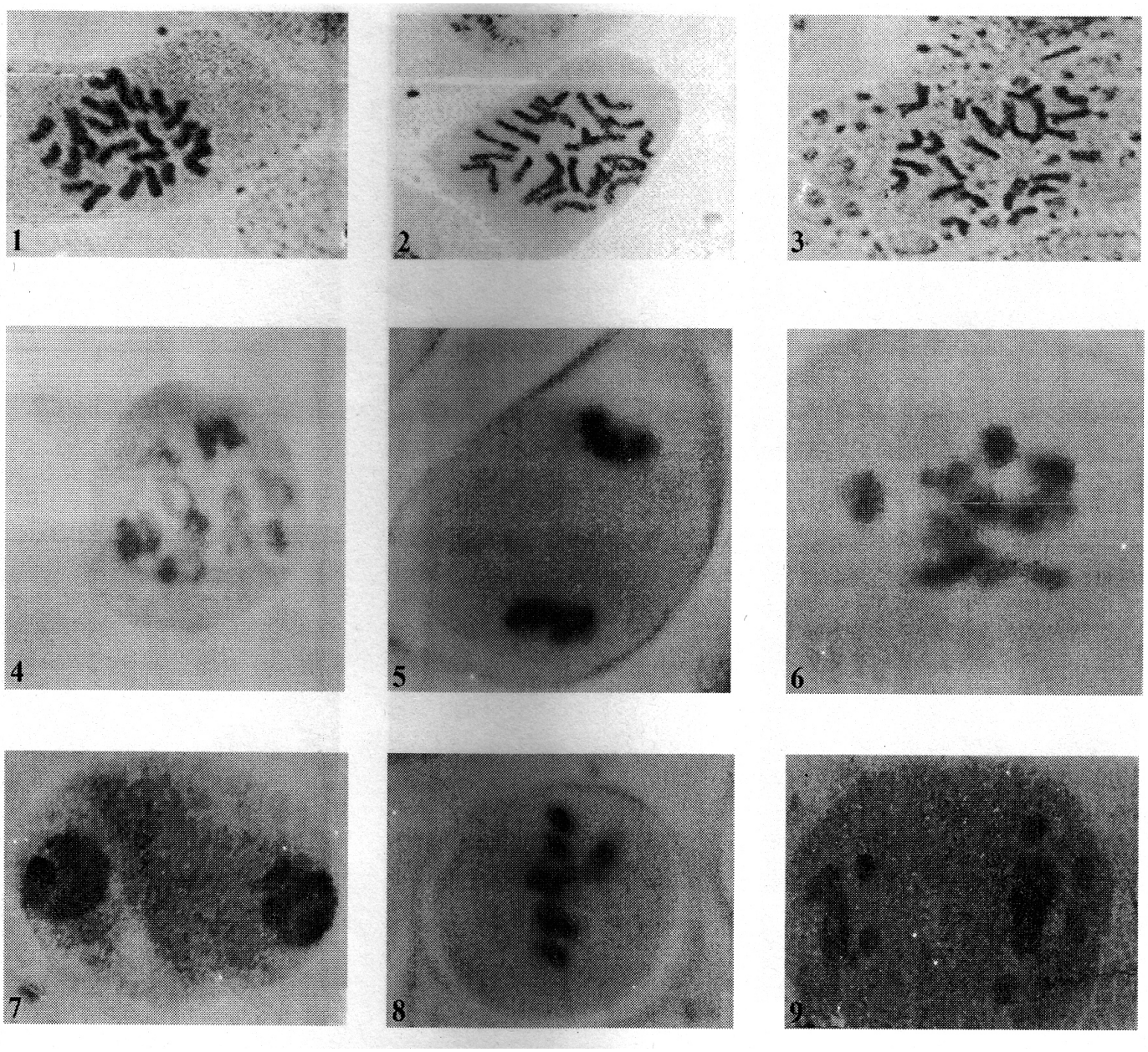

Fig. Cytological configuration in control and treated plant of *Capsicum annuum* (Jaya cultivar) : Figure (1-9) **1.** Control plant showing normal metaphase 2n = 24. **2.** 10 KR Plant showing metaphase stage 2n = 24 **3.** EMS treated Plant showing normal metaphase 2n = 24. **4.** Control plant at metaphase I showing twelve bivalents. **5.** Control plant at anaphase I showing clumping of chromosomes. **6.** 10 KR (R_1) plant at metaphase I showing translocation ring as well as chains. **7.** 10 KR (R_2) plants at anaphase I showing clumping of chromosomes at two poles. **8.** 20 KR (R_1) Plants at metaphase I showing multivalent. **9.** 20 KR (R_2) Plants showing chromosomal laggards at anaphase I.

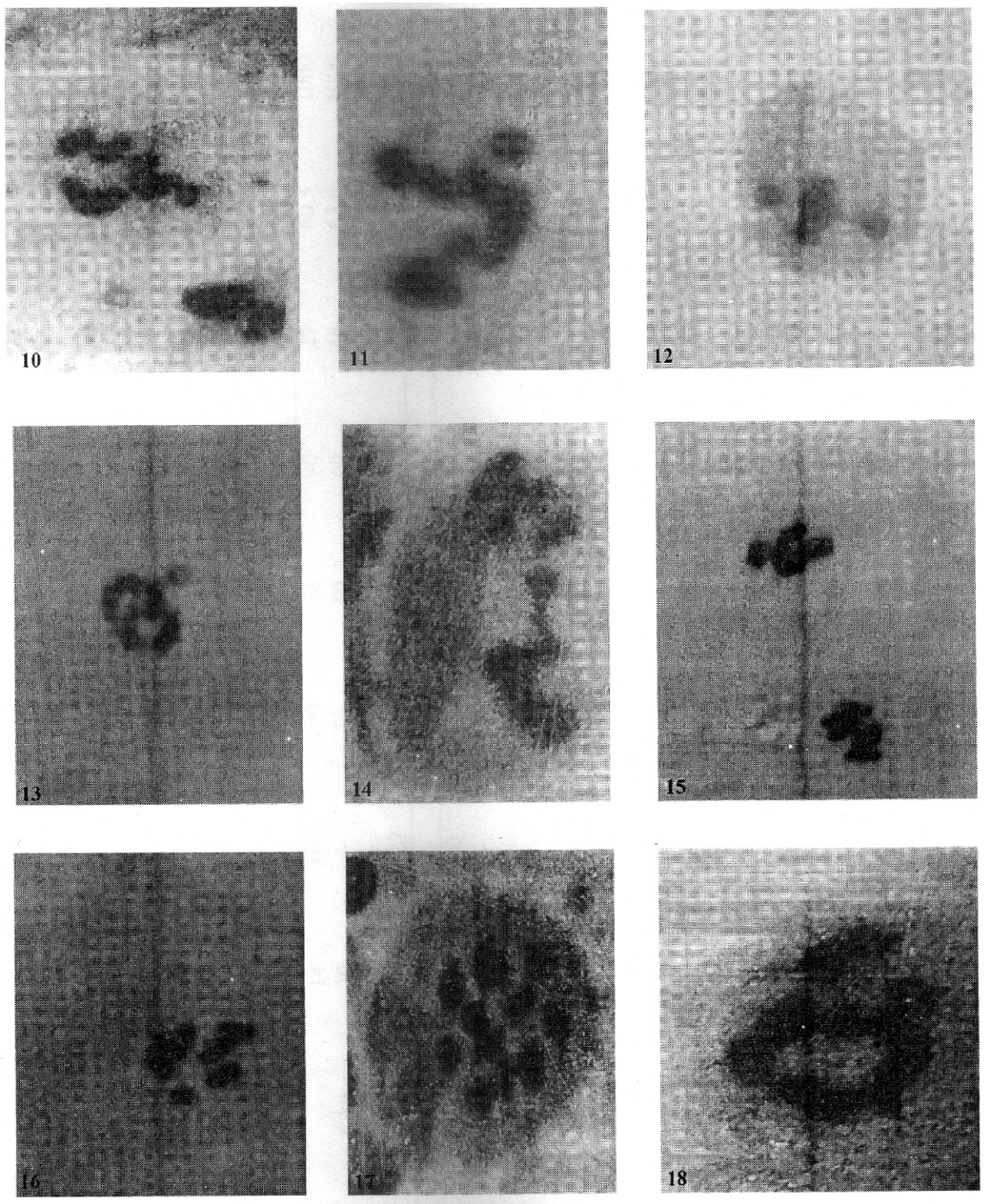

Fig. Meiotic configuration in treated plant of *Capsicum annuum* (Jaya cultivar) : Figure (10-18). **10.** 30 KR (R_1) plant metaphase I showing translocation forming γ-shaped structure. **11.** 30 KR (R_2) Plant metaphase I showing translocation chain. **12.** 40 KR Plant metaphase I showing precocious chromosome. **13.** 0.10% (C_1) Plant metaphase I showing translocation ring. **14.** 0.10% (C_2) Plant anaphase I showing chromosomal bridges. **15.** 0.15% (C_1) plant anaphase I showing clumping of chromosomes. **16.** 0.20% (C_1) Plant metaphase I showing univalent, bivalent and multivalent. **17.** 0.20% (C_2) Plant metaphase I showing multivalent. **18.** 0.30% (C_1) Plant metaphase I showing translocation chains and rings.

TABLE-4

Frequency of abnormalities at metaphase I in control and treated plants of "Jaya" cultivator

Control and treated plants	Uni-valent	Multi-valent	Multi- +Bi+uni valent	Multi- + Bi-valent	Multi + univalent	Number of Cells with Translo-cation chains	Translo-cation rings	Clumped chrom-osomes	Precocious separation	Percentage of affected cells	Total No. of PMCs studied
Control	74	42	–	–	–	11	6	130	28	29.1	1000
Gamma rays doses in KR						**R_1 Generation**					
10 KR	82	178	–	14	100	100	12	90	29	60.5	1000
20 KR	70	240	80	20	–	40	80	60	46	63.6	1000
30 KR	58	360	60	35	–	120	30	50	22	73.5	1000
40 KR	42	380	–	22	122	134	90	52	38	88.0	1000
						R_2 Generation					
10 KR	78	160	–	10	60	50	12	52	48	47.0	1000
20 KR	60	190	62	–	–	–	60	30	36	43.8	1000
30 KR	50	298	41	28	–	90	15	40	–	56.2	1000
40 KR	35	340	–	36	100	120	78	22	25	75.6	1000
EMS construction						**C_1 Generation**					
0.10%	80	92	60	8	80	70	22	60	30	50.2	1000
0.15%	70	180	–	6	70	80	65	40	–	51.1	1000
0.20%	50	320	–	–	–	110	48	28	–	55.6	1000
0.30%	30	370	80	5	110	120	25	20	48	80.8	1000
						C_2 Generation					
0.10%	62	72	40	4	62	40	8	50	18	35.6	1000
0.15%	52	160	–	3	46	42	38	42	–	38.3	1000
0.20%	38	240	–	–	–	70	30	40	–	41.8	1000
0.30%	20	310	60	1	80	72	15	25	30	61.3	1000

TABLE–5

Frequency of abnormalities at anaphase I in control and treated plants of "Jaya" cultivar

Control and treated plants	Total No. of cells studied	Number of affected cells showing				
		Frangments	Unequal separation	Chromosomal laggards + lagging bivalent	Chromosomal bridges	Percentage of affected cells
Control	800	-	-	30	20	6.2
Gamma rays doses in KR				**R_1 Generation**		
10 KR	800	15	10	35	25	10.6
20 KR	800	20	4	40	30	11.7
30 KR	800	120	70	22	12	28.0
40 KR	800	220	30	25	20	36.0
				R_2 Generation		
10 KR	800	8	6	18	16	6.0
20 KR	800	14	2	22	18	7.0
30 KR	800	90	55	17	8	21.2
40 KR	800	180	20	16	9	28.1
EMS Construction				**C_1 Generation**		
0.10%	800	8	30	50	30	14.7
0.15%	800	10	180	110	40	42.5
0.20%	800	60	240	98	48	55.7
0.30%	800	65	340	72	55	66.5
				C_2 Generation		
0.10%	800	–	20	30	–	6.2
0.15%	800	06	80	80	–	20.7
0.20%	800	40	150	50	20	32.5
0.30%	800	50	210	40	25	40.6

TABLE-6

Study of pollen grain in control and treated plants of "Jaya" cultivar

Control and treated plants	No. of Pollen grains studied	Sterile pollen grain		Normal Pollen	Fertile pollen grain		
		No. of Sterile pollens	Percentage of sterile pollens		No. of Micro Pollens	No. of Macro Pollens	Percentage of fertile pollens
Control	1000	65	6.5	932	2	1	93.5
Gamma rays doses in KR				**R_1 Generation**			
10 KR	1000	192	19.2	807	1	–	80.8
20 KR	1000	208	20.8	790	2	–	79.2
30 KR	1000	320	32.0	677	–	3	68.0
40 KR	1000	384	38.4	611	–	5	61.6
				R_2 Geeneration			
10 KR	1000	98	9.8	901	1	–	90.2
20 KR	1000	110	11.0	888	2	–	89.0
30 KR	1000	173	17.3	824	–	3	82.7
40 KR	1000	217	21.7	783	–	–	78.3
EMS Construction				**C_1 Generation**			
0.10%	1000	165	16.5	832	2	1	83.5
0.15%	1000	183	18.3	814	3	–	82.7
0.20%	1000	205	20.5	793	1	1	79.5
0.30%	1000	298	29.8	700	–	2	70.2
				C_2 Generation			
0.10%	1000	137	13.7	859	3	1	86.3
0.15%	1000	165	16.5	835	–	–	83.5
0.20%	1000	242	24.2	758	–	–	75.8
0.30%	1000	290	29.0	707	2	1	71.0

DISCUSSION

It was noticed that the seeds of control plant germinated in five days whereas in treated plants time taken for germination varied from 6 to 10 days. In the similar fashion the time taken by seeds in forming seedlings was much more in treated materials as compared to control one. The morphological characters of treated plants showed certain interesting deviations (Table-2). Considerable variation in the height of the irradiated plant was observed. Plants at certain doses showed decrease in the overall length while at certain other doses it showed increase in the overall length. On the other hand , the EMS treated plants showed stunted growth with less height as compared to the control plant. Another remarkable variation was noted in the bitterness of the fruit. It was found to be considerably less in the treated plants than in the control one. The overall reduction in morphological parameters have also been reported by many other workers like Pushpalatha *et al.* (1992), Reddy *et al.* (1992) and Jabeen and Mirza (2004). Reduction has been attributed to various factors including changes in balance in growth regulators and metabolic activity (Aman, 1968). When the effect of gamma ray and EMS was comparatively examined, the effect of EMS was found to be more systematic than gamma rays.

The cytological observations showed somatic chromosome number as 2n = 24. The meiotic anomalies reported were chromosomal breaks and stickiness of chromosomes. The first anomaly was common in gamma ray treated plants and second in EMS treated plants. Similar observations have been reported by Mackenzie *et al.* (2005), Botticella *et al.* (2011) and Parthirana (2012). Stickiness of chromosome may occur due to disturbances in cytochemically balanced reaction in the nucleic acid (Jayabalan and Rao, 1987) or due to partial dissociation the nucleoprotein and alterations in their pattern of organization. The chromosomal breaks are very much common in irradiated plants because the gamma rays target the chromosomes in haphazard manner which results in chromosome break (Matsumara and Fujii, 1958 and Newcombe, 1971).

During meiosis the gametic number was reported as n = 12 in control as well as treated plants. In treated plants meiotic anomalies were quite pronounced. At metaphase I, anomalies like multivalent, univalent, translocation chains and rings, clumping of chromosomes and precocious separation of chromosomes were prominent (Table-4). Similarly at anaphase I chromosomal laggards, lagging bivalents, chromosomal bridges, unequal separation of chromosomes and chromosome fragments were of common occurrence (Table-5). Pollen sterility was also quite noticeable (Table-6). All these anomalies have also been reported by many workers including Bansal and Dalmirsingh (1972), Reddy *et al.* (1992). Meshram *et al.* (1992) and others. Multivalent formation is attributed to association of chromosome due to radiations or EMS treatment. According to Katiyar (1978) alteration in chromosome association of different types of multivalents are possibly the outcome of irregular pairing of chromosomes due to translocation. Presence of simple chromosomal bridges may arise due to failure of trerminalization of the chaismata or due to the stickiness of the chromosome (Tripathi *et al.* 2008). Unequal separation of chromosomes and formation of chromosomal laggards may be the result of desynaptic effect. The pollen sterility was found to increase with the increase in different doses or concentration of mutagens. The increasing pollen sterility could be attributed to increase in different doses or concentration of mutagens. The increasing pollen sterility could also be attributed to increase in cytological abnormalities. The degree of chromosome pairing has been found to be mainly responsible for increased sterility in mutagenic population. Gaul *et al.* (1996) and Ekberg (1969) are of the opinion that mutagenic treatment might be attributed to detectable chromosomal aberrations and cryptic deficiency, which finds a logical extension in the present investigation.

When mutagenic effectiveness and efficiency are analyzed, it is found that Gamma ray is more effective than EMS because here effectiveness means rate of mutation related to the dose, and mutation rate has been found to increase considerably with the increase in the dose of Gamma ray. However, EMS treatment is more efficient than Gamma ray because efficiency is related to the point mutation rate in relation to other biological effects such as gross chromosomal aberrations. In the present investigation the EMS treated plants showed changes but with lesser chromosomal aberrations and we know that plants with point mutation also show lesser aberration. More precisely, it may be remarked that the effect of gamma ray is haphazard while that of EMS is systematic.

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