Gamma rays and EMS induced chromosomal abnormalities and pollen fertility in *Brassica campestris* (L.) genotypes

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Abstract: *Mutagenesis has come up with a hope as an efficient tool for creating genetic variability and boosting up of characters under consideration. Degree of cytological aberration either in mitosis or meiosis is regarded as one of the dependable criteria to estimate the radio-sensitivity of the species and the effect of mutagens .With this view, to understand effect of various doses of gamma rays and EMS on the meristmatic root cells of two genotypes T-9 and PT-303 of Brassica campestris (L.) (2n=20), this work was undertaken. It was found to mutagens caused declining trends in mitotic indices with increasing doses/concentrations in both the cultivars. The percentages of abnormalities is directly proportional to doses/concentrations, abnormalities included stickiness, clumping, c-metaphase, laggard and bridges at anaphase and telophase were observed in dividing cells. The pollen fertility among all the mutagenic treatments shows gradual decreases with respect to the increase in concentrations. It may be attributed due to induced chromosomal aberration. The percentage of chromosomal aberrations observed in case of EMS treated set was higher than gamma rays treated sets suggesting that EMS could be successfully employed for creating novel genetic variability in oilseed Brassicas.*

Key words: *Chromosomal aberration, EMS, gamma rays, pollen fertility,*

1. INTRODUCTION:

Crop improvement is a key route to ensuring continued benefits arising from food and plant products. Wide range of genetic, biochemical and metabolic variation needs to be generated for effective crop improvement. With the discovery of ionizing and chemical mutagens a new field of science known as "mutation breeding" was developed. These mutagens may cause genetic changes in an organism, break the linkages and produce many new promising traits for the improvement of crop plants. Induced mutations provide beneficial variations for practical plant breeding purpose. During the past seven decades, more than 2252 mutant varieties have been officially released in the world (Maluszynski, *et al*., 2000).[1] Khatri *et al.,* (2005)[2] reported that gamma rays and EMS could be fruitfully applied to develop new varieties with high yield and other improved agronomic traits in *Brassica juncea* . Thus to administer successful mutagenesis, selection of efficient mutagen and treatment is a pre-requisite. Mutagens are the potent tool to create novel variation particularly in those crops with restricted or lack of genetic diversity. The cytological abnormality serves as the primary effect of phenotype variation. Degree of cytological aberration either in mitosis or meiosis is regarded as one of the dependable criteria to estimate the radio-sensitivity of the species and the effect of mutagens (Ignacimuthu, 1989).[3] Pollen sterility is also used for calculating the mutation index which is a good indicator to forecast the spectrum of genetic variability that can arise from the mutated sectors. Considering these researches attributes, the present investigation documents mutagenic effectiveness of gamma rays and EMS on chromosome biology of *Brassica campestris* (L.) genotypes T-9 and PT-303.

2. MATERIALS AND METHODS:

Two varieties of *Brassica campetris* (L.) T-9 and PT-303, physical mutagen (gamma rays) and chemical mutagen Ethyl Methane Sulphonate (EMS) were employed in the present study. The seeds of these varieties were procured from U.P. State seed corporation, Lucknow. The healthy seeds of T-9 and PT-303 were treated with gamma rays (25 KR, 35 KR and 45 KR) through 60Co source (at the dose rate of 1.8 KR/sec) done at National Botanical Research Institute (N.B.R.I. Lucknow, India) and three different doses/concentrations of EMS such as 0.3%, 0.6% and 0.9% in laboratory. Both these treatments were done to create genetic variability for selection of desirable genotypes. After each treatment the irradiated seeds were sown in experimental field along with their suitable control counterparts for pollen studies. However, for mitotic studies treated seeds were grown in petriplates (lined with moist filter paper) along with their controls. The healthy root tips were fixed in carnoy's fixative (1:3 glacial acetic acid + alcohol), preserved in 70 % alcohol and stained in 2 % acetocarmine. For the pollen studies, floral buds of appropriate size were collected and stained in 2 % acetocarmine. Photomicrographs were taken and analyzed using Nikon image capturing system.

Mitotic indices and percentages of abnormally dividing cells were calculated. Different types of chromosomal abnormalities such as laggards, fragments, bridges etc. were detected from the desired preparations. They were detected from metaphase; anaphase and telophase stages of mitosis and data were recorded and analyzed statistically.

3. RESULT AND DISCUSSION:

The mutagenic effect of physical (γ-rays) and chemical Mutagen (EMS) have been studied on chromosomal behavior and mitotic activity on of the *Brassica campestris* (L.) root-tips. The mitotic index and abnormality (%) were calculated and presented in Table 1a, 1b, 2a and 2b and figures (Plate.1). In *Brasssica campestris* (L.), the somatic complement consists of 20 chromosomes (2n = 20). The results revealed that mitotic indices noted in the treated cell were lower than the indices observed for their respective control except at 0.9% EMS treatment where an increase in mitotic indices was observed. In var.T-9, the mitotic indices showed a declining trend with the increasing dose while in var. PT303, it showed fluctuating trend. The mitotic index was highest for var.T-9 at 0.9% EMS and minimum at 45 kR. Mean value for mitotic indices shows significant reduction at 25, 35kR and 0.3% EMS, 0.6% EMS than that of control while in var. PT-303; it was noted maximum at control and minimum at 35kR dose of gamma rays. Significant reduction in mitotic indices was observed at all doses of gamma rays and at 0.6%EMS treatment as compared to control. These findings are in close agreement with the earlier reports of (Narsinghani *et al*., 1976).[4] in Pea and (Patil *et al*., 2011)[5] in soyabean. The existence of such varietal differences with respect to mutagen sensitivity in present study has also been reported by Khan and Goyal (2009)[6] in mungbean.

In both the varieties the percentage of various mitotic abnormalities gradually increased with increasing doses/concentrations of mutagens. In var. T-9 maximum 66 % abnormal cell observed at 0.9% EMS while minimum 2.2% noted at control. In case of var. PT-303 maximum 68 % abnormality in dividing cells found at 45kR while abnormal cell absent (0%) at control. Several chromosomal abnormalities including unequal divisions, multinucleated cells, clumping, stickiness, laggard formation, fragmentation of chromosomes, disturbed metaphase(c-metaphase) persistent nuclei, cleft, bridges at anaphase and telophase observed in mutagens treated meristmatic cells. Condensation was found more in EMS and gamma rays treatments in var. PT-303 compared to var. T-9 and C-metaphases present only in gamma rays treatment. Clumping was more prominent in both the varieties at EMS treatments. Bridges at anaphase were more in gamma radiation than EMS treatment in both the genotypes. Laggards were present in all the treatments of both the varieties. Stray, fragmentation, disturbed anaphase and binucleated telophase were observed in gamma ray treatments while cleft at metaphase were more in both the varieties in EMS treatments. A broad range of chromosomal aberrations were induced by both the treatments but higher proportion has been attributed to stickiness of chromosomes, laggard, clumping and bridges at anaphase. Stickiness might have been arisen either due to depolymerization of nucleic acid caused by mutagenic treatment or due to partial dissociation of the nucleoproteins and alterations in their pattern of organization (Kumar *et al.*,2003).[7] The behavior of laggard chromosome is characteristic in that might result from late chiasma terminalization (Pagliarini.,1990).[8] Bridges reported at higher doses in both varieties in this study might have arisen through breaks in two chromosomes followed by union of the centric fragments (Shreekrishna,2006).[9] The formation of bridges at anaphase indicates the occurrence of exchange between chromosomes. C-metaphase at lower doses in both the varieties is one of the consequences of inactivation of spindle apparatus connected with the delay in division of centromere. Fragmentation at different stages observed also reported by Mikaelson *et al.,* (1968).[10] The formation of bi and multinucleated cell in treated root cells may be due to metabolic disorder and inhibit cytokinesis (Braun and Dayer.1972).[11] Disturbed metaphase and anaphase where the chromosome spread irregularly over the cells observed more frequently in different stages of cell division.

Pollen fertility was gradually decreased with increase in doses of mutagens in both of the varieties. However in var.T-9 maximum percentage of pollen fertility (96.03%) was noted at 25 kR and minimum (58.20%) at 0.9% EMS while in case of PT-303 maximum (94.20%) at control and minimum (46.05%) at 45 kR. The pollen fertility is decreased as doses increase in both the mutagens. The maximum fertility was observed at control while minimum was noted at 0.9% EMS in both the varieties. The increasing pollen sterility has been mainly attributed to the chromosomal interchange, chromosomal aberration and gene mutation (Gautam *et al.,*1992).[12] As more and more abnormalities accumulate, the process of gamete formation is affected and it will leads to non- viable gametes that could considerably reduce the plant fertility (Kumar and Rai., 2007).[13]

4. CONCLUSION:

It is evident from above study that mutagenic treatments caused declining trends in mitotic indices with increasing doses. Comparative study of the two treatment showed that mitotic indices were much retarded by gamma rays in comparison to EMS and abnormality percentage was higher in gamma irradiated set in comparison to EMS. The development of these abnormalities indicates that mutagens gamma rays and EMS caused variability which could be beneficial in developing the cultivars with desirable alleles for further improvement programs.

5. ACKNOWLEDGEMENTS:

The author is grateful to U.P. State Seed Corporation, Lucknow for providing germplasms, and the National Botanical Research Institute (NBRI), Lucknow, for providing gamma irradiation facility.

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Table 1a: Effect of mutagens on mitotic cell division (Mean ± SE, n=5) and pollen fertility in var. T-9 at different doses/ concentrations.

Treatments	Mitotic index (%)	% of cells in prophase	% of cells in metaphase	% of cells in anaphase	% of cells in telophase	Abnormality % aberrant cells	Pollen Fertility %
Control	11.23±0.81	39.47±3.89	19.13±1.93	20.23±1.95	21.16±2.04	0 2.0	87.03
25kR	8.88±0.63	37.75±3.66	13.91±3.39	22.25±0.79	26.09±2.90	27.85	96.03
35kr	7.02±0.25	32.30±3.11	19.22±1.32	27.76±1.25	20.72±2.12	39.05	80.25
45kR	7.60±0.43	33.21±1.41	12.50±3.62	26.90±5.34	27.38±3.10	57.00	78.25
0.3% EMS	10.69±0.82	37.64±5.03	14.62±2.04	24.77±4.83	22.97±4.80	35.00	83.20
0.6% EMS	8.42±0.53	47.22±1.83	19.95±2.08	18.36±2.01	14.47±2.73	43.33	60.20
0.9% EMS	12.54±1.59	42.88±2.82	22.08±1.91	19.43±2.57	15.62±2.76	60.33	58.20
F6,28DF	6.09^{**}	2.49^{*}	2.15^{*}	1.45^{ns}	2.55^{*}	-	-

**-p<0.01,*-P<0.05, ns-p>0.05

Table 1b : Percentage of different abnormalities in var. T-9 at different doses/concentrations of mutagens.

Treatments	C-meta	Conden-sation - stray	Clumpi-ng	Cleft	Ana-bridges	Bridges-telo	Disturbed-ana	Laggard	Fragme-ntation	Stickin-ess	Micronu-clei	Others
Control	65.00	-	-	-	-	-	-	-	-	-	-	35.00
25kR	15.38	7.60	1.05	9.73	11.53	8.00	3.00	7.70	3.84	3.84	8.33	20.00
35kR	7.31	7.31	11.40	3.05	13.51	8.34	11.25	12.31	7.31	9.19	6.53	2.49
45kR	13.45	11.62	12.00	8.65	13.56	5.25	2.40	12.25	4.65	4.65	10.19	1.33
0.3% EMS	13.75	10.20	9.50	4.50	10.00	2.00	3.50	8.75	10.32	14.75	9.30	3.43
0.6% EMS	15.45	8.00	12.50	9.00	10.65	2.00	5.25	10.65	4.50	7.00	10.00	5.00
0.9% EMS	10.00	10.34	10.33	1.20	4.50	2.42	1.50	15.00	5.33	10.00	13.20	16.18

Table 2a: Effect of mutagens on mitotic cell division (Mean ± SE, n=5) and pollen fertility in var. PT-303 at different doses/ concentrations.

Treatments	Mitotic index (%)	% of cells in prophase	% of cells in metaphase	% of cells in anaphase	% of cells in telophase	Abnormality % aberrant cells	Pollen Fertility %
Control	12.09±1.01	41.79±4.88	13.65±3.20	22.34±4.99	22.22±3.35	0.0	94.20
25kR	6.44±.33	40.03±2.34	17.74±2.59	23.10±4.47	19.13±4.58	33.33	70.97
35kR	5.27±0.59	33.16±2.31	18.77±3.08	33.31±3.33	14.76±1.73	40.10	56.39
45kR	5.18±0.07	32.15±2.13	24.40±1.86	26.33±3.02	17.12±2.01	61.00	46.05
0.3% EMS	7.17±0.36	26.30±2.54	26.94±1.49	25.73±0.57	21.03±3.41	40.12	78.40
0.6% EMS	6.74±0.41	30.04±2.26	19.69±1.68	24.83±1.48	25.44±1.51	47.00	63.80
0.9% EMS	8.81±0.38	36.23±1.78	14.52±1.99	27.27±2.97	21.98±2.51	68.00	46.60
F6,28DF	21.11^{**}	2.43^{ns}	4.33**	1.17^{ns}	1.43^{ns}		

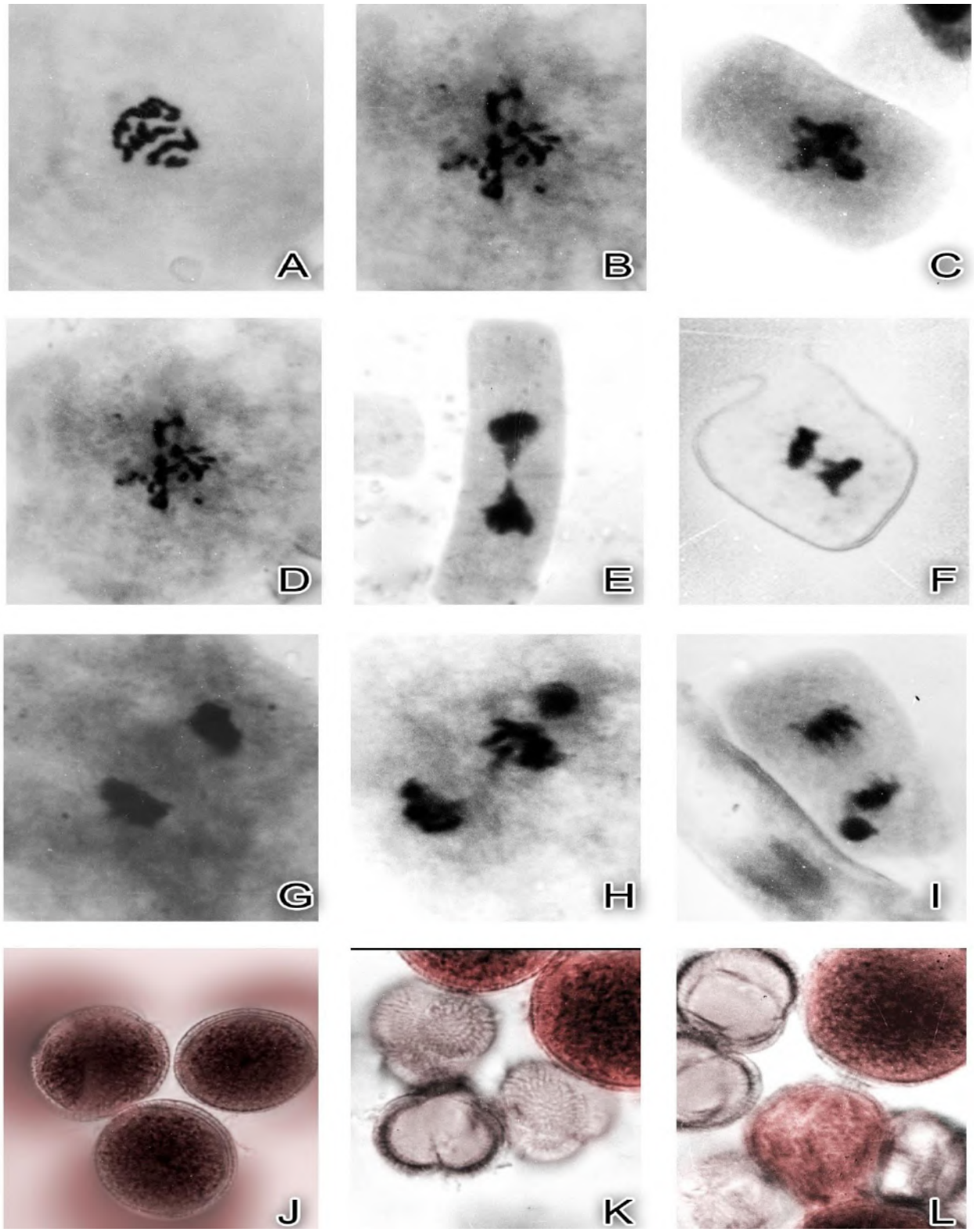
**-p<0.01,*-P<0.05, ns-p>0.05

Table 2b: Percentage of different abnormalities in var. PT-303 at different doses / concentrations of mutagens.

Treatments	C-meta	Condensation - Stray	Clumping	Cleft	Ana-Bridges	Bridges-Telo	Dist-Ana.	Laggard	Fragme-ntation	Stickin-ess	Micro-nuclei	Others
Control	-	-	-	-	-	-	-	-	-	-	-	-
25kR	10.40	20.00	1.20	2.30	20.33	2.50	2.50	12.00	3.84	10.00	12.27	3.20
35kR	7.40	11.33	4.50	7.40	24.62	7.30	2.00	11.11	4.00	3.33	7.40	9.61
45kR	9.80	14.50	8.33	4.50	25.00	5.00	1.20	10.18	4.64	11.63	4.50	0.72
0.3%EMS	4.10	10.40	10.50	8.00	8.00	-	5.20	11.05	12.65	15.20	10.00	4.90
0.6%EMS	11.50	1.50	12.50	12.33	10.00	2.00	8.20	8.05	2.00	12.45	13.50	5.97
0.9% EMS	9.50	8.50	15.33	11.50	10.33	5.86	12.20	12.08	5.25	8.33	5.00	7.20

Plate 1: Figure description:

A. Abnormal metaphase.
B. Fragmentation at metaphase.
C. Persistent nucleolus at metaphase.
D. Cleft.
E. Stickiness and bridges.
F. Bridges at anaphase.
G. Condensation
H. Abnormal Anaphase
I. Bridges and persistent nucleolus at anaphase.
J. Fertile pollen grains
K. Bridge at anaphase
L. Sterile pollen grains.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