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Comparative assessment of Gamma rays and EMS induced chromosomal aberrations in *Brassica* Genotypes

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Abstract
Mutagenic effect of gamma rays and ethyl methane sulphonate (EMS) on mitotic indices and structural chromosomal aberrations were studied using three different doses/concentrations of mutagens along with a control. It was found to mitotic indices decrease with increasing the doses of mutagens in all the two genotypes T-9 and PT-303 of *Brassica campestris*. The mutagens were also found to induce high frequency of chromosomal changes like c-metaphase, chromatid bridges, chromosome fragments, laggards etc. Their frequencies were also found to increase with increase of the doses. Comparative study of the two treatments showed that mitotic indices were much retarded by gamma rays in comparison to EMS and abnormality percentage was higher in EMS in comparison to gamma rays.

Keywords: *Brassica campestris*, chromosomal aberration, EMS, mutagen.

1. Introduction
Cytological studies provide information regarding the role and effect of mutagens on various genotypes. Mutagen generally induces high frequency of chromosomal changes and mitotic abnormalities. Degree of cytological aberration either in mitosis and meiosis is regarded as one of the dependable criteria for estimate the radio sensitivity of the species and the effect of mutagenesis. Gamma rays are the most energetic form of electromagnetic radiation and they are considered as the most penetrating in comparison to other radiation such as alpha and beta rays. A group of chemicals are reported to induce chromosomal aberration. These chemicals are commonly known as chemical mutagens having specific and limited action, and found to induce specific mutation or aberration in organisms. Chemical and physical mutagens are being used in inducing variability in plant breeding program. Geneticists are using chemical mutagens as potential tools and it is reported that a number of chemicals influence the sensitivity as well as increase the frequency and spectrum of mutations. The studies of a number of compounds and the development of mutagenesis have been reported by different workers (Prasad 1972; Rao and Rao 1983) [1, 2,]. In view of these research aspects, many mutant varieties have been developed through mutagenesis. Among them 94% were following the treatments of physical mutagen, 5% through chemical mutagen and the remaining 1% through a combined treatment of physical and chemical mutagens (Singh 1993) [3]. Considering these research attributes the present study was undertaken to investigate the mutagenic effect of a chemical mutagen *i.e.* Ethyl methane sulphonate and gamma irradiation on somatic cells of *Brassica campestris* var. toria *i.e.* PT-303 and T-9.

Materials and Methods
Two cultivars of *Brassica campetris* var. toria *i.e.* PT-303 and T-9 and 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. Three different doses of EMS such as 0.3%, 0.6% and 0.9% and gamma irradiation 25kR, 35kR and 45kR were used. The treated seeds of both the varieties along with respective control were kept in petridishes containing moist paper for germination. When the roots grew up to 1.0-1.5 cm in length root tips of different varieties from each treatment were collected separately by a pair of fine forceps and fixed in carnoy's fixative between 1.15 to 1.45pm. Then, they were transferred to 90% ethyl alcohol. The root tip cells were stained by squash method using Haematoxylin as stain. Detailed cytological observations were made and the numbers of dividing, non - dividing cells as well as cells with division abnormalities in treated material along with control were counted. Scoring was done from five slides for each treatment taking five fields from each slide.

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.

Results and discussion: 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. The Mitotic index of PT-303 was comparatively lower than the T-9 and the magnitude of reduction was higher in gamma rays followed by EMS. 25kR, 35 kR, 45kR doses of gamma rays and 0.3%, 0.6% concentrations of EMS significantly reduced mitotic index in var. T-9 and PT-303. In var.T-9, the mitotic indices showed a declining trend with the increasing dose while in var. PT-303, it a showed fluctuating trend. The mitotic index was maximum for var.T-9 at control and minimum at 45 kR (Table1a). Mean value for mitotic indices shows significant reduction at 25, 35kR and 0.3% EMS and 0.6%EMS than that of control while in var.PT303; it was noted maximum at 0.9% EMS and minimum at 45kR dose of gamma rays in treated cells. Significant reduction in mitotic indices was observed at all doses of gamma rays and at 0.6%EMS treatment as compared to control (table2a). The similar result of reduction in mitotic indices also reported by Narsinghani *et al. (*1976) [4] and Patil *et al* (2011) [5]. The existence of such varietal differences in sensitivity to mutagens in present study has also been reported by Khan and Goyal (2009) [6].

Chromosomal abnormalities (Plate.1) in root tip cells of two varieties treated with different doses of EMS and gamma irradiation were determined. The percentage of various abnormalities gradually increased with increasing doses of mutagens. Results showed non-significant variation among the different varieties. In case of different doses there is significant variation. In both the varieties the percentage of various mitotic abnormalities gradually increased with increasing doses/concentrations of mutagens. In T-9 maximum 66% abnormal cell observed at 0.9% EMS while minimum 2.2% noted at control (Table1b). In case of var.PT-303 maximum 68% abnormality in dividing cells found at 45kR while abnormal cell absent (0%) at control (Table 2b) The actions of ionizing radiations and chemical mutagens on somatic cell division have been reported by some investigators in a number of plant species (Clowes and Hall 1970; Reiger and Michaelis 1972) [7, 8]. Several chromosomal abnormalities including disturbed metaphase (C-metaphase), condensation, persistent nucleolus, clumping, laggard formation, fragmentation of chromosomes, bridge at anaphase and telophase, micronuclei, multinucleated observed in mutagen treated meristmatic cells. Similar results were also reported by Alam *et al.,* (1980) [9]. Gamma irradiation and EMS treatments induces c-metaphase at lower doses in both the varieties. Somashekhar (1984) [10] suggested that c-metaphase is one of the consequences of inactivation of spindle apparatus connected with the delay in division of centromere. Condensation was found more in EMS and gamma rays treatments in var.PT-303 compared to var. T-9. 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 varieties of *B. campestris*. Bridges at anaphase and telophase stages observed at higher doses in both varieties. The formation of bridges at anaphase indicates the occurrence of exchange between chromosomes. The numbers of bridges depend upon the number of chromosomes take part in exchange. Due to sticky nature of chromosomes, chromatin bridges may be found at anaphase and telophase stages (Jain and Sarbhoy, 1987) [11] and that is also caused due to mutagenesis. According to Dempong (1973) [12] production of bridges at anaphase and telophase can be attributed to breakage and reunion of chromatids or sub-chromatids. Laggards were present in all the treatments of both the varieties. Das and Roy (1989) [13] were of the opinion that due to effect of mutagens spindle fibres failed to carry the respective chromosome to polar region and resulting in the lagging chromosome appeared at anaphase. 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 treatment (Table1b, 2b). The formation of bi and multinucleated cell in treated meristmatic root cells may be due to metabolic disorder and inhibit cytokinesis. According to Honna *et al.,* (2000) [14], the development of these abnormalities indicates that mutagens gamma rays and EMS partial inhibit the mitotic apparatus and caused variability.

Conclusion

It is evident from above study that abnormalities were induced by gamma rays and EMS, comparative study of the two treatments showed that mitotic indices were much retarded by gamma rays in comparison to EMS and abnormality percentage higher in EMS treatments than gamma rays. Genotypic variation also observed, var. PT-303 was more sensitive than T-9.

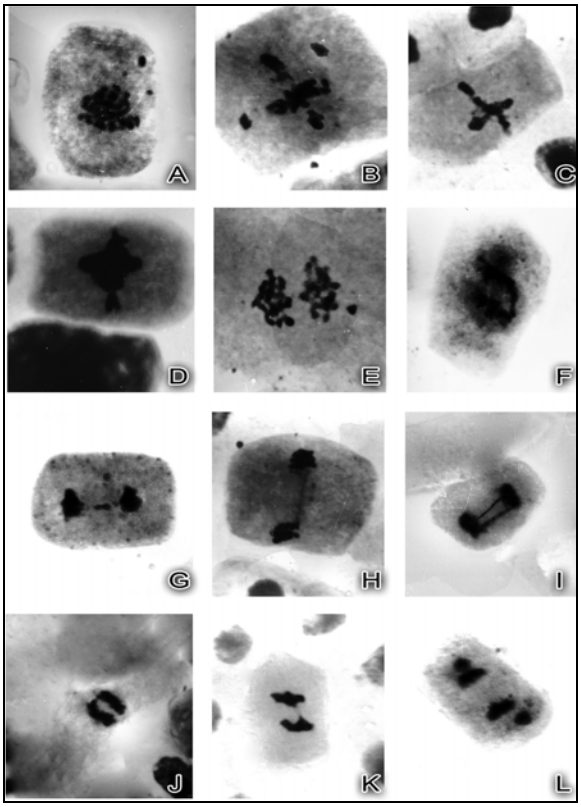

Plate 1: Figure description **A.** C-Metaphase. **B.** Desynchronized metaphase. **C.** Persistent nucleolus at metaphase. **D.** Stickiness at metaphase. **E.** Fragmentation at anaphase. **F.** Clumping at anaphase. **G**. Laggard at telophase**. H.** Bridge at telophase. **I.** Di-centric bridges at telophase. **J.** Abnormal anaphase. **K.** Bridge at anaphase **L.** Micronuclei at early telophase

Table 1a: Effect of mutagens on mitotic cell division (Mean ± SE, n=5) of root cells 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
Control	11.23±0.81	39.47±3.89	19.13±1.93	20.23±1.95	21.16±2.04	0 2.0
25kR	8.88±0.63	37.75±3.66	13.91±3.39	22.25±0.79	26.09±2.90	27.85
35kr	7.02±0.25	32.30±3.11	19.22±1.32	27.76±1.25	20.72±2.12	39.05
45kR	7.60±0.43	33.21±1.41	12.50±3.62	26.90±5.34	27.38±3.10	57.00
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
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
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
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 of mutagens.

Treatments	C-meta.	Condensation - stray	Clump-ing	Meta-Claft	Ana-bridges	Telo-bridges	Disturbed -anaphase	Laggard	Fragmentations	Stickiness	Micro-nuclei.	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) of root meristem 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
Control	12.09±1.01	41.79±4.88	13.65±3.20	22.34±4.99	22.22±3.35	0.0
25kR	6.44±.33	40.03±2.34	17.74±2.59	23.10±4.47	19.13±4.58	33.33
35kR	5.27±0.59	33.16±2.31	18.77±3.08	33.31±3.33	14.76±1.73	40.10
45kR	5.18±0.07	32.15±2.13	24.40±1.86	26.33±3.02	17.12±2.01	61.00
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
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
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
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: Percent of different abnormalities in var. PT-303 at different doses of mutagens

Treatments	C-meta	Condesation - Stray	Clump-ing	M-Claft	Ana-Bridge	Telo-Bridge	Dis.-Ana.	Laggard	Fragment.	Stickin-ess	Micro-nuclei	Oth.
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

References

1. Parsad MVR. A comparison of mutagenic effectiveness and efficiency of gamma rays, EMS, NMV and NG. Indian J Genetics and Plant Breeding.1972; 32:361-367.
2. Rao GM, Rao MV. Mutagenic efficiency, effectiveness and factor of effectiveness of physical and chemical mutagen in rice, Cytologia.1983; 48:427-436.
3. Singh DN, Godward MBE. Radiation studies in *Eleusine coracana* (L.) Cytologia. 1974; 39:729-740.
4. Narsinghani VG, Kumar S. Mutation studies in gamma rays treated peas. Mysore J. Agric. Sci. 1976; 10:396-403.
5. Patil GP, Wakode MM. Effect of physical and chemical mutagens on soybean. Curr. Bot. 2011; 2(4):12-14
6. Khan S, Goyal S. Mutation genetic studies in mungbean IV. Selection of early maturing mutants. Thai Journal of Agricultural Science. 2009; 42(2):109-113.
7. Clowes FAL, Hall EJ. The immediate response of the quiescent center to X-rays. New Phytol. 1970; 69:1-18.
8. Reiger R, Michaelis A. Effect of chromosome repatterning in *viciafaba*L. 1. Aberration distribution, aberration spectrum and karyotype sensitivity after treatment with ethanol of differently reconstructed chromosome complements. BiotZb. 1972; 91:151-169.
9. Alam S, Khan MR, Banu N, Daruzzaman M. Cytological effects of insecticides (Dimecron – 100 and Vapona) on wheat. Bangladesh Journal of Agriculture. 1980; 5:176-181.
10. Somashekhar RK, Gowada. MTG. Effect of fungicide vitavax on *Allium cepa*. Cytologia. 1984; 49:177-181.
11. Jain HK, Sarbhoy RK. Cytogenetical studies on the effect of some chlorinated pesticides. I. Effect on Somatic chromosomes of *Lens* and *Pisum*. Cytologia. 1987; 52:47-63.

12. Dempong, Maxwell An Cytological effect of nogolamycin on *Tradaschntia* poludum microspore. Mutation Research. 1973; 21:223-226.
13. Das SK, Roy SK. Radio Cytologiacal studies on *Solanum* meiotic abnormalities. Cytologia. 1989; 54:477-481.
14. Hanna HES, Sheikh IAE. Arrested mitotic cycle and induction of chromosomal aberrations by aflatoxin B2 in root cells of Vicia faba L. Cytologia. 2002; 65:113-122.