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COMPARATIVE CLASTOGENIC EFFECTS OF INDIVIDUAL AND COMBINED TREATMENTS OF GAMMA RAYS AND EMS ON *NIGELLA SATIVA* L.

G. KUMAR* and PRIYANKA GUPTA**
Plant Genetics Laboratory, Department of Botany, University of Allahabad, Allahabad-211002, India.
* girjesh.kumar1@gmail.com
** priyanka2302@gmail.com

Mutations are extremely useful for producing crops with better yield and improved quantitative traits. But it has often been seen that the type of mutation varies with the plant and also the variety used. In some plants, Gamma ray exposure was more beneficial while in some other plants EMS showed a better response. Alternatively in some other cases the combined treatment of mutagens was found to be more effective. In case of *Nigella sativa* L. var Azad, the combined treatment of Gamma rays and EMS was more genotoxic than their individual treatments. So, it can be envisaged to bring about phenotypic variations also which might be beneficial for the plant.

Keywords: Combined treatment, EMS, Gamma rays, Genotoxic, *Nigella sativa* L.

Introduction

Mutations can be beneficially utilized for tailoring better varieties of crop plants. But in general, ionizing radiations and chemical mutagens like Ethyl methane sulphonate (EMS) affect a wide range of chromosomal alterations resulting into abnormal behaviour during meiosis, leading to various degree of sterility. Further the cytological abnormalities during meiosis have also been regarded as one of the dependable parameters for estimating mutagenic sensitivity of a species. Various workers have compared the mutagenic efficiencies of different plants. Their results seem to be entirely specific for particular species and even varieties. While many workers like Rao and Siddiq[1], Rao and Rao[2], Kumar and Dubey[3], Dhamyanthi and Reddy[4] found chemical mutagens to be more effective than physical ones, many others like Siddiq[5], Tarar and Dhyansagar[6], Zeerak[7] found the reverse to be the case. It has also been seen that treatments with certain chemical substances just after irradiation may prove to be more potent than the individual mutagens[8]. Since 1952, several chemical agents have been found to be able to protect plants as well as animals against radiation induced chromosomal damage. So, the present study has been undertaken on *Nigella sativa* L. to assess the effect of individual and combined treatments of Gamma rays and EMS.

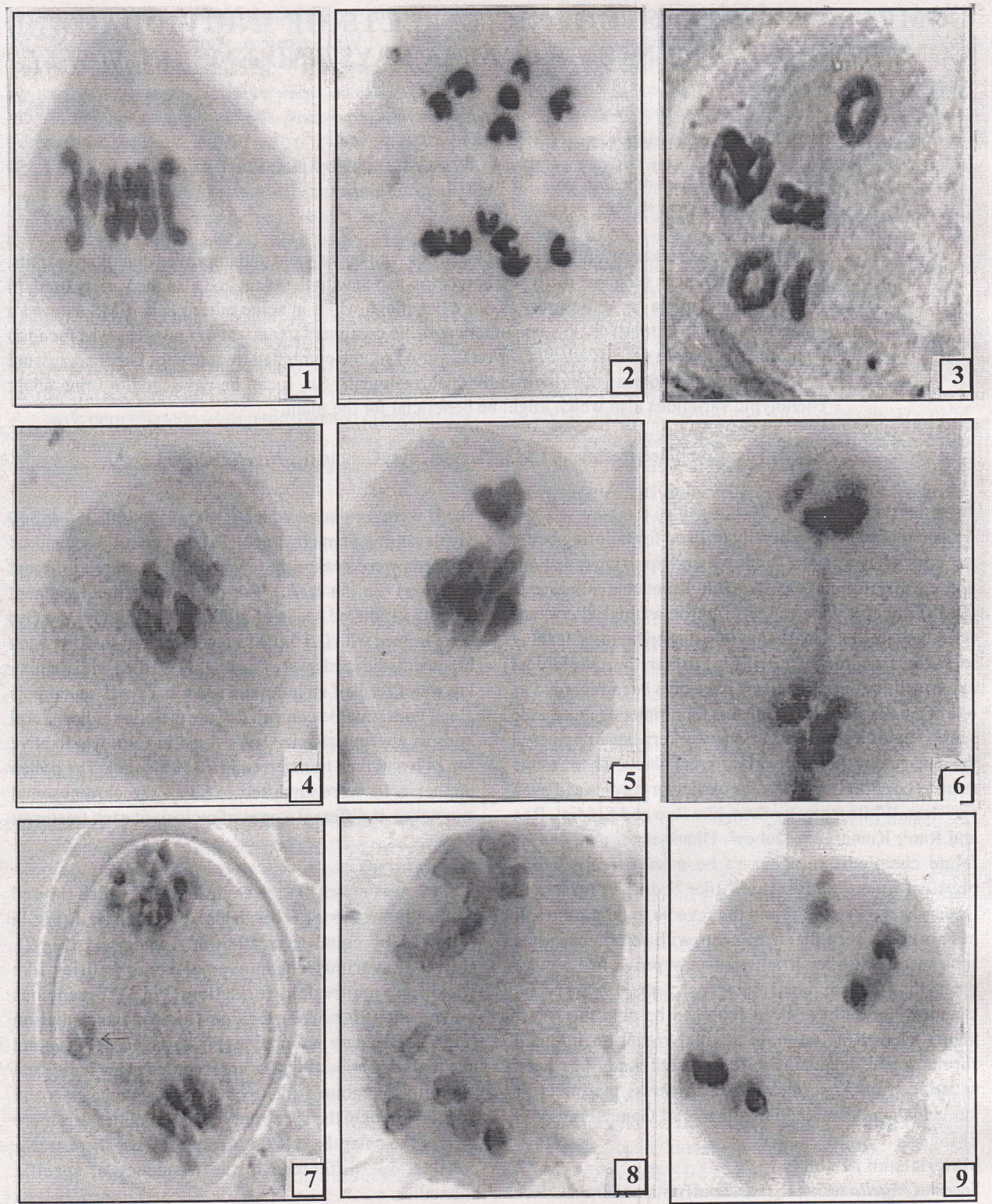
Material and Methods

Seeds of *Nigella sativa* L. (var Azad) obtained from C.S.A University of Agriculture & Technology, Kanpur. These seeds were irradiated with 50, 100, 150, 200, 250 & 300 Gy doses of gamma rays at NBRI, Lucknow. A second set of seeds were soaked in 0.5 % EMS for three different durations (3h, 5h & 7h). For the combination treatment gamma irradiated seeds of all the doses (50 Gy-300 Gy) were treated with 0.5 % EMS for 5 hr duration. After each treatment, the seeds were thoroughly washed with running tap water for half an hour and sown in experimental pots along with suitable control. For the meiotic studies, floral buds of appropriate size were fixed in Carnoy's fixative for 24 h and then transferred in 70 % alcohol. The pollen mother cells were analysed to score the chromosomal anomalies at different stages of meiosis at each treatment dose.

Observations

Meiosis was normal in the control plants, which showed regular formation of 6 bivalents at Metaphase I (Fig.1) followed by equal separation at Anaphase I (Fig.2). However, various chromosomal abnormalities like stickiness, multivalent formation (Fig.3), precocious movement (Figs.5&9), bridge (Fig.6), unorientation (Fig.9), secondary association (Fig.4), laggard (Fig.7) and unequal separation (Fig.8) were recorded in the individual as well as combined treatment sets of gamma rays and EMS. Although, the spectrum of abnormalities was more or less the same for all the treatment sets, there was a considerable difference in the frequency of specific anomalies.

In the gamma ray treated set, all the major



Figs.1-9. 1-6 bivalents at Metaphase I; 2 - 6 : 6 separations at Anaphase I; 3 - Multivalent at Metaphase I; 4 - Secondary association at Metaphase I; 5- Precocious movement at Metaphase I; 6 - Bridge at Anaphase I; 7 - Laggard at Anaphase I; 8 - Unequal separation at Anaphase I.

Table 1. Meiotic abnormalities induced by Gamma rays in *Nigella sativa* L.

Treatment	Tt. No. of PMCs scored	Metaphase						Anaphase					T. Ab (%)	Pollen fertility (%)
		Mv	St	Pm	Un	Uni	Sec	Br	Lg	St	Us	Dis		
Control	396	-	-	-	-	-	-	-	-	-	-	-	-	98.4
50 Gy	348	1.83	-	0.18	-	-	-	-	1.12	-	-	0.45	3.58	95.8
100 Gy	342	2.48	0.28	0.56	-	-	0.38	0.48	1.87	-	-	0.78	6.83	92.6
150 Gy	312	3.12	0.42	0.87	0.23	-	0.67	1.23	2.21	0.24	0.36	2.41	11.76	88.7
200 Gy	352	3.62	0.96	1.23	0.64	0.20	0.72	1.74	2.79	0.68	0.78	2.85	16.21	83.5
250 Gy	386	3.98	1.12	1.72	0.89	0.36	0.92	2.18	3.12	0.94	1.38	3.26	19.87	79.2
300 Gy	248	4.45	1.42	2.26	1.34	0.71	1.09	2.75	3.72	1.38	1.93	3.54	24.59	75.9

Abbreviations: PMCs-Pollen mother cells, Mv-Multivalent formation, St-Stickiness, Pm-Precocious movement, Un-unorientation, Uni-Univalents, Sec- Secondary association, Br-Bridge, Lg-Laggard, Us-Unequal separation, Dis-Disturbed polarity

Table 2. Meiotic abnormalities induced by 0.5 % EMS in *Nigella sativa* L.

Treatment	Tt. No. of PMCs scored	Metaphase					Anaphase					T. Ab (%)	Pollen fertility (%)
		Mv	St	Pm	Un	Sec	Br	Lg	St	Us	Dis		
Control	382	-	-	-	-	-	-	-	-	-	-	-	98.8
3 hr	345	0.64	1.49	0.49	0.48	0.25	1.32	0.79	0.60	-	0.86	7.12	97.6
5 hr	412	0.83	1.86	0.67	0.65	0.42	2.13	0.92	1.23	0.23	0.98	9.94	90.2
7 hr	321	0.98	2.13	0.86	0.82	0.69	3.76	1.12	2.14	0.49	1.20	14.19	88.5

Abbreviations: PMCs-Pollen mother cells, Mv-Multivalent formation, St-Stickiness, Pm-Precocious movement, Un-unorientation, Sec-Secondary association, Br-Bridge, Lg-Laggard, Us-Unequal separation, Dis-Disturbed polarity

abnormalities displayed an increasing trend along with the increasing doses of treatment (Table 1). At the lowest dose *i.e.* 50 Gy the Total abnormality percentage (T.Ab %) was 3.58 % whereas at the highest dose of 300 Gy the T. Ab % was found to be 24.59 %. The most dominant abnormality was multivalent (4.45 %) followed by laggards (3.72 %). Univalents, which were not found at lower doses, were evident at higher doses (150-300 Gy). Pollen fertility was found to decrease from 98.4 % in control to 75.9 % at 300 Gy dose.

In the EMS treatment (Table 2), the Total percentage of abnormal cells at 3 hr dose was 7.12 % while at 7 hr dose it was 14.19 %. Stickiness (2.13 % at metaphase and 2.14 % at anaphase) was the most dominant abnormality followed by bridge (3.76 %). Pollen fertility was found to vary from 98.8 % in control to 88.5% in 7 hr

Table 3. Meiotic anomalies induced by combined treatment of Gamma rays and EMS in *Nigella sativa* L.

Treatment	Tt. No. of PMCs scored	Metaphase					Anaphase					T. Ab (%)	Pollen fertility (%)
		Mv	St	Pm	Un	Sec	Br	Lg	St	Us	Dis		
Control	386	-	-	-	-	-	-	-	-	-	-	-	97.6
50 Gy +0.5 %EMS	540	0.24	0.63	0.89	-	-	-	0.49	0.74	-	0.58	3.50	92.3
100 Gy +0.5%EMS	372	0.42	0.94	1.56	0.32	-	0.46	0.72	1.14	-	0.82	5.56	88.7
150 Gy +0.5%EMS	326	0.86	1.78	2.63	0.86	0.42	0.98	1.34	2.18	0.31	1.58	11.36	80.2
200 Gy +0.5%EMS	412	1.37	2.31	3.18	1.44	0.87	1.23	1.92	2.89	0.68	1.98	17.87	73.5
250 Gy +0.5%EMS	470	2.68	3.12	4.23	1.88	1.24	1.78	2.21	3.26	1.20	2.74	27.02	68.4
300 Gy +0.5%EMS	386	2.84	4.35	5.27	2.42	1.83	2.19	3.22	4.17	1.71	3.82	31.82	60.9

Abbreviations: PMCs-Pollen mother cells, Mv-Multivalent formation, St-Stickiness, Pm-Precocious movement, Un-unorientation, Uni-Univalents, Sec- Secondary association, Br-Bridge, Lg-Laggard, Us-Unequal separation, Dis-Disturbed polarity

dose.

In the combined treatment of gamma rays and EMS (Table 3), the T.Ab % was found to increase from 3.50 % at 50 Gy + 0.5 % EMS dose to 31.82 % at 300 Gy + 0.5 % EMS dose. In this case stickiness was also predominant (4.35 % at metaphase and 4.17 % at anaphase) followed by precocious movement (5.27 %). Pollen fertility decreased from 97.6 % in control to 60.9 % at 300 Gy + 0.5 % EMS dose. In all the three treatment sets *i.e.* Gamma rays, EMS and their combination, the combination treatment registered a maximum abnormality of 31.82 % at 300 Gy+ 0.5 % EMS dose followed by 24.59 % abnormality at 300 Gy Gamma rays and 14.19 % abnormality at 7 hr dose of EMS. Pollen fertility was also affected much in case of 300 Gy + 0.5 % EMS dose (60.9 %) as compared to 75.9 % and 88.5 % in case of 300 Gy Gamma rays and 7 hr EMS dose, respectively. This indicates that the combination treatment is the most genotoxic. In both, the combination treatment as well as EMS, stickiness was the predominant abnormality while in the Gamma rays treated set multivalent was predominantly observed. Univalents were observed only at higher doses of gamma rays *i.e.* 150 - 300 Gy.

Discussion

As a result of individual and combined mutagenic treatment of seeds with gamma rays and EMS, the plants showed varying degree of meiotic irregularities. Rao and Laxmi[9] attributed univalent formation to the partial and complete lack of homologous chromosomal pairing. Multivalent formations have been reported in various plants like barley[10], tomato[11] etc. According to Katiyar[12], alteration in the chromosome associations comprising of uni, tri, tetra and multivalents were possibly the outcome of non-or irregular pairing of chromosomes due to translocations.

Precocious movement and unorientation of chromosomes at metaphase I seem to be a manifestation of improper functioning of the spindle. Secondary association of chromosomes in any diploid species has been interpreted as a result of modified chromosome arrangement due to duplication, interchanges or stickiness[13,14]. Stickiness of chromosomes was the most common abnormality observed in case of EMS and combined treatments. Jayabalan and Rao[15] reported stickiness due to disturbances in the cytochemically-balanced reactions. Gaulden[16] attributes chemically induced stickiness to direct action of mutagen on the histone proteins leading to improper folding of DNA. Saylor and Smith[17] suggested that the formation of bridges can be due to the failure of chiasmata in a bivalent to terminalize and the chromosomes get stretched between

poles. According to Sinha and Godward[18], the presence of bridges with or without fragments both at anaphase I and II could be interpreted as due to the paracentric inversions.

Laggards at anaphase can be attributed to the delayed terminalization or perhaps to stickiness of chromosome ends. Unorientation of chromosomes at metaphase I sometimes lead to unequal separation at anaphase I[19]. Pollen sterility was higher in combination treatments than the single mutagenic treatment. Chary and Bhalla[20] also reported an increase in pollen sterility with an increase in mutagenic treatment.

As more and more abnormalities accumulate the process of gamete formation is affected and it leads to non-viable gametes that considerably reduce the plant fertility as well as the yield. Our investigation clearly reveals that as far as chromosome associated genetic changes are concerned, combined treatment was more effective than the individual ones. The production of cytological variations in *Nigella sativa* L. due to the combined treatment may be considered as indicators to their corresponding mutagenic efficiency at the first instance that may lead to production of greater phenotypic variations, although not necessarily in terms of viable mutations.

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References

1. Rao G M and Siddiq E A 1977, Induced variation for yield and its components in rice. *Indian J. Genet.* **37** 12-21.
2. Rao G M and Rao V M 1983, Mutagenic efficiency, effectiveness and factor of effectiveness of physical and chemical mutagens in rice. *Cytologia* **48** 427-436.
3. Kumar S and Dubey D K 1998, Mutagenic efficiency and effectiveness of separate and combined treatments with Gamma rays, EMS and DES in Khesari (*Lathyrus sativus* L.). *J. Indian Bot. Soc.* 77 1-4.
4. Dhamyanthi K P M and Reddy V R K 2000, Cytogenetic effects of Gamma rays and Ethyl Methane Sulphonate in Chilli pepper (*Capsicum annuum* L.). *Cytologia* **65** 129-133.
5. Siddiq E A 1973, Cytogenetical effects of physical and chemical mutagens on rice. *Indian J. Genet.* **37** 162-171.
6. Tarar J L and Dhyansagar V R 1980. Comparison of Ethyl Methane Sulphonate and Radiation induced meiotic abnormalities in *Turnera umlifolia* Linn. Var. *augustifolia* Willd. *Cytologia* **45** 221-231.
7. Zeerak N A 1991, Cytogenetical effect of Gamma Rays and Ethyl Methane Sulphonate in Brinjal (*Solanum melongena* L.). *Cytologia* **56** 639-643.
8. Reddy V R K and Annadurai M 1992, Cytological effects of different mutagens in Lentil (*Lens culinaris* Medik). *Cytologia* **57** 213-216..
9. Rao N B and Laxmi N 1980, Gamma ray induced meiotic abnormalities in *Capsicum annuum* L. *Caryologia* **33** 509-518.
10. Burnham C R, White F H and Livers R 1954, Chromosomal interchanges in barley. *Cytologia* **19** 191-202.
11. Gill B S, Burnham C R and Stringam G R 1980, Cytogenetic analysis of chromosomal translocation in tomato. *Can. J. Genet. Cytol.* **22** 333-341.
12. Katiyar R B 1978, Radiocytogenetical studies on *Capsicum* Meiotic anomalies. *Cytologia* **43** 415-421.
13. Stebbins G L 1950, *Variation and evolution in plants.* Columbia Univ. Press, New York.
14. Darlington C D 1928, Studies in Prunus I and II. *J. Genet.* **19** 215-221.
15. Jayabalan N and Rao G R 1987, Gamma radiation induced cytological abnormalities in *Lycopersicon esculentum* Mill. var. Pusa Ruby. *Cytologia* **52** 1-4.
16. Gaulden M E 1987, Hypothesis: some mutagens directly alter specific chromosomal proteins to produce chromosomal stickiness. *Mutagenesis* **2** 357-365.
17. Saylor L G and Smith B N 1966, Meiotic irregularities in species of interspecific hybrids in *Pisum. Am. J. Bot.* **53** 453-468.
18. Sinha S S N and Godward M B E 1972, Radiation studies in *Lens culinaris. Indian J. Genet.* **32** 331-339.
19. Khan I A 1996, Meiotic irregularities induced by DES in chilli pepper (*Capsicum annuum* L.) var. NP46A. *Prog. Hort.* **28** 36-40.
20. Chary S N and Bhalla J K 1985, Mutagenic effectiveness and efficiency of gamma rays and EMS on pigeon pea *Cajanus cajan* L. Mill sp. *J. Cytol. Genet.* **23** 174-182.