COMPARATIVE RADIOCYTOLOGICAL EFFECT OF GAMMA RAYS AND LASER RAYS ON SAFFLOWER

G. KUMAR, P. SRIVASTAVA[1]

In the present study we investigated the comparative genotoxic potential of laser rays and gamma rays on somatic and gametic cells of safflower (*Carthamus tinctorius* L.). Mutagenic parameters like mitotic and meiotic consequences were accessed from the plants that showed a linear relationship between dose absorbed and chromosomal anomalies. A wide spectrum of chromosomal aberrations was encountered in both the laser and gamma ray irradiated set but the most frequent anomaly dominated was the stickiness of chromosomes. Some noble cytological mutants were also isolated like chain and multivalent formation at metaphase and cytoplasmic bridge formation at anaphase. The percentage of chromosomal aberrations observed in case of gamma rays treated set was higher than laser rays treated sets suggesting that gamma rays could be successfully employed for creating additional genetic variability in safflower.

Key words: Safflower, gamma rays, laser rays, genotoxic potential, chromosomal anomalies.

INTRODUCTION

Although induced mutations have great relevance for raising superior plant types in different crop plants, most mutations are lethal or semi-lethal and do not have any practical value possibly due to doses monitored or mutagens employed. Thus to administer successful mutagenesis, selection of efficient mutagen and treatment is a pre-requisite as mutagens are the potent tools for bringing direct improvement and certain qualitative and quantitative changes in crop plants (Paul and Datta, 2006). Furthermore, the conventional method of breeding has not been effective in boosting the per hectare yield of either oil or seed (Khadeer and Anwar, 1991) therefore mutagenesis has come up with a hope as an efficient tool for creating genetic variability and boosting up of characters under consideration.

Cytological analysis with respect to both mitotic and meiotic behavior is one of the most dependable indices to estimate the potency of mutagen (Bhat *et al.*, 2007). Present investigation documents mutagenic effectiveness of gamma rays and laser on safflower chromosome biology, which is considered as a traditional crop of arid and semi-arid parts of India, Iran, Egypt, Pakistan and other Mediterranean countries. It belonged to the family Compositae (Oad *et al*., 2002, Tunkturk and Ciftici, 2004). It is an annual broad leaf, oilseed crop adapted chiefly

[1] Department of Botany, Plant Genetics Laboratory, University of Allahabad, Allahabad-211002, U.P., India. Address of corresponding author: E-mail: prisri.aks1985@gmail.com

to small grain production area (Oelke *et al*., 1990). Today this crop supplies oilmeal, birdseed and foots for food and industrial products markets, although this crop is now primarily grown for oil (Oelke *et al.*, 1990, Essendel, 2001). Since not much work on cytology of the plant has been done so far due to the poor staining capability and chromosome stickiness, the present work is an attempt to induce genetic variability which may be employed as an asset by the plant breeders and cytogeneticists for the improvement of safflower.

MATERIAL AND METHODS

Procurement of seeds. Seeds of safflower (*Carthamus tinctorius* L. var A1) were obtained from National Bureau of Plant Genetic Resources (I.A.R.I.), New Delhi.

Treatment of seeds. The healthy seeds of safflower were treated with gamma rays (15 KR, 30 KR, 45 KR, 60 KR and 80 KR) through ^{60}Co source (at the dose rate of 1.8 KR/sec) done at National Botanical Research Institute (N.B.R.I., Lucknow, India) and with laser rays (½ h, 1h and 1½ h) at the wavelength of 635 nm with helium-neon laser (especially prepared for irradiation of biological materials) in Physics Department, University of Allahabad. The light power of the laser was 24 mW and the intensity of light falling on the seeds was about 1 mW cm^{-2}.

Cytological studies. Both these treatments were done to create genetic variability for selection of desirable genotypes. After each treatment the irradiated seeds were sown in experimental pots along with their suitable control counterparts for meiotic 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. Mitotic index and frequency of cytological abnormalities from dividing cells were estimated. For the meiotic studies, floral buds of appropriate size were fixed in carnoy's fixative preserved in 70 % alcohol and stained in 2 % acetocarmine. Photomicrographs were taken and analyzed using Nikon image capturing system.

RESULTS

Mitotic Analysis In safflower, the somatic complement consists of 24 chromosomes (2n = 24). Mitotic index was recorded to be 12.72 % in control set with no chromosomal anomalies (Figures 1, 2). However, a gradual reduction in mitotic index (from 12.72 % in control to 6.15 % at 1½ h in case of laser rays treated set and 5.36 % at 45 KR in case of gamma ray treated set) was recorded. Treatments with both the mutagens not only reduced the frequency of dividing

cells but a wide spectrum of chromosomal abnormalities was also recorded. The individual abnormalities and the total abnormal cells increased along with the increment in the treatment doses. The maximum abnormality percentage was recorded to be 14.02 % at 45 KR of gamma rays treated set. The most frequent chromosomal aberrations encountered in both the treatment set was stickiness of chromosomes (Figs. 3, 4) and rest of the percentage has been shared between scattering, unorientation, bridge formation (Fig. 5), laggards and micronuclei at anaphase (Figure 6) as presented in Table 1.

Table 1

Comparative account of mitotic indices and chromosomal anomalies in laser and gamma rays irradiated set

Treatments	No. of cells analyzed	No. of cells with type of abnormalities							T Ab (%)	MI (%)
		Metaphase			Anaphase		Telophase			
		Sc	St	Un	Sgbr	St	Br	Lg		
Control	500	-	-	-	-	-	-	-	-	12.72
Laser ray										
½ h	465	0.75	0.51	0.60	0.52	0.45	0.42	0.65	3.90	11.15
1h	422	2.02	1.87	0.95	1.12	1.32	0.99	0.61	8.88	8.55
1½ h	323	2.80	2.05	1.98	1.30	1.64	1.19	0.77	11.73	6.15
Gamma ray										
150Gy	560	0.98	1.12	1.00.	0.75	1.11	0.32	-	5.28	10.22
300Gy	428	1.88	2.25	1.33	1.00	1.63	1.00	0.98	10.07	8.01
450Gy	400	2.32	3.41	1.72	2.02	2.25	1.28	1.12	14.02	5.36
600Gy	-	**MITOTIC INHIBITION**								
800Gy	-	**MITOTIC INHIBITION**								

Abbreviations: Sc – scattering, St – stickiness, Un – unorientation, SgBr – single bridge, DbBr – double bridge, Br – bridge, Lg – laggard, T Ab% – total abnormality %, MI – mitotic Index

Meiotic Analysis. Evaluation of PMCs revealed perfectly normal meiosis in control plants with nearly 12 bivalents at diakinesis and metaphase I (Fig. 7) and 12:12 separation at anaphase (Fig. 8). The plants in both treatment sets displayed varying degrees of chromosomal anomalies distributed in all phases of division. A dose-based increase in meiotic abnormalities was observed in both the laser and gamma rays treatment sets. Stickiness being the most frequent anomaly was found to be associated with other anomalies like stickiness at metaphase II with unorientation and chain formation (Fig. 9), multivalent formation (Fig. 10), stickiness at anaphase II with laggard and forward movement as simultaneously in

same PMC as shown by arrow (Fig. 11). A considerable amount of cytoplasmic bridge formation (Fig. 12) was also reported along with other anomalies like tripolarity, secondary association, etc. as presented in Table 2.

Table 2

Effect of laser rays and gamma rays on meiosis and total stickiness percentage in safflower

Treatments	Total PMCs Scored	Number of cells with type of Abnormalities										T St %	T Ab %
		Metaphase (I/II)					Anaphase (I/II)						
		St	Pr	Ns	Cl	Oth	St	Br	Lg	Tp	Oth		
Laser ray													
Control	252	-	-	-	-	-	-	-	-	-	-	-	-
½ h	275	2.65	1.85	2.11	0.82	1.25	3.02	1.30	1.18	0.92	0.48	21.0	16.58
1h	310	4.00	-	3.18	2.11	-	4.22	2.42	-	1.72	0.92	23.6	19.29
1½ h	282	7.28	2.01	3.70	2.68	2.21	6.22	3.62	0.84	-	-	25.9	25.03
Gamma ray													
Control	246	-	-	-	-	-	-	-	-	-	-	-	-
150Gy	268	2.91	1.52	1.12	1.38	1.01	3.67	1.03	0.80	0.52	0.58	22.4	13.54
300Gy	254	5.00	2.40	1.01	2.06	0.82	3.35	2.50	1.85	-	1.81	25.6	20.70
450Gy	237	7.75	2.73	1.48	2.94	1.24	5.33	1.88	-	0.98	-	30.6	28.04
600Gy													
800Gy													
					NO	**GERMINATION**							
					NO	**GERMINATION**							

Abbreviations: St – stickiness, Pr – precocious movement, Ns – non synchronous division, Cl – clumping, Oth – Other Abnormalities, Br – bridge, Lg – laggard, Tp – tripolarity, T.St. % – total stickiness, T.Ab% – total abnormality

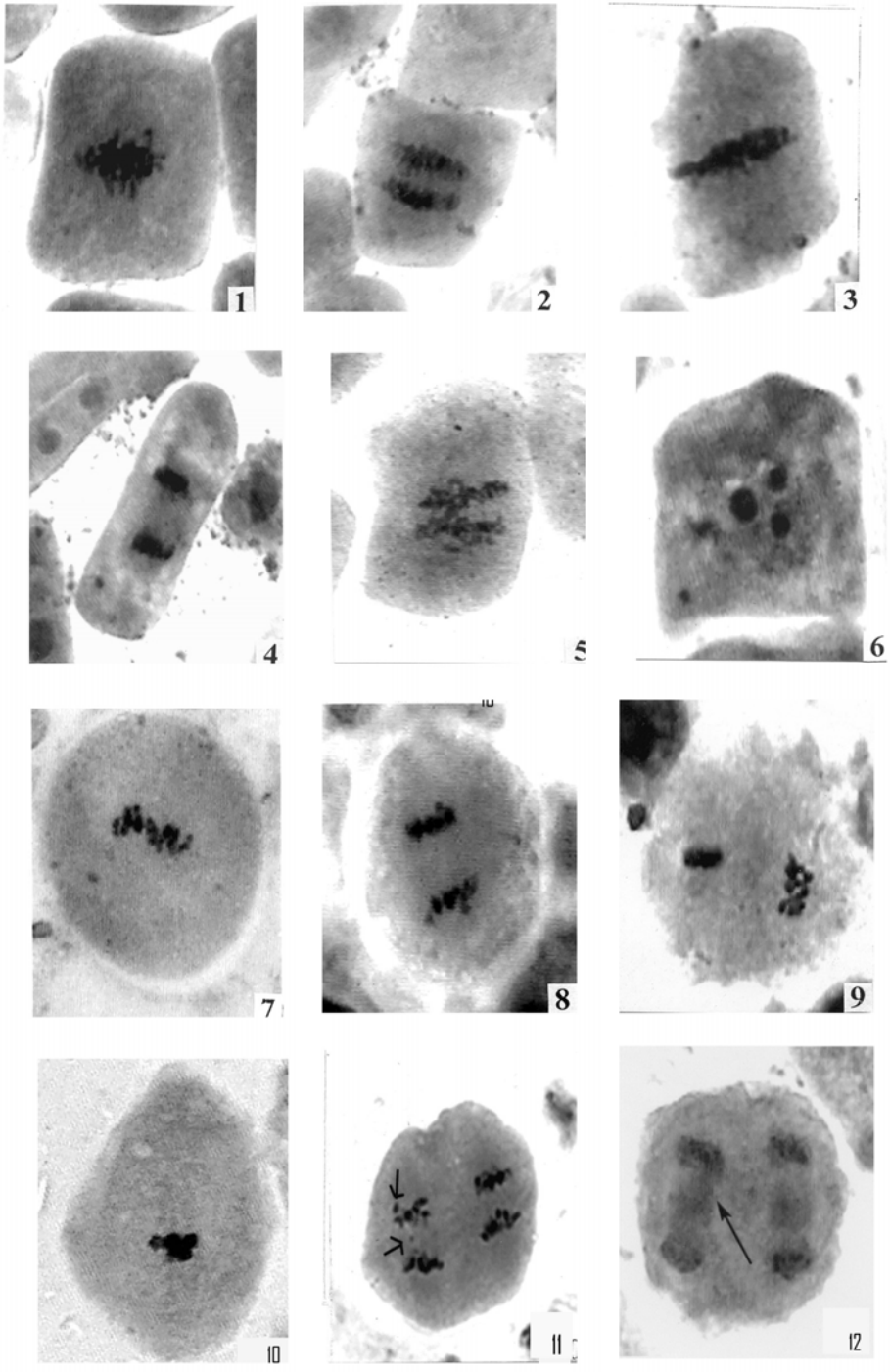
The most prominent abnormality induced was stickiness at metaphase (I/II) and at anaphase (I/II). The range of stickiness was dose dependant. It was recorded to be the highest 25.90 % in case of 1½ h dose of laser ray treated set and 30.60 % in case of 45 KR of gamma ray treated set. However, total abnormality percentage varied from 16.58 %–25.03 % in laser rays treated set and 13.54 %–28.04 % in case of gamma rays treated set. From the aforesaid results it becomes clear that gamma rays elicited a much greater genotoxic response than the laser rays.

Mitotic cells

Fig. 1 – Normal Metaphase. Fig. 2 – Normal Anaphase. Fig. 3 – Stickiness at metaphase. Fig. 4 – Stickiness at anaphase. Fig. 5 – Single lateral bridge at anaphase. Fig. 6 – Micronuclei

Meiotic cells

Fig. 7 – Normal metaphase I. Fig. 8 – Normal Anaphase I. Fig. 9 – Stickiness & unorientation at metaphase II with chain formation. Fig. 10 – Multivalent formation. Fig. 11 – Stickiness at anaphase II with laggard and forward movement. Fig. 12 – Cytoplasmic bridge formation at anaphase II.


1
2
3
4
5
6
7
8
9
10
11
12

DISCUSSION

In the present investigation both the mutagenic treatments exhibited similar types of aberrations but the percentage of anomalies differed showing that different mutagens have different mutagenic potential for safflower. Such induced chromosomal variations have been widely investigated from the point of view of understanding the mechanics of mutagen induced chromosomal damage and biological dosimetry in safflower. The induction of cytological disturbances in the mitotic as well as meiotic cells is of great value, as it results in genetic damage that is handed over to the next generation (Kumar and Rai, 2007).

A broad range of chromosomal aberrations were induced by both the treatments but higher proportion has been attributed to stickiness of chromosomes which 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). It may also arise due to improper clustering of chromosomes at any phase of cell cycle, which makes the chromatids connected by subchromatid bridges (Mc Gill *et al*., 1974). The behavior of laggard chromosome is characteristic in that they generally lead to micronuclei formation (Koduru and Rao, 1981, Kumar and Rai, 2006). Micronuclei also arise if laggard or non-oriented chromosomes fail to reach the poles in time to be in main telophase nucleus (Utsunomiya *et al*., 2002). Bridges reported might have arisen through breaks in two chromosomes followed by union of the centric fragments (Shreekrishna, 2006) or due to stickiness of chromosome at metaphase and their failure to separate at anaphase or due to breakage and reunion of chromosome (Badr, 1988, Grant, 1978). Pagliarini (1990) reported that laggards might result from late chiasma terminalization. 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).

CONCLUSION

Mutation breeding offers great prospects for crop improvement by incorporation of various micro- and macromutations (Kumar *et al.*, 2003), but before any mutagen is selected for extensive usage, a preliminary screening of the potential mutagen should be done on mitotic cells before carrying its study generation wise. Thus, from our study it can be concluded that it is worthwhile to use gamma rays for creating additional genetic variability in safflower which could be favorably exploited by plant geneticists and breeders.

Acknowledgements. The authors are grateful to NBPGR (I.A.R.I.), New Delhi, for providing inbred seeds of safflower, and the National Botanical Research Institute (NBRI), Lucknow, for providing gamma irradiation facility and Department of Physics, University of Allahabad for doing laser irradiation. Sincere thanks to all the members of the Plant Genetics Laboratory for their encouragement and support. Thanks are also to the Head of the Department of Botany, University of Allahabad, for providing necessary facilities.

REFERENCES

1. Badr, A., 1988, Cytogenetic activities of some Fungicides. *Cytologia* **53**: pp. 635-640.
2. Bhat, T.A., Sharma, M., Anis, M., 2007, Comparative analysis of mitotic aberrations induced by diethyl sulphonate (DES) and sodium azide (SA) in *Vicia faba* L. (Fabaceae). *Asian Journal of Plant Sciences*, **6(7)**, pp. 1051-1057.
3. Esendal, E., 2001, Safflower production and research in Turkey. *5th* Intl. Safflower Conf. Williston North Dakota and Sydney, Montana U.S.A.
4. Grant, W.F., 1978, Chromosomal aberrations in plants as monitoring system. *Environment Health Perspective*, **27**: pp. 27-43.
5. Khadeer, M.A., Anwar, S.Y., 1991, Induction and characterization of seed mutants for oil and fatty acid composition in safflower. *Cytologia*, **56**, pp. 517-522.
6. Koduru, P.R.K. and Rao, M.K., 1981, Cytogenetics of synaptic mutants in higher plants. *Theoretical Applied Genetics*, **59**, pp. 197-214.
7. Kumar, G. and Rai, P., 2007, Comparative genotoxic potential of mercury and cadmium in soybean. *Turkish Journal of Biology*, **31**, pp. 13-18.
8. Kumar, G., and Rai, P., 2006, Partial genome elimination through micronuclei in soybean (*Glycine max*). *National Academy of Science Letters*, **29**, pp. 417-421.
9. Kumar, G., Kesarwani, S.and Sharma, V., 2003, Clastogenic effect of individual and combined treatment of Gamma rays and EMS in *Lens culinary. Journal of Cytology and Genetics*, **4**, pp. 149-154.
10. McGill, M., Pathak, S. and Hsu, Tc., 1974, Effects of Ethidium Bromide on Mitosis and chromosomes. A possible material basis for chromosome stickiness. *Chromosoma*, **47**, pp.157-167.
11. Oad, F.C., Samo, M.A., Quayyum, S.M., Oad, N.L., 2002, Inter and intra row spacing effect on the growth, seed yield and oil content of safflower. *Asian Journal of Plant Sciences*, **1**, pp. 18-19.
12. Oelke, E.A., Oplinger, T.M., Teynor, D.M., Putnam, D.H., Doll, J.D., Kelling, K.A., Durgan, B.R., and Noetzal, D.M., 1990, Safflower alternative field crop. Manual Ext. Univ. of Minnesota center of alternative plant and animal products and Minnesata ext.
13. Pagliarini, M.S., 1990, Meiotic behaviour and pollen fertility in *Aptenia cordifolia*. Aizoaceae. *Carylologia,* **43**, pp. 157-162.
14. Paul, R., Datta, A.K., 2006, Mutagenic efficiency and effectiveness of Gamma rays and EMS in some seed spices. *Journal of Cytology and Genetics*, **7**, pp. 91-99.
15. Shreekrishna, V., 2006, Cytological Abnormalities in *Amaranthus paniculatus* treated with Ethyl Methyl sulphonate. *Journal of Cytology and Genetics*, **7**, pp. 101-104.
16. Tunkturk, M., Ciftici, V., 2004, Relationships among traits using correlation and path coefficient analysis in safflower (*Carthamus tinctorius* L.) sown in different fertilization levels and row spacing. *Asian Journal of Plant Sciences*, **3**, pp. 683-686.
17. Utsunomiya, K.S., Bione, N.C.P., and Pagliarini, M.S., 2002, How many different kinds of abnormalities could be found in unique endogamous maize plant? *Cytologia*, **67**, pp. 69-176, 2002.