OPEN ACCESS Asian Journal of Biological Sciences

ISSN 1996-3351
DOI: 10.3923/ajbs.2019.

Research Article
Frequency and Spectrum of Chlorophyll Mutations Induced by Single and Combination Treatments of Gamma Rays and EMS in Urdbean

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Abstract

Background and Objective: Chlorophyll mutation frequency is useful in assessing the potency of a mutagen. Hence, scoring of chlorophyll mutations are dependable indices for evaluating the genetic effects of mutagenic treatments. Present investigation was undertaken to understand the response of urdbean varieties T-9 and Pant U-30 to individual and combined treatments of gamma rays and EMS for identifying such mutagenic treatments causing maximum chlorophyll mutations which could be exploited for inducing beneficial viable mutations in subsequent generations. **Materials and Methods:** Seeds of two urdbean varieties were irradiated with 100, 200, 300 and 400 Gy doses of gamma rays. For chemical treatments, seeds were treated with 0.1-0.4% of EMS for 6 h and for combination treatments, dry seeds of each variety were first irradiated with gamma rays at 200 and 300 Gy followed by the treatment with 0.2 and 0.3% of EMS. The mutagen treated seeds were sown in complete randomized block design to raise M_1 generation. The M_1 seeds were sown in next season in plant progeny rows for raising M_2 generation. Chlorophyll mutations were observed when seedlings were 8-15 days old in M_2 generation. They were identified and classified according to Gustafson's method. **Results:** Six kinds of chlorophyll mutants viz., albina, chlorina, maculata, xantha, virescent and viridis were recorded in M_2 generation when seedlings were 7-15 days old. Out of the six chlorophyll mutation types, albina, chlorina and xantha survived upto 7-15 days only, while the other three types were viable and set seeds. Gamma rays alone as well as in combination with EMS produced large number of albina mutations, while EMS alone induced the maximum number of chlorina mutations in both the varieties. Frequency of chlorophyll mutations was dose dependent and increased with increasing dose of each mutagen. The combined treatments of gamma rays+EMS produced higher frequency of chlorophyll mutations as compared to their individual treatments. **Conclusion:** Chlorophyll mutations could be exploited for identifying the threshold dose of a mutagen that would increase the probability of retrieving economically useful mutations in ensuing generations.

Key words: Chlorophyll mutations, gamma rays, EMS, combination treatments, urdbean

Received: Accepted: Published:

Citation: Sonu Goyal, Mohammad Rafiq Wani and Samiullah Khan, 2019. Frequency and spectrum of chlorophyll mutations induced by single and combination treatments of gamma rays and EMS in Urdbean. Asian J. Biol. Sci., CC: CC-CC.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Enhancement of mutation frequency and alteration of mutation spectrum in a predictable manner are the two important aspects of mutation research. In the past, varied approaches have been tried to achieve these goals[1-2]. To ensure a speedy generation of variability for a specific trait to be improved, a mutation breeder has to go through all basic events met in the methodology to ensure reliable information about the mutagenic sensitivity of biological material and the extent of effectiveness and efficiency of a mutagen in question. Mutagens vary in their mode of action, effectiveness, efficiency and the spectrum of mutations induced. Similarly, genotypes show differential sensitivity towards mutagens even at varietal level[3-4]. Because of their easy identification, chlorophyll mutations form a reliable index in the evaluation of mutagenic effects and estimation of mutational events. From breeders' point of view, the frequency of chlorophyll mutants expressed as percent of M_2 population seems to be more realistic and helpful. Therefore, results were explained on M_2 plant basis in the present investigation.

Scoring of chlorophyll mutations in M_2 generation has proved to be the most dependable index for evaluating the genetic effects of mutagenic treatments. Several authors have reported the occurrence of different types of chlorophyll mutations such as albina, xantha, chlorina, viridis, virescent, tigrina, etc., in M_2 generation following treatments with various mutagenic agents[5-10]. Ionizing radiations generally produce a higher proportion of albina mutations than chemical mutagens[11-15]. Chemical mutagens induce higher frequency of chlorophyll mutations than radiations as observed in mungbean[16], chickpea[17], pea[18], lentil[19], grasspea[20] and soybean[15]. In recent years, much emphasis has been laid on the use of combination treatments of physical and chemical mutagens. Chemical mutagens are not only mutagenic themselves but also affect mutation in specific ways when combined with radiation[21].

Urdbean (*Vigna mungo* (L.) Hepper) possesses low genetic variability due to cleistogamous nature of its flower. The present investigation was undertaken with the aim to study the individual and combined effects of gamma rays and EMS on the frequency and spectrum of chlorophyll mutations in M_2 generation of urdbean varieties T-9 and Pant U-30 which could be utilized as markers for retrieving certain economically useful mutants in subsequent generations.

MATERIALS AND METHODS

Two varieties of urdbean (*Vigna mungo* (L.) Hepper) namely T-9 and Pant U-30 were used in the present study. Seeds of both the varieties were obtained from G.B. Pant University of Agriculture and Technology, Pantnagar, Uttaranchal, India. Both varieties were well adapted to agro-climatic conditions of Uttar Pradesh including the site of study. Dry seeds of each variety with moisture content 12%, were irradiated with 100, 200, 300 and 400 Gy doses of gamma rays from a ^{60}CO source at the National Botanical Research Institute, Lucknow, Uttar Pradesh, India. For chemical treatments, healthy seeds of uniform size of each variety were presoaked for 9 h in distilled water and treated with 0.1, 0.2, 0.3 and 0.4% of EMS for 6 h with intermittent shaking at room temperature of 25±1°C. The solution of EMS was prepared in the phosphate buffer of pH 7. After treatment the seeds were thoroughly washed in running tap water to remove the excess of mutagen.

For combination treatments, dry seeds of each variety were first irradiated with gamma rays at 200 and 300 Gy doses and then treated with 0.2 and 0.3% of EMS. (i.e., 200 Gy+0.2% EMS, 300 Gy+0.2% EMS, 200 Gy+0.3% EMS and 300 Gy+0.3% EMS). The procedure adopted was similar to that for the individual treatment. For each variety, 350 pre-soaked seeds were again soaked in phosphate buffer for 6 h to serve as controls. Three replications of 100-seeds each were sown for every treatment and control in each variety in a randomized complete block design (RCBD) at the Agriculture Farm, Aligarh Muslim University, Aligarh, India. The spacing was maintained at 30 cm (seed to seed in a row) and 60 cm (between the rows) in the field. The experiment was conducted during zaid (summer) season of 2008. Recommended agronomic practices were employed for preparation of field, sowing and subsequent management of the population of urdbean. About 25 healthy seeds from each normal looking M_1 plant of all different treatments with their respective controls in both varieties were planted in plant progeny rows in M_2 generation, during summer season of 2009. Different treatments and controls comprised of 50 progenies. Chlorophyll mutations were scored when seedlings were 7-15 days old. They were identified and classified according to Gustafsson[22]. The frequency of chlorophyll mutations was calculated by the following equation:

$$\text{Mutation frequency (\%)} = \frac{\text{Number of mutant seedlings}}{\text{Total number of } M_2 \text{ seedlings}} \times 100$$

The effect of combined treatments on chlorophyll mutations frequency was analyzed following the method of Sharma[23]:

$$\text{Coefficient of interaction (k)} = \frac{(a+b)}{(a)+(b)}$$

Where:

(a+b) = Mutation frequency induced by the two mutagens in combination treatments

(a)+(b) = Mutation frequencies induced by the two mutagens when applied alone

k = Hypothetical interaction coefficient

The value of 'k' should be one, if the interaction is additive. Any deviation from this value would show synergistic or less than additive effects.

RESULTS

Different chlorophyll mutations were scored in M_2 generation when seedlings were 7-15 days old. These chlorophyll deficient mutants were grouped into lethal and non-lethal types. The lethal group included albina, chlorina and xantha, while maculata, virescent and viridis were non lethal in nature. The description of such chlorophyll mutations was given as under:

Albina : White leaves of seedlings with relatively smaller size as compared to normal seedlings of the same age. Mutants survived for about a week

Chlorina : Yellowish green in colour, seedlings survived for about 15 days

Maculata : Seedlings showed whitish dots on their leaves. Plants were vigorous, late in maturity and produced few seeds

Xantha : Straw yellow colour seedlings with normal growth in the beginning but started withering after 10 days, died within 15 days

Virescent : Seedlings which became dark green at later stage of development were light green in early stages. They were as vigorous as the normal plants and set seeds

Viridis : Seedlings showed dark green colour of leaves. The plants were slow in growth and had low seed yield

Results on chlorophyll mutations in M_2 generation following individual and combination treatments of gamma rays and EMS showed that the chlorophyll mutation frequency increased with increasing doses of the mutagens (Table 1, 2; Fig. 1). Among individual mutagens, EMS was found to be more effective in inducing chlorophyll mutations than gamma rays. However, combination treatments of gamma rays+EMS overall yielded higher frequency of chlorophyll mutations as compared to their individual treatments in both the varieties of urdbean. Moreover, the varieties responded differently to mutagenic treatments vis-à-vis the frequency and induction of chlorophyll mutations. The var. Pant U-30 was more sensitive in producing more chlorophyll mutations than the var. T-9 (Table 3, Fig. 2). Both the varieties showed the predominance of chlorina type of chlorophyll mutations followed by albina types (Table 3). A perusal of Table 4 and Fig. 3 showed that gamma rays alone (1.17%) and combination of gamma rays+EMS (2.56%) produced large

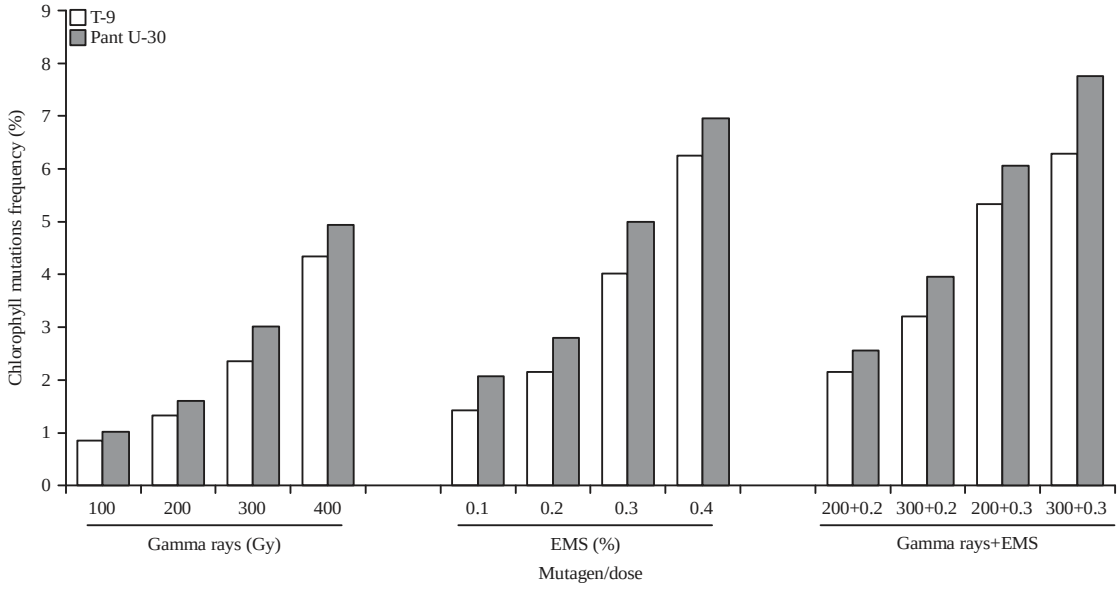

Fig. 1: Chlorophyll mutations frequency in two varieties of urdbean

Table 1: Frequency and spectrum of chlorophyll mutants induced by gamma rays, EMS and their combination in M_2 generation of urdbean var. T-9

Treatments	No. of M_1 plant progenies	No. of plant progenies segregating in M_2	Mutated plant progenies (Mp %)	No. of M_2 seedlings	Chlorophyll mutant types						Total	Frequency (%)	Synergism based on M_2 chl. mut. frequency (%)
					Albina	Chlorina	Maculata	Xantha	Virescent	Viridis			
Control													
Gamma rays	50	-	-	1196	-	-	-	-	-	-	-	-	-
100 Gy	50	1	2.00	1160	4	-	-	6	-	-	10	0.86	-
200 Gy	50	5	10.00	1121	3	2	3	-	5	2	15	1.34	-
300 Gy	50	6	12.00	1058	2	8	6	5	-	4	25	2.36	-
400 Gy	50	7	14.00	809	15	5	5	2	5	3	35	4.33	-
EMS (%)													
0.1	50	2	4.00	1169	2	7	3	-	5	-	17	1.45	-
0.2	50	8	16.00	1145	5	7	3	3	2	5	25	2.18	-
0.3	50	8	16.00	1093	5	12	-	8	10	9	44	4.02	-
0.4	50	10	20.00	815	6	16	11	9	5	4	51	6.26	-
Gamma rays+EMS (%)													
200 Gy+0.2	50	7	14.00	1150	4	7	3	5	2	4	25	2.17	0.62
300 Gy+0.2	50	10	20.00	1119	12	6	5	4	9	-	36	3.22	0.71
200 Gy+0.3	50	10	20.00	1032	16	8	14	5	3	9	55	5.33	0.99
300 Gy+0.3	50	11	22.00	779	14	12	3	10	1	9	49	6.29	0.98

Table 2: Frequency and spectrum of chlorophyll mutants induced by gamma rays, EMS and their combination in M_2 generation of urdbean var. Pant U-30

Treatments	No. of M_1 plant progenies	No. of plant progenies segregating in M_2	Mutated plant progenies (Mp %)	No. of M_2 seedlings	Chlorophyll mutant types						Total	Frequency (%)	Synergism based on M_2 chl. mut. frequency (%)
					Albina	Chlorina	Maculata	Xantha	Virescent	Viridis			
Control													
Gamma rays	50	-	-	1212	-	-	-	-	-	-	-	-	-
100 Gy	50	2	4.00	1179	3	2	1	-	3	3	12	1.02	-
200 Gy	50	6	12.00	1125	4	3	2	2	4	3	18	1.60	-
300 Gy	50	8	16.00	1100	4	10	6	7	-	6	33	3.00	-
400 Gy	50	10	20.00	850	14	8	6	3	6	5	42	4.94	-
EMS (%)													
0.1	50	3	6.00	1204	4	8	5	1	-	7	25	2.08	-
0.2	50	9	18.00	1133	6	9	4	5	3	5	32	2.82	-
0.3	50	10	20.00	1121	9	21	8	-	14	4	56	4.99	-
0.4	50	10	20.00	862	8	23	10	10	4	5	60	6.96	-
Gamma rays+EMS (%)													
200 Gy+0.2	50	7	14.00	1171	6	6	8	3	-	7	30	2.56	0.58
300 Gy+0.2	50	11	22.00	1112	12	9	5	8	10	-	44	3.96	0.68
200 Gy+0.3	50	11	22.00	1054	19	13	12	7	4	9	64	6.07	0.92
300 Gy+0.3	50	14	28.00	800	22	15	9	4	2	10	62	7.75	0.97

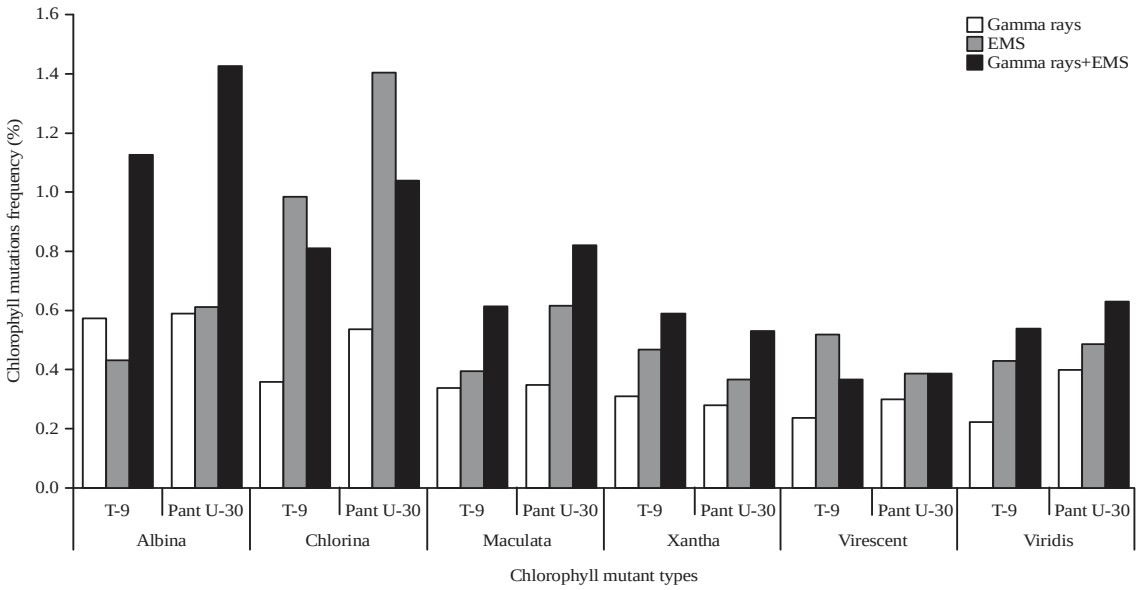

Fig. 2: Comparative chlorophyll mutations frequency and spectrum in two varieties of urdbean

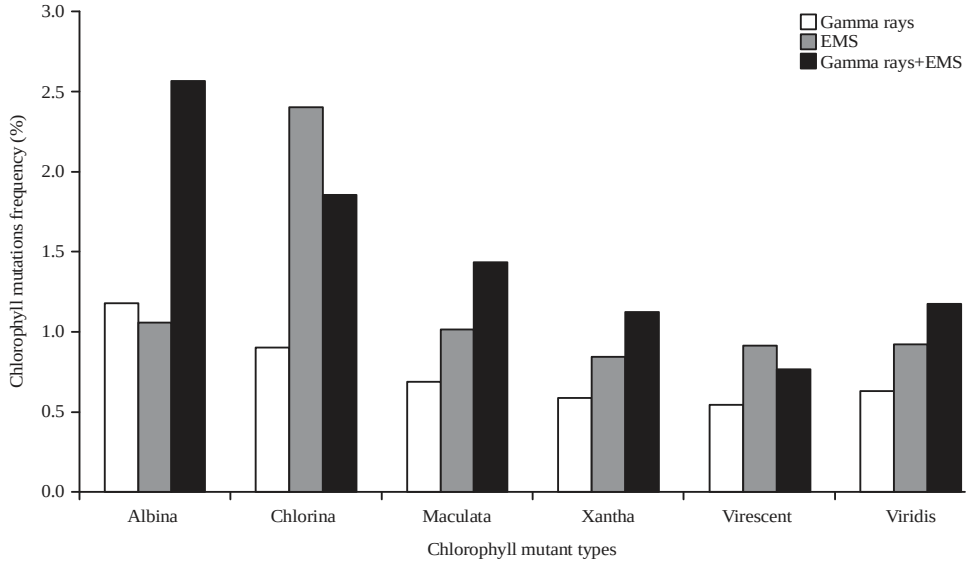

Fig. 3: Chlorophyll mutations frequency on mutagen basis in urdbean

Table 3: Mutagen induced chlorophyll mutation frequency and spectrum in two varieties of urdbean

	Comparative frequency (%) of chlorophyll mutation spectrum					
Mutagen*/variety	Albina	Chlorina	Maculata	Xantha	Virescent	Viridis
Var. T-9						
Gamma rays	0.58	0.36	0.34	0.31	0.24	0.22
EMS	0.43	0.99	0.40	0.47	0.52	0.43
Gamma rays+EMS	1.13	0.81	0.61	0.59	0.37	0.54
Total	2.14	2.16	1.35	1.37	1.13	1.19
Var. Pant U-30						
Gamma rays	0.59	0.54	0.35	0.28	0.30	0.40
EMS	0.62	1.41	0.62	0.37	0.39	0.49
Gamma rays+EMS	1.43	1.04	0.82	0.53	0.39	0.63
Total	2.64	2.99	1.79	1.18	1.08	1.52

*Data based on pooled values of four doses

Table 4: Comparative mutagen induced chlorophyll mutation spectrum in urdbean

	Comparative frequency (%) of chlorophyll mutation spectrum					
Mutagen*	Albina	Chlorina	Maculata	Xantha	Virescent	Viridis
Gamma rays	1.17	0.90	0.69	0.59	0.54	0.62
EMS	1.05	2.40	1.02	0.84	0.91	0.92
Gamma rays+EMS	2.56	1.85	1.43	1.12	0.76	1.17

*Data based on pooled values of two varieties T-9 and Pant U-30

number of albina mutations followed by chlorina (0.90 and 1.85%), maculata (0.69 and 1.43%), viridis (0.62 and 1.17%), xantha (0.59 and 1.12%) and virescent (0.54 and 0.76%), while EMS alone produced maximum number of chlorina (2.40%) types of chlorophyll mutations followed by albina (1.05%), maculata (1.02%), viridis (0.92%), virescent (0.91%) and xantha (0.84%).

DISCUSSION

Chlorophyll mutations represent reliable indices for evaluation of genetic effects of the mutagens. In the present study, the frequency of chlorophyll mutations recorded in M_2 generation was concomitant with the dose in both the varieties of urdbean. However, higher frequency of chlorophyll mutations with medium or lower doses of mutagens was also reported in chickpea[24] and *Cajanus cajan*[25]. Of the six types of chlorophyll mutants recorded in M_2 generation, chlorina followed by albina types were predominant in both the varieties. Occurrence of chlorina mutants had been attributed to different causes such as impaired chlorophyll biosynthesis, degradation of chlorophyll and deficiency of carotenoids[26]. The frequency of albina mutants was more in gamma rays alone as well as in combination with EMS, whereas EMS alone produced maximum number of chlorina mutants. Recovery of higher number of albina mutations from gamma rays singly and in combination with EMS was contrary to the contention of Arora and Kaul[27] who reported that gamma rays alone and in combination with EMS induced more number of chlorina mutants. Athwal *et al*.[28] in chickpea and Karthika and Subbalakshmi[15] in soybean reported that albina constituted the largest single category of mutants observed in gamma ray treated population. Kharkwal[17] obtained higher frequency of albina followed by chlorina and xantha mutants among the EMS treated population of chickpea. Gupta and Yashvir[12] in *Setaria italica*, Vanniarajan *et al*.[29] in *Vigna mungo* and Khan *et al*.[7] in *Cicer arietinum* reported that chemical mutagens produced high frequency of chlorina type of chlorophyll mutants. The frequency and spectrum of mutations recorded by Ignacimuthu and Babu[30] in the var. T-9 of urdbean subjected to similar EMS doses differ from the present results. Mutant types like maculata and virescent were not recovered in EMS treatments by these authors. The disparity could be due to altered treatment conditions since they used 6 hours presoaked T-9 seeds for mutagen treatment. Presoaking enhances metabolic activity and initiates DNA synthesis and its subsequent replication. Both G and S phases of the cell cycle were sensitive to mutagens[27]. Hence, both enhancements in frequency and types of mutations recovered after presoaking may be expected.

The combined gamma rays + EMS treatments produced highest frequency of chlorophyll mutations followed by EMS and gamma rays. These results were contrary to the findings of Arora and Kaul[27] who observed that gamma ray treatment was the most potent mutagen in inducing the highest chlorophyll mutations frequency followed by EMS and combined treatments of gamma rays and EMS in *Pisum sativum*. The greater effectiveness of combined treatments in inducing chlorophyll mutations has been reported by many workers[31-33]. Individual treatments of EMS induced comparatively higher frequency of chlorophyll mutations than gamma rays. The EMS is supposed to be specific to certain chromosomal regions[34] containing genes for chlorophyll development and has been reported to induce high frequency of chlorophyll mutations[11].

The combined treatments showed a considerable degree of synergism for increase in the frequency of chlorophyll mutations. A probable reason for synergism is that the mutagen first applied may expose the protected mutable sites to the second mutagen and the repair enzymes may be rendered non-functional by the second mutagen, thereby promoting the fixation of already induced pre-mutational changes[23]. In the present study, negative synergism (less than additive) was observed in combination treatments of gamma rays+EMS. Less than additive effect may result in case, the two mutagens compete for the same site[35]. These results confirmed the findings of Reddy *et al*.[36] in rice and Khan[37] in mungbean. Positive synergistic effects for chlorophyll mutations were reported in combined treatments of gamma rays and EMS in barley[38]. Synergistic effects of physical and chemical mutagens have been reported in various crops[39-42].

The higher number of mutant seedlings recorded after each treatment in Pant U-30 variety compared to that of T-9 variety is an indicative of differential response of these two

varieties to the mutagens. Many genetic differences are expected between the two varieties as var. T-9 is a local selection and Pant U-30 was developed through hybridization. Genetic differences even of a single gene may induce significant changes in mutagen sensitivity which influences not only the rate but also the spectrum of recoverable mutations[43,1]. Induction of 0.99% chlorina mutations in the var. T-9 and 1.41% in the var. Pant U-30 by EMS and 0.36% chlorina in T-9 and 0.54% in Pant U-30 by gamma rays indicated mutagen specificity by the two urdbean varieties as far as chlorophyll deficient mutations were concerned. Despite the fact, that different frequencies of similar mutations are induced by different mutagens, the chief limiting factor in the induction and recovery of mutations was the genetic constitution of the experimental material[44-45].

CONCLUSION

Chlorophyll mutants though do not have much economic importance due to their lethal nature, however they could be helpful in recognizing the threshold dose of a mutagen that would increase the genetic variability and number of economically useful mutants in subsequent generations.

SIGNIFICANCE STATEMENT

Augmentation of mutation frequency and the alteration of mutation spectrum in a predictable manner remain all time important aspects of mutation research. In this study, the chlorophyll mutants induced by single and combination treatments of physical (gamma rays) and chemical (EMS) mutagens could be utilized as genetic markers in different mutation breeding research programmes for inducing viable mutations of greater economic and agronomic importance.

ACKNOWLEDGMENTS

We are grateful to the Chairman, Department of Botany, Aligarh Muslim University, Aligarh for providing necessary research and laboratory facilities.

REFERENCES

1. Gustafsson, A., 1963. Productive mutations induced in barley by ionizing radiations and chemical mutagens. Hereditas, 50: 211-262.
2. Swaminathan, M. S. and N.P. Sarma, 1968. Alteration of the mutation spectrum in barley through treatments of different periods in the S phase of DNA synthesis. Curr. Sci., 37: 685-686.
3. Das, P.K. and S. Kundagrami, 2000. Frequency and spectrum of chlorophyll mutations in grasspea induced by gamma rays. Indian J. Genet. Plant Breed., 60: 239-241.
4. Devi, T.R., H.S. Prodhan, N. Brajendra and M. Rohinikumar, 2002. Effectiveness and efficiency of gamma rays in inducing chlorophyll mutations in M_2 generation of three rice bean genotypes (*Vigna umbellata* (L.) Thunb. Ohwi and Ohashi). Environ. Ecol., 20: 890-893.
5. Paul, A. and D.P. Singh, 2002. Induced chlorophyll mutations in lentil (*Lens culinaris* Medik). Indian J. Genet. Plant Breed, 62: 263-264.
6. Toker, C. and M.I. Cagirgan, 2004. Spectrum and frequency of induced mutations in chickpea. Int. Chickpea Pigeonpea Newslett., 11: 8-10.
7. Khan, S., M.R. Wani, M. Bhat and K. Parveen, 2005. Induced chlorophyll mutations in chickpea (*Cicer arietinum* L.). Int. J. Agric. Biol., 7: 764-767.
8. Yamaguchi, H., T. Morishita, K. Degi, A. Tanaka, N. Shikazono and Y. Hase, 2006. Effect of carbon-ion beams irradiation on mutation induction in rice. Plant Mutat. Rep., 1: 25-27.
9. Khan, M.H. and S.D. Tyagi, 2009. Studies on induction of chlorophyll mutations in soybean, *Glycine max* (L.) Merrill. Front. Agric. China, 3: 253-258.
10. Wani, M.R., 2017. Induced chlorophyll mutations, comparative mutagenic effectiveness and efficiency of chemical mutagens in lentils (*Lens culinaris* Medik). Asian J. Plant Sci., 16: 221-226.
11. Swaminathan, M.S., V.L. Chopra and S. Bhaskaranl, 1962. Chromosomes aberrations and the frequency and spectrum of mutations induced by EMS in barley and wheat. Indian J. Genet., 22: 192-207.
12. Gupta, P.K. and Yashvir, 1975. Induced mutations in foxtail millet (*Setaria italica* Beauv.): I. Chlorophyll mutations induced by gamma rays, EMS and DES. Theor. Applied Genet., 45: 242-249.
13. Subramanian, D., 1980. Effect of gamma irradiation in *Vigna*. Indian J. Genet., 40: 187-194.
14. Cheema, A.A. and B.M. Atta, 2003. Radiosensitivity studies in basmati rice. Pak. J. Bot., 35: 197-207.
15. Karthika, R. and B.S. Lakshmi, 2006. Effect of gamma rays and EMS on two varieties of soybean. Asian J. Plant Sci., 5: 721-724.
16. Singh, G.R., P.K. Sareen and R.P. Saharan, 2000. Induced chlorophyll and morphological mutations in mungbean. Indian J. Genet. Plant Breed., 60: 391-393.
17. Kharkwal, M.C., 1998. Induced mutations in chickpea (*Cicer arietinum* L.). II. Frequency and spectrum of chlorophyll mutations. Indian J. Genet. Plant Breed., 58: 465-474.
18. Filippetti, A., C.F. Marzano, L.M. Monti and G.T.S. Mungozza, 1977. Studies on breeding commercial varieties of peas for industrial use by experimental mutagenesis. I. Frequency and types of mutations induced with X-rays and diethyl sulphate. Genet. Agric., 31: 40-48.

19. Vandana, T.A. and D.K. Dubey, 1994. Frequency and spectrum of mutations induced by ethyl methane sulphonate (EMS) and diethyl sulphate (DES) in lentil Var. K-85. LENS Newslett., 21: 16-19.
20. Waghmare, V.N. and R.B. Mehra, 2001. Induced chlorophyll mutations, mutagenic effectiveness and efficiency in *Lathyrus sativus* L. Indian J. Genet. Plant Breed., 61: 53-56.
21. Reddy, C.S. and J.D. Smith, 1981. Mutagenic effects of combination treatments of hydrazine, ethyl methanesulphonate and gamma rays in *Sorghum bicolor* (L.) Moench. Indian J. Bot., 4: 5-14.
22. Gustafsson, A., 1940. The mutation system of the chlorophyll apparatus. Acta Lund. Univ., 36: 1-40.
23. Sharma, R.P., 1970. Increased mutation frequency and wider mutation spectrum in barley induced by combining gamma rays with ethylmethane sulphonate. Indian J. Genet., 30: 180-186.
24. Srivastava, L S., H. Chad and S. Kumar, 1973. Dose response studies on EMS and MMS treated gram. Sci. Cult., 39: 345-347.
25. Nadarajan, N., R. Sathupati and N. Shivaswamy, 1982. Investigation on induced macromutations in *Cajanus cajan*. Madras Agric. J., 69: 713-717.
26. Bevins, M., C.M. Yang and J. Markwell, 1992. Characterization of a chlorophyll-deficient mutant of sweetclover (*Melilotus alba*). Plant Physiol. Biochem., 30: 327-331.
27. Arora, R. and M.L.H. Kaul, 1989. Mutagen induced chlorophyll deficiency in *Pisum sativum*. Cytobios, 57: 189-199.
28. Athwal, D.S., S.K. Bhalla, S.S. Sandhu and H.S. Brar, 1970. A fertile dwarf and three other mutants in *Cicer*. Indian J. Genet. Plant Breed., 30: 261-266.
29. Vanniarajan, C., P. Vivekanandan and J. Ramalingam, 1993. Spectrum and frequency of chlorophyll and viable mutations in M_2 generation of blackgram. Crop Improve., 20: 215-218.
30. Ignacimuthu, S. and C.R. Babu, 1988. Radiosensitivity of the wild and cultivated urd and mung beans. Indian J. Genet. Plant Breed., 48: 331-342.
31. Gautam, A.S., K.C. Sood and A.K. Ricarria, 1992. Mutagenic effectiveness and efficiency of gamma-rays, ethyl methane sulphonate and their synergistic effects in black gram (*Vigna mungo* L.). Cytologia, 57: 85-89.
32. Singh, V.P., M. Singh and J.P. Pal, 1999. Mutagenic effects of gamma rays and EMS on frequency and spectrum of chlorophyll and macromutations in urdbean (*Vigna mungo* L. Hepper). Indian J. Genet., 59: 203-210.
33. Lal, G.M., B. Toms and S.S. Lal, 2009. Mutagenic sensitivity in early generation in black gram. Asian J. Agric. Sci., 1: 9-11.
34. Goud, J.V., 1967. Induced mutations in bread wheat. Indian J. Genet., 27: 40-45.
35. Aastveit, K., 1968. Effects of Combinations of Mutagens on Mutation Frequency in Barley. In: Mutations in Plant Breeding II, IAEA (Ed.). International Atomic Energy Agency, Vienna, pp: 5-14.
36. Reddy, T.P., C.S. Reddy and G.M. Reddy, 1973. Mutational studies with some base specific chemicals in *Oryza sativa* L. Indian J. Exp. Biol., 11: 222-224.
37. Khan, I.A., 1981. Effect of single and combination treatments of gamma rays and hydrazine hydrate on the induction of mutations in mungbean (*Phaseolus aureus* Roxb.). J. Cytol. Genet., 16: 125-130.
38. Patil, S.S., 1988. Interaction of physical and chemical mutagens for induction of chlorophyll mutations in barley. J. Maharashtra Agric. Univ., 13: 134-136.
39. Agarkova, S.N. and A.G. Yakovlev, 1977. Effects of gamma rays and EMS on mutation frequency in *Phaseolus vulgaris*. Referationyl Zhurnal, 3: 55-56.
40. Venkateswarlu, M., B.N. Susheelamma, P. Kumar and K. Subhash, 1988. Studies on induced mutation frequency in *Catharanthus roseus* (L.) G. don by gamma rays and EMS individually and in combination. Indian J. Genet. Plant Breed., 48: 313-316.
41. Reddy, V.R.K., 1992. Mutagenic parameters in single and combined treatments of gamma rays, EMS and sodium azide in triticale, barley and wheat. Adv. Plant Sci., 5: 542-553.
42. Singh, A.K., 2007. Mutagenic effectiveness and efficiency of gamma rays and ethylmethane sulphonate in mungbean. Madras Agric. J., 94: 7-13.
43. Bhan, A.K. and M.L.H. Kaul, 1976. Frequency and spectrum of chlorophyll-deficient mutations in rice after treatment with radiation and alkylating agents. Mutat. Res./Fund. Mol. Mech. Mutagen., 36: 311-317.
44. Gregory, W.C., 1965. Mutation Frequency, Magnitude of Changes and the Probability of Improvement in Adaptation. In: The Use of Induced Mutations in Plant Breeding, Favret, E.A. (Ed.). Pergamon Press, Oxford, pp: 429-441.
45. Kaul, M.L.H. and A.K. Bhan, 1977. Mutagenic effectiveness and efficiency of EMS, DES and gamma-rays in rice. Theor. Applied Genet., 50: 241-246.