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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 M₁ generation. The M₁ seeds were sown in next season in plant progeny rows for raising M₂ generation. Chlorophyll mutations were observed when seedlings were 8-15 days old in M₂ 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 M₂ 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

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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 goals¹⁻². 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 level³⁻⁴. 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 M₂ population seems to be more realistic and helpful. Therefore, results were explained on M₂ plant basis in the present investigation.

Scoring of chlorophyll mutations in M₂ 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 M₂ generation following treatments with various mutagenic agents⁵⁻¹⁰. Ionizing radiations generally produce a higher proportion of albina mutations than chemical mutagens¹¹⁻¹⁵. Chemical mutagens induce higher frequency of chlorophyll mutations than radiations as observed in mungbean¹⁶, chickpea¹⁷, pea¹⁸, lentil¹⁹, grasspea²⁰ and soybean¹⁵. 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 radiation²¹.

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 M₂ 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 ⁶⁰CO 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 presoaked 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 M₁ plant of all different treatments with their respective controls in both varieties were planted in plant progeny rows in M₂ 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 Gustafsson²². 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 Sharma²³:

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

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.

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

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

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

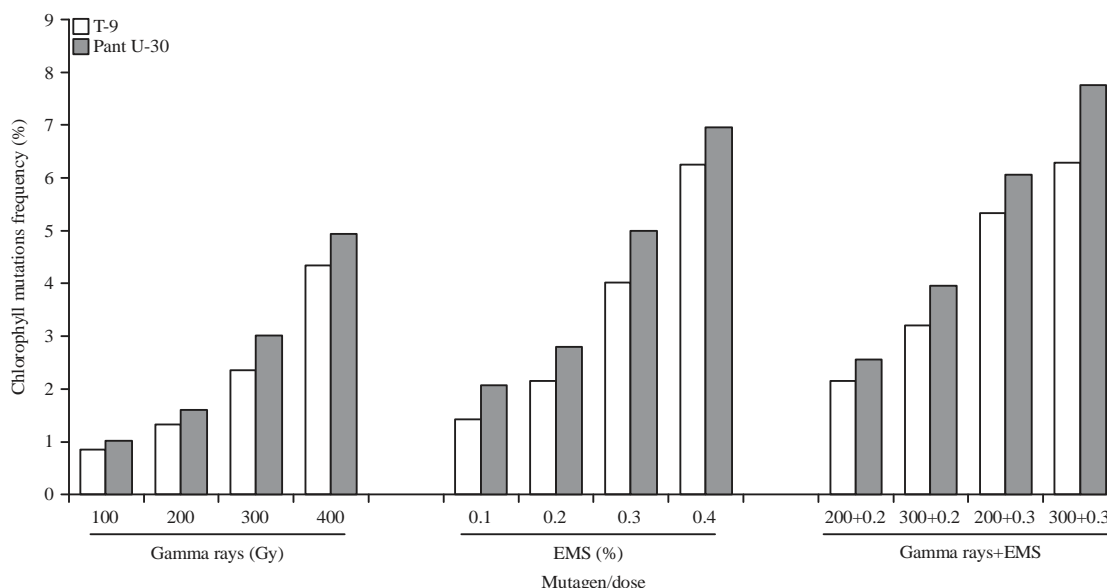


Fig. 1: Chlorophyll mutations frequency in two varieties of urdbean

Table 1: Frequency and spectrum of chlorophyll mutants induced by gamma rays, EMS and their combination in M₂ 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 M₂ 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	0.58
300 Gy+0.2	50	11	22.00	1112	12	9	5	8	10	-	44	3.96	0.68	0.68
200 Gy+0.3	50	11	22.00	1054	19	13	12	7	4	9	64	6.07	0.92	0.92
300 Gy+0.3	50	14	28.00	800	22	15	9	4	2	10	62	7.75	0.97	0.97

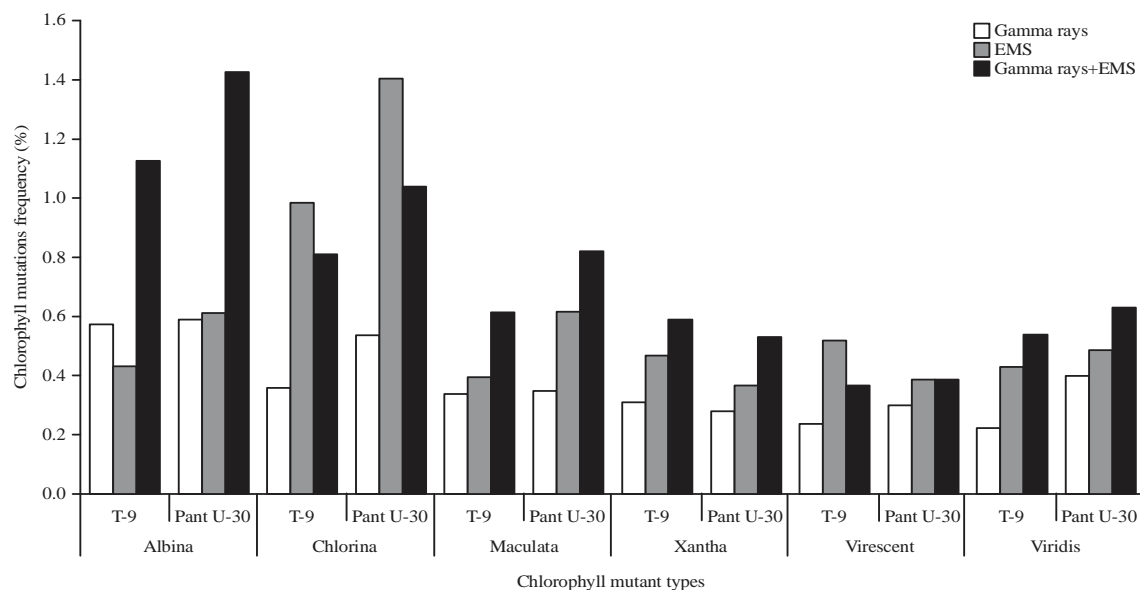


Fig. 2: Comparative chlorophyll mutations frequency and spectrum in two varieties of urdbean

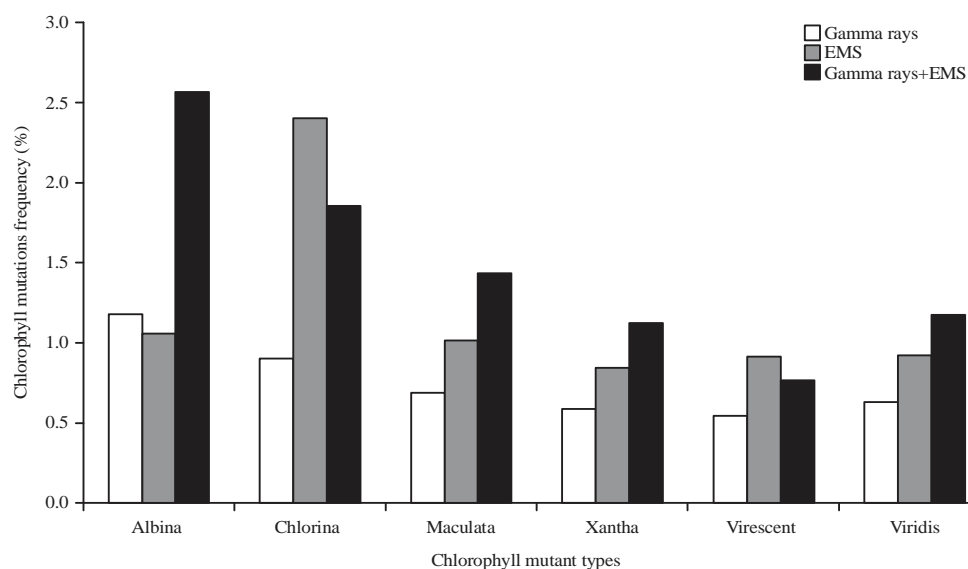


Fig. 3: Chlorophyll mutations frequency on mutagen basis in urdbean

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

Mutagen*	Comparative frequency (%) of chlorophyll mutation spectrum					
	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 chickpea²⁴ 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 carotenoids²⁶. 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 Kaul²⁷ 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 Subbalakshmi¹⁵ in soybean reported that albina constituted the largest single category of mutants observed in gamma ray treated population. Kharkwal¹⁷ obtained higher frequency of albina followed by chlorina and xantha mutants among the EMS treated population of chickpea. Gupta and Yashvir¹² 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 Babu³⁰ 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 mutagens²⁷. 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 Kaul²⁷ 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 workers³¹⁻³³. Individual treatments of EMS induced comparatively higher frequency of chlorophyll mutations than gamma rays. The EMS is supposed to be specific to certain chromosomal regions³⁴ containing genes for chlorophyll development and has been reported to induce high frequency of chlorophyll mutations¹¹.

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 changes²³. 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 site³⁵. These results confirmed the findings of Reddy *et al.*³⁶ in rice and Khan³⁷ in mungbean. Positive synergistic effects for chlorophyll mutations were reported in combined treatments of gamma rays and EMS in barley³⁸. Synergistic effects of physical and chemical mutagens have been reported in various crops³⁹⁻⁴².

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 material⁴⁴⁻⁴⁵.

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.

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