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Induced mutagenic frequency and spectrum of chlorophyll mutants in French bean

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Abstract

M_2 generation was raised from EMS, SA and gamma ray treated M_1 seeds of French bean varieties Varun and Waghya. M_2 generation was screened for the frequency and spectrum of chlorophyll mutations. Chemical mutagens appeared to be more effective in inducing maximum frequencies than physical mutagen in both the varieties. 0.020% concentration of SA induced the highest frequency of chlorophyll mutants in Varun (9.47%) and Waghya (8.29%). Broad spectrum of chlorophyll mutants was recorded in M_2 generation. Different chlorophyll mutants obtained in present investigation such as *albina, xantha, chlorina* and *viridis* revealed diversity in their frequencies in both the varieties. The spectrum of chlorophyll mutants in both the varieties indicated the dominance of *chlorina* and *viridis* mutants as compared to *albina* and *xantha.*

Keywords: EMS, SA, gamma ray, mutation, French bean

INTRODUCTION

Chlorophyll mutations are used as markers in genetic, physiological and biochemical investigations. Chlorophyll mutations can be used as an index in evaluating the mutagenic action of different mutagens in several crops [1]. They are the most frequently observed and easily identified factorial mutations in M_2 generation. The chlorophyll mutations have been utilized as a measure for assessing the effectiveness and efficiency of mutagens as well as indicators to predict the size of vital factor mutations. Chlorophyll mutants provide genetic information and are often used in finding out the metabolic functions and biochemical pathways in plants [2].

The present investigation was undertaken to study the effects of three mutagens, ethyl methanesulphonate (EMS), sodium azide (SA) and gamma rays on induction of frequency and spectrum of chlorophyll mutants in French bean varieties Varun and Waghya.

MATERIALS AND METHODS

Two varieties of French bean, Varun and Waghya were treated with physical (gamma-rays) and chemical mutagens (EMS and SA). Healthy and well dried seeds of the varieties Varun and Waghya of French bean having uniform size and 10% moisture content were employed for irradiation with gamma rays .Seeds were packed in small polythene bags and sealed for gamma ray treatment. The seed samples were exposed to doses 05 kR, 10 kR and 15 kR of gamma rays from Co^{60}, 1000 curie source of the gamma irradiation unit of the Department of Biophysics, Government Institute of Science, Nipat Niranjan, Aurangabad. (M. S.) India. The dose rate was 24,578 rads per hour. For chemical mutagenic treatment, healthy and uniform seeds of French bean varieties Varun and Waghya were surface sterilized with 0.1 % mercuric chloride solution for about one minute and washed thoroughly with distilled water. They were presoaked in distilled water for 6 hours. The presoaked seeds were later immersed in the mutagenic solution for 4 hours in case of EMS and for 6 hours in case of SA and the treatments were given with intermittent shaking. The volume of the chemical mutagenic solution used was three times as that of seeds so as to facilitate uniform conditions. All the chemical mutagenic treatments were given at room temperature of 25±2°C. Seeds soaked in distilled water for 12 hours served as control. The different concentrations used for chemical mutagenic treatment were 0.05%, 0.10% and 0.15% for EMS and 0.010%, 0.015% and 0.020% for SA, respectively. Immediately after the completion of treatment, the seeds were washed thoroughly under running tap water to remove excess of mutagens. Later on treated seeds were post soaked in distilled water for 2 hours. The post soaked seeds were dried in folds of filter paper. The treated seeds were planted in the field according to randomized block design with three replications along with control. Each plot (10 x10 feet) consisted of 6 rows with a distance of 30 cm between the rows and 15 cm between the plants. Each M_1 plant was harvested separately. Seeds of selected M_1 plants were planted in a single row to raise M_2 generation with three replications following randomized block design. Spacing and the experimental area were the same as those used for growing M_1 plants. The M_2 population of each treatment was subjected to screening for different chlorophyll mutations. The pertinent values were calculated and the number of mutations per 100 plants of M_2 generation was estimated according to the method of Gaul (1957) [3]. The different types of chlorophyll mutations scored were: *xantha*, *chlorina, albina* and *viridis* in different treatments. The spectrum of chlorophyll mutants was classified according to the terminology of Gustafsson (1940) [4] and Blixt (1961) [5].

RESULTS AND DISCUSSION

In present investigation various types of chlorophyll mutants such as *xantha*, *chlorina, albina* and *viridis* could be detected at seedling stage in M_2 generation. The data pertaining to the mutation frequency and spectrum of chlorophyll mutants recorded in all the treatments of both the varieties of French bean are presented in tables 1 to 4. It could be observed that the frequency of chlorophyll

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mutants was concentration/dose dependent. The increase in chlorophyll mutation frequency was recorded with increased concentrations/doses of all mutagens. This is in agreement with earlier report [6] in French bean. Similar trend was also reported in other crops by several workers Arvind Kumar et al., (2007) [7] in black gram, Dhanevel et al., (2008 [8] in cowpea, Singh and Chaturvedi (1988) [9] in *Lathyrus sativus* and Reddy and Annadurai (1991) [10] in lentil. 0.020% concentration of SA induced the highest frequency of chlorophyll mutants in Varun (9.47%) and Waghya (8.29%). In present study SA treatments induced more chlorophyll mutants than EMS and gamma rays in both the varieties. Effectiveness of SA in induction of chlorophyll mutants was also recorded by Silva and Barbosa (1996) [11] in French bean.

Table1. Effect of mutagens on the frequency of chlorophyll mutants in M_2 generation of French bean variety Varun

Treatment	Concentration (%) / Dose (kR)	Frequency of chlorophyll mutants (%) (MF)
Control	----	----
EMS	0.05	2.90
	0.10	3.69
	0.15	5.18
SA	0.010	6.52
	0.015	8.09
	0.020	9.47
Gamma rays	05kR	1.17
	10kR	2.13
	15kR	3.07

SE = Standard Error

Table 2. Effect of mutagens on the frequency of chlorophyll mutants in M_2 generation of French bean variety Waghya

Treatment	Concentration (%) / Dose (kR)	Frequency of chlorophyll mutants (%) (MF)
Control	----	----
EMS	0.05	2.81
	0.10	3.18
	0.15	4.48
SA	0.010	5.60
	0.015	7.11
	0.020	8.29
Gamma rays	05kR	1.11
	10kR	2.08
	15kR	2.85

SE = Standard Error

Table 3. Effect of mutagens on the spectrum of chlorophyll mutants in M_2 generation of French bean variety Varun

Treatment	Concentration % /Dose (kR)	Relative % of chlorophyll mutants			
		Albina	***Xantha***	***Chlorina***	***Viridis***
Control	----	----	----	----	----
EMS	0.05	----	22.22	44.44	33.333
	0.10	08.33	25.00	50.00	16.66
	0.15	14.28	28.57	35.71	21.42
SA	0.010	20.00	20.00	26.26	33.33
	0.015	05.88	47.05	35.29	11.76
	0.020	11.11	22.22	38.88	27.77
Gamma rays	05kR	----	25.00	50.00	50.00
	10kR	14.28	----	57.14	28.57
	15kR	12.50	25.00	50.00	12.50

Table 4. Effect of mutagens on the spectrum of chlorophyll mutants in M_2 generation of French bean variety Waghya

Treatment	Concentration %/Dose (kR)	Relative % of chlorophyll mutants			
		Albina	***Xantha***	***Chlorina***	***Viridis***
Control	----	----	----	----	----
EMS	0.05	11.11	----	44.44	44.44
	0.10	9.09	27.27	44.45	18.18
	0.15	15.38	30.76	38.46	15.38
SA	0.010	----	07.14	50.00	42.85
	0.015	06.25	12.50	25.00	56.25
	0.02	17.64	23.52	17.64	41.17
Gamma rays	05kR	25.00	----	25.00	75.00
	10kR	28.57	14.28	14.28	42.85
	15kR	12.50	25.00	25.00	37.50

Broad spectrum of chlorophyll mutants was recorded in M_2 generation. The spectrum of chlorophyll mutations became broader with increasing concentrations/doses of all mutagens in both the varieties. Similar trend was also reported by Gaikwad and Kothekar (2004) [12] in lentil. All chlorophyll mutants such as *albina, xantha, chlorina* and *viridis* revealed diversity in their frequencies in both the varieties. *Albina* mutants were detected in all the treatments except at 0.010% SA treatment in Waghya. It could not be detected at 0.05% EMS concentration and 05kR gamma ray dose in Varun. 0.010% SA concentration induced highest frequency (20%) of *albina* in Varun while highest frequency (28.57%) of *albina* in Waghya was recorded at 10kR dose of gamma ray. Among all treatments lowest frequency (05.88) of *albina* was induced by 0.015% SA in Varun. Except at 10kR gamma ray dose in Varun and 05kR dose in Waghya, *xantha* mutants were noticed in all the treatments. Highest frequency (47.05%) of *xantha* was recorded in Varun at 0.015% SA while 0.010% SA induced the lowest frequency (7.14%) of *xantha* mutant in Waghya. All the concentrations/doses induced *chlorina* mutants in higher frequencies as compared to *albina* and *xantha.* The highest frequency induced being 57.14% after gamma ray dose (10kR) in Varun and the lowest being 14.28% at 10kR gamma ray dose in Waghya. *Viridis* mutants were also noticed in all treatments in both the varieties. Lowest induction (11.76%) of *viridis* mutant could be detected in Varun at 0.015% SA treatment. Highest frequency (75%) of *viridis* was found at 05kR dose of gamma rays in Waghya. The spectrum of chlorophyll mutants in both the varieties indicated the dominance of *chlorina* and *viridis* mutants as compared to *albina* and *xantha.* Dominance of *viridis* mutants as compared to *albina* and *xantha* was reported by Al-Rubeai (1982) [6] in French bean. From present study it can be concluded that chemical mutagens are more effective in inducing maximum frequencies of chlorophyll mutants than physical mutagen in French bean varieties Varun and Waghya.

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