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Research Article

Efficiency and effectiveness of physical and chemical mutagens and their combination in inducing chlorophyll mutations in M_2 generation of Lablab [*Lablab purpureus* (l.) Sweet var. *Typicus*]

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Abstract

A comparative study of the frequency and spectrum of chlorophyll mutations induced by gamma rays (150, 200, 250, 300 and 350 Gy), sodium azide (0.5, 1.0, 1.5 and 2.0 mM) and combination treatments of gamma rays and sodium azide (100 Gy + 1.5 mM, 150 Gy + 1.5 mM, 200 Gy + 1.0 mM, 250 Gy + 1.0 mM and 300 Gy + 0.5 mM) in M_2 generationA was made in the lablab var. CO 9. Four different types of chlorophyll mutants *viz*., albina, viridis, chlorina and xantha were identified in the treated population and mutation frequency was calculated on plant population basis. Frequency of chlorina mutants was highest followed by albina, xantha and viridis. Combination treatments in general proved to be very effective followed by sodium azide and gamma rays in inducing maximum frequency of chlorophyll mutations. Although the chlorophyll mutations do not have any economic value due to their lethal nature, such a study could be useful in identifying the threshold dose of a mutagen that would increase the genetic variability and number of economically useful mutants in the segregating generations.

Key words: Lablab, physical and chemical mutagens, chlorophyll mutations

Introduction

Lablab purpureus (L.) Sweet has two distinct botanical forms namely *Lablab purpureus* (L.) Sweet Var. *typicus* (garden bean) and *Lablab purpureus* (L.) Sweet Var. *lignosus* (field bean). Garden bean is mainly cultivated for its green pods, while the field bean is grown for its seeds.

The primary objective of the mutation breeding is to enlarge the frequency and spectrum of mutations, and also to increase the incidence of viable mutations, as an approach towards directed mutagenesis. Mutation breeding can be a valuable supplement to conventional plant breeding methods. A systematic and comparative study of mutagenic effectiveness and efficiency of gamma rays, sodium azide and their combination based on frequency and spectrum of chlorophyll mutations in M_2 generation in lablab has been undertaken in the study.

Materials and Methods

Three hundred well dried (10 – 12 % moisture content), fully matured, disease and insect-free seeds with uniform shape, size and colour were irradiated with the help of gamma cell installed at IGCAR, Kalpakkam. The source material kept inside the gamma chamber is ^{60}Co. The seeds were soaked in double distilled water before treating them with the mutagen sodium azide for a period of 6 h. Then the pre-soaked seeds were treated with the chemical mutagen for 4 h. The volume of the mutagen was 3 times of the seeds utilized. The treatment was carried out with intermittent shaking at room temperature. The seeds were then washed in running water for about half an hour to remove the residues of the chemical, if any, and the excess moisture on seed coat was removed by using folds of blotting paper. For combination treatments, seeds irradiated with gamma rays were soaked prior to sodium azide treatment. Chlorophyll mutants were scored and classified following Gustafsson (1940). Mutation frequency was calculated as percentage of M_2 plants and mutagenic effectiveness and efficiency were calculated on the basis of formula suggested by Konzak et al. (1965).

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Results and Discussion

Konzak et al. (1965) have presented a detailed account of the mutagenic effectiveness and efficiency. The term “effectiveness” is a measure of gene mutation in relation to dose and “efficiency” is an estimate of biological effects induced, such as lethality, sterility and injury. To obtain high efficiency the mutagenic effect must greatly surpass the damage in the cells, such as chromosomal aberrations and toxic effects as proposed by Gaul et al. (1972). According to them, the effectiveness of mutagens was of theoretical importance, but did not have any immediate practical implication. Further, they reported that low biological damage of mutagens permitted the use of high dose of mutagens, but for practical purpose, the aim was to obtain high efficiency.

Usually, chlorophyll mutations occur up to 15 days from the time of seed germination and in them the primary leaves will be deficient of chlorophyll (Stummann and Henningsen, 1980). In M_2 generation, it was noted that the frequency of chlorophyll mutations increased with increase in dose or concentration in both the mutagens and also in combination treatments (Table 1). This was in agreement with the results obtained by Singh et al. (2001) in rice.

Based on the intensity of pigmentation at the seedling stage, four types of chlorophyll mutants viz., albina, chlorina, xantha and viridis were recorded in the segregating M_2 plants (Table 2). Most of the chlorophyll mutant types (albina, chlorina and xantha) were lethal and survived for 20 – 25 days only. Viridis survived till maturity, but produced no flowers and seeds. In sodium azide treated population, xantha occurred more frequently followed by chlorina, albina and viridis, whereas, in gamma treated population, albina occurred more frequently. Vanniarajan et al. (1993) reported the same in black gram. In case of combination treatments, chlorina occurred more frequently followed by albina, xantha and viridis. This is in agreement with the reports of Lourdusamy and Rathnasamy (1995) in lablab. It is generally believed that ionizing radiations produced a high frequency of albina type, whereas, chemical mutagens produce higher frequency of chlorina and xantha types (Gupta and Sharma, 1990).

Combination treatments, except 300 Gy + 0.5 mM showed less than additive effects. Rao and Ayengar (1964) reported similar results in rice. The absence of additive effect may be due to low concentration of chemical in the combination treatment, as reported by Krishnaswami (1977). The additive effect can be attributed to pre-mutational lesions and mutations of favourable modifications in either one or both the processes (Sharma and Swaminathan, 1969). Combination treatment 300 Gy + 0.5 mM, showed synergistic effect. The synergistic effect of combined treatments has been attributed by Sharma (1970) to either (i) the first mutagen treatment denatures DNA (Cox et al., 1955 and Scholes and Weiss, 1962) and make accessible otherwise non-available sites for reaction to the second mutagen (Arnason et al., 1963) or (ii) the repair enzymes (Sobels, 1966) may be rendered non-functional (Roberts and Warwick, 1958) by the second mutagen, facilitating the fixation of already induced pre-mutational lesions. Both these pathways should yield a frequency of mutations higher than the total of two mutagens.

Chlorophyll mutation frequency increased along with increase in dose or concentration in all the mutagenic treatments. However, a slight decline was observed at the highest concentration of 2.0 mM of sodium azide. It seems that strong mutagens reach their saturation point even at a lower dose in the genotypes having highly mutable allelic sites, and any further increase in the dose does not add to their frequency. It has also been suggested that with the increase in the mutation dose beyond a certain point, the strong mutagens become more toxic than higher doses of relatively weak mutagens. Similar observations have been made in chickpea by Kharkwal (1998) and Wani and Anis (2004). Combination treatments in general proved to be very effective followed by sodium azide and gamma rays, in inducing maximum frequency of chlorophyll mutations and has been reported by Singh et al. (1999).

It is, therefore, concluded that although the chlorophyll mutations do not have any economic value due to their lethal nature, such a study could be useful in identifying the threshold dose of a mutagen that would increase the genetic variability and number of economically useful mutants in the segregating generations.

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Table 1. Frequency of Chlorophyll mutations in M_2 generation

Treatments	Number of M_2 seedlings examined	Mutation frequency on M_2 seedling basis	
		Number of mutants	Percentage
Gamma rays			
Control	1136	---	---
150 Gy	965	8	0.83
200 Gy	781	10	1.28
250 Gy	682	12	1.76
300 Gy	629	13	2.07
350 Gy	564	15	2.66
Sodium azide			
Control	1059	---	---
0.5 mM	933	9	0.96
1.0 mM	749	15	2.00
1.5 mM	678	17	2.51
2.0 mM	622	16	2.57
Gamma rays + Sodium azide			
Control	1077	---	---
100 Gy + 1.5 mM	859	19	2.21
150 Gy + 1.5 mM	792	21	2.65
200 Gy + 1.0 mM	763	22	2.88
250 Gy + 1.0 mM	698	24	3.44
300 Gy + 0.5 mM	579	28	4.84

Table 2. Spectrum of Chlorophyll mutations in the M_2 generation

Treatments	Total chlorophyll mutations	Spectrum (relative percentage) of chlorophyll mutations			
		Albina	Viridis	Chlorina	Xantha
		Gamma rays			
150 Gy	8	50.00	25.00	---	25.00
200 Gy	10	60.00	---	40.00	---
250 Gy	12	58.33	8.33	33.33	---
300 Gy	13	46.15	30.77	23.08	---
350 Gy	15	60.00	---	13.33	26.67
		Sodium azide			
0.5 mM	9	---	---	33.33	66.67
1.0 mM	15	20.00	6.67	20.00	53.33
1.5 mM	17	11.76	11.76	29.42	47.06
2.0 mM	16	18.75	12.50	25.00	43.75
		Gamma rays + Sodium azide			
100 Gy + 1.5 mM	19	26.32	10.52	63.16	---
150 Gy + 1.5 mM	21	23.81	9.52	42.86	23.81
200 Gy + 1.0 mM	22	18.18	22.73	40.91	18.18
250 Gy + 1.0 mM	24	20.83	16.67	45.83	16.67
300 Gy + 0.5 mM	28	25.00	10.71	46.43	17.86

Table 3. Mutagenic effectiveness and efficiency – Chlorophyll mutations

Treatments	% Survival reduction at 30 days (Lethality, L)	% Height reduction at 30 days (Injury, I)	% Seed fertility reduction (Sterility, S)	Mutation per 100 M_2 plants (M)	Effectiveness	Mutagenic efficiency		
					$\frac{M \times 100}{kR \text{ or } C \times t}$	$\frac{M \times 100}{L}$	$\frac{M \times 100}{I}$	$\frac{M \times 100}{S}$
Gamma rays								
150 Gy	23.08	8.51	11.33	0.83	5.53	3.60	9.75	7.33
200 Gy	29.35	11.39	16.65	1.28	6.40	4.36	11.24	7.69
250 Gy	33.66	15.46	19.10	1.76	7.04	5.23	11.38	9.21
300 Gy	41.72	19.23	25.51	2.07	6.90	4.96	10.76	8.11
350 Gy	48.21	23.65	28.49	2.66	7.60	5.52	11.25	9.34
Sodium azide								
0.5 mM	14.32	6.46	9.58	0.96	48.00	6.70	14.86	10.02
1.0 mM	28.40	10.31	14.99	2.00	50.00	7.04	19.40	13.34
1.5 mM	42.89	18.75	22.03	2.51	41.83	5.85	13.39	11.39
2.0 mM	51.56	24.03	30.88	2.57	32.13	4.98	10.69	8.32
Gamma rays + Sodium azide								
100 Gy + 1.5 mM	30.77	10.09	15.80	2.21	---	7.18	21.90	13.99
150 Gy + 1.5 mM	39.35	14.44	18.78	2.65	---	6.73	18.35	14.11
200 Gy + 1.0 mM	45.42	18.23	26.35	2.88	---	6.34	15.80	10.93
250 Gy + 1.0 mM	53.19	22.29	30.66	3.44	---	6.47	15.43	11.22
300 Gy + 0.5 mM	58.08	28.71	36.12	4.84	---	8.33	16.86	13.40