

# Induced chlorophyll mutations. I. Mutagenic effectiveness and efficiency of EMS, HZ and SA in mungbean

Mohd Rafiq WANI<sup>1</sup>, Samiullah KHAN (✉)<sup>2</sup>, Mohammad Imran KOZGAR<sup>2</sup>

<sup>1</sup> Department of Botany, Govt. Degree College (Boys), Anantnag- 192 231, (J&K), India

<sup>2</sup> Mutation Breeding Laboratory, Department of Botany, Aligarh Muslim University, Aligarh- 202 002, (U.P.), India

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**Abstract** A systematic and comparative study on the frequency and spectrum of chlorophyll mutations induced by ethylmethane sulphonate (EMS) – an alkylating agent, hydrazine hydrate (HZ) – a base analogue and sodium azide (SA) – a respiratory inhibitor, was carried out in two mungbean varieties, namely, PDM-11 and NM-1. A wide spectrum of chlorophyll mutants was obtained in the M<sub>2</sub> generation. All these chlorophyll-deficient mutants were lethal except maculata, viridis and virescent. Chlorina followed by xantha types were predominant in both the varieties. EMS treatments induced the highest frequency of chlorophyll mutations followed by HZ and SA. The frequency of chlorophyll mutations was dose-dependent and increased with the mutagen concentration. Based on effectiveness in both varieties, the order of mutagens was HZ > SA > EMS. Two criteria viz., pollen sterility (Mp/S) and seedling injury (Mp/I) were taken into consideration to determine the efficiency of the mutagens. EMS was found to be the most efficient mutagen followed by HZ and SA. Moderate concentrations of the mutagens were the most effective and efficient in inducing mutations.

**Keywords** mungbean, chemical mutagens, chlorophyll mutations, mutagenic effectiveness, efficiency

## Introduction

The enhancement of mutation frequency and alteration of mutation spectrum in a predictable manner are the two important goals of mutation research. Chlorophyll mutation frequency is useful in assessing the potency of a mutagen. Hence, scoring of chlorophyll mutations has proved to be a much more dependable index for evaluating the genetic effects of mutagenic treatments. Several authors have reported the occurrence of different types of chlorophyll mutants in different crop plants such as chickpea (Kharkwal, 1998; Khan et al., 2005), urdbean (Singh et al., 1999), lentil (Wani and Khan, 2003), limabean (Kumar et al., 2003), cowpea (John, 1999) and grasspea (Das and Kundagrami, 2000; Waghmare, 2001). The chlorophyll deficient mutants have been successfully used as genetic markers in plant

breeding programmes for obtaining information regarding the role and effect of different mutagens to find out the response of a particular genotype to a particular mutagen. Before the start of any sound breeding programme, a knowledge of relative biological effectiveness and efficiency of various mutagens and their selection is essential to recover high frequency of desirable mutations (Smith, 1972; Kumar and Mani, 1997). It is not necessary that an effective mutagen should be an efficient one also (Gaikward and Kothekar, 2004). Mutagenic effectiveness is an index of the response of a genotype to the increasing doses of the mutagen, whereas mutagenic efficiency indicates the extent of genetic damage recorded in the M<sub>2</sub> generation in relation to the biological damage caused in M<sub>1</sub>. Both of these though are two different properties, but the usefulness of any mutagen in a plant breeding programme depends on both of them. The present investigation was undertaken with the aim to study the effects of EMS, HZ and SA on the frequency and spectrum of chlorophyll mutants and to evaluate the relative effectiveness and efficiency of these chemical mutagens on two mungbean varieties in the M<sub>2</sub> generation.

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Correspondence: Samiullah KHAN

E-mail: mbl.amu@gmail.com

## Materials and methods

Uniform and healthy seeds of two mungbean (*Vigna radiata* (L.) Wilczek) varieties, PDM-11 and NM-1, pre-soaked in distilled water for 9 hours, were treated with three chemical mutagens, EMS, HZ and SA for 6 hours at  $(25 \pm 1)^\circ\text{C}$ . The concentrations used for EMS ranged from 0.1%–0.4%, whereas these were 0.01%–0.04% for HZ and SA. The solutions of EMS and HZ were prepared in phosphate buffers of pH 7, whereas SA solution was prepared in a phosphate buffer adjusted to pH 3. To facilitate uniform absorption, large quantities of the solution of mutagens, approximately three times the volume of seeds (Konzak et al., 1965), were used. Following these treatments, the seeds were thoroughly washed in running tap water to remove the residue mutagens from the seed surface. The treated seeds were directly sown in the field along with untreated controls. Three replications of 100 seeds per treatment were sown in a randomized complete block design to raise the  $M_1$  generation. The  $M_1$  plants were harvested separately and the seeds sown the next season in plant progeny rows to raise the  $M_2$  generation. The distance between the seeds in a row and between the rows was kept at 30 and 60 cm, respectively. Chlorophyll mutants were scored in the  $M_2$  when seedlings were 7–15 days old and classified according to Gustafsson (1940). Frequency of chlorophyll mutants was calculated as percentage of  $M_2$  segregating progenies and that of mutant seedlings in the  $M_2$  generation. The effectiveness and efficiency of the different treatments of all the three mutagens were calculated by the method suggested by Konzak et al. (1965). Pollen fertility was estimated by staining the pollen grains with 1% acetocarmine solution. Pollen grains, which took the stain and had a regular shape, were considered to be fertile, with the shrunken, empty and unstained ones as sterile.

## Results and discussion

### Frequency and spectrum of chlorophyll mutations

In the present study, six different types of chlorophyll mutants were recorded in the field when seedlings were 7–15 days old. The spectrum of  $M_2$  chlorophyll mutants included albina, chlorina, maculata, xantha, virescent and viridis in both varieties (Tables 1 and 2). The seedlings, which initially appeared to be normal, began to manifest different types of chlorophyll deficiencies at the later stage of growth. Maculata, viridis and virescent survived up to maturity and produced few seeds, while albina, xantha and chlorina died at the seedling stage.

A brief description of these chlorophyll mutants is given below:

**Albina:** The mutants were white, lethal and survived for up to 8–10 d after germination.

**Chlorina:** These were characterized by the presence of a light green colour of the first pair of leaves in the seedlings. The emerging leaves were also light green and became darker with the approach of maturity, but never regained the normal green colour. These seedlings died within 15 d.

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

**Xantha:** Leaves were yellow in colour. Seedlings survived for 10–15 d only.

**Virescent:** The leaves were white or light yellow in colour, with patches of yellowish green or green colour that appeared gradually. In most cases, these yellowish or light green patches completely disappeared and the normal colour was regained. They were as vigorous as the normal plants and set seeds.

**Table 1** Frequency and spectrum of chlorophyll mutants in the  $M_2$  generation of mungbean var. PDM-11

Treatment	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							Frequency (%)
					Albina	Chlorina	Maculata	Xantha	Virescent	Viridis	Total	
Control	35	–	–	1050	–	–	–	–	–	–	–	–
0.1% EMS	39	5	12.82	1131	–	14	3	11	3	7	38	3.36
0.2% EMS	37	12	32.43	1147	5	21	8	13	7	10	64	5.58
0.3% EMS	36	14	38.89	1152	6	20	9	14	8	9	66	5.73
0.4% EMS	35	15	42.86	1015	9	20	11	10	10	10	70	6.89
0.01% HZ	34	3	8.82	1122	4	13	–	8	–	4	29	2.58
0.02% HZ	39	11	28.20	1170	–	18	6	9	4	9	46	3.93
0.03% HZ	38	11	28.95	1216	3	21	7	12	6	8	57	4.69
0.04% HZ	35	12	34.28	1073	5	18	9	16	7	13	68	6.34
0.01% SA	34	2	5.88	1156	–	10	–	7	–	6	23	1.99
0.02% SA	37	6	16.21	1200	–	12	–	8	4	7	31	2.58
0.03% SA	37	8	21.62	1147	1	14	8	10	–	6	39	3.40
0.04% SA	35	8	22.86	1155	1	17	3	13	1	8	43	3.72

**Table 2** Frequency and spectrum of chlorophyll mutants in M<sub>2</sub> generation of mungbean var. NM-1

Treatment	No. of M <sub>1</sub> plant progenies	No. of plant progenies seg-regating in M <sub>2</sub>	Mutated plant progenies (Mp) (%)	No. of M <sub>2</sub> seedlings	Chlorophyll mutant types							Frequency (%)
					Albina	Chlorina	Maculata	Xantha	Virescent	Viridis	Total	
Control	37	–	–	1184	–	–	–	–	–	–	–	–
0.1% EMS	40	5	12.50	1190	1	18	4	10	2	9	44	3.70
0.2% EMS	39	15	38.46	1170	3	19	10	15	8	13	68	5.81
0.3% EMS	37	15	40.54	1184	5	23	9	16	7	12	72	6.08
0.4% EMS	37	16	43.24	1110	6	21	13	15	9	14	78	7.03
0.01% HZ	39	5	12.82	1209	–	16	2	11	1	3	33	2.73
0.02% HZ	39	12	30.77	1205	2	20	7	10	5	8	52	4.31
0.03% HZ	35	11	31.43	1195	4	19	9	15	5	10	62	5.18
0.04% HZ	38	12	31.58	1102	5	20	10	17	9	14	75	6.80
0.01% SA	40	3	7.50	1160	1	9	1	8	1	5	25	2.15
0.02% SA	36	6	16.66	1180	1	13	1	7	2	9	33	2.79
0.03% SA	39	9	23.07	1170	–	15	7	10	2	7	41	3.50
0.04% SA	38	9	23.68	1102	–	18	6	10	–	9	43	3.90

Viridis: These mutants were characterized by reduced height and viridine green colour of leaves. The plants were slow growing and had a low seed yield.

The chlorophyll mutation frequency was calculated on the M<sub>1</sub> progeny basis as well as on the M<sub>2</sub> seedling basis. The trend of mutation frequency was similar in both methods. Both varieties were found to respond to the mutagenic treatments differently. Var. NM-1 appeared to produce a slightly higher number of chlorophyll mutants than var. PDM-11. The frequency of chlorophyll mutations was found to be dose-dependent. A higher frequency of chlorophyll mutations with medium or lower doses of mutagen were reported by Nadarajan et al. (1982) in *Cajanus cajan*, while several other reports indicated a dose-dependent increase in chlorophyll mutation frequency (Kharkwal, 1998; Das and

Kundagrami, 2000; Barshile et al., 2006). The frequency of chlorina mutants was the highest, followed by xantha, viridis, maculata, virescent and albina (Table 3). Arora and Kaul (1989) in *Pisum sativum*, Vanniarajan et al. (1993) in *Vigna mungo* and Kharkwal (1998) in *Cicer arietinum* also reported that the chemical mutagens produced a high frequency of chlorina and xantha types of chlorophyll mutants. The occurrence of chlorina mutants in a large number of crops has been attributed to different causes such as impaired chlorophyll biosynthesis, further degradation of chlorophyll and bleaching because of a deficiency of carotenoids (Bevins et al., 1992).

Among the mutagens used in the present study, EMS induced the highest frequency and widest spectrum of chlorophyll mutations compared with HZ and SA (Table 4).

**Table 3** Mutagen induced chlorophyll mutations spectrum in mungbean

Mutagen*/Variety	Relative frequency of chlorophyll spectrum (%)						
	Albina	Chlorina	Maculata	Xantha	Virescent	Viridis	
Var. PDM-11	EMS	0.45	1.69	0.69	1.08	0.63	0.81
	HZ	0.26	1.53	0.48	0.98	0.37	0.74
	SA	0.04	1.14	0.23	0.81	0.11	0.58
	Total	0.75	4.36	1.40	2.87	1.11	2.13
Var. NM-1	EMS	0.32	1.74	0.77	1.20	0.56	1.03
	HZ	0.23	1.59	0.59	1.12	0.42	0.74
	SA	0.04	1.19	0.32	0.76	0.11	0.65
	Total	0.59	4.52	1.68	3.08	1.09	2.42

\* means data based on pooled values of four concentrations.

**Table 4** Comparative mutagen induced chlorophyll mutations spectrum in mungbean

Mutagen*	Relative frequency of chlorophyll spectrum (%)					
	Albina	Chlorina	Maculata	Xantha	Virescent	Viridis
EMS	0.77	3.43	1.46	2.28	1.19	1.84
HZ	0.49	3.12	1.07	2.10	0.79	1.48
SA	0.08	2.33	0.55	1.57	0.22	1.23

\* means data based on pooled values of two varieties PDM-11 and NM-1.

This confirmed that EMS is a more potent chemical mutagen in inducing chlorophyll mutants and supports the earlier report of Khan et al. (2005) in *Cicer arietinum*. EMS is supposed to be specific to certain chromosomal regions (Goud, 1967) containing genes for chlorophyll development and has been reported to induce a high frequency of chlorophyll mutations (Swaminathan et al., 1962). The frequency of chlorophyll mutants induced by SA was much less compared with the other two mutagens. This may be because of the inhibition of catalase and peroxidase and an increase in peroxide concentration in the cell.

### Mutagenic effectiveness and efficiency

Data on the effectiveness and efficiency of EMS, HZ and SA in varieties PDM-11 and NM-1 are presented in Tables 5 and

6. It was found that effectiveness and efficiency were generally higher at moderate concentrations of all three mutagens used. Mutagenic effectiveness was as high as 32.05 in EMS treatments, while the highest effectiveness of HZ and SA treatments was 256.42 and 138.83, respectively, in var. NM-1. Mutagenic effectiveness decreased at the highest concentrations of all the three mutagens in both varieties. The order of mutagenic effectiveness as determined on the basis of mutated plant progenies was HZ > SA and EMS. Contrary to earlier reports of various workers (Reddy, 1992; Kumar and Dubey, 1998; Waghmare and Mehra, 2001), EMS proved to be less effective than HZ and SA. The difference in mutagen concentration and/or genotypic response seemed to be the reasons for the low values of its effectiveness. The decline in the mutagenic effectiveness recorded at higher doses shows that the increase in mutation rate was not proportional to the

**Table 5** Effectiveness and efficiency of the mutagens in M<sub>2</sub> generation of mungbean var. PDM-11

Treatment	Pollen sterility (S) (%)	Seedling injury (I) (%)	Mutated plant progenies (Mp) (%)	Mutagenic effectiveness Mp/t.c	Mutagenic efficiency	
					Mp/S	Mp/I
0.1% EMS	11.42	4.38	12.82	21.36	1.12	2.93
0.2% EMS	20.01	8.50	32.43	27.02	1.62	3.81
0.3% EMS	24.01	14.03	38.89	21.60	1.62	2.77
0.4% EMS	26.82	21.66	42.86	17.86	1.60	1.98
Overall	20.56	12.14	31.75	21.96	1.49	2.87
0.01% HZ	12.42	6.66	8.82	147.00	0.71	1.32
0.02% HZ	17.67	9.56	28.20	235.00	1.59	2.95
0.03% HZ	20.76	18.50	28.95	160.83	1.39	1.56
0.04% HZ	23.14	26.93	34.28	142.83	1.48	1.27
Overall	18.49	15.41	25.06	171.41	1.29	1.77
0.01% SA	8.85	9.12	5.88	98.00	0.66	0.64
0.02% SA	11.50	13.86	16.21	135.08	1.41	1.17
0.03% SA	18.74	22.72	21.62	120.11	1.15	0.95
0.04% SA	20.64	31.14	22.86	95.25	1.11	0.73
Overall	14.93	19.21	16.64	112.11	1.08	0.87

**Table 6** Effectiveness and efficiency of the mutagens in M<sub>2</sub> generation of mungbean var. NM-1

Treatment	Pollen sterility (S) (%)	Seedling injury (I) (%)	Mutated plant progenies (Mp) (%)	Mutagenic effectiveness Mp/t.c	Mutagenic efficiency	
					Mp/S	Mp/I
0.1% EMS	11.11	7.85	12.50	20.83	1.12	1.59
0.2% EMS	22.87	13.08	38.46	32.05	1.68	2.94
0.3% EMS	25.47	18.14	40.54	22.52	1.59	2.23
0.4% EMS	27.41	26.16	43.24	18.01	1.58	1.65
Overall	21.71	16.30	33.68	23.35	1.49	2.10
0.01% HZ	13.79	10.38	12.82	213.67	0.93	1.23
0.02% HZ	19.36	16.11	30.77	256.42	1.59	1.91
0.03% HZ	22.87	20.25	31.43	174.61	1.37	1.55
0.04% HZ	23.75	32.40	31.58	131.58	1.33	0.97
Overall	19.94	19.78	26.65	194.07	1.30	1.41
0.01% SA	8.69	13.92	7.50	125.00	0.86	0.54
0.02% SA	11.97	20.67	16.66	138.83	1.39	0.80
0.03% SA	17.62	28.69	23.07	128.17	1.31	0.80
0.04% SA	20.49	37.55	23.68	98.66	1.15	0.63
Overall	14.69	25.20	17.72	122.66	1.18	0.69

increase in the doses of various mutagens.

Two criteria, pollen sterility (Mp/S) and seedling injury (Mp/I), were taken into consideration to determine the efficiency of the mutagens. Efficiency varied depending upon the criterion selected for its estimation. Moderate concentrations of the mutagens proved to be more efficient than the lowest and the highest ones. Based on Mp/S and Mp/I, EMS proved to be the most efficient mutagen followed by HZ and SA in both varieties (Tables 5 and 6). Gautam et al. (1992) and Ratnam and Rao (1993) reported that mutagenic efficiency increased with an increase in the dose of mutagens, but Khan (1999), in blackgram, reported higher mutagenic efficiency at lower doses. The efficiency at lower doses was greater because the biological damage (seedling injury and pollen sterility) generally increased with the enhancement in the dose at a faster rate than the mutations yielded in M<sub>2</sub> at the same dose (Konzak et al., 1965). The results indicated that the mutagenic efficiency calculated on the basis of seedling injury was higher when compared with that based on pollen sterility for both EMS and HZ. This may be because induced seedling injury was less for both the mutagens than the amount of pollen sterility. In SA treatments, the efficiency calculated on the basis of sterility was higher than seedling injury. This confirms the findings of Kumar and Dubey (1998) in *Lathyrus sativus*.

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