J Indian bot Soc Vol 77 (1998) 143-145

MUTAGEN INDUCED BIOLOGICAL DAMAGE AND CHLOROPHYLL MUTATIONS IN *VIGNA RADIATA* L. WILCZEK

SAMIULLAH KHAN, MUJEEB-UR-REHMAN, BAHAR A. SIDDIQUI AND SHAMIM A. AZAD
Mutation Breeding Lab., Department of Botany, Aligarh Muslim University, Aligarh 202002 U.P., India
(Accepted December, 1998)

The biological damage (like effect on germination, seedling height and pollen fertility) in M_1 and chlorophyll mutation frequency, effectiveness and efficiency of Ethyl-methane sulphonate (EMS) and sodium azide (SA) was evaluated in two mungbean varieties viz., K-851 and T-44 in M_2 generation. The biological damage increased with the increasing concentrations of both the mutagens. EMS was found most effective and efficient in inducing chlorophyll mutation frequency in both the varieties. The spectrum of chlorophyll mutations consisted of albina, xantha, chlorine and viridis, chlorina type being predominant.

Key Words: Chemical mutagens, biological damage, chlorophyll mutations, effectiveness and efficiency.

Induced mutagenesis has now been accepted as a significant tool to break through the limitations of variability and to create variability in a short period of time. Chlorophyll mutations although not useful for plant breeding purpose, may be used to assess the potency of mutagens. In the present investigation, chlorophyll mutation frequency, effectiveness and efficiency of the mutagens was estimated in two varieties of mungbean using EMS and SA.

MATERIALS AND METHODS

Four hundred seeds of mungbean varieties K-851 and T-44 were treated with 0.1 and 0.3 percent and 0.01 and 0.03 percent concentrations of Ethylmethane sulphonate (EMS) and sodium azide (SA) for 6 hours at room temperature. Prior to mutagenic treatment seeds were presoaked in distilled water for 9 hrs. Three replications of 100 seeds each were sown for every treatment in each variety at the University agricultural Farm. The distance between seeds in a row and between the rows was kept 30 x 60 cm. respectively. In order to determine the percentage of seed germination and seedling height, the remaining lot of 100 seeds of each treatment with their respective controls of both the varieties were spread over moist cotton in petriplates. Finally, the petriplates were kept in the BOD incubator at 27 ± 1°C temperature.

For raising M_2 generation, healthy seeds of both the varieties from each normal looking M_1 plant of all different treatments with their respective controls were planted in the plant progeny rows. Chlorophyll mutations were scored when seedlings were 8-15 days old. They were identified and classified as per Gustafsson (1940)'s classification. Formulae suggested by Konzak *et al.* (1965) were used to evaluate the mutagenic effectiveness and efficiency of the mutagens used.

OBSERVATIONS

M_1 generation: Effect of chemical mutagens on biological damage in M_1 generation is given in Table 1. Irrespective of the varieties involved the effect of EMS and SA on biological damage viz. seed germination, seedling height and pollen fertility was observed in M_1 generation. A dose dependent reduction was noticed for all the treatments in both the varieties (K-851 and T-44) of mungbean. Sodium azide was found to be more effective in reducing seed germination and seedling height in both the varieties. However,

Table 1. Effect of chemical mutagens on seed germination, seedling height and pollen fertility in M_1 generation of mungbean.

Variety	Treatment	Germination *(%)	Pollen fertility (%)	Seedling height (cm) **Mean ± S.E.
K-851		94.66	97.00	6.90 ± 0.017
	0.1% EMS	89.33	89.65	6.50 ± 0.015
	0.3% EMS	85.00	85.00	6.09 ± 0.024
	0.01% SA	88.66	90.00	6.23 ± 0.033
	0.03%	81.33	83.00	6.00 ± 0.073
T-44	Control	92.00	96.55	6.84 ± 0.007
	0.1% EMS	88.33	90.20	6.16 ± 0.005
	0.3% EMS	84.66	86.45	5.92 ± 0.005
	0.01% SA	86.00	92.50	6.10 ± 0.042
	0.03%	79.66	89.65	5.50 ± 0.015

* Three hundred seeds were sown in each treatment and control.
** Mean ± S.E. of 10 randomly selected seedlings.

Received January, 1998

Tabl;e 2. Frequency and spectrum of chlorophyll mutation in M_2 generation of mung bean.

Variety	Treatment	No. of M_1 plant progenies	No. of plant progenies segregating in M_2	% mutant plant progenies (Mp)	No. of M_2 seedling	Chlorophyll mutant types				Total	Frequency (%)
						Albina	Xantha	Chlorina	Viridis		
K-851	Control	25	-	-	674	-	-	-	-	-	-
	0.1% EMS	25	16	64.00	661	1	5	4	2	13	1.96
	0.3% EMS	25	18	72.00	613	2	4	4	5	15	2.44
	0.01% SA	25	11	44.00	623	-	3	5	1	9	1.44
	0.03% SA	25	13	52.00	611	2	-	4	-	6	0.98
T-44	Control	25	-	-	710	-	-	-	-	-	-
	0.1% EMS	25	15	60.00	621	-	2	3	4	9	1.44
	0.03% EMS	25	16	64.00	502	3	1	5	3	12	1.99
	0.01% SA	25	8	32.00	610	-	3	2	3	8	1.31
	0.03%	25	11	44.00	548	1	1	4	3	9	1.64

Table 3. Effectiveness and efficiency of chemical mutagens in mungbean.

Variety	Treatment	(%) seedling injury (I)	(%) Pollen sterility (S)	(%) Mutated plant progenies (Mp)	Mutagenic effectiveness	Mutagenic efficiency Mp/I	Mp/S
K-851	0.1% EMS	5.79	7.57	64.00	1.06	6.59	8.45
	0.3%	11.73	12.37	72.00	3.60	6.21	5.82
	0.01% SA	9.71	7.21	44.00	0.07	3.89	6.10
	0.03% SA	13.04	9.27	52.00	0.26	3.77	5.60
T-44	0.1% EMS	21.24	6.57	60.00	1.00	2.82	9.13
	0.3% EMS	24.48	10.46	64.00	3.20	2.61	6.11
	0.01% SA	22.19	4.19	32.00	0.05	1.44	7.63
	0.03% SA	29.84	7.14	44.00	0.22	1.47	6.16

pollen fertility was affected more in both the treatments of ethylmethane sulphonate. Variety, T-44 was found to be most sensitive with regards to seed germination and seedling height whereas var. K-851 gave maximum reduction in pollen fertility.

M_2 generation

Frequency and spectrum of chlorophyll mutations: Chlorophyll mutations were recorded at the seedling stage in the mutagen treated population (Table 2). The mutation frequency was the highest (1.99%) at 0.3 percent EMS in var.T-44 followed by 0.1 percent EMS (1.96%) in var. K-851. EMS induced maximum frequency of chlorophyll mutations in both the varieties. The spectrum of chlorophyll mutations unduced includes albina, xantha, chlorina and viridis. Chlorina type was predominant in both the varieties followed by xantha viridis and albina types.

Effectiveness & efficiency:

Effectiveness was higher at higher concentrations of both the mutagen while the efficiency decreased with the increase in mutagenic concentrations (Table 3). EMS proved to be more efficient than SA in both the varieties of mungben.

DISCUSSION

Various mutagenic treatments in the present investigation reduced germination, seedling height and pollen fertility at higher concentrations. These findings are in agrement with those reported by earlier workers in various crops like barley (Nilan *et al.*, 1968), lentil (Sinha and Singh 1983, Reddy *et al.*, 1992) green gram (Narang and prakash 1983, Grover and Virk 1984). Reduction in germination following mutagenic treatment may be due to the difference in the balance between promotors and inhibitors of seed germination present in seed coat (Amen 1968) or due to the inhibitory effects of the mutagens on germination (Sree Ramulu, 1972). Both the mutagenic treatments delayed the germination process. Similar observations were made by Chowdhury (1978) in wheat, Kleinhofs *et al.* (1978) reported the delay in initiation of metabolism following germination, resulting in uniform delay in mitotic activity, seedling growth ATP and DNA synthesis. The delay was interpreated as being due to the ATP deficiency. Kumar and Gupta (1978) reported that pollen sterility in *Vigna mungo* was associated with asybaogus and/ or desynapsis,

The frequency of chlorophyll mutations increased with the increasing concentrations of th mutagens. Similar observations were made by subramanian (1980). Chlopophyll mutations may result from alteration in the DNA in the chloroplasts and EMS is known to react preferentially with guanine (Freese 1963). With regard to the effectiveness and efficiency, EMS was more effective as well as efficient than SA in both the varieties. The high efficiency of EMS over physical mutagens was also advocated by Goud (1967) and Uhlik (1972). The high efficiency at lower doses may be attributed to less toxic effects of low doses.

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