

Induced Chlorophyll Mutations in *Vigna marina* (Burm.) Merr.

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A total number of 14, 377 M₂ seedlings from 280 M₁ plant progenies obtained through different doses of gamma irradiation and EMS treatments was studied in M₂ generation. Among the different doses and concentrations tried, 10 Krad was most effective and efficient based on lethality, whereas 25 mM of EMS was most efficient based on injury and sterility. The occurrence of *viridis* and *chlorina* was frequent in treatments with both the above mutagens. The spectrum of chlorophyll mutants consisted of *albina*, *viridis*, *chlorina* and *xantha*.

A vast literature is available on induced chlorophyll mutations in various pulse crops. However, the legumes of fodder value have not been studied adequately and there are no reports on *Vigna marina* (Burm.). So an attempt has been made in this direction in this study.

MATERIAL AND METHODS

Vigna marina (Burm.) Merr. is a short lived perennial legume. Self fertilised seeds with a moisture content of 9.5 per cent were treated with 10, 20, 30, 40, 50, 60 and 70 krad of gamma rays through the gamma cell at the Sugarcane Breeding Institute, Coimbatore, by exposing the seeds to a cobalt 60 gamma source. The dose rate was 0.3×10^6 rads per hour.

The chemical mutagen, Ethyl Methane Sulphonate (CH₂SO₂OC₂H₅) having a molecular weight of 124.16

obtained from East man Kodak Ltd., USA, was used for the treatment of seeds. The pre-soaked seeds (12 hours) were treated with EMS at room temperature $26 \pm 2^\circ\text{C}$ with intermittent shaking. After four hours EMS treatment the seeds were thoroughly washed with running tap water for half an hour and were sown in pots under glass house condition. Thirty days old seedlings were transplanted in the field.

Twenty M₁ plants in each treatment and in control were advanced to M₂ generation and raised as individual progeny rows. The M₂ seedling progenies of individual M₁ plants were examined from 8th to 15th day, for the chlorophyll mutants which were classified according to the system proposed by Gustafsson (1940). The effectiveness and efficiency of mutagens in inducing chlorophyll mutants were estimated after Konzak *et al.* (1965)

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RESULTS AND DISCUSSION

a) Chlorophyll mutation frequency

To estimate the frequency of chlorophyll mutation 14,377 M_2 seedlings raised from 280 M_1 plants were studied and the results are presented in Table I, when expressed as percentage on M_1 plant progeny, the rate of mutation was, in general, higher than that on M_2 seedling basis. For seven doses of gamma rays the rate of mutation on M_1 plant basis ranged from 35.0 to 75.0 per cent and on M_2 seedling basis it varied from 1.8 to 5.6 per cent the highest rate observed being at 20 Krad. In treatments with EMS, the mutation rate on M_1 plant basis was from 25.0 to 65.0 per cent and as M_2 seedling basis, it ranged from 2.05 to 5.80 per cent the highest being in the concentration of 25 mM. The frequencies were high in low doses of both the mutagens and were slightly decreased at high doses, thereby pointing to the elimination of mutations at the highest doses both by haplontic and diplontic sieves.

b) Segregation ratio :

In gamma rays, the mean segregation ratio ranged from 4.08 to 8.92. There was a gradual decrease upto 60 Krad. The relative percentage of segregation, (excepting at 20 krad) was less than 20. In none of the treatments, the percentage exceeded 25. The mean segregation ratio was 6.21 to 8.75, the highest being at the concentration of

25 mM. Lowest doses of both mutagens recorded higher ratios and it decreased with increase in dosage. The reasons for not realising the expected segregation ratio of 25 per cent are minute chromosomal aberrations which affected the gene transmission (Moh and Alan, 1968). The small mutated sector involving more number of initial cells and lethality of mutated cells may be the other cause for the realisation of a lower segregation ratio.

c) Effectiveness and efficiency

The effectiveness and efficiency were the highest at the lowest doses of both the mutagens (10 krad and 25 mM). The greater efficiency of low doses of mutagens appeared to result from the fact that injury, lethality and sterility tended to increase with increase in dosage of any mutagen at faster rates than mutations themselves (Konzak *et al.*, 1965). The usefulness of any mutagen depends upon the mutagenic effectiveness as well as efficiency. Gamma rays were found to be more effective than EMS with respect to the induction of chlorophyll mutations. Similar trend was observed in rice by Siddiq and Swaminathan (1968) and Swaminathan (1969). At the most effective dose of Gamma rays (10 krad) and of EMS (25 mM) the mutagenic efficiency was higher in gamma irradiation when estimated on the basis of lethality. On the other hand, based on injury and sterility, EMS was more efficient than gamma rays.

TABLE I Relative effectiveness and efficiency of gamma rays and EMS in *Vigna marina*

Mutagen	Dose/ Conc.	Mutation rate		Mean segrega- tion ratio	Effective- ness M×100 tc or Krad	Efficiency		
		M1 Plant	M2 basis			M×100 L	M×100 I	M×100 S
Gamma rays :	10 Krad	60.00	5.56	8.92	55.6	556.0	27.3	41.6
	20 Krad	70.00	5.60	7.97	28.0	34.1	22.8	35.6
	30 Krad	65.00	4.95	7.27	16.5	26.2	17.4	22.3
	40 Krad	50.00	3.96	7.08	9.9	15.6	11.3	13.1
	50 Krad	75.00	5.09	6.59	10.2	11.6	8.5	15.5
	60 Krad	50.00	2.19	4.08	3.7	4.0	3.0	6.3
	70 Krad	35.00	1.82	5.73	2.6	2.3	2.2	4.6
	Control	—	—	—	—	—	—	—
EMS	25 mM	65.00	5.80	8.75	5.8	23.6	51.2	59.7
	50 mM	25.00	2.36	7.62	1.2	9.8	13.6	14.3
	75 mM	50.00	3.99	7.80	1.3	11.3	18.2	13.1
	100 mM	35.00	2.05	6.21	0.5	3.6	6.9	5.2
	125 mM	40.00	2.76	6.40	0.6	4.2	7.2	6.4
	150 mM	35.00	2.54	6.79	0.4	3.0	4.6	6.0
	Control	—	—	—	—	—	—	—

M = Mutation rate, t = time, c = concentration, Krad = Kilorad.

L = percentage lethality, I = percentage injury, S = percentage Survival.

TABLE II Spectrum of Chlorophyll Mutation in the M₂ Generation

Mutagens	Dose/ conc.	Total number of chlorophyll mutants	Relative % of chlorophyll mutants			
			Albina	Chlorina	Viridis	Xantha
Gamma rays	10 krad	61	—	42.62	55.74	1.64
	20 krad	66	1.52	56.06	40.90	1.52
	30 krad	49	—	38.78	61.22	—
	40 krad	49	8.16	42.86	38.78	10.20
	50 krad	52	1.92	36.54	57.69	3.85
	60 krad	25	—	60.00	32.00	8.00
	70 krad	15	—	80.00	13.33	6.57
	Control	—	—	—	—	—
EMS	25 mM	59	—	45.76	52.55	1.69
	50 mM	23	—	43.48	52.17	4.35
	75 mM	39	—	56.41	43.59	—
	100 mM	19	—	47.37	42.10	10.53
	125 mM	27	—	59.26	37.04	3.70
	150 mM	25	12.00	52.00	32.00	4.00
	Control	—	—	—	—	—

d) Mutation Spectrum :

The chlorophyll mutants observed were of four types, namely, *albina*, *chlorina*, *xantha* and *viridis*. The relative percentages are presented in Table II. In both the mutagens, the occurrence of *viridis*, and *chlorina* was most frequent. *Xantha* occurred in all treatments except 30 krad of gamma rays and 75 mM of EMS, but the frequency was very low. *Albina* appeared only in the middle doses of gamma rays namely 20, 40 and 50 krad and it is specific to 150 mM of EMS. Rare induction of *albina* by EMS has been reported by Ramaswamy (1973) in black gram. Westergaard (1960) found that the alkylating chemicals induced the less drastic mutations such as *viridis* instead of extreme mutations such as *albina* in higher proportions than those induced by radiations possibly because of their apparently less drastic effect on chromosomes.

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