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RADIOSENSITIVITY OF THE WILD AND CULTIVATED URD AND MUNG BEANS

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ABSTRACT

Seeds of *Vigna sublobata*, *V. radiata* cv. PS 16, and *V. mungo* cv. T 9 were used for the induction of mutations with EMS and gamma rays. Four doses each of these mutagens (EMS: 0.1, 0.2, 0.3 and 0.4%; gamma rays: 10, 20, 30 and 40 kR; EMS + gamma rays: 0.1% + 10 kR, 0.2% + 20 kR, 0.3% + 30 kR, and 0.4% + 40 kR) were employed. Mutagenesis lowered the dehydrogenase activity in M_1 and M_2 plants of all three species. Dose-dependent decrease was observed in seedling emergence, seedling height, survival, and pollen fertility in M_1 and M_2 plants. The spectrum of chlorophyll mutations was narrow; the spectrum and frequencies of chlorophyll mutations increased with dose of each mutagenic treatment. The observations suggested that i) *V. sublobata* is more radioresistant as compared to *V. radiata* and *V. mungo*, ii) *V. radiata* is more radiosensitive than *V. mungo*, and iii) *V. sublobata* is more closely related to *V. mungo* than to *V. radiata*.

Key words: Radiosensitivity, lethality, *Vigna sublobata, V. radiata, V. mungo.*

Genetic variability is the basis for plant breeding, and mutation induction has been used to create variation for utilization in crop improvement. Radiations have been used successfully to induce useful mutations for plant breeding [1–3]. Radiosensitivity is known to vary not only among cultivars, but also among taxonomic groups at different levels of taxonomic hierarchy [4–7]. The properties of the genetic system and the chemical and physical aspects of the mutagens influence radiosensitivity; the genetic control of radiosensitivity has been demonstrated in several crops [8–10].

In the present work, radiosensitivity of *Vigna radiata* (L.) Wilczek, *V. mungo* (L.) Hepper (cultivars), and *V. sublobata* (Roxb.) Babu and Sharma (a wild relative) has been compared using parameters such as dehydrogenase activity, seedling emergence, seedling height (components of seedling vigour), survival, chlorophyll mutations, pollen sterility, and mutagenic effectiveness and efficiency. The effects induced by gamma rays were compared with those induced by a chemical mutagen

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(ethyl methanesulphonate, EMS) used alone and in combination with gamma rays. The term radiosensitivity includes sensitivity due to chemical and physical mutagens.

MATERIALS AND METHODS

Seeds of *Vigna radiata* cv. PS 16, *V. mungo* cv. T 9 (obtained from Pulse Research Laboratory, IARI, New Delhi) and *V. sublobata* (harvested from the garden-grown progenies of a natural population collected from the Palney Hills of Western Ghats, Tamil Nadu) were used for mutation induction. Three mutagenic treatments, EMS alone, gamma rays alone, and EMS + gamma rays combined, were employed in the doses: 0.1, 0.2, 0.3, and 0.4%; 10, 20, 30 and 40 kR; and 0.1%+10 kR, 0.2%+20 kR, 0.3%+30 kR, and 0.4%+40 kR, respectively. The chemical EMS was dissolved in distilled water and 6–8 presoaked seeds were treated for 10 h, thoroughly washed in running water for 12 h, and the excess moisture was blotted off. The moisture content of the seeds for γ-ray treatment was adjusted to 10.5-11.0% by differential drying in an air oven [11]. ^{60}Co was used as the source of gamma rays for the irradiation of seeds at IARI, New Delhi, at the dose rate of 2500 R/min. For the combined treatment, the seeds were first irradiated with gamma rays and then treated with EMS; a control was also maintained. Two replicates of 150 seeds each were maintained for each treatment. The M_1 and M_2 populations were raised in radomized block design.

Lethality was expressed in terms of dehydrogenase activity in germinating seeds, seedling emergence, seedling growth, survival, spectrum and frequency of chlorophyll mutations, and pollen sterility. Dehydrogenase activity was determined by the tetrazolium chloride test [12]. Seedling emergence was recorded 15 days after sowing in the field. Seedling height was measured from 16-day-old seedlings; GR_{50} values were calculated for gamma-ray treatments [13] and survival was estimated in 45-day-old plants. The spectrum and frequencies of chlorophyll mutations were determined following Gustafsson [14] and Blixt and Gottschalk [15]. The vital stain method [16] was used to evaluate pollen sterility. Mutagenic effectiveness and efficiency were calculated according to Konzak et al. [17].

RESULTS

DEHYDROGENASE ACTIVITY

In M_1 and M_2 plants of all the species, the EMS + gamma-ray treatments were the ones which consistently reduced the enzyme activity as compared to the control (Table 1). The enzyme activity was lower in M_2 plants of all three species than in M_1 plants.

SEEDLING EMERGENCE

In M_1 and M_2 plants of all three species, the seedling emergence decreased with increase in the mutagenic dose; the combined EMS + gamma-ray treatment markedly decreased seedling emergence (Fig. 1A and B). The treatments of EMS and gamma rays were more or less equally detrimental in all the three species. In M_1 and M_2 plants of the wild relative, both low and high doses reduced seedling

Table 1. Dehydrogenase activity (expressed in O.D. per 100 ml of the extract) in M_1 and M_2 generations of the three species at different doses

Mutagen	Dose, % or kR	M_1 Generation			M_2 Generation		
		V. sublobata	*V. radiata*	*V. mungo*	*V. sublobata*	*V. radiata*	*V. mungo*
Control	—	14.0 ± 0.3	29.0 ± 0.6	30.0 ± 0.3	13.0 ± 0.7	29.0 ± 0.3	30.0 ± 0.6
EMS	0.1	12.0 ± 0.3	29.0 ± 0.3	20.0 ± 0.6	11.0 ± 0.6	26.0 ± 0.6	22.0 ± 0.6
	0.2	13.0 ± 0.3	26.0 ± 0.7	22.0 ± 0.6	10.0 ± 0.5	20.0 ± 0.7	18.0 ± 0.3
	0.3	14.0 ± 0.7	29.0 ± 0.6	26.0 ± 0.3	9.0 ± 0.5	22.0 ± 0.3	17.0 ± 0.7
	0.4	14.0 ± 0.3	29.0 ± 0.6	24.0 ± 0.9	12.0 ± 0.6	20.0 ± 0.8	25.0 ± 0.8
Gamma rays	10	11.0 ± 0.4	24.0 ± 0.3	24.0 ± 0.7	12.0 ± 0.7	17.0 ± 0.4	28.0 ± 0.7
	20	10.0 ± 0.6	26.0 ± 0.6	18.0 ± 0.6	11.0 ± 0.3	22.0 ± 0.9	24.0 ± 0.8
	30	13.0 ± 0.6	27.0 ± 0.9	23.0 ± 0.8	8.0 ± 0.6	19.0 ± 0.8	21.0 ± 0.7
	40	12.0 ± 0.5	26.0 ± 0.6	20.0 ± 0.3	9.0 ± 0.9	24.0 ± 0.3	26.0 ± 0.6
EMS + gamma rays	0.1 + 10	11.0 ± 0.9	24.0 ± 0.3	24.0 ± 0.6	10.0 ± 0.3	22.0 ± 0.3	30.0 ± 0.3
	0.2 + 20	10.0 ± 0.6	22.0 ± 0.5	22.0 ± 0.7	11.0 ± 0.5	21.0 ± 0.6	26.0 ± 0.6
	0.3 + 30	10.0 ± 0.5	20.0 ± 0.6	24.0 ± 0.8	12.0 ± 0.6	18.0 ± 0.5	26.0 ± 0.6
	0.4 + 40	11.0 ± 0.6	24.0 ± 0.6	19.0 ± 0.6	9.0 ± 0.6	23.0 ± 0.6	24.0 ± 0.5

emergence as compared to the controls, but the values did not differ substantially from the controls in M_1 and M_2 plants of the cultigens at low-dose treatments. Seedling emergence was higher at all doses in M_2 as compared to M_1 plants.

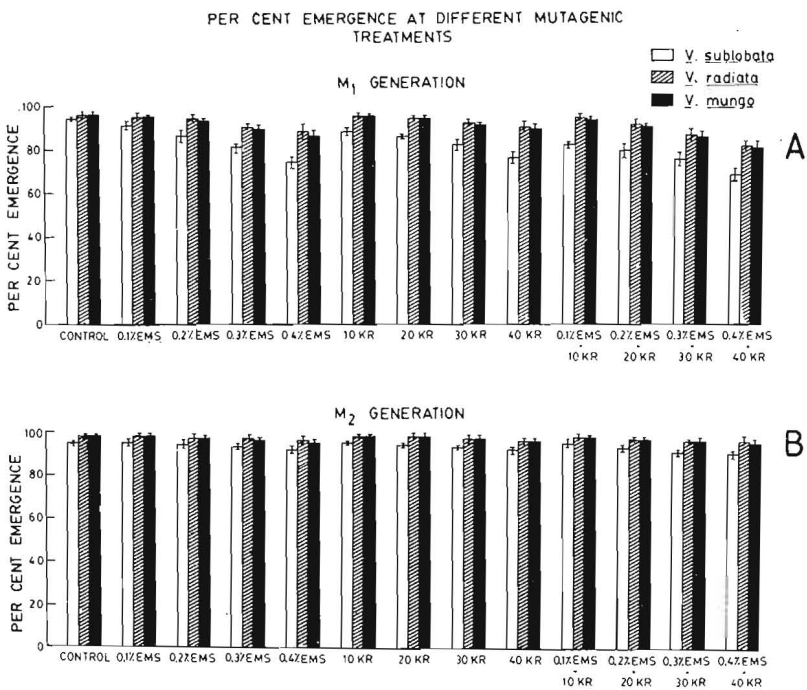

Fig. 1. Percent seedling emergence in the three species at different dose treatments of the mutagens and control. (A) M_1 generation, (B) M_2 generation.

SEEDLING HEIGHT

Seedling height was reduced with all doses as compared to the controls (Fig. 2A and B). The differences between species, treatments, and species × treatments were statistically significant at $P < 0.05$ in M_1 as well as M_2 plants (Table 2). The reduction was markedly higher at EMS + gamma-ray combined treatments. The mean seedling height was lower in the wild species than in the two cultigens at all doses. In M_2 generation of all three species, the pattern of induced variation was similar to that of M_1 plants, except at low dose treatments, where the mean values were higher and almost equal to the controls. The GR_{50} was highest (79 kR) for the wild species; for *V. mungo* it was relatively closer (75 kR) to that of the wild species, whereas *V. radiata* showed the lowest (54 kR) value.

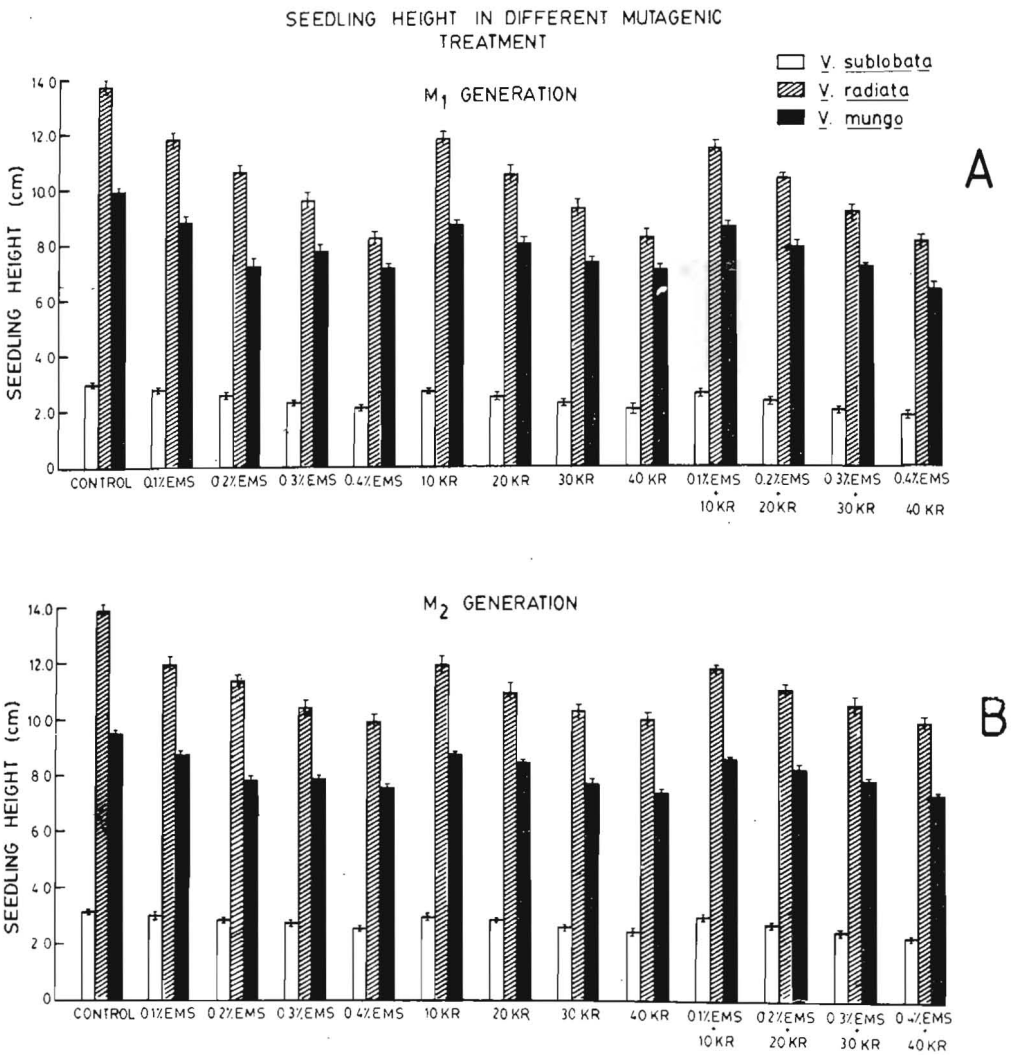

Fig. 2. Mean seedling height in the three species at different dose treatments of the mutagens and control (A) M_1 generation, (B) M_2 generation.

Table 2. Two-way analysis of variance for seedling height in M_1 and M_2 generations of three *Vigna* species

Generation	Source of variation	d.f.	SS	MS	F
M_1	Species and treatments	38	9198.6	242.0	0.059
	Between species	2	8262.4	4131.2	70.34**
	Between treatments	12	704.7	58.7	6.09**
	Species × treatments	24	231.5	9.6	8.93*
	Error	703	765.2	1.0	
	Total	779			
M_2	Species and treatments	38	9714.7	255.6	0.055
	Between species	2	9293.2	4646.6	175.08**
	Between treatments	12	318.4	26.5	6.17**
	Species × treatments	24	103.0	4.2	4.38
	Error	703	692.3	0.9	
	Total	779			

*, ** Significant at $P < 0.05$ and $P < 0.01$, respectively.

PLANT SURVIVAL

All treatments reduced survival of M_1 and M_2 plants in all three species, but the decrease was relatively more for the wild relative than in the cultigens (Fig. 3A and B). The survival of M_2 plants was better than that of M_1 plants in all treatments.

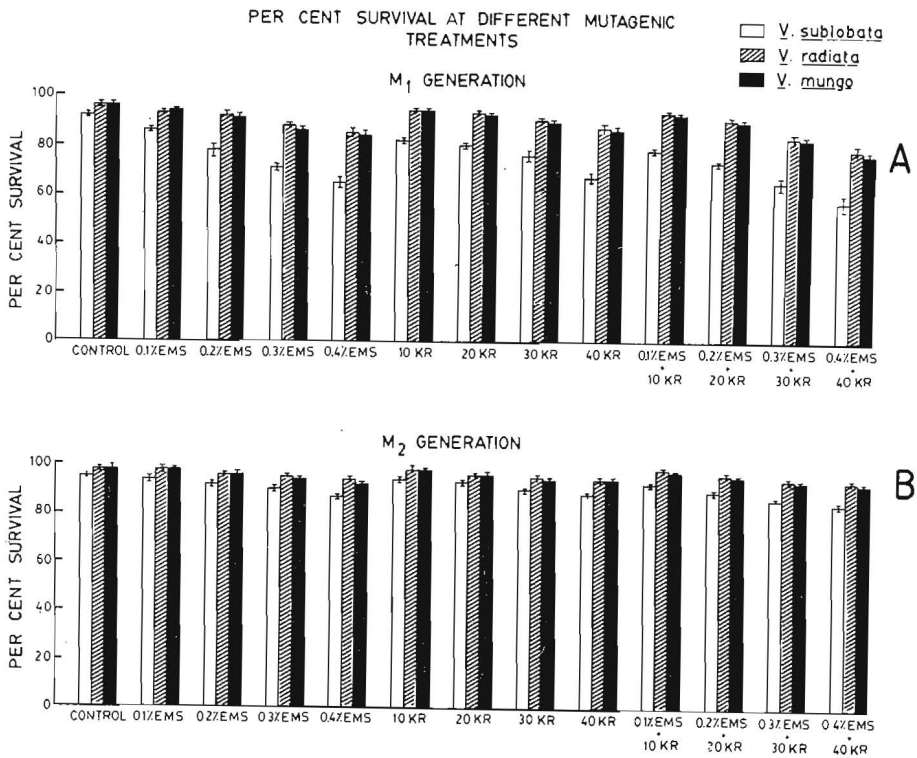

Fig. 3. Percent survival in the three species at different dose treatments of the mutagens and control. (A) M_1 generation, (B) M_2 generation.

POLLEN STERILITY

The pollen sterility increased with the increase in doses in both M_1 and M_2 plants of all the three species; and pollen sterility was lower in M_2 plants as compared to M_1 plants (Table 3). The wild species, *V. sublobata*, showed less pollen sterility both in M_1 and M_2 plants as compared to the cultigens.

Table 3. Induced pollen sterility (% shrivelled, nonstained pollen grains) in M_1 and M_2 generations of three *Vigna* species in different treatments (mean ± SE)

Mutagen	Dose, % or kR	M_1 Generation			M_2 Generation		
		V. sublobata	*V. radiata*	*V. mungo*	*V. sublobata*	*V. radiata*	*V. mungo*
Control	—	2.4 ± 0.2	4.9 ± 0.1	3.5 ± 0.2	2.1 ± 0.2	4.2 ± 0.1	3.5 ± 0.1
EMS	0.1	4.3 ± 0.3	9.7 ± 0.2	8.6 ± 0.3	3.6 ± 0.2	6.5 ± 0.1	5.6 ± 0.2
	0.2	7.2 ± 0.3	14.1 ± 0.3	11.5 ± 0.3	4.2 ± 0.2	9.7 ± 0.2	8.1 ± 0.2
	0.3	9.0 ± 0.3	19.6 ± 0.3	16.4 ± 0.3	6.5 ± 0.4	12.1 ± 0.3	10.6 ± 0.3
	0.4	12.6 ± 0.4	22.9 ± 0.4	19.2 ± 0.5	7.5 ± 0.4	14.4 ± 0.3	13.5 ± 0.5
Gamma rays	10	7.2 ± 0.3	8.9 ± 0.2	6.7 ± 0.2	3.2 ± 0.1	5.4 ± 0.2	5.2 ± 0.2
	20	10.2 ± 0.2	13.6 ± 0.3	9.1 ± 0.2	5.9 ± 0.2	8.8 ± 0.2	6.6 ± 0.2
	30	13.9 ± 0.3	18.9 ± 0.2	12.5 ± 0.3	8.0 ± 0.3	11.1 ± 0.3	9.8 ± 0.2
	40	15.7 ± 0.4	21.7 ± 0.3	15.6 ± 0.4	10.9 ± 0.4	13.5 ± 0.3	17.8 ± 0.3
EMS + gamma rays	0.1 + 10	10.2 ± 0.4	13.2 ± 0.2	12.8 ± 0.5	5.8 ± 0.2	9.8 ± 0.2	7.9 ± 0.3
	0.2 + 20	13.3 ± 0.3	18.4 ± 0.3	17.0 ± 0.5	8.4 ± 0.3	12.4 ± 0.3	11.3 ± 0.3
	0.3 + 30	15.7 ± 0.5	22.7 ± 0.4	20.8 ± 0.5	11.1 ± 0.5	15.3 ± 0.3	14.5 ± 0.4
	0.4 + 40	19.7 ± 0.5	25.6 ± 0.5	23.1 ± 0.8	14.1 ± 0.5	18.5 ± 0.5	17.4 ± 0.5

SPECTRUM AND FREQUENCY OF CHLOROPHYLL MUTATIONS

Altogether six kinds of chlorophyll mutants (albina, xantha, chlorina, chlorotica, viridis and vario-maculata) were recorded from the segregating M_2 plants of the three species; out of the six mutation types, albina and xantha survived only for 10–15 days, and chlorina was lethal or sterile; the other three were viable and fertile. Vario-maculata was recovered only in the wild species (Fig. 4A). The chlorophyll mutation rates were lower in the M_2 generation of the wild relative as compared to the cultigens. For example, *V. radiata* showed 9.6%, *V. mungo* showed 7.5%, and *V. sublobata* showed 5.8% chlorophyll mutations. In all the three species, mutation frequency increased with increasing dose of mutagens, and the increase was 1 to 5 times at high doses (Fig. 4C). The combined EMS + gamma-ray treatment produced higher frequency of mutations than the other two treatments (Fig. 4B). The proportion of M_2 families segregating for chlorophyll mutants increased with increasing dose in all the species tested, and there was a marked difference between the three species, with *V. radiata* recording the highest and *V. sublobata* the lowest values (Fig. 4D).

MUTAGENIC EFFECTIVENESS AND EFFICIENCY

In all three species, the mutagenic effectiveness and efficiency of mutagenic treatments varied not only between treatments of a mutagen, but also between mutagens (Table 4). The most effective mutagenic treatment for the wild species was 0.1% EMS + 10 kR gamma-rays, but the most efficient treatments were 40 kR gamma rays in terms of seedling lethality and pollen sterility, and 30 kR gamma rays in terms of seedling height reduction; for *V. radiata*, the most effective mutagenic

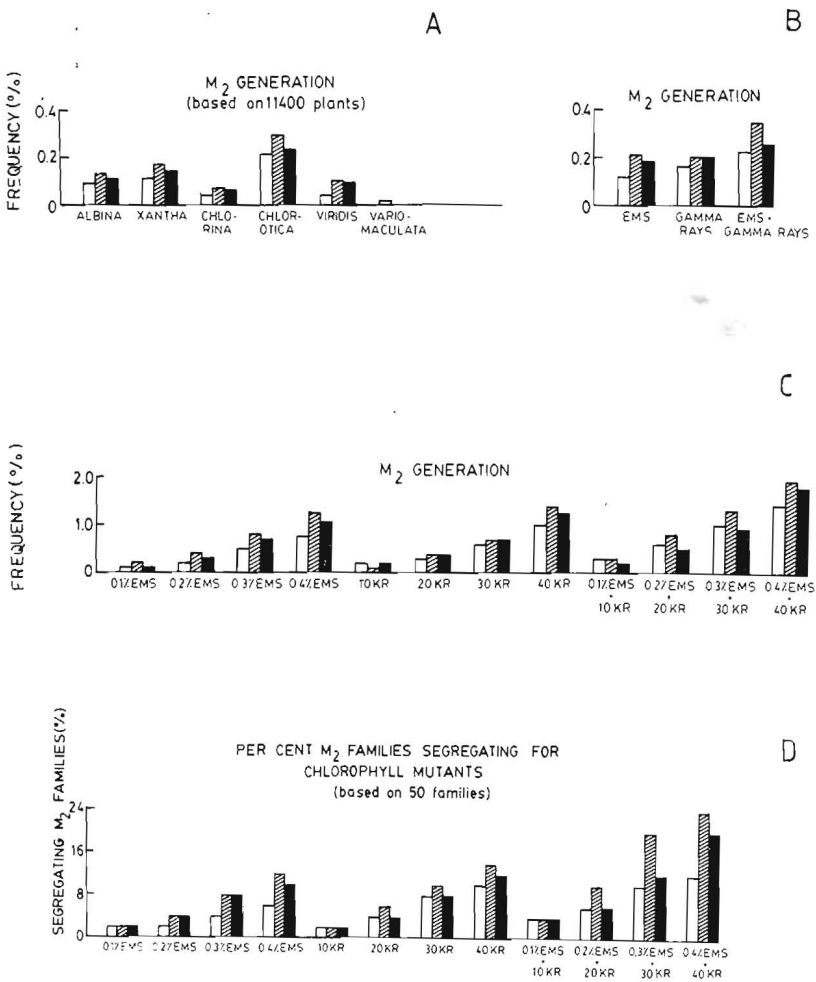

Fig. 4. Chlorophyll mutation frequencies in the species (*Vigna sublobata* □, *V. radiata* ▨ *V. mungo* ■).

A. Mutation frequencies of different chlorophyll mutants.

B. Mutation frequencies based on different mutagens.

C. Mutation frequencies based on different dose treatments.

D. Percent M_2 families segregating for chlorophyll deficient mutants.

treatment was 0.3% EMS + 30 kR gamma rays, but the efficiency was maximum in terms of seedling lethality, seedling height reduction, and pollen sterility with 20 kR gamma rays, 0.3% EMS + 30 kR gamma rays, and 0.4% EMS + 40 kR gamma rays, respectively. For *V. mungo*, the most effective treatment was 0.4% EMS + 40 kR gamma rays, but the most efficient mutagenic treatment in terms of seedling lethality was 40 kR gamma rays and in terms of seedling height reduction and pollen sterility 0.4% EMS + 40 kR gamma rays.

DISCUSSION

The response of plant species to chemical and physical mutagens appears as modification of phenotypes. Some of the mutagen-induced modifications are neutral or beneficial, others detrimental, and still others lethal to the organism. Consequently, the effectiveness and efficiency of the mutagen, and the utility of induced variability depend, to a large extent, on induced lethality, which, in turn, depends on radiosensitivity [18, 19].

Table 4. Relative mutagenic effectiveness and efficiency of different mutagenic treatments in three *Vigna* species (based on M_2 families segregating for chlorophyll mutants)

Mutagen	Dose, % or kR	Mutagenic effectiveness Mf/tc or Mf/kR or Mf/tc + kR			Mutagenic efficiency								
					lethality Mf/L			seedling height reduction Mf/l			pollen sterility Mf/S		
		V.s.	*V.r.*	*V.m.*	*V.s.*	*V.r.*	*V.m.*	*V.s.*	*V.r.*	*V.m.*	*V.s.*	*V.r.*	*V.m.*
EMS	0.1	2.00	2.00	2.00	0.33	0.67	1.00	10.00	1.04	1.03	1.12	0.49	0.62
	0.2	1.00	2.00	2.00	0.14	1.00	0.80	5.00	1.29	1.58	0.53	0.46	0.71
	0.3	1.33	2.67	2.67	0.19	1.00	0.80	6.25	1.92	2.29	0.61	0.57	0.88
	0.4	1.50	3.00	2.50	0.22	1.09	0.83	7.23	2.17	2.51	0.64	0.72	0.83
Gamma rays	10	0.20	0.20	0.20	0.20	1.00	1.00	8.00	1.02	0.92	0.51	0.47	0.39
	20	0.20	0.30	0.20	0.33	2.00	1.00	9.09	1.85	1.34	0.58	0.65	0.50
	30	0.27	0.33	0.27	0.50	1.67	1.14	11.76	2.24	2.22	0.75	0.68	0.62
	40	0.25	0.35	0.30	0.63	1.56	1.20	11.11	2.54	3.05	0.76	0.77	0.76
EMS + gamma rays	0.1 + 10	4.40	4.40	2.20	0.29	1.33	0.50	11.11	1.69	0.94	0.54	0.48	0.23
	0.2 + 20	3.30	5.50	3.30	0.32	1.67	0.86	9.34	2.90	2.06	0.55	0.74	0.44
	0.3 + 30	3.66	7.32	4.40	0.37	1.54	0.86	10.20	4.28	3.51	0.75	1.12	0.69
	0.4 + 40	3.30	6.60	5.50	0.34	1.33	1.00	11.65	4.15	4.89	0.69	1.16	1.02

V.s. — *Vigna sublobata*, *V.r.* — *Vigna radiata*, *V.m.* — *Vigna mungo*; Mf — per cent frequency of chlorophyll mutations; t — duration of time for chemical mutagenic treatment; c — concentration of chemical mutagen; kR — dose of the physical mutagen; L — per cent lethality as estimated by survival rate; l — reduction in seedling height; and S — per cent pollen sterility.

SEEDLING VIGOUR, SURVIVAL, CHLOROPHYLL MUTATIONS AND LETHALITY

Mutagenesis altered dehydrogenase activity in both M_1 and M_2 plants of all three species. The enhanced activity at high doses is perhaps due to gene interactions which arise due to the positive effect of mutated genes and the low levels of activity as compared to controls, particularly in M_2 plants of the wild relative, might be

due to deletions. The pattern of induced variability suggests that mutations might have also occurred at regulatory loci rather than in structural genes.

There was a dose-dependent decrease in seedling emergence, seedling height, and survival of M_1 and M_2 plants of all the three species. Similar observations are reported in several crops including grain legumes [7, 10, 20, 21]. However, Rathnaswamy et al. [22] did not find such a linear relationship in *V. radiata*. The higher seedling vigour of M_2 plants as compared to M_1 plants does suggest that natural selection eliminated the recessive homozygous lethals. The detrimental effects of mutagenesis at seedling stage have been reported in a number of crops [22–24]. The reduction in mean seedling height in M_1 is mainly due to physiological injury in the seed and seedlings. This is evident from the approximation of the mean values of M_2 plants to those of the controls and the increase of variance in M_2 as compared to M_1. The statistically significant variance ratios between species and between treatments in M_1 and M_2 plants suggest differential responses of the species to different mutagenic treatments. Similar observations have been reported in wild and cultivated rice [25] and in different cultivars of *V. radiata* [26].

In all three species, the spectrum of chlorophyll mutations was narrow, but some previous studies [7, 27, 28] revealed as many as 7–8 types of mutations (only 6 types observed in the present study). In legumes, fairly high proportion of chlorophyll mutants belong to chimeras [20]. The conflicting reports on the spectrum and frequency of chlorophyll mutations in a single species may be due to genotypic differences between the cultivars used by different workers. There is a dose-dependent increase in the spectrum and frequency of chlorophyll mutations in M_2 of all the three species. Similar observations have been reported in other legumes [7, 26]. The low frequency of albina and the induction of vario-maculata mutations in the wild relative but high frequency of albina with total absence of vario-maculata in the cultigens support the genotypic specificity of mutagens. However, the similarity in frequency and spectrum of chlorophyll mutations among all the species suggests homology among the genomes of the three species. The recessive nature of chlorophyll mutations has been demonstrated earlier [27, 28].

RADIOSENSITIVITY AND MUTAGENIC EFFECTIVENESS AND EFFICIENCY

Mutagenic sensitivity depends on the genotype, while mutagenic effectiveness and efficiency depend on mutagenic sensitivity, which, in turn, is influenced by the kind and dose of mutagen. The nonrandom pattern of variation in mutagenic effectiveness and efficiency demonstrates that the genotypic responses to different mutagens are of genetic origin and depend on the physical and chemical properties of the mutagens. In M_1 plants, there is a dose-dependent increase in the effectiveness and efficiency. Similar observation was reported in *V. radiata* [7, 29].

Among the different mutagenic treatments, the combination of EMS + gamma rays was the most potent both in terms of effectiveness and efficiency. This can be explained by synergistic effect of EMS and gamma rays [28, 30, 31], as i) the first mutagenic treatment makes accessible otherwise nonavailable sites for reaction to the second mutagen; and ii) premutational lesions induced by the first treatment are

fixed due to an inhibitory effect of the subsequent treatment [32]. However, Khalatkar and Bhatia [33] argued that the synergistic effect is not due to increased uptake of the chemical mutagen as a result of radiation-induced alterations in the membranes, but probably due to the interaction of radiation and chemically induced lesions.

Gamma rays are more effective than EMS, but the latter is more efficient than the former. A similar observation was recorded by Siddiq and Swaminathan [34]. Bahl and Gupta [28] found that chemical and physical mutagens are equally effective in mungbean. The dose-dependent increase in mutagenic effectiveness and efficiency in all three species demonstrates that the mutation rate per unit dose of radiation has not reached the saturation point. The mutagenic specificity can be explained in terms of the concept of selection sieves [35] or that the active genes are more accessible to certain mutagens than repressible or inactive genes.

In terms of chlorophyll mutations and pollen sterility, the wild relative is more radioresistant than the cultigens. *V. radiata* is the most radiosensitive, whereas the radiosensitivity of *V. mungo* is close to that of the wild relative; for dehydrogenase activity, *V. mungo* is more radiosensitive than *V. radiata* and *V. sublobata;* and for seedling emergence and plant survival, the wild relative is more radiosensitive than the cultigens. Thus, the extent of radiosensitivity varies depending on the character used for comparison. Therefore, it is essential to evaluate radiosensitivity of the species based on the total mutagenic effect on the phenotype rather than based on a single trait, such as, germination or seedling height alone [36].

The low effectiveness and efficiency of all three mutagenic treatments in the wild relative and the high effectiveness and efficiency in *V. radiata* confirm that the wild relative is more and *V. radiata* least radioresistant; the effectiveness and efficiency of mutagens in *V. mungo* were higher than in the wild relative, suggesting that *V. mungo* is also less radioresistant than the wild relative. These observations are in agreement with those deduced from meiotic chromosomal abnormalities, nuclear volume and nuclear DNA amounts [37].

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