

## Mutagenicity of gamma rays and E. M. S. and their combination in Bengalgram (*Cicer arietinum L.*)\*

K. K. VADIVELU<sup>1</sup> and M. RATHINAM<sup>2</sup>

Physical (gamma rays) and chemical (EMS) mutagens and combination of these two (Gamma+EMS) reduced the percentage of germination, survival, seedling growth, drymatter production and seed fertility in bengalgram with increase in dose of mutagens. EMS produced greater reduction than gamma rays. Combination treatments showed enhanced effect compared to single treatments. Among the varieties CO. 1 was more sensitive than G 62404. A linear dependence for all the characters studied on dosage was evident in both the varieties. The same trend was noted in the induction of chlorophyll mutations. Chemical mutagen was more potent in inducing chlorophyll mutations.

The relative effectiveness and efficiency of the radiations and chemicals in inducing mutations are being studied on an intensive scale on a variety of crop species to find out the potentialities of mutation breeding for crop improvement. Such studies on induced mutations in pulse crops have been comparatively few, particularly in bengalgram (*Cicer arietinum*), in which the natural variability is very much restricted. As such, a study was made on the relative sensitivity of two cultivars of bengalgram and on the frequency of chlorophyll mutations induced by the chemical and physical mutagens and their combinations.

### MATERIALS AND METHODS

The particulars of the two varieties (CO 1 and G 62404) of bengalgram and the mutagens (gamma rays and EMS) used are presented in Table I and II,

respectively. Treatments with gamma rays were given with a <sup>60</sup>Co r cell of 1000 curie, delivering 5000 rads/minute. The treatments with EMS were given in phosphate buffer (pH 7). The seeds were presoaked for 4 hours and treated with the chemical mutagen for 8 hours, with continuous shaking, the volume of the chemical being 10 times that of the seeds. For combination treatments, the seeds treated with gamma rays at required doses were then soaked in chemical solutions for 8 hours.

After treatment, the seeds were washed for 30 min. in running water and sown in germination trays or in field. The M<sub>1</sub> was studied for the following characteristics to determine the relative effects of the mutagens: (i) germination and seeding height on the 10th day; (ii) survival of seedlings on the 30th day; (iii) dry matter production

---

\* Part of Ph. D. thesis submitted by the first author to Tamil Nadu Agricultural University, Coimbatore.

1. Department of Seed Technology and (2) Department of Forestry, Tamil Nadu Agricultural University, Coimbatore.

of 10 matured plants; and, (iv) seed fertility. The mean values of 10 plants selected at random were considered for the seedling growth, dry matter production and seed fertility. Chlorophyll mutations were scored in  $M_2$  when the seedlings were 10 to 15 days old.

## RESULTS AND DISCUSSION

Mutagenic sensitivity has been known to be influenced by a variety of factors, of which the type of mutagen and dose, moisture content of the seed, treatment conditions and genotype of the material are important. The effect of physical and chemical mutagens on the one hand and their combination treatments on the other, as measured by reduction of germination, survival, seedling growth, drymatter production and seed fertility, were considered as the main indices for over all response.

### (i) *Germination and survival :*

The percentage of germination and survival in the  $M_1$  generation decreased with increasing dose of the mutagens (Table I) in the varieties CO 1 and G 62404. The germination and survival indicated the varietal response to mutagenic treatments through progressive reduction with increasing doses of gamma rays. Such reduction was noticed at higher doses of gamma rays in bengalgram (Athwal, 1963; Anon., 1971; Mujeeb, 1974), greengram (Krishnaswamy, 1977). However, in the present study, increased germination was observed in the lower doses (10, 20, and 30 k rad) of gamma rays in G 62404. Stimulatory effects were reported in ben-

galgram (Mujeeb, 1974) and *Phaseolus vulgaris* (Hussain and Disoski, 1976).

The chemical mutagen, EMS reduced the germination and survival at higher concentrations and the inhibition of germination and survival showed linearity with increasing concentrations. Negative association between EMS concentration and germination and survival was reported in peas (Selima *et al.*, 1974), blackgram (Ramaswamy, 1973) and greengram (Krishnaswamy, 1977). The reduction in germination and survival due to combination treatments was more than that in the individual treatments of the mutagens. Such effects due to combination treatments were reported in pea (Selima *et al.* 1974). The reduction in germination and survival was more drastic due to combination treatments than single treatments, thus indicating an additive effect of enhanced toxicity.

### (ii) *Seedling growth :*

The height of the seedlings decreased with increasing doses of the mutagens, showing that seedling injury is positively correlated with dose (Table I). The chemical mutagen (EMS) and combination treatments showed enhanced effect as compared to gamma rays treatment. Similar findings were reported with gamma rays in redgram (Nadarajan, 1976); with EMS in redgram (Shrivastava, 1975; Srinivasan, 1977); and with combination treatments in redgram (Srinivasan, 1977).

### (iii) *Dry matter production*

The dry matter decreased with increasing doses of the mutagens and it

was found to be more drastic under combinations treatments (Table 1).

#### (iv) Seed fertility

The seed fertility decreased with increase in dose of the mutagens (Table I) revealing a linear dependence of fertility on dose as found by Krishnaswamy (1977) in greengram. EMS caused more drastic effects on fertility than gamma rays. The dose range of 50 to 60 m/M caused more than 50% sterility, while gamma rays at 60 krad did not induce 50% sterility, which shows that the bengalgram varieties are sensitive to chemical mutagens (EMS). Combination treatments showed antagonistic effect on seed sterility in the varieties. Similar response to combination treatments was noticed by Ramaswamy (1973) in blackgram, Krishnaswamy (1977) in greengram and Srinivasan (1977) in redgram. The sterility in  $MM_1$  plants might have resulted from cytological aberrations of very low order.

#### (v) Chlorophyll mutations in $M_2$

A critical comparison of the chlorophyll mutations indicates that mutation rate, in general, increased with an increase in dose of gamma rays, EMS concentration and strength of combination (Table II). This trend was observed in both the varieties. Such effects were reported in several crop plants. It was attributed to the rigour of both diplantic and haplantic selection in the biological material (Swaminathan, 1961). Among the mutagens used in the present study, EMS was found to be the most potent and induced more number of mutations.

Combination treatments were found to be more effective in inducing more number of mutations than single mutagens.

The results suggested that the frequency of mutations is governed by the genetic architecture of the material used, as reported by Davies (1962) and Chao and Chai (1961). The two varieties CO 1 and G 62404 differed considerably in their mutagenic response as evidenced from the rate of survival, seedling growth and sterility. When each variety was considered independently, it was further obvious that the mutation frequency increased with increase of seedling injury or lethality in  $M_1$ .

#### REFERENCES

- ATHWAL, D. S. 1963. Some X-ray induced and spontaneous mutations in *Cicer*. *Indian J. Genet.*, **23**: 50-57.
- ANON. 1971. *New Vistas in Pulse Production*. Indian Agricultural Research Institute, New Delhi. pp. 67-70.
- CHAO, C. Y. and S. W. CHAI 1961. Cytological and genetical changes induced by X-rays and thermal neutrons in rice. *Botan. Bull Acad. Sinica*, **2**: 15-25.
- DAVIES, D. R. 1962. The genetical control of radio sensitivity. II. Growth measurement in *Lycopersicum* and *Melandrium*. *Radiation Botany*, **1**: 277-95.
- HUSSAIN, E. C. and I. A. M. DISOSKI 1976. Mutation breeding experiments in *P. Vulgaris* L. EMS and 'r' ray induced seed coat colour mutants. *Zeitschrift fur. Pflanzl Zuchtung*, **76**: 190-99.
- MUJEEB, K. A. 1974. Gamma radiation induced variation in some morphological and nutri-

- tional components of *Cicer arietinum* L. (Cv. Chhola). *Experientia*. **30**: 819-92.
- KRISHNASWAMY, S. 1977. Studies on induced mutations in greengram. (*Vigna radiata* (L) Wilczek.). Ph. D. Thesis, Tamil Nadu Agric. Univ., Coimbatore.
- NATARAJAN, N. 1976. Induced mutagenesis in redgram (*Cajanus cajan* (L.) Millsp.) micro and macromutants. M. Sc. (Ag.) Thesis. Tamil Nadu Agric. Univ., Coimbatore.
- RAMASWAMY, N. M. 1973. Investigations on induced mutagenesis in black gram (*Phaseolus mungo* L.) Ph. D. Thesis, Tamil Nadu Agric. Univ., Coimbatore.
- SELIMA, A. R., H. A. S. HUSSAIN, and I. I. S. El. SHAWARF I. I. S. 1974. EM and gamma ray induced mutation in *Pisum Sativum* L. II. Effects of EMS and gamma rays on  $M_1$  germination seedling height and fertility. *Egyptian J. Genet. and Cytol.*, **3**: 172-92.
- SHRIVASTAVA, M. P. 1975. Effect of gamma irradiation on diploid and tetraploid seeds of *Cajanus cajan* (L.) Millsp. *Curr. Sci.* **44**: 167-68.
- SRINIVASAN, K. 1977. Studies on induced mutagenesis in redgram (*Cajanus Cajari*. (L.) Millsp.). M. Sc. (Ag.) Thesis, Tamil Nadu Agric. Univ., Coimbatore.
- SWAMINATHAN, M. S. 1961. Effect of ciplantic selections on the frequency and spectrum of mutations induced in polyploids following seed irradiations. *Symp. Effects of Ionizing Radiations on Seeds and Significance for Crop Improvement*, IAEA, Vienna, pp. 279-88.

TABLE I. Effect of gamma rays and EMS on various characters in M<sub>1</sub> (Per cent over control)

Treatments	Germination		Survival		Seedling height		Dry matter		Seed fertility																																																								
	CO.1	G62-404	CO.1	G62-404	CO.1	G62-404	CO.1	G62-404	CO.1	G62-404																																																							
Control	100.0	100.0	100.0	100.00	100.0	100.0	100.0	100.0	100.0	100.0																																																							
<i>Gamma rays</i>																																																																	
10	96.0	100.7	95.0	96.0	105.9	102.3	103.1	100.0	81.6	79.7																																																							
20	91.4	101.9	89.2	96.8	90.2	99.0	98.5	97.3	73.4	71.9																																																							
30	86.9	115.4	81.6	96.5	97.7	98.4	90.3	85.7	71.1	66.3																																																							
40	75.7	87.2	66.4	70.5	75.2	93.1	71.3	69.2	64.3	66.4																																																							
50	44.9	72.8	38.0	47.4	72.8	81.3	49.9	38.0	64.2	63.8																																																							
60	47.8	61.5	28.9	37.8	61.8	69.4	42.5	36.2	55.1	57.8																																																							
<i>EMS</i>																																																																	
10	86.7	102.3	90.2	96.5	83.0	80.4	91.3	80.5	73.8	72.7																																																							
20	82.0	97.8	85.6	96.2	82.4	59.9	79.3	77.5	70.5	70.5																																																							
30	78.5	85.7	77.3	80.7	79.4	53.6	71.8	69.9	70.2	67.6																																																							
40	64.1	64.6	51.7	51.6	69.1	53.6	63.0	57.9	59.9	61.5																																																							
50	55.4	57.9	39.9	43.2	54.5	46.1	41.5	34.8	55.1	54.9																																																							
60	48.0	48.7	32.2	35.7	49.7	42.7	39.3	46.2	46.2	47.1																																																							
<i>Combination</i>																																																																	
10+10	82.6	104.6	80.6	91.6	77.9	73.1	90.3	88.6	69.7	67.5																																																							
10+20	79.9	92.2	80.3	90.9	74.8	68.4	81.2	76.5	68.9	70.2																																																							
10+30	64.3	64.3	36.7	42.8	50.0	54.2	45.8	28.5	57.5	55.4																																																							
20+10	78.5	87.6	73.9	78.8	53.8	55.2	64.2	70.8	60.2	59.9																																																							
20+20	62.5	85.1	63.6	74.3	55.6	49.9	58.0	49.6	54.9	54.1																																																							
30+10	52.1	63.7	24.5	33.3	47.0	39.7	28.1	15.4	48.5	48.9																																																							
<table border="0" style="width:100%; border-collapse: collapse;"> <tr> <td style="width:10%;"></td> <td style="width:10%;">SE</td> <td style="width:10%;">CD</td> <td style="width:10%;">SE</td> <td style="width:10%;">CD</td> <td style="width:10%;">SE</td> <td style="width:10%;">CD</td> <td style="width:10%;">SE</td> <td style="width:10%;">CD</td> <td style="width:10%;">SE</td> <td style="width:10%;">CD</td> </tr> <tr> <td>Varieties</td> <td>0.81</td> <td>1.60</td> <td>0.39</td> <td>0.78</td> <td>0.11</td> <td>0.32</td> <td>0.17</td> <td>0.35</td> <td>0.42</td> <td>0.84</td> </tr> <tr> <td>Treatments</td> <td>2.91</td> <td>5.76</td> <td>1.37</td> <td>2.73</td> <td>0.37</td> <td>0.73</td> <td>0.59</td> <td>1.18</td> <td>1.43</td> <td>2.86</td> </tr> <tr> <td>Doses</td> <td>2.05</td> <td>4.07</td> <td>1.00</td> <td>1.98</td> <td>0.26</td> <td>0.52</td> <td>0.59</td> <td>1.18</td> <td>1.01</td> <td>2.02</td> </tr> <tr> <td>Varieties X Doses</td> <td>2.91</td> <td>5.76</td> <td>1.36</td> <td>2.74</td> <td>0.36</td> <td>0.74</td> <td>0.34</td> <td>0.68</td> <td>1.43</td> <td>2.86</td> </tr> </table>												SE	CD	SE	CD	SE	CD	SE	CD	SE	CD	Varieties	0.81	1.60	0.39	0.78	0.11	0.32	0.17	0.35	0.42	0.84	Treatments	2.91	5.76	1.37	2.73	0.37	0.73	0.59	1.18	1.43	2.86	Doses	2.05	4.07	1.00	1.98	0.26	0.52	0.59	1.18	1.01	2.02	Varieties X Doses	2.91	5.76	1.36	2.74	0.36	0.74	0.34	0.68	1.43	2.86
	SE	CD	SE	CD	SE	CD	SE	CD	SE	CD																																																							
Varieties	0.81	1.60	0.39	0.78	0.11	0.32	0.17	0.35	0.42	0.84																																																							
Treatments	2.91	5.76	1.37	2.73	0.37	0.73	0.59	1.18	1.43	2.86																																																							
Doses	2.05	4.07	1.00	1.98	0.26	0.52	0.59	1.18	1.01	2.02																																																							
Varieties X Doses	2.91	5.76	1.36	2.74	0.36	0.74	0.34	0.68	1.43	2.86																																																							

TABLE II. Frequency of Chlorophyll mutations in M<sub>2</sub> generation

Treatments (KR/mM)	CO.1				G62-404			
	Number of M <sub>1</sub> plants scored and segregating	No. of M <sub>2</sub> Plants scored and segregating	Mutation frequency (%)		Number of M <sub>1</sub> plants scored & segregating	No. of M <sub>2</sub> plants scored & segregating	Mutation frequency (%)	
			M <sub>1</sub> plant basis	M <sub>2</sub> plant basis			M <sub>1</sub> plant basis	M <sub>2</sub> plant basis
Control	100	1015	—	—	100	3600	—	—
Gamma 10	100	3635	16	06.9	100	3560	22	0.75
" 20	100	3736	24	0.86	100	2946	25	0.90
" 30	100	3512	31	1.08	100	3112	27	0.97
EMS 20	100	2966	40	2.09	100	3122	24	1.15
" 30	100	2796	42	2.43	100	3086	45	1.81
Combination								
Gamma + EMS								
10 + 10	100	3010	36	2.26	100	2948	47	2.04
10 + 20	100	2946	46	2.41	100	2888	57	2.36

S. E. = M<sub>1</sub> 6.25 M<sub>2</sub> 0.19  
 C. D. = M<sub>1</sub> 14.14 M<sub>2</sub> 0.43