

## An Appraisal of Indices for Optimal Dose Range in $M_1$ of *Vigna marina*, a Forage Legume

P. VINDHIYA VARMAN<sup>1</sup>, P. CHANDRASEKARAN<sup>2</sup> and S. KRISHNASAMY<sup>3</sup>

The sensitivity of the fodder legume *vigna marina* (Burm.) Merr. to Physical and chemical mutagens were studied by treating dry seeds with gamma rays and presoaked seeds with EMS in  $M_1$  generation. The  $LD_{50}$  for survival ranged between 50 to 60 krad of gamma rays and between 75 to 100 mM of EMS. The reduction in seedling height and fertility was proporsinate to the increase in dosage in both the mutagens. The doses, where the inhibition of plumule length was 20 to 35 Per cent, were found to be an optimal for breeding purposes. The use of 10 to 20 krad of gamma rays and a concentration less than 25 mM of EMS may be optimal dose for this crop.

*Vigna marina* (Burm.) Merr. is a short lived perennial legume. It 's rapid early growth is an out standing feature. It is highly palatable at all stages of growth and therefore a potentially useful fodder legume. In mutation breeding a pre-requisite is the basic informations on the type and doses of mutagens. Information on the use of physical and chemical mutagens on *Vigna marina* is not available at present. Investigations have, therefore, been taken up on induction of mutations in this useful forage.

### MATERIAL AND METHODS

Gamma rays and Ethyl Methane Sulphonate were selected for the present study. Gamma rays irradiation was done at the gamma cell installed at the Sugarcane Breeding Institute, Coimbatore by exposing the seeds to a Cobalt<sup>60</sup> Gamma source for the required duration. The chemical mutagen Ethyl Methane Sulphonate ( $CH_3SO_3OC_2H_5$ ) having a molecular weight of 124.16

obtained from East man Kodak Ltd., USA was used for the treatment of seeds  
a) Gamma Irradiation:

Well filled seeds with a moisture content of 9.5 per cent were chosen for irradiation. The doses were 10 krad to 70 krad with 10 krad interval. Three hundred seeds were irradiated in each dose and sown immediately in three replications, along with control.

### b) EMS

The Seeds were pre-soaked for 12 hours in distilled water. The solution of EMS concentrations ranging from 25 to 150 mM with 25mM interval was prepared in glass distilled water. The volume of the mutagen solution was three times to the volume of the seed. The mutagen solution was prepared without any buffer. The duration of chemical treatment was four hours and performed at room temperature

\* Part of M.Sc. Ag. Theseis submitted to Tamil Nadu Agricultural University, Coimbatore.

1. Research Associate, Cashew Research Station, Vridhachalam. 2. Dean (Post Graduate)  
3. Associate Professor, Forest Research Station, Mettupalayam.



( $26 \pm 2.0$ ) with intermittent shaking during the treatment. The seeds were thoroughly washed with running tap water for half an hour after the treatment. Immediately after washing, the seeds were sown in three replications, along with control.

Germination counts were taken on 10th day and survival on 30th day. Seedling height was measured on 30th day. pollen fertility determined by acetocarmine stainability method. Those taking the stain were considered as fertile and expressed in percentage. Seed fertility was estimated from ten pods selected at random. The percentage values were transformed into angular sines and subjected to statistical analysis.

In a laboratory experiment, radicle and plumule length were measured on 10th day after sowing. Thirty seedlings from each treatment were taken from paper media. The length of radicle and plumule was measured individually for all seedlings. The plumule length was measured from collar region to the tip of the cotyledon leaf and radicle length from the collar region to the tip of the root.

## RESULTS AND DISCUSSION:

### a) GERMINATION AND SURVIVAL

In gamma rays, the germination in the lowest dose of 10 krad showed stimulatory effect (105.2 per cent). But the germination was seen to reduce with the increasing dosage to an extent of 65.9 per cent in the highest dose of 70 krad (Table 1). A similar decreasing trend was noticed for survival also, The doses above 50 krad markedly affected the survival. Fifty per cent survival was observed in 50 to 60 krad.

In EMS, a linear effect was evident in both germination and survival. But a stimulatory effect in germination was noticed in 25 mM. Fifty percent germination was observed around 100 mM, where as 50 per cent survival was observed between 75 to 100mM.

The germination and survival reduction with increase in the doses of both the mutagens were already reported by Ramaswamy (1973) in *Vigna mungo* Palanisamy (1975) in *Vigna sinensis*. Stimulatory effects at lower doses are not of uncommon occurrence as observed by Rao (1968) in *Vigna sinensis* and Mujeeb (1974) in *Cicer arietinum*.

### b) SEEDLING HEIGHT:

There is significant reduction in the seedling height in gamma rays treatment as the dose increased, the inhibition in growth being more pronounced at higher doses (17.4 per cent in 70 krad). In EMS, the reduction in the mean height was gradual and maximum at 150 mM with 47.2. per cent (Table 2). Various explanations have been offered for the reduced growth following mutagenic treatment of seeds. Inhibition of DNA synthesis (Mikaelson, 1968) and reduction of reactivity to IAA (Miura *et al* 1974) were considered responsible.

### c) POLLEN FERTILITY:

The reduction in Pollen fertility was substantial in treatment with both mutagens. In gamma rays, the decrease was drastic recording 52.4 per cent at the highest dose of 70 krad. In chemical mutagen also the trend was same and at 150 mM the fertility was 53.8 per cent.



## d) SEED FERTILITY:

As in the case of pollen fertility, in seed fertility also the increase in doses resulted in reduction in fertility. The decrease was more (60.7 per cent and 57.3 per cent) in 70 krad and 150mM respectively.

The causes for a greater degree of  $M_1$  sterility under radiation treatment are mainly chromosomal, both detectable and cryptic (Gaul *et. al.* 1966.)

## I) RADICLE AND PLUMULE LENGTH:

The mean values of length of radicle and plumule following mutagen treatments are given in Table 3. A gradual reduction in radicle length was observed in gamma rays treatments. The length was drastically affected in higher doses. It was 77.3 per cent in 10 krad and 33.3 percent in 70 krad dose of gamma rays. Similarly, plumule length also showed a linear trend with doses. It was 91.2 per cent in the lowest dose (10 krad) and 40.7 per cent in the highest dose (70 krad).

In EMS treatment also, similar dose event relationship was seen. The reduction of radicle length was very drastic in the highest concentration (1.3 per cent in 150 mM). The same was the trend in plumule length also.

Though both radicle and plumule length were affected, the effect was greater in EMS treatment for both characters than in gamma rays irradiation. However, the reduction in radicle length was found to be more when compared to that of plumule length. Thus the roots may be more sensitive to radiation

damage than shoots as reported by prasad and Das (1973) in *Lathyrus sativus*.

Brunner (1975, 1977) reported that in the species with epicotylous type of germination, epicotyl length was the most sensitive criterion for assessing radiation damage; where for species with hypocotylous type of germination total shoot height was most useful criterion. The shoot or epicotyl reduction to 20 to 35 per cent in  $M_1$  is considered indicative of efficient dose range used for inducing mutations.

Hence, the type of germination in *Vigna marina* is *hypogeal*, the doses, where the inhibition of plumule length to an extent of 20 to 35 per cent may be considered an optimal for breeding purposes. In the case of gamma rays 29.7 per cent reduction was observed at 20 krad and 44.5 per cent at 25mM. Low doses of 10 and 20 krad induced high amount of genetic variation in most of the characters studied. Where as the lowest concentration of EMS 25mM also induced moderate amount of variation. Besides, in that dose range chlorophyll mutations, segregation ratio, viable mutations and micro mutations, also high (Those particulars were published elsewhere in the same journal) Since, the reduction was higher even in the lowest concentration of 25 mM, the dose itself seems to be high for economic breeding purposes. So, by considering all these facts, for *Vigna marina* 10 to 20 krad of gamma rays and a concentration less than 25mM of EMS to induce mutations for the improvement of characters was indicated from the present study.



## REFERENCE

- BRUNNER, H. 1975 Radio sensitivity of grain legume species, *Mutat. Breeding News letter* 5, 7-9.
- BRUNNER, H. 1977 Mutagenicity of ethyl methane sulfonate and Sodium azide in grain legumes, *Mutat. Breeding News letter* 9, 8-9.
- GAUL, H., K. BENDER, E. ULONSKA and M. SATO. 1966 EMS-Induced genetic variability in barley, the problem of EMS-induced sterility and a method to increase the efficiency of EMS treatment. *Mutations in plant Breeding*. (Proc. Panel. Vienna, 1966) IAEA Vienna 63-84.
- MIKAELSON, K. 1968 Effects of fast neutrons on seedling growth and metabolism in barley. *Neutron irradiation of seeds II* (Tech. Rep. series No. 92) IAEA, Vienna 63-70.
- MIURA, K., I. HASHIMOTA, and H. YAMAGUCHI 1974. Effect of gamma irradiation on cell elongation and auxin level in *Avena coleoptiles*. *Radiat. Bot.* 14: 207-215.
- MUJEEB, K. A. 1974. Gamma radiation induced variation in some morphological and nutritional components of *Cicer arietinum* L. Cv. *Chhola*. *Experientia* 30: (8) 891-92.
- PALANISAMY, G. A. 1975 Investigation on the induction of mutations in Gowpea (*Vigna sinensis* L. SAVI) M.Sc. Ag. Thesis submitted to Tamil Nadu Agricultural University Coimbatore.
- PRASAD, A. B. and A. K. DAS. 1973. Induced mutations in different varieties of *Lathyrus sativus* L. *Genetics* 74: (211) S. 218.
- RAMASWAMY, N. M. 1973 Investigations on induced mutagenesis in blackgram (*Phaseolus mungo* L.) Ph. D. THESIS submitted to Tamil Nadu Agrl. University, Coimbatore.
- RAO, J. P. 1968 Studies on the mutagenic effects of X-rays and diethyl sulphate on cowpea. M.Sc. Ag. Dissertation, Madras Univ.



TABLE I Effect of Mutagens on Germination and Survival in  $M_1$  Generation

Mutagen (Dose/Conc)	Germination		Survival	
	Mean percentage (Transformed)	Percentage of control	Mean percentage (Transformed)	Percentage of control
<b>GAMMA RAYS (krad)</b>				
Control	57.0 (70.3)	100.0	55.0 (67.0)	100.0
10	59.4 (74.0)	105.2	54.5 (66.3)	99.0
20	51.6 (61.3)	87.2	48.5 (56.0)	83.6
30	52.7 (63.3)	90.1	47.5 (54.3)	81.1
40	50.0 (58.7)	83.4	44.7 (50.0)	74.6
50	44.9 (50.3)	71.6	37.6 (37.7)	56.2
60	45.0 (50.0)	71.1	29.1 (23.7)	35.3
70	42.9 (46.3)	65.9	21.9 (14.0)	20.9
'F' Value	25.01**		71.12**	
SE	1.59		2.12	
CD	4.67		6.23	
<b>EMS (mM)</b>				
Control	57.7 (71.3)	100.0	56.4 (69.3)	100.0
25	61.8 (77.7)	100.9	47.2 (54.3)	78.4
50	49.4 (57.7)	80.9	45.4 (50.7)	73.1
75	40.6 (42.3)	59.3	40.6 (42.3)	61.1
100	35.0 (33.0)	46.3	30.6 (26.0)	37.5
125	32.0 (28.3)	39.7	26.2 (19.7)	28.4
150	25.6 (19.3)	27.1	21.1 (13.0)	18.8
'F' Value	33.17**		83.89**	
SE	2.36		1.38	
CD	7.25		4.24	

\*\* Significant at 1 per cent level.

In parenthesis : Retrans formed mean per cent.



TABLE 2 Pollen and seed Fertility (M<sub>1</sub>)

Mutagen (Dose/Conc)	Height of seedling (30th day)		Pollen Fertility		Seed Fertility	
	Mean (cm)	% of control	Mean% (Transformed)	% of control	Mean% (Transformed)	% of control
GAMMA RAYS (krad)						
Control	22.8	100.0	70.38	(88.7)	63.6	(80.2)
10	18.1	79.6	65.81	(83.2)	56.5	(69.5)
20	17.2	75.4	62.30	(78.4)	55.3	(67.6)
30	16.3	71.6	60.35	(75.5)	52.3	(62.4)
40	14.8	65.0	52.12	(62.3)	48.4	(55.9)
50	9.1	39.7	52.00	(62.1)	47.2	(53.8)
60	5.9	25.9	49.65	(58.1)	46.5	(52.5)
70	4.0	17.4	42.99	(46.5)	44.2	(48.7)
'F' Value	4.44**		40.74**		9.80**	
SE	3.10		1.44		2.06	
CD	9.39		4.37		6.36	
EMS (mM)						
Control	21.3	100.0	67.9	(85.9)	63.6	(80.2)
25	19.1	89.6	58.7	(72.9)	58.4	(72.4)
50	17.2	80.8	56.1	(68.8)	55.9	(66.9)
75	16.2	75.8	51.2	(60.7)	48.4	(55.9)
100	14.3	67.3	47.2	(53.9)	44.2	(48.7)
125	12.4	57.9	45.1	(50.2)	42.5	(45.7)
150	10.1	47.2	42.8	(46.2)	42.7	(46.0)
'F' Value	22.92**		4.68**		27.58**	
SE	0.81		4.08		1.61	
CD	2.48		12.57		4.96	

In paranthesis : Retransformed mean percentage:

\*\* Significant at 1% level

\* Significant at 5% level



TABLE 3 Effect of Mutagens on Radicle and plumule length  
(Laboratory experiment)

Mutagen Dose/Conc)	Radicle length		Plumule length	
	Mean(cm)	%of mean	Mean(cm)	%of mean
<b>GAMMA RAYS (krad)</b>				
Control	6.6	100.00	9.1	100.00
10	5.1	77.27	8.3	91.21
20	4.3	65.15	6.4	70.33
30	3.6	54.55	5.5	60.44
40	3.3	50.00	5.1	56.04
50	2.4	36.36	4.4	48.35
60	2.8	42.42	4.1	45.05
70	2.2	33.33	3.7	40.66
'F' Value	98.0**		506.33**	
SE	0.16		0.10	
CD	0.48		0.29	
<b>EMS (mM)</b>				
Control	8.0	100.00	9.9	100.00
25	4.7	58.75	5.5	55.55
50	2.3	28.75	2.5	25.25
75	1.5	18.75	1.8	18.18
100	0.7	8.75	1.3	13.13
125	0.3	3.75	0.6	6.06
150	0.1	1.25	0.4	4.04
'F' Value	356.42**		597.67**	
SE	0.15		0.14	
CD	0.47		0.34	

\*\* Significant at 1 per cent level.