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Effect of Physical and Chemical Mutagens on Seed Germination and Seedling growth of Garden Bean

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Abstract: The present investigation was carried out to find the LD_{50} value, seed germination and seedling growth induced by Gamma rays and EMS in *Lablab purpureus* (L.) Sweet var. *typicus* cv. CO (Gb) 14. The study was performed by exposing the seeds with various doses of Gamma rays (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50kR) and different concentrations of EMS (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50mM) along with control. The effect of mutagens on germination was observed on 7^{th} day and seedling characters like, shoot length and root length were recorded on 7^{th} day and 15^{th} day after sowing. The germination percentage and seedling growth decreased with increase in doses/concentrations of the mutagens. Mean performance of different quantitative traits were better in control when compared with the treated plants. The lethal dose 50 (LD_{50}) value was found at 25kR of gamma rays and 30mM of EMS.

Key Words: LD_{50} value, Gamma rays, EMS, Seed germination, Seedling growth and *Lablab purpureus* (L.) Sweet var. *typicus* cv. CO (Gb) 14

INTRODUCTION

Lablab purpureus (L.) Sweet (2n = 22) belongs to the family Fabaceae and is one of the most ancient crops among cultivated plants. The wild forms of *Lablab* are believed to have originated in India[1] and

were introduced into Africa from Southeast Asia during the eighth century[2]. It has been widely distributed to many tropical and subtropical countries where it has become naturalised[3].Within India, *Lablab* as a field crop mostly confined to the peninsular region and cultivated in a large extent in Karnataka and adjoining districts of Tamil Nadu, Andhra Pradesh and Maharashtra[4]. *Lablab* bean is an important food source in tropical Africa and Asia where it is being used as a grain legume and vegetable as well as for animal fodder and green manure in mixed crop-livestock systems[5]. *Lablab* grains and pods are rich in proteins (20-28%) and vitamins[6].

The protein in *Lablab* has high levels of amino acids like lysine (6.2%) which is low in cereal grains. It can therefore play a major role in improving the diets of vulnerable rural communities in developing countries especially in sub-Saharan countries who mainly rely on starch-based diets with minimal animal protein. Protein isolated from the bean can be used as a food additive for improving cake quality[7]. *L. purpureus* is used as a nitrogen-fixing green manure to improve soil quality. It not only produces nitrogen through fixation, but returns nitrogen through leaf decay[8]. It is cultivated either as a pure crop or intercropped with finger millet, groundnut, castor, corn, pearl millet or sorghum. It is an excellent quality crop for fattening both sheep and cattle, and is also regarded as good feed for milking cows.

In recent years, induction of mutations employing mutagens has widely been accepted as an excellent tool for creating genetic variability and as supplementary approach in the crop improvement programmes in number of plants[9]. Induced mutagenesis has assumed an improvement role in plant breeding by increasing variability in plants[10]. The main advantage of mutation breeding is the possibility of improving one or two characters without changing the rest of the genotype[11].

In mutagenesis, mutagens *viz*., physical (Gamma rays) and chemicals such as Ethyl Methane Sulphonate (EMS) and Methyl Methane Sulphonate (MMS) are most frequently used[12]. In view of these research aspects, many mutant varieties have been developed through mutagenesis. Among them, 94% were following the treatments of physical mutagen, 5% through chemical mutagen and the remaining 1% through a combined treatment of physical and chemical mutagens[13].

Gamma rays are electromagnetic radiations with the shorter wavelength (shorter than X-rays). It is one of the important physical agents used to improve the characters and productivity of many plants[14]. Irradiation also been successfully used for mutation breeding of various crops and ornamental plants[15] and has proven an adept means of encouraging the expression of recessive genes and producing new genetic variations[16]. Ethyl Methane Sulphonate (EMS) is a well potential mutagen, widely employed in induction of genetic variability; it is an alkylating agent and induces high frequencies of base pair substitutions.

Mutation breeding has contributed significantly to plant improvement. According to Lagoda[17], 2,700 mutant varieties were officially released from 170 different plant species in more than 60 countries and recorded in the FAO/IAEA Mutant Varieties Database (MVD).

MATERIALS AND METHODS

Experimental plant material selected for the present investigation was *Lablab purpureus* (L.) Sweet var. *typicus* cv. CO (Gb) 14. Seeds of this variety were procured from Tamilnadu Agricultural University (TNAU), Coimbatore. 350 seeds were used for each each dose/concentration. Dry, uniform seeds were irradiated with different doses of gamma rays (5KR, 10KR, 15KR, 20KR, 25KR, 30KR, 35KR, 40KR, 45KR and 50KR) with a radioisotope ^{60}Co at Sugarcane Breeding Institute (ICAR), Coimbatore. For chemical mutagen treatments, healthy seeds of uniform size were presoaked for 6 hours in distilled water and treated with different concentrations of EMS (5mM, 10mM, 15mM, 20mM, 25mM, 30mM, 35mM, 40mM, 45mM and 50mM) for 4 hours with intermittent shaking at room temperature.

After treatment, the seeds were thoroughly washed in running tap water to remove the excess of mutagen. Untreated dry seeds were presoaked in distilled water for 6 h and used as control. Out of 350 seeds in each treatment, 50 seeds were kept in Petri - dishes over the moist germinating paper. The seeds were sown in a field at a spacing of 30 x 15 cm in randomized block design replicated thrice. Three replications with 100 seeds / replication sown in field were used for recording field experiment data. The assessment of seed germination percentage was made on 7th day. Shoot length and root length of treated and control plants were recorded on 7th and 15th day after sowing.

Seed germination and seedling growth: The assessment of seed germination of treated and control seeds were observed. The Germination was considered to have occurred when the hypocotyls emerged out from the seed. The germinated seeds for each treatment and control were counted on 7th day and the germination percentage was worked out. The shoot length was measured from the cotyledonary node to the tip of the top-most leaf of the plant and the root lengths were measured from the cotyledonary node to the tip of the primary root of randomly selected seedlings on 7th and 15th day after sowing and expressed in cm /seedling.

RESULTS AND DISCUSSION

In control, the percentage of germination was 92. Among the concentrations 5KR treated seeds showed the highest germination percentage (Table-1) than the other treatments. Gradual reduction in germination percentage was observed from lower to higher dosage of gamma rays and EMS treatments and it is also reported in *Sesame*[18] and Okra (Bhendi) [19]. Among the two mutagens, EMS was proved to be more effective in reducing the seed germination than the Gamma rays. Similar inhibitory effect on seed germination by the mutagens has also been reported earlier in chickpea[20], cowpea[21], urdbean[22] and mungbean[23]. 25KR of gamma rays and 30mM of EMS treated seeds showed 50 per cent of germination **(Table – 1)**. Hence, it was considered as LD_{50} value for gamma rays and EMS treated seeds respectively.

Reduction in shoot length and root length were recorded in the control and treated plants. The highest reduction of shoot length and root length was observed in 50KR of gamma rays and 50mM of EMS **(Tables 2&3)**. The decreased seedling height with increased dose of gamma rays was also observed in

Lablab purpureus[24]. The reduction in length of root and shoot was attributed to the effects of mutagens on the physiological system[25]. Such a reduction in length of root and shoot arising out of mutagenic treatments was previously reported in crop plants[26].

Table-1: Effect of Mutagens on Seed Germination in *Lablab purpureus* (L.) Sweet var. *typicus* cv. CO (Gb) 14

Mutagens	Treatments dose/ conc.	No. of Seeds Sowed	No. of Seeds Germinated On 7th day	Percentage Of Seed Germination	Reduction over Control
Control	-	50	46	92	-
Gamma Rays	5KR	50	43	86	6.5
	10KR	50	38	76	17.3
	15KR	50	34	68	26.0
	20KR	50	28	56	39.1
	25KR	50	25	50	45.6
	30KR	50	22	44	52.1
	35KR	50	20	40	56.5
	40KR	50	17	34	63.0
	45KR	50	15	30	67.3
	50KR	50	12	24	73.9
EMS	5mM	50	41	82	10.8
	10mM	50	38	76	17.3
	15mM	50	34	68	26.0
	20mM	50	32	64	30.4
	25mM	50	29	58	36.9
	30mM	50	25	50	45.6
	35mM	50	20	40	56.5
	40mM	50	18	36	60.8
	45mM	50	14	28	69.5
	50mM	50	11	22	76.0

Table-2: Effect of Gamma rays and EMS on Shoot length (cm / Seedling) in *Lablab purpureus* (L.) Sweet var. *typicus* cv. CO (Gb)14

Mutagen	Treatment dose / conc	7th day Shoot length			15th day Shoot length		
		Range	Mean ±SE	Reduction over control	Range	Mean± SE	Reduction over control
Control	-	21.2 – 25.2	24.14±0.52	-	29.8 – 35.4	33.97±0.69	-
Gamma Rays	5KR	20.8 – 24.5	23.46±0.39	2.81	29.3 – 35.1	32.59±0.67	4.06
	10KR	20.3 – 24.1	22.78±0.54	5.63	28.9 – 34.7	31.61±0.76	6.94
	15KR	19.9 – 23.8	22.28±0.49	7.70	28.2 – 34.2	31.31±1.05	7.83
	20KR	19.5 – 23.2	21.98±0.58	8.94	27.7 – 33.8	30.18±0.74	11.15
	25KR	19.0 – 22.9	21.39±0.45	11.39	27.2 – 33.1	29.97±0.82	11.77
	30KR	18.5 – 22.4	20.72±0.44	14.16	26.7 – 32.7	29.06±0.69	14.45
	35KR	17.9 – 22.1	19.99±0.38	17.19	26.4 – 32.2	28.87±0.65	15.01
	40KR	17.2 – 21.7	18.84±0.64	21.95	26.0 – 31.7	27.88±0.74	17.92
	45KR	16.5 – 21.1	17.84±0.62	26.09	25.4 – 31.2	27.09±0.90	20.25
	50KR	15.7 – 20.8	17.15±0.65	28.95	24.9 – 30.7	26.24±0.74	22.75
EMS	5mM	19.3 – 24.0	21.64±0.70	10.35	28.7 – 34.6	31.97±0.65	5.88
	10mM	18.5 – 23.7	20.49±0.72	15.12	28.3 – 34.2	31.32±0.84	7.80
	15mM	18.0 – 23.1	20.15±0.73	16.52	27.8 – 33.9	30.77±0.81	9.42
	20mM	17.7 – 22.6	19.86±0.57	17.72	27.3 – 33.4	29.43±0.74	13.36
	25mM	17.2 – 22.1	18.84±0.53	21.95	26.9 – 32.9	29.00±0.78	14.63
	30mM	16.6 – 21.8	18.65±0.59	22.74	26.3 – 32.3	27.71±1.03	18.42
	35mM	16.1 – 21.2	18.32±0.59	24.10	25.9 – 31.8	27.22±0.74	19.87
	40mM	15.7 – 20.6	17.96±0.64	25.60	25.2 – 31.2	26.22±1.05	22.81
	45mM	15.3 – 20.1	17.52±0.47	27.42	24.8 – 30.7	26.16±0.34	23.16
	50mM	14.8 – 19.8	16.71±0.64	30.77	24.2 – 30.2	25.78±0.66	24.10

Table-3: Effect of Gamma rays and EMS on Root length (cm / Seedling) in *Lablab purpureus* (L.) Sweet var. *typicus* cv. CO (Gb)14

Mutagen	Treatment dose / conc	7th day Root length			15th day Root length		
		Range	Mean ±SE	Reduction over control	Range	Mean± SE	Reduction over control
Gamma Rays	Control	8 – 15	13.34±0.75	-	9 – 17.0	14.44±0.45	-
	5KR	7.9 – 14.5	12.95±0.66	2.92	8.5 – 16.7	14.12±0.99	2.21
	10KR	7.6 – 14.0	12.59±0.66	5.62	8.0 – 16.3	13.94±0.41	3.46
	15KR	7.4 – 13.9	12.13±0.66	9.07	7.7 – 16.0	13.45±0.97	6.8
	20KR	7.1 – 13.7	11.6±0.95	13.04	7.4 – 15.7	12.93±1.14	10.45
	25KR	6.5 – 13.4	11.3±0.90	15.29	7.0 – 15.0	12.55±0.70	13.08
	30KR	6 – 13.0	10.72±0.98	19.64	6.6 – 14.6	11.73±0.97	18.76
	35KR	5.5 – 12.5	9.93±1.10	25.5	6.4 – 14.2	11.36±0.96	21.32
	40KR	5.3 – 12.0	9.57±0.90	28.2	6.0 – 13.7	11.03±0.91	23.6
	45KR	5.0 – 11.5	9.11±0.89	31.70	5.6 – 13.1	10.74±0.84	25.62
	50KR	4.5 – 11.0	8.77±0.81	34.25	5.2 – 12.6	10.48±0.82	27.42
EMS	5mM	7.5 – 14.1	12.51±0.97	6.22	8.1 – 16.2	13.79±0.99	4.50
	10mM	7.3 – 13.7	11.92±0.77	10.64	7.7 – 16.0	13.19±1.18	8.65
	15mM	7 .0 –13.3	11.11±0.78	16.71	7.4 – 15.6	12.68±1.32	12.18
	20mM	6.5 – 12.7	10.57±0.98	20.76	7.0 – 15.4	12.05±1.00	16.55
	25mM	6 – 12.3	9.90±1.32	25.78	6.7 – 14.6	11.77±1.35	18.49
	30mM	05.8 – 12	9.46±0.74	29.08	6.2 – 14.0	11.40±0.98	21.05
	35mM	5.5 – 11.7	9.19±1.01	31.10	5.9 – 13.6	10.53±1.03	27.07
	40mM	5.1 – 11.3	8.87±0.89	33.50	5.4 – 13.1	10.23±1.23	29.15
	45mM	4.7 – 11	8.39±0.48	37.10	5.1 – 12.7	9.87±1.12	31.64
	50mM	4.3 – 10.7	7.83±0.43	41.30	4.8 – 12.2	9.64±0.81	33.24

CONCLUSION

The present investigation was carried out to study the significant effect of Gamma rays and EMS on seed germination and seedling growth in *Lablab purpureus* (L.) Sweet var. *typicus* cv. CO (Gb) 14. Seed germination and seedling growth decreased with increase in dose / concentration of mutagens. Based on the seed germination percentage, LD_{50} value was found out as 25KR of Gamma rays and 30mM of EMS.

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