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Effect of gamma irradiation and EMS on germination, seedling length and seedling vigour index in vegetable Mochai (*Lablab purpureus* var. *lignosus* L.)

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Abstract
The experiment was carried out in the department of Vegetable Science, TNAU, Coimbatore with an objective to determine the LD_{50} value and the effect of mutagen on germination percentage, seedling length and seedling vigour index with respect to varying doses of Gamma rays and Ethyl methane sulfonate. The seeds of HA-3 were treated with six doses of gamma rays *viz.*, 100 Gy, 150 Gy, 200 Gy, 250 Gy, 300 Gy, 350 Gy and six concentrations of Ethyl Methane Sulfonate *viz.*, 10 mM, 20 mM, 30 mM, 40 mM, 50 mM and 60 mM. The treated seeds were sown in protray and sand tray which was placed in the germination chamber to fix the lethal dose value (LD_{50}) by using probit analysis. Based on the values obtained, it is concluded that the treatment 277.678 Gy of gamma irradiation and 41.541 mM of EMS concentration was identified as LD_{50} value.

Keywords: Gamma irradiation, Ethyl Methane Sulfonate, LD_{50}, *Lablab purpureus* var. *lignosus*

Introduction
Field Bean (*Lablab purpureus* var. *lignosus* (L.)) belongs to the family Fabaceae. It is a cool-season versatile legume crop. It was grown in the tropical areas of Asia, Africa, and Australia. According to Naeem *et al*., (2009) [12] and Bello-Perez *et al*., (2007) [3], dolichos bean is also referred to as Field bean, Hyacinth bean, Country bean, Indian bean, Egyptian bean, Sem, Wal, Avare, Avarai, etc.

There are two types of dolichos beans which was distinguished based on the appearance and texture of the pods along with the angle at which the seeds are attached to the suture of the pods. These are (1) *Lablab purpureus* var. *typicus* and (2) *Lablab purpureus* var. *lignosus*.

Lablab purpureus var. *typicus* which is mainly cultivated for the consumption of pods as vegetable. *Lablab purpureus* var. *lignosus* is bushy type annual which is mainly cultivated for dry seeds. The pods have the characteristic fragrance and aroma. According to Uday Kumar *et al*. (2016) [15], trans-2-dodecenoic and trans-2-tetradecenoic acids are two main fatty acids that are responsible for the pod's fragrance.

Existence of genetic variability is a pre-requisite to the breeder for any crop improvement programme. Being a highly self-pollinated crop, there exist a low degree of variability in lab-lab so mutation serves as a potential method of crop improvement. Besides, mutation breeding has gained much popularity in recent years. A mutation refers to the changes in the genetic material, often leading to a structural modification within a gene. Genetic variations arise due to alterations in the fundamental gene sequence.

In this study the main objective is to standardise the LD_{50} value in lab-lab by employing gamma rays and EMS as the source of mutagens.

Materials and Methods
The experiment was carried out in Department of Vegetable Science, TNAU, Coimbatore during (February to June) 2023. Lethal dose (LD_{50}) value and its impact on shoot length, root length and seedling vigour index were observed. Based on the mortality percentage probit analysis was calculated. The variety HA-3 were used for the present study. The treated seeds were sown at the spacing of 30x45cm.

Gamma rays and Ethyl Methane Sulfonate (EMS) were used as a source of mutagens to induce the mutation and the procedure was explained clearly in the detail given below.

Gamma rays

The seeds of variety HA-3 were irradiated with Gamma rays at National Research Centre for Banana, Trichy. Cesium-137 (^{137}Cs) was used as the source of radiation. The seeds were irradiated with series of doses *viz*., 100Gy, 150Gy, 200Gy, 250Gy, 300Gy, 350Gy. The untreated seeds were considered as control.

Ethyl methane sulfonate

The seeds of HA-3 were soaked in distilled water for 6hrs. Then the pre-soaked seeds were treated with EMS and allowed for 6hrs. The treated seeds were washed with running water for 30 mins. Different concentration of EMS *viz*., 10 mM, 20 mM, 30 mM, 40 mM, 50mM and 60mM were used for the treatments. The seeds which were soaked in phosphate buffer were considered as control.

Lethal dose (LD_{50}) value

To study the sensitivity of *Lablab purpureus* var. *lignosus* for the gamma irradiation and Ethyl methyl sulfonate, LD_{50} value was identified. The treated seeds were sown in the portray and sand tray method. The experimental design followed in this study was CRD with 3 replications. The seeds were sown immediately after treated with mutagen. The portrays were placed in the shade net house and sand tray method was carried out in the germination room where temperature was maintained at 25±1 °C with a light intensity of 1000 lux and relative humidity of 95%. The germination percentage was recorded after seven days of sowing.

Seedling vigour index(I)

Seedling vigour index for every treatment was determined according to the following formula (Abdul-Baki and Anderson, 1973) [1].

Vigour index = [Mean of root length (cm) + Mean of shoot length (cm)] × Percentage of seed germination.

Results and Discussion

The germination percentage of vegetable mochai seeds was notably affected by varying levels of irradiation. This impact was observed seven days after planting, both in sandtray and protray. The results obtained for seedling length and vigour index were furnished below

Determination of LD_{50} value

Based on mortality percentage, LD_{50} value was determined by using probit analysis. It was found to be that LD_{50} value for gamma rays was 277.678Gy and for EMS it was expected to be 41.541 mM. From the table 1. it is observed that mortality percentage increased with increase in dose of gamma rays. The values ranged from 28% (100Gy) to 60% (350Gy). Table.2. indicated that the mortality percentage of EMS was found to be maximum in 50mM (60%). The 20mM showed the maximum number of abnormalities in sand tray method. The decrease in germination percentage could be due to the impact of mutagens on the seed meristematic tissues as reported by (Laskar *et al.,* 2023) [8]. Similar results were reported by the earlier publications of Prasath *et al*., (2019) [13] and Bonde *et al.*, (2020) [5] in mung bean.

Table 1: Probit analysis for the determination LD_{50} value of gamma irradiation in *Lablab purpureus* var. *lignosus* (L.)

Treatments	No. of seeds sown	No. of dead seeds	Mortality percentage	Log_{10} dose	Probit value
T_0 -Control	25	3	12		
T_1 - 100 Gy	25	7	28	2.00	4.42
T_2 - 150 Gy	25	8	32	2.18	4.53
T_3 - 200 Gy	25	10	40	2.30	4.75
T_4 - 250 Gy	25	11	44	2.40	4.85
T_5 - 300 Gy	25	13	52	2.48	5.05
T_6 - 350 Gy	25	15	60	2.54	5.25

Table 2: Probit analysis for the determination LD_{50} value of EMS in *Lablab purpureus* var. *lignosus* (L.)

Treatments	No. of seeds sown	No. of dead seeds	Mortality percentage	Log_{10} dose	Probit value
T_0 -Control	25	2	8		
T_1 - 10 mM	25	7	28	1.00	4.42
T_2 - 20 mM	25	12	48	1.30	4.95
T_3 - 30 mM	25	10	40	1.48	4.75
T_4 - 40 mM	25	10	40	1.60	4.75
T_5 - 50 mM	25	15	60	1.70	5.25
T_6 - 60 mM	25	14	56	1.78	5.15

Shoot length(cm)

The data on shoot length observed in sandtray method and portray was represented in table.3. The 100Gy irradiated seedlings observed the maximum shoot length in sandtray (14.80 cm) and portray (14.50 cm). Higher dose of 350Gy recorded the minimum of 11.00cm in sandtray and 11.80cm in portray. In EMS treated seedlings, observed the maximum shoot length in minimum dose of 10mM in both sandtray (12.00 cm) and protray (12.40 cm). The minimum shoot length of 7.50 cm (sandtray) and 7.60cm (protray) was observed respectively. Chemical mutagens are more regional specific in contrast to physical mutagens which were gene specific (Umavathi and Mullainathan, 2014) [16]. Similar findings were reported by Ramya and Nallathambi (2014) [14] in black gram, Mahla *et al.*, (2018) [9] in cluster bean and Atteh and Adeyeye (2022) [2] in broad bean.

Table 3: Effect of mutagen on shoot length (cm)

Mutagen	Treatment	Sand tray method	Roll towel method
Gamma rays	T_0 - Control	16.40	15.70
	T_1 - 100 Gy	14.80	14.50
	T_2 - 150 Gy	14.20	14.40
	T_3 - 200 Gy	13.10	13.60
	T_4 - 250 Gy	13.70	13.90
	T_5 - 300 Gy	12.30	12.80
	T_6 - 350 Gy	11.00	11.80
	Mean	13.72	13.80
	S.E(m)	0.67	0.47
EMS	T_0 - Control	14.20	14.00
	T_1 - 10 mM	12.00	12.40
	T_2 - 20 mM	10.50	11.20
	T_3 - 30 mM	10.80	10.50
	T_4 - 40 mM	10.40	10.30
	T_5 - 50 mM	9.50	9.20
	T_6 - 60 mM	7.50	7.60
	Mean	10.73	10.77
	SE(m)	0.77	0.78

Root length (cm)

The data on root length was presented in table.4. In gamma rays irradiated seedling, maximum root length was observed in sand tray (14.30cm) and protray (14.70 cm) whereas, minimum root length was observed in 350 Gy with lengths of 11.40cm and 11.20cm respectively. The EMS treated seedlings showed the reduced root length when compared to gamma rays. The minimum root length was observed in 60 mM as 8.10 cm (sandtray) and 8.20cm (protray). The results were similar to the findings of Girija and Dhanavel, 2013 [6] in cowpea, Bind and Devmani (2014) [4], Monica and seetharaman (2015) [10] in garden bean.

Table 4: Effect of EMS on root length(cm)

Mutagen	Treatment	Sand tray method	Roll towel method
Gamma rays	T_0 - Control	16.90	16.20
	T_1 - 100 Gy	14.30	14.70
	T_2 - 150 Gy	13.20	13.60
	T_3 - 200 Gy	13.10	13.50
	T_4 - 250 Gy	12.00	12.40
	T_5 - 300 Gy	11.50	12.10
	T_6 - 350 Gy	11.40	11.20
	Mean	13.21	13.44
	S.E(m)	0.72	0.61
EMS	T_0 - Control	15.60	15.20
	T_1 - 10 mM	13.40	13.40
	T_2 - 20 mM	11.50	11.20
	T_3 - 30 mM	10.60	10.80
	T_4 - 40 mM	9.60	9.50
	T_5 - 50 mM	9.10	8.60
	T_6 - 60 mM	8.10	8.20
	Mean	11.12	11.02
	S.E(m)	0.98	0.96

Fig 1: Seedling height of 300 Gy gamma irradiated observed in both sand tray method and protray

Fig 2: Seedling height of 40 mM EMS treated observed in both sandtray method and protray

Vigour index

The vigour index obtained were presented in the table.5. There exists a significant difference with increase in dose of mutagen. The maximum vigour index was observed in control rather than the treatments. For gamma rays, the vigour index ranged from 2095.20 to 899.20 in sandtray whereas, in protray it ranged from 2080.80 to 933.60. It is concluded that with the increase in the dose of gamma rays, the vigour index of seedlings were reduced. In EMS treated seedling, the maximum vigour index was observed in lower dose of 10mM as 1828.80 in sandtray and 1857.60 in protray and minimum was observed in 60mM as 875.84 in sandtray and 889.28 in portray. The results obtained were in accordance with the findings of Kathiravan *et al.*, (2008) [7] and Parashiva and Kumar (2004) [11].

Table 5: Effect of mutagens on vigour index(I)

Mutagen	Treatment	Sandtray method	Roll towel method
Gamma rays	T_0 - Control	2930.40	2087.20
	T_1 - 100 Gy	2095.20	2080.80
	T_2 - 150 Gy	1904.68	1921.00
	T_3 - 200 Gy	1572.00	1626.00
	T_4 - 250 Gy	1439.20	1472.80
	T_5 - 300 Gy	1144.32	1195.20
	T_6 - 350 Gy	899.20	933.60
EMS	T_0 - Control	2741.60	2686.40
	T_1 - 10 mM	1828.80	1857.60
	T_2 - 20 mM	1146.08	1183.52
	T_3 - 30 mM	1292.40	1277.40
	T_4 - 40 mM	1201.20	1191.60
	T_5 - 50 mM	744.00	712.00
	T_6 - 60 mM	875.84	889.28

Conclusion

Based on the above findings, it can be inferred that the germination percentage, shoot length, root length, and seedling vigor index (I) in vegetable mochai decreased with increase in dose of mutagens. Additionally, Ethyl Methane Sulfonate appears to be an effective method for inducing morphological, physiological, and biochemical alterations in vegetable mochai. Therefore, the LD50 value which was determined based on mortality percentage and seedling length, was fixed at 277.678 Gy for gamma rays and 41.541 mM for EMS, respectively.

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