

## Influence of mutagenic effects on seed germination and linear growth of M<sub>1</sub> generation in Grass pea (*Lathyrus sativus* L.).

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### Abstract

The M<sub>1</sub> generation of Grasspea (*Lathyrus sativus* L.) was raised by treating the seeds with Gamma rays (doses 15, 30, 45 GY) and various doses of chemical mutagen [EMS (0.05, 0.10 and 0.15%); SA (0.05, 0.10 and 0.15%) and NMU (0.15, 0.30 and 0.45%)] *in vitro*. The gradual reduction in seed germination percentage and linear growth of root and shoot length in all mutagen treated doses of M<sub>1</sub> generation as compared to control was noticed. The doses of SA and NMU show more effective than Gamma rays and EMS in linear growth. Mean values in mutagen treated doses, in these parameters significantly reduced and deleterious effects were more pronounced in higher doses, indicating almost a linear relationship.

### INTRODUCTION

Grass pea *Lathyrus sativus* L. belongs to Fabaceae family (2n= 14), cultivated in Rabi season, in tribal areas of Maharashtra. Though, grass pea is an important crop, showing less-significance in India, Bangladesh, Pakistan, Nepal and Ethiopia. It is cultivated and extensively naturalized in European countries viz. Germany, Portugal, Spain and east to the Balkans and South Russia; in Crete, Rhodes, Cyprus and in West Asian and North African countries viz. Syria, Lebanon, Palestine, Egypt, Iraq, Afghanistan, Morocco, and Algeria. The genus *Lathyrus* is recognize as one of the largest group includes 187 species and subspecies (Allkin *et al.*, 1983) and number of species are found in the World wide distribution (Zeven and de W *et al.*, 1982).

It is a highly self-pollinated crop with cleistogamous nature. Creation of variability through pollination and artificial hybridization is very difficult as the flowers are cleistogamous and very delicate to handle. Even if hybridization is carried out the seed set is less than five per cent. In these crop proper male sterility system were not showing, which is helpful for hybridization. Hence,

the main objective of present investigation was creation of variation by induction of mutation through physical and chemical mutagens.

### MATERIALS AND METHODS

The variety of *Lathyrus* seeds was procured from the Indira Gandhi Krishi Vishvavidyalaya, Raipur (C.G.) India and treated by various doses of Chemical mutagen Ethyl methane sulphonate (EMS-CH<sub>3</sub>SO<sub>2</sub>OC<sub>2</sub>H<sub>5</sub>) (0.05, 0.10 and 0.15%); Sodium Azide (SA-Na<sub>3</sub>N) (0.05, 0.10 and 0.15%) and N-Nitroso-N-methylurea (NMU-C<sub>2</sub>H<sub>5</sub>N<sub>3</sub>O<sub>2</sub>) (0.15, 0.30 and 0.45%) as well as physical mutagen Gamma rays (15, 30, 45 GY) [Co<sup>60</sup> gamma source (irradiation source capacity to release 3000 Ci delivery 7200 r/min curie), irradiated at the Department of Biophysics, Government Institute of Science, Aurangabad. (M.S.) India].

Six hundred (for three replication) healthy and uniform size of seeds were pre-soaked in distilled water for six hours, then these seeds were immerse in the chemical mutagenic solution for six hours, with the different concentration 0.05, 0.10 and 0.15% for EMS; 0.05, 0.010 and 0.015 % for SA and 0.15, 0.30 and 0.45% for

NMU, while for the physical mutagen (Gamma rays) treated seeds soaked in water for 6 hrs (15, 30 and 45 KR doses).

To ensure uniform absorption of the mutagen, the volume of mutagen solution was maintained at proportion of ten times to that of the seed volume. The whole treatment was carried out at a room temperature of  $28\pm 2^{\circ}\text{C}$  for four hours after washed in running water, untreated seeds soaked in water were used as control.

**Seed Germination and linear growth percentage:**

In order to find out the effect of Gamma rays, EMS, SA and NMU on seed germination and linear growth, 100 seeds (100 X 3 = Triplicate for each

dose) were spread over moist Whatman's filter paper no.1 in Petri plates. Similarly 100 seeds (100 X 3 = Triplicate for each dose) were sown in field area. Observations were recorded daily till seven days. The mean as well as variance length of root and shoot of treated as well as control seedlings were measured separately using seven days old seedlings of three replicates from each mutagenic doses.

The germination percent was counted and recorded on seventh day per treatment, three replicate were used. Percent inhibition /stimulation over control were calculated as follows (Maluszynski *et al.* 2009):

**Seed germination (%)** = [(Number of seeds germinated/ Total number of seeds sown) x 100]

**Plant survival (%)** = [(Number of plants survived / Total number of seeds germinated) x 100]

**Emergence reduction (%)** =  $100 - [(Average\ emergence\ in\ the\ dose \times 100) / Average\ emergence\ in\ the\ control]$

**Statistical Analysis:** Collected data were subjected to Analysis of variance technique (Fisher, 1985)

**RESULTS AND DISCUSSION:**

**Seed germination:** The mutagen doses of Gamma rays, EMS, SA and NMU treated seeds of *Lathyrus*, shows gradually decrease in seed germination percentage with increasing doses, in both the experimental conditions, viz. *in Vivo* and *in Vitro* in  $M_1$  generation. All the treated lowest doses of mutagens resulted less seed germination percentage as compared to control. In Gamma rays it shows 72.34, 58.81 and 48.62 (in concentration of 15, 30 and 45 GY respectively); in Ethyl Methane Sulphonate (EMS), it was 67.32, 58.30 and 47.39 (in concentration of 0.05, 0.10 and 0.15% respectively); in Sodium Azide (SA), it was 70.33, 63.00 and 52.67 (in concentration of 0.05, 0.10 and 0.15% respectively) whereas in N-Nitroso-N-methylurea (NMU) 65.43, 54.12, 46.37 at 0.15, 0.30 and 0.45% doses respectively (Table 1) (Fig. 1 and Photo plate 1).

It had also noticed that in present investigation, there is gradual increase in reduction of emergence as the treated doses of mutagen increased. It was reduced from 07.62 to 37.91 in doses of Gamma rays 15 to 45 KR; from 14.03 to 39.48 in doses of EMS 0.05 to 0.15 %; from 10.19

to 32.74 in doses of SA 0.05 to 0.15 %; and from 16.45 to 40.78 in doses of NMU it was 0.15 to 0.45 % respectively.

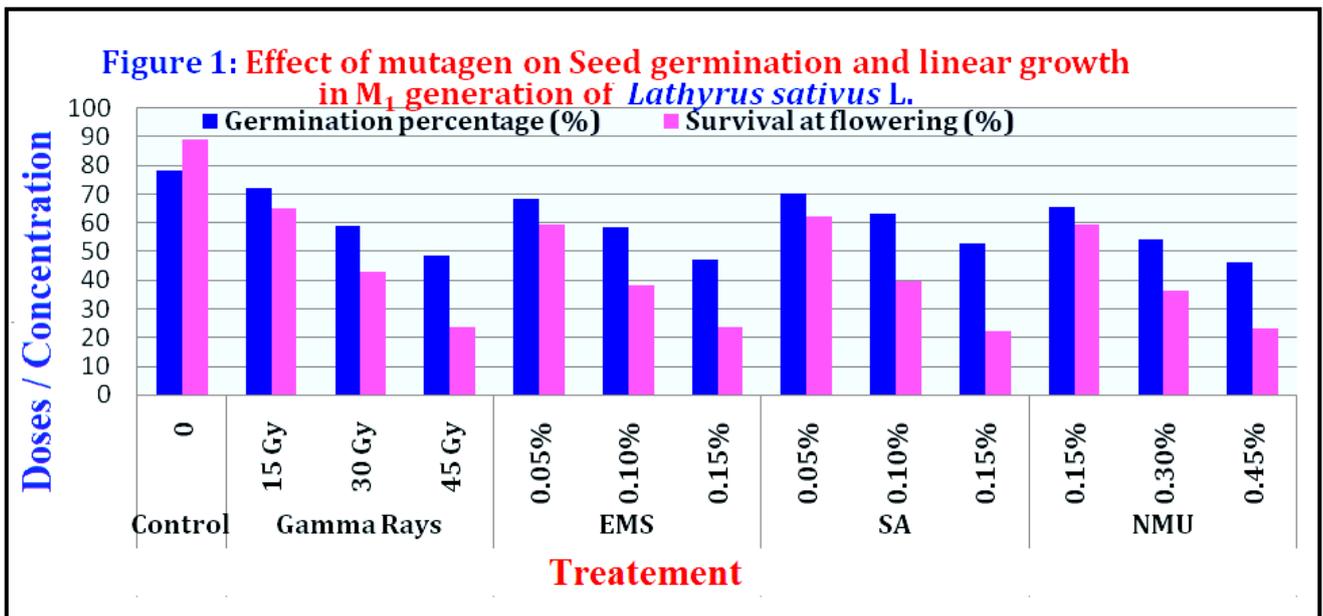
In present investigation, the reduction in seed germination percentage was associated with the increase in the concentration of mutagens. Similar results were also reported for EMS by Pedavai and Dhanavel (2004) in Soybean and Singh and Kole (2005) in Mung beans and Sharma *et al.* (2005) in Urdbean. The lowest germination was due to delay in the one set of mitosis and chromosomal aberration induced some enzyme activity such results in reduced (Ramya *et al.*, 2014) in black gram germination (Ananthaswamy *et al.*, 1971). The activity of seed germination and linear growth decreased appreciably in the mutagenic treatments; however, there was no direct relationship between the doses of mutagens and the parameters. Earlier studies of Mahna *et al.* (1989) in Black gram, Lal *et al.* (2009) in Lentil, Goyal and Khan (2010), and Makeem and Babu (2010) in Urdbean, Roychowdhury and Tah (2011) in *Dianthus*, Bhat *et al.* (2011) in different legumes and Ariraman (2014) on Pigeon Pea have shown the adverse effects of mutagens on seed germination parameters.

**Table 1: Effect of mutagen on Seed germination and linear growth in M<sub>1</sub> generation of *Lathyrus sativus* L.**

Treatment	Concentration/Doses	Total Seeds Soaked	Germination seedling	Germination Percentage (%)	Survival at flowering	Plant Survival at flowering (%)	Reduction in emergence over Control
Control	--	300	235	78.31	209	89.02	--
Gamma Rays	KR 15	300	217	72.34	153	65.05	07.62
	KR 30	300	174	58.81	101	42.92	24.90
	KR 45	300	146	48.62	55	23.58	37.91
EMS	0.05%	300	205	67.32	140	59.62	14.03
	0.10%	300	175	58.30	90	38.34	25.55
	0.15%	300	142	47.39	55	23.41	39.48
SA	0.05%	300	211	70.33	147	62.46	10.19
	0.10%	300	189	63.00	93	39.70	19.55
	0.15%	300	158	52.67	52	21.97	32.74
NMU	0.15%	300	197	65.43	140	59.54	16.45
	0.30%	300	162	54.12	86	36.49	30.89
	0.45%	300	139	46.37	54	23.01	40.78

The decrease in seed germination was mainly due to all the mutagen's action causing point mutations, enzyme inhibitions as well as chromosomal aberrations (Auti, 2005). The effects of physical and chemical mutagens resulted

metabolic activities causes disturbance in the seed physiology (Kulkarni, 2011). The reduction in seed germination was noticed due to injuries caused to the genetic material which may lead to decrease in seed germination (Undirwade and Kulkarni, 2019).



The 50% reduction of germination was recorded at higher doses of gamma rays (48.62% of 15 KR dose) and Chemical mutagen EMS (47.39 % of 0.15% Concentration), SA (52.57% of 0.15% Concentration) and NMU (46.37% of 0.15% Concentration). It indicated that germination percentage was reduced under the influence of physical and chemical mutagenic treatment at higher doses in M<sub>1</sub> population. Similar results were reported earlier in red gram by Jayanthi (1986), in winged bean by Veeresh *et al.* (1995) and in blackgram by Thilagavathi and Mullainathan (2011), in horse gram by Kulkarni (2011), in black gram by Nallathambi and Ram (2014). The significant survival reduction was observed in the higher dose / concentration of all mutagen.

**Linear Growth:** In effect of physical (Gamma Rays) and chemical (EMS, SA and NMU) mutagens root and shoot length in Grass pea, shows the gradual decreased with increasing dose noticed (Table 2). The average root length (seven days old) of mutagen treated seedlings recorded and noticed that there was significantly gradually decreased. It had noticed 9.84, 7.31 and 4.62 cm in 15, 30 and 45 GY of Gamma rays doses; 10.35, 8.96 and 5.45 cm in 0.05, 0.10 and 0.15% of EMS doses; 2.77, 1.62 and 0.85 cm in 0.05, 0.1 and 0.15 % of SA doses and 5.63, 2.97 and 1.08 cm in 0.15, 0.30 and 0.45% of NMU doses respectively, as against 14.43 cm in control.

Table 2: Showing the effects of mutagen on Linear growth on average Length (cm) of radical and Shoot of M<sub>1</sub> *Lathyrus sativus* L after seven days.

Mutagen		Root			Shoot		
Treatments	Doses /Concentration	Length	STDEV	SE ±	Length	STDEV	SE ±
Control	--	14.43	1.94	0.73	12.46	1.03	0.39
Gamma rays	15 Gy	9.84	77	0.29	8.76	1.11	0.42
	30 Gy	7.31	1.09	0.14	7.68	1.08	0.4
	45 Gy	4.62	0.82	0.31	3.52	0.46	0.17
EMS	0.05 %	10.35	1.05	0.39	8.43	0.69	0.38
	0.1 %	8.96	1.01	0.38	6.05	0.62	0.23
	0.15 %	5.45	0.67	0.26	4.87	0.81	0.3
SA	0.05 %	2.77	0.72	0.27	2.34	0.35	0.13
	0.1 %	1.62	0.16	0.64	0.92	0.17	0.06
	0.15 %	0.85	0.17	0.06	0.53	0.11	0.04
NMU	0.15 %	5.63	0.98	0.84	3.72	0.43	0.42
	0.3 %	2.97	1.08	0.56	2.04	0.75	0.34
	0.45 %	1.08	0.62	0.42	0.93	0.51	0.24

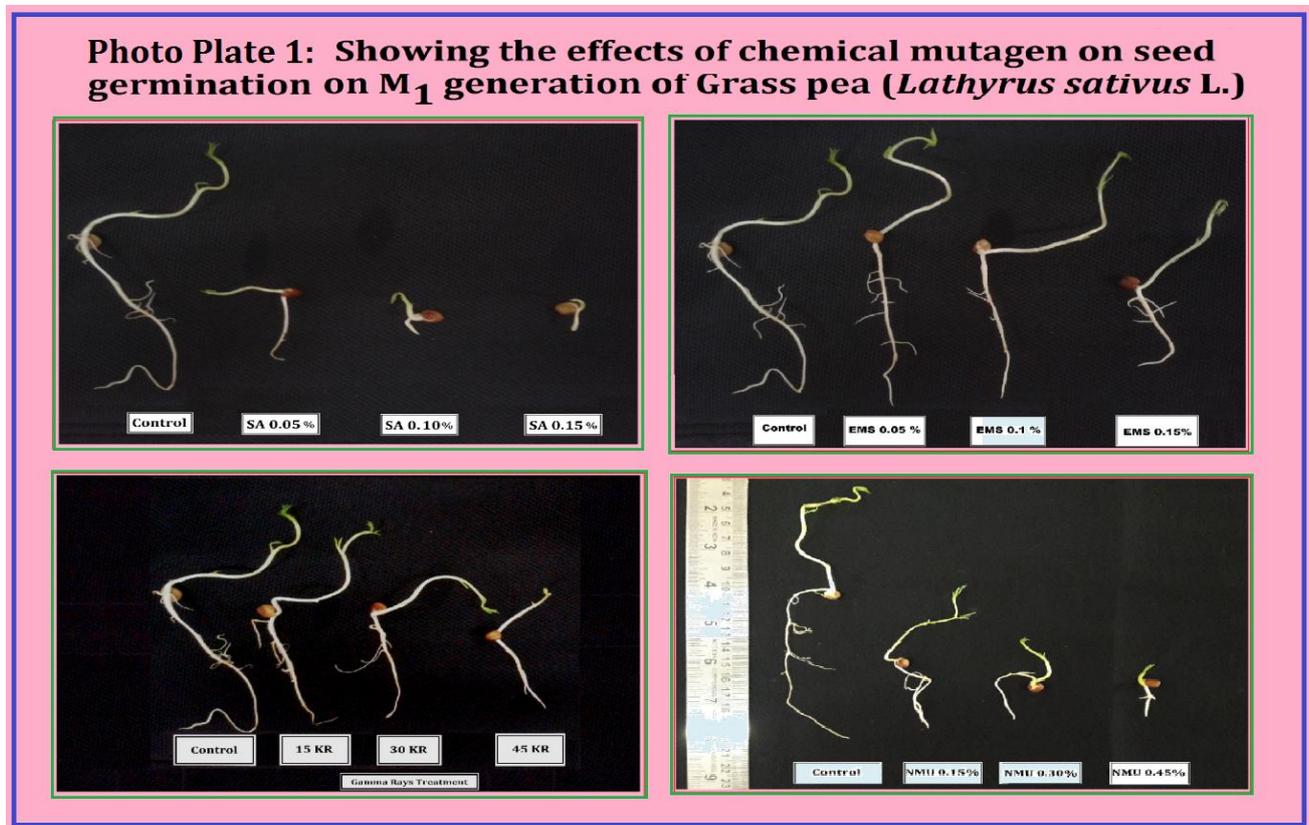
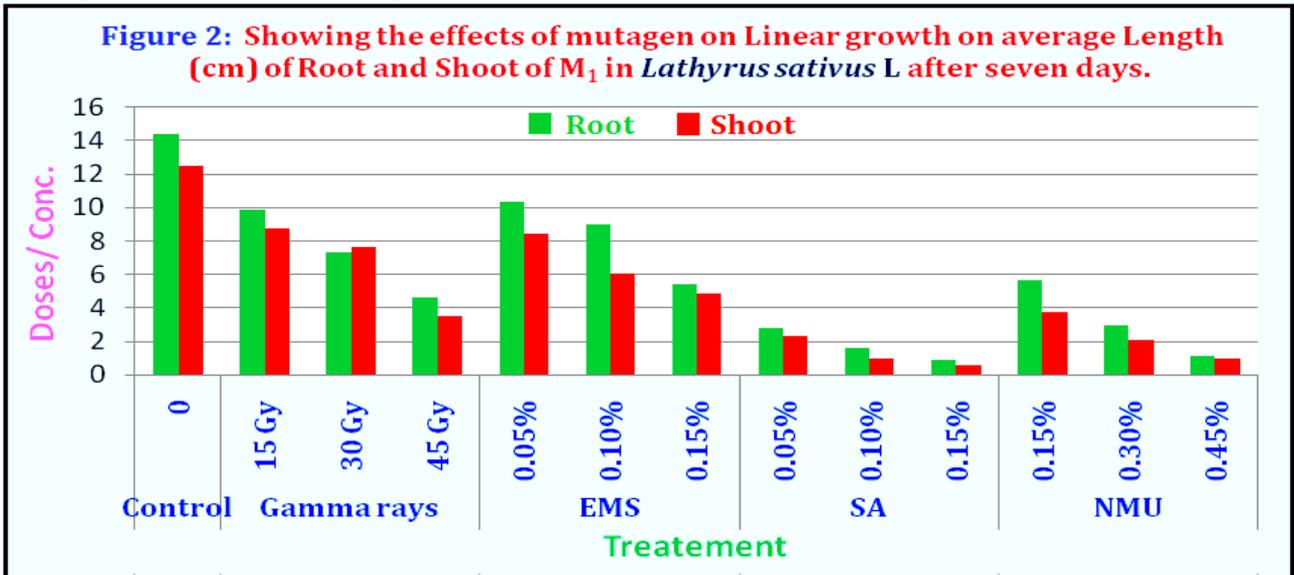
Similarly seven days old mutagens treated seeds resulted that there was decrease in the length of shoots of all doses of mutagen (Figures 1 and Table 2). The average length of shoot was 8.76, 7.68 and 3.52 cm in 15, 30 and 45 GY of Gamma rays doses; 8.43, 6.05 and 4.87 cm in 0.05, 0.10 and 0.15% of EMS doses; 2.34, 0.92 and 0.53 cm in 0.05, 0.1 and 0.15 % of SA doses and 3.72, 2.04 and 0.93 cm in 0.15, 0.30 and 0.45% of NMU doses respectively, as against 12.46 cm in control (Table 2, Figure 2).

Similar findings were observed by Ignacimuthu and Babu (1988) on urd and mung beans, Zaka *et al.* (2004) on Pea, Toker *et al.* (2005) in *Cicer* seeds, Makeem and Babu (2010) in Urdbean, Ramya *et al.*, (2014) on Black gram, Sathees *et al.* (2020) on Cowpea. In present investigation, it had been noticed that the doses of SA and NMU shows more reduction in root and shoot length as compared to Gamma rays and EMS (Table 2, Photo Plate1).

The gradual decrease in root and shoot could be due to the damage to the meristematic cells as a consequence of physiological injury in the seeds and seedlings were correlated with reduction in seedling height after mutagenic treatments (Kulkarni, 2011). SA, NMU treatments showed

great reduction in length of shoot and root of seedlings than EMS and Gamma rays treatments of doses. The doses SA, EMS Gamma rays were more sensitive as manifested by reduction in the seven days old seedlings length of root as well as shoots, as increased in mutagen doses.

Figure 2. Effect of mutagens on average length (cm) of root and shoot of mutagen treated seeds ( $M_1$ ) of *Lathyrus sativus* L. after 7 days.



## CONCLUSION

The present study reveals that, the effect of physical and chemical mutagen on seed germination and linear growth in all the treated doses of mutagens showed gradually increases in an inhibitory effect as the doses increased, on seed germination percentage and linear growth root and shoot length. The concentration / dose used in present study will be effective in induction of wide range of mutants. It had also observed that the result of the field experiment was different as compared to laboratory experiment.

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## REFERENCES

- Allkin R, Macfarlane TD, White RJ, Bisby FA and Adey ME, 1983. Viciae Database Project Publ no. 2, Southampton and the Ethiopian chronicles, vol. II. Methuen, London
- Ananthaswamy HN, Vakil UK, Srinivasan A, 1971. Biochemical and physiological changes in gamma irradiated wheat during germination. *Rad. Bot.* **11**: 1-12.
- Ariraman M, Gnanamurthy S, Dhanavel D, Bharathi T and Murugan S 2014. Mutagenic Effect on Seed Germination, Seedling Growth and Seedling Survival of Pigeon Pea (*Cajanus cajan* (L.) Millsp). *International Letters of Natural Sciences* **21**: 41-49
- Auti SG, 2005. Mutational Studies in mung (*Vigna radiata* (L.) Wilczek). Ph D Thesis Pune University Pune (MS), India.
- Bhat MUD, Khan S and Kozgar MI, 2011. Studies on induced mutations in chickpea (*Cicer arietinum* L.) I. Responses of the mutagenic treatments in M<sub>1</sub> biological parameters. *Elec. J. of Plant Breeding*. **2**: 422-424.
- Ignacimuthu S, Babu CR, 1988. Radio sensitivity of the wild and cultivated urd bean and mung beans. *Indian J of Gen. and Plant Breeding*. **48**(3): 331-342.
- Ignacimuthu S and Babu C R (1989). Induced chromosomal and pollen sterility in wild and cultivated urd beans and mungbean. *Cytologia.*, **51**(1) : 159-167.
- Jayanthi S, 1986. Biological effects of gamma rays and Ethyl Methane Sulphonate in the M<sub>1</sub> generation of Red gram (*Cajanus Cajan* (L.)) M.Sc.,(Agric) Thesis, Kerala University, India
- Kulkarni GB, 2011. Effect of mutagen on Pollen fertility and seed germination in Horse gram [*Macrotyloma uniflorum* (Verdecourt)] *Bioscience Discovery*, **2** (1):146-150.
- Lal GM, Toms B and Lal SS. 2009 Mutagenic sensitivity in early generation in black gram. *Asian J. of Agricultural Sciences*. **1**: 9-11.
- Mahla H, Kumar D and Shekhawat A, 2010. Effectiveness and efficiency of mutagens and induced variability in clusterbean (*Cyamopsis tetragonoloba*) *Indian J. of Agricultural Sciences*, **80** (12): 1033-1037.
- Mahna SK, Garg R and Parvteesam M, 1989. Mutagenic effects of Sodium Azide in black gram. *Curr. Sci.* **58**: 582-584.
- Makeen K, Babu GS, 2010. Mutagenic Effectiveness and Efficiency of Gamma Rays, Sodium Azide and Their Synergistic Effects in Urd Bean (*Vigna mungo* L.). *World J Agric Sci*. **6** (2): 234-237.
- Ramya B, Nallathambi G and Ram SG 2014. The effect of mutagens on M<sub>1</sub> population of black gram (*Vigna mungo* L. Hepper). *African J. of Biotechnology* **13** (8):951-956.
- Ramya B and Nallathambi G, 2014. Effect of mutagenesis on germination, survival, pollen and seed sterility in M<sub>1</sub> generation of black gram [*Vigna mungo* (L.) Hepper] *Plant Archives* **14** (1): 499-501.
- Roychowdhury R, Tah J. 2011. Chemical mutagenic action on seed germination and related agro-metrical traits in M<sub>1</sub> *Dianthus* generation. *Current Botany*; **2** (8): 19-23.
- Sathees N, Gowthami K, Pramila Devi R, Gowtham T, Mounika K, Nivesh M and Mohan S, 2020. Effects of Induced Physical and Chemical Mutagen in Cowpea (*Vigna unguiculata* L. walp). *Int.J.Curr. Microbiol. App. Sci.* **9** (10): 693-703.
- Sharma SK, Sood R and Pandey DP, 2005. Studies on mutagen sensitivity, effectiveness and efficiency in urdbean (*Vigna mungo* (L.) Hepper.). *Indian J Genet.*, **65**: 20-22.

**Thilagavathi C and Mullainathan L, 2011.** Influence of physical and chemical mutagens on quantitative characters of (*Vigna mungo* (L.) Hepper). *Inter. Multidisciplinary Res. J.* **2**:06-08.

**Veeresh LC, Shivashankar G and Shailaja H, 1995.** Effect of seed irradiation on some plant characteristics of winged bean. *Mysore. J. Agric. Sci.* **29**:1-4.

**Zaka R Chenal C and Misset MT, 2004.** Effect of low doses of short-term gamma radiation on growth and development through two generations of *Pisum sativum*. *Science of the Total Environment.* **320**:121-129.

**Zeven AC and de Wet JMJ, 1982.** In: Dictionary of cultivated plants. Pudoc, Wageningen

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