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Optimization of doses for Ethyl Methane Sulphonate (EMS) and analysis of M_1 generation of fodder cowpea [*Vigna unguiculata* (L.) Walp]

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Abstract
Mutation breeding is an important tool for creating variability and the source of new breeding materials. Ethyl Methane Sulphonate (EMS) is a very potent chemical mutagen. The present study envisages the optimization of doses of mutagen for fodder cowpea and analysis of M_1 generation. Fodder cowpea seeds were treated with different concentrations of EMS (0.1%, 0.2%, 0.3%, 0.4% and 0.5%) and lethal dose 50 (LD_{50}) value was determined using probit analysis. Effective doses were fixed according to LD_{50} value. Analysis of M_1 generation was carried out on cowpea, treated with effective doses of mutagen. Germination parameters like seedling shoot length, root length, vigour index and the quantitative characters like number of pods/plant, pod length, number of seeds/plant and seed yield/plant and presence of chlorophyll mutants were recorded from M_1 generation. All the biometric characters showed wide variation among the mutant population. Chlorophyll mutant plants were observed in all mutated populations. Presence of chlorophyll mutants were recorded higher in 0.51% of EMS treatment. Generally, a reduction in the quantitative parameters was noted with the increase of concentration of the mutagen.

Keywords: LD_{50}, fodder cowpea, mutation, germination analysis, EMS, M_1 generation

1. Introduction
Indian Livestock sector contributes to the economy in a significant manner. For the development of livestock sector, good quality feed and fodder is mandatory. Among the fodder, legume fodder is favorable because of its higher crude protein content and high quality protein (Singh *et al*., 2018) [39]. Cowpea [*Vigna unguiculata* (L.) Walp] is an excellent choice for leguminous fodder. This can be used for green fodder, dry fodder, and also as silage along with cereals like sorghum or maize. Grains can be utilized as both human food and animal feed. Cowpea is also a green manure crop and a cover crop (KAU, 2016) [21].
Mutation breeding is a fruitful tool for crop improvement. Mutagenesis is more effective in autogamous crops than allogamous (Micke, 1988) [32]. Also, mutagenesis increases the variability and thus long lost phenotypes may reappear (Khursheed *et al*., 2019) [23]. Mutagenesis can be induced by both physical and chemical mutagens. Physical mutagens include gamma rays, X rays, UV rays, fast neutrons, etc and alkylating agents and azides are classified under chemical mutagens (Kodym and Afza, 2003) [24]. Chemical mutagen accounts for greater gene mutation and lesser chromosomal aberration (Acquaah, 2006) [2]. Ethyl Methane Sulphonate (EMS) is the most commonly used chemical mutagen. EMS was found more effective and efficient in inducing mutation, compared with gamma rays and their combined treatments (Girija and Dhanavel, 2009) [16].
The accomplishment of mutation breeding mainly depends on the optimum dose of mutagen. Optimum dose of mutagen creates maximum mutation and minimum lethality. Many scientists believe that optimum dose should be approximate to Lethal Dose 50 (LD_{50}) (Singh, 2003) [38]. LD_{50} is the dosage that can cause the death of half of the dosed population (Gad, 2014) [15]. Thus, LD_{50} is an important parameter which determined the optimum doses of mutagen for mutation induction (Alvarez-Holguin *et al*., 2019). So, LD_{50} determination can be considered as the first and foremost step in mutation breeding.

2. Materials and Methods
2.1 LD_{50} estimation
Seeds of fodder cowpea variety Aiswarya were collected from College of Agriculture, Vellayani. The seeds were well dried with 12% of moisture content.

Each treatment consists of 100 seeds. Seeds were soaked in water for six hours as pre-treatment. This was followed by the soaking of those seeds in EMS solutions with respective concentrations for six hours. The concentrations of EMS solutions were 0.1%, 0.2%, 0.3%, 0.4% and 0.5%. Seeds were kept in distilled water for control. After six hours, the seeds were washed under running water to remove the traces of chemical. Later, the seeds were placed on petriplates. Seed germination percent was noted from fifth to twelfth day after placing them on petriplates. Number of seedlings survived fifteen days after sowing was taken for seedling survival percentage.

2.2 Determination of effective doses

Considering the LD_{50} value, effective doses were fixed. Generally, concentrations near to LD_{50} value are taken as effective doses. The fodder cowpea seeds were treated with effective doses of EMS and germination analysis was done. Seedling shoot length and root length were measured in laboratory condition. Seedling shoot length and root length was measured on the seventh day after sowing. Seedling vigour index was also calculated using Abdul-Baki and Anderson (1973) [1] method.

Vigour Index I = Total seedling length × Germination percent

2.3 M_1 generation

Seeds treated with effective doses of EMS were sowed in field. Each treatment including control consists of 300 seeds, were grown in the field of College of Agriculture, Vellayani during January 2019 to March 2019. The design was Randomized Block Design with three replications and spacing was 0.30m * 0.15m. The observations like seed germination percent, seedling survival percent, plant survival upto maturity, number of pods per plant, pod length, number of seeds per plant and seed yield per plant were recorded. Seedlings which exhibited the appearance of white spots, streaks or patches on leaves were observed as chlorophyll mutants.

3. Results and Discussion

3.1 Seed germination percent

Seed germination was affected with mutation. Seed germination was decreasing with the increase of concentration of the mutagen upto 0.4% and showed a slight increase in 0.5% of EMS. Bind and Dwivedi (2014) [8] and Nair and Mehta (2014) [34] reported the same kind of relationship between mutagen dosage and germination percent in cowpea. Similar results were recorded by Mini (1989) [33] in cowpea and Desai and Rao (2014) [12] in pigeon pea. Along with reduced germination percent, higher concentrations of mutagen showed delayed germination. Seed germination was found delayed for 2 days in 0.5% EMS. Kurobane *et al*., (1979) [28] proposed that germination is affected because mutation hinders the enzyme activity. Mutation may affected the production of enzymes responsible for germination. According to the findings of Ariraman *et al*., (2014) [6] the reduction of germination may resulted from the action of mutagens on meristematic regions of seeds. Similarly, Kozgar (2012) [25] also reported the reduced germination after mutagen treatment. They suggested that presoaking of seeds may affect the respiration and metabolism, leading to decreased germination. Data on seed germination was given in Table 1.

3.2 Seedling survival percent

Like seed germination, seedling survival was also affected by mutation. Seedling survival also showed a negative shift with respect to the increasing mutagen concentration. Among the mutagen treatments, highest seedling survival percent of 90% was observed in 0.1% EMS and lowest of 26% was recorded in 0.5% EMS (Table 1). Concentration of the mutagen and seed survival percent was inversely related. Similar results were mentioned in cowpea (Kumar *et al*., 2009: Gnanamurthy *et al*., 2013) [26, 18] in kidney bean (Ali *et al*., 2014) [4] and in chick pea (Umavathy and Mullainathan, 2014). EMS creates random point mutation (Sikora *et al*., 2011) [37]. That is, higher concentration of EMS can result in more efficient mutation. This may leads to harmful effects on seedling survival in higher doses.

Table 1: Effect of mutagen on seed germination and seedling survival

Treatment	Germination percent	Seedling survival percent	Relative percentage	Percent reduction in survival
Control	97	93	100	0
0.35% EMS	90	90	96.77	3.22
0.39% EMS	87	83	89.25	10.75
0.43% EMS	84	76	81.72	18.28
0.47% EMS	64	61	65.59	34041
0.51% EMS	74	26	27.96	72.04

3.3 LD_{50}

From the data of seed germination and seedling survival, LD_{50} value was estimated using probit analysis. The obtained probit curve was represented in Fig. 1. From the curve, LD_{50} value was obtained as 0.4275%, which was rounded to 0.43% EMS. Based on the LD_{50} value, effective doses were fixed as 0.35%, 0.39%, 0.43%, 0.47% and 0.51%.

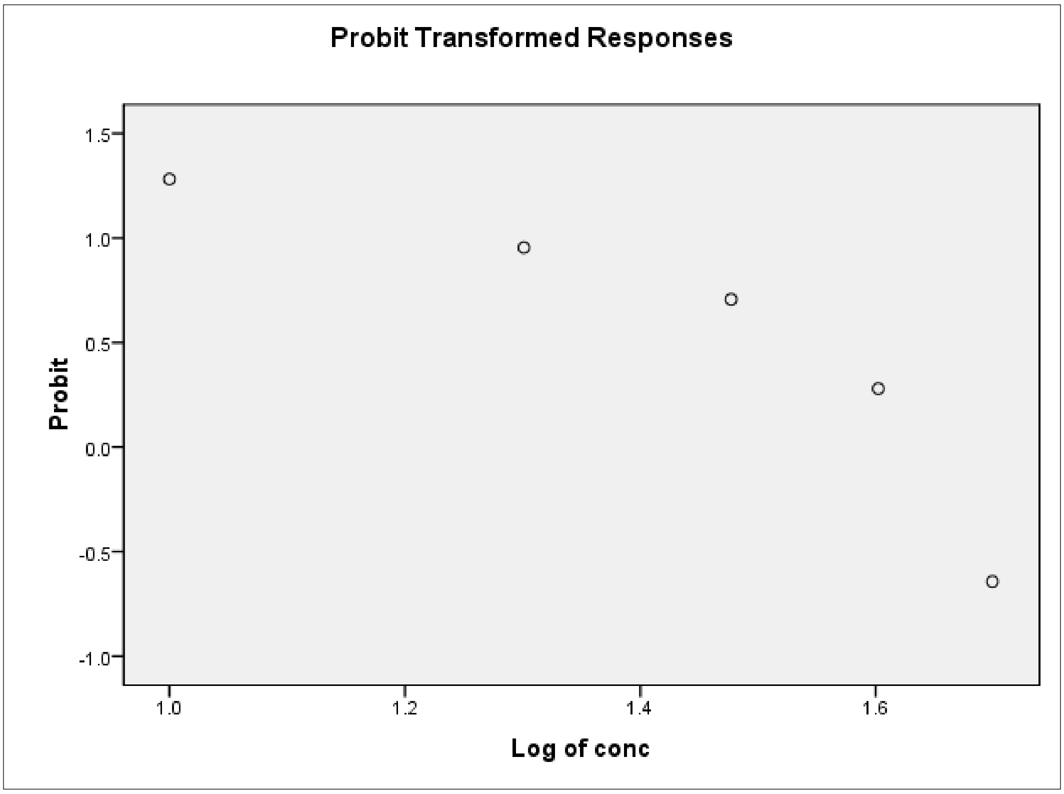

Fig 1: ProBit curve for LD_{50} determination

3.4 Biological effects in M_1

Seed germination of M_1 generation of cowpea affected very much by mutation. Germination percent decreased among the mutagenized population. Germination percent decreased with the increase in concentration of the mutagen upto 0.43% EMS and then showed an increase along with increase of the concentration of EMS. Thus, percent decrease over control was maximum in 0.43% EMS with 38.04% reduction in germination.

Seedling survival was noted after fifteen days of sowing. Seedling survival also represented similar pattern as seed germination. Survival percent decreased with the EMS dosed populations till 0.43% and then recorded increased survival percent in higher concentrations. Among the mutagen treated population, 0.51% EMS exhibited higher seedling survival percent.

Similarly, percentage of plant survival maturity showed a decreasing plant survival upto maturity and then noted with increased survival percentage. All the three characters of seed germination percent, seedling survival percent and percent plant survival upto maturity followed same trend. Olasupo *et al*., (2016) [35] observed that seed germination and plant survival of cowpea recorded an inverse relationship with the concentration of the mutagen. Same result was recorded by Dhanavel *et al*., (2008) [13] in cowpea. Mini (1989) [33] mentioned the reduction in seedling survival percent as an effect of both cytological and physiological disturbances. Enhancement in germination and survival may resulted from the stimulatory action of mutagen. Data of seed germination, seedling survival and plant survival upto maturity was represented in Table 2.

Table 2: Effect of mutagen on seedling growth parameters

Treatment	Shoot length (cm)	Root length (cm)	Germination percent	Vigour Index
Control	17.54	8.515	92	2397.06
0.35% EMS	7.045	6.945	89.66	1254.34
0.39% EMS	22.085	6.705	79.66	2293.41
0.43% EMS	10.28	5.71	57	911.43
0.47% EMS	6.48	5.295	78.66	926.22
0.51% EMS	7.58	5.56	88.66	1164.9

Seedling shoot length and root length was measured and analysed. Seedling shoot length was found highest in 0.39% EMS (22.085) than control (17.540). The lowest shoot length was observed in 0.47% EMS with 6.480. This treatment showed 36.94% of reduction in seedling height comparing to control. Treatments other than 0.39% EMS showed greater reduction in the shoot length. Similarly, reduction of shoot length was noted in broad bean mutants (Laskar and Khan, 2014) [29]. Same result was reported in fenugreek by Bashir *et al*., 2013 [7]. Similarly, seedling root length was measured. Root length was affected by the action of mutagens. Root length was found higher in control than mutagenized populations with 8.505 cm and 0.47% EMS showed smallest. Root length decreased with the increase of concentration of EMS. Comparing to root length, percent reduction in shoot length was more. That is, shoot length was more affected by mutagen than root length. Vigour Index was measured using both seedling height and germination percent. Similar to shoot length and root length, treatment means of Vigour Index (VI) were not significantly different for treated and untreated population. Maximum VI was noted in control, followed by 0.39% EMS and minimum VI was recorded in 0.43% EMS. Data were represented in Table 2. Shaikh *et al*., (1980) [36] observed reduction in both root length and shoot length of the mutants of black gram, mung bean and lentil. They found out that, root lengths were more affected by radiation than shoot length. Evans (1962) [14] reported that radiation and radiomimetic chemicals can induce chromosome aberrations. This may also affects the seedling growth and vigour.

Table 3: Effect of EMS on seed germination, seedling survival and plant survival upto maturity in M_1 generation

Treatment	Seed germination %		Seedling survival percentage	Lethality %	% Plant survival upto maturity
	Mean	Percent decrease over control			
Control	92	-	95.667	0.00	95.333
0.35% EMS	89.67	2.53	84.667	11.5	79.333
0.39% EMS	79.67	13.40	70.333	26.48	67
0.43% EMS	57	38.04	54	43.55	53
0.47% EMS	78.67	14.49	75.333	21.25	70.333
0.51% EMS	88.67	3.62	87	9.06	80.667

3.5 Quantitative characters

Yield attributing characters like number of pods per plant, pod length, seeds per plant and seed yield per plant was recorded and found variation among the mutant population (Table 4). All the characters except number pods per plant showed an increased value in mutant population, especially in 0.51% EMS. The variability obtained in these characters was very promising. For number of pods per plant, control showed highest mean value, followed by 0.51% EMS. Khan *et al*., (2004) [22] recorded increased pod number among the treated population in *Vigna radiata* and Waghmare and Mehra (2000) [41] in grasspea. In case of pod length, 0.39%, 0.43%, and 0.51% EMS reported higher mean value than control. Ahmad and Yaqoob (1993) [3] reported lesser variability in pod length than other characters. Considering seeds per pod, mean value of 0.51% EMS was higher than untreated population. For seed yield per plant, 0.51% EMS with highest mean was on par with control and other treatments showed decrease in seed yield. Waghmare and Mehra (2000) [41] suggested that reduction in seed yield may be resulted from the toxic effects of mutagen. Wani and Anis (2001) [42] observed reduction in number of seeds per pod and increase in seed weight in chickpea mutants.

According to Girija and Dhanavel (2013) [17], quantitative characters decreased in M_1 generation. But in this study, the variation among the characters were prominent than reduction in the characters. In the experiment of Deepalakshmi and Kumar (2004) [11], number of pods per plant, pod length and seed yield exhibited both positive and negative shift in the mutagen treated population of blackgram. Yasmin *et al*. (2020) [43] reported a negative effect on number of pods per plant, pod length and seed yield per plant in blackgram mutants. Mahtab *et al.* (2015) [31] obtained a rapeseed mutant with more number of pods per plant, seeds per pod and seed weight, comparing to control. Wani and Anis (2001) [42] concluded that mutagen affected these traits independently. Wider range of these characters provides the opportunity to carry over the research and so as to practice selection.

Table 4: Effect of EMS on the quantitative characters of M_1 generation

Treatment	NPP	PL	SPP	SY
Control	7.33	12.95	14	17.53
0.35% EMS	5	12.56	12	12.4
0.39% EMS	3.3	15.14	12.33	4.26
0.43% EMS	4.3	14.10	12.33	15.67
0.47% EMS	4.3	12.63	10.33	3.90
0.51% EMS	6.3	14.72	15	18.43

NPP-Number of pods per plant, PL-Pod length, SPP-Seeds per pod, SY-Seed yield per plant

3.6 Presence of chlorophyll mutants

Kumari *et al*., (2019) [27] suggested that leaf colour mutation is an index of mutagenic effectiveness and efficiency. Plants with chlorophyll deficiency were found in all mutagenized populations. The occurrence of chlorophyll mutants was higher in T_5 (0.51%), followed by T_3 (0.43%). Kumari *et al*., (2019) [27] also observed an increase in the frequency of chlorophyll mutants with increasing concentration in cowpea. Commonly found chlorophyll mutants were Albina, maculate, alboviridis and viridis. Albina and xantha plants did not survive and died before branching. Viridis plants showed slow growth, while maculata plants developed normally with normal green leaves later. Plant with one chimeric branch and rest with normal branches were found in M_1. John (1987) [20] also shared same observation. She hypothesized that may be a part of embryo gets affected with mutation, causing the occurrence of such chimera. Chlorophyll deficient plants are presented in Fig 2.

Fig 2: Chlorophyll mutants observed in M_1 generation

4. Conclusion

Fodder cowpea seeds were mutated using various doses of EMS. LD_{50} as estimated as 0.43% using seed germination percent and seedling survival percent. Germination analysis of seedlings was carried out in seeds treated with effective doses of mutagen. Seedling shoot length, root length and vigour index was showed a reduction with increasing concentration of mutagen. Induction of mutation can affect the germination of seeds. So, germination analysis can be considered as a measure for indicating variations due to mutation. M_1 population was observed for several yield attributing characters like number of pods per plant, pod length, number of seeds per pod and seed yield per plant. All these characters exhibited wide variation among the mutagen treated population. Presence of chlorophyll mutants can be regarded as an indicator of mutation. Thus, analysis of M_1 population can help in assessing the variation caused by mutation.

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6. References

1. Abdul-Baki AA, Anderson JD. Vigor determination in soybean seed by multiple criteria. Crop Sci. 1973;13(6):630-633.
2. Acquaah G. Principles of Plant Genetics and Breeding (2nd Ed.). Wiley-Blackwell, New Jersey, 2006, 760p.
3. Ahmad B, Yaqoob M. Radiation for induced mutation in mungbean (*Vigna radiata* (L. Wilczek)). Sarhad J Agric. 1993;9(5):423-427.
4. Ali A, Talukdar B, Naik B. Induced genetic variability for seed germination and other yield parameters in kidney bean (*Phaseolus vulgaris* L.). Indian Streams Res. J 2014;4(7):2230-7850.
5. Álvarez-Holguín A, Morales-Nieto CR, Avendaño-Arrazate CH, Corrales-Lerma R, Villarreal-Guerrero F, Santellano-Estrada E, *et al*. Mean lethal dose (LD_{50}) and growth reduction (GR_{50}) due to gamma radiation in Wilman lovegrass (*Eragrostis superba*). Rev. Mex. Cienc. Pecu. 2019;10(1):227-238.
6. Ariraman M, Gnanamurthy S, Dhanavel D, Bharathi T, Murugan S. Mutagenic effect on seed germination, seedling growth and seedling survival of Pigeon pea *(Cajanus cajan* (L.) Millsp Int. Lett. Nat. Sci. 2014;16:41-49.
7. Bashir S, Wani AA, Nawchoo IA. Mutagenic sensitivity of Gamma rays, EMS and Sodium azide in *Trigonella foenumgraecum* L. Sci. res. report. 2013;3(1):20-26.
8. Bind D, Dwivedi VK. Effect of mutagenesis on germination, plant survival and pollen sterility in M_1 generation of in cowpea [*Vigna unguiculata* (L.) Walp]. Indian J Agr. Res. 2014;*48*(5):398-401.
9. Bonde PJ, Thorat BS, Gimhavnekar VJ. Effect of gamma radiation on germination and seedling parameters of mung bean (*Vigna radiata*). Int. J Curr. Microbiol. App. Sci. 2020;11:1582-1587
10. Chaudhary L, Sharma R, Kumar M. Estimation of LD_{50} and effect of sodium azide on germination and seedling parameters of different cultivars of *Cajanus cajan* (L.) Millspaugh. Toxicol. Environ. Health Sci. 2021;13:1-7. https://doi.org/10.1007/s13530-021-00105-6
11. Deepalakshmi AJ, Kumar CRA. Creation of genetic variability for different polygenic traits in blackgram {*Vigna mungo* (L.) Hepper} through induced mutagenesis. Legum. Res. 2004;27(3):188-192
12. Desai AS, Rao S. Effect of gamma radiation on germination and physiological aspects of pigeon pea (*Cajanus cajan* (L,) Millsp) seedlings. Int. J. Appl. Res. Nat. Soc. Sci. 2014;2(6):47-52.
13. Dhanavel D, Pavadai P, Mullainathan L, Mohana D, Raju G, Girija M, *et al*. Effectiveness and efficiency of chemical mutagens in cowpea (*Vigna unguiculata* (L.) Walp). Afr. J. Biotechnology. 2008;*7*(22):4116-4117.
14. Evans HJ. Chromosome aberrations induced by ionizing radiations. Int. Rev. Cyt. 1962;*13*:221-321.
15. Gad SC. LD_{50}/LC_{50} (Lethal Dosage 50/Lethal Concentration 50). Encyclopedia of Toxicology, 2014. 10.1016/B978-0-12-386454-3.00867-8.
16. Girija M, Dhanavel D. Mutagenic effectiveness and efficiency of gamma rays, ethyl methane sulphonate and their combined treatments in cowpea (*Vigna unguiculata* L. Walp). Global J Mol. Sci, 2009;*4*(2):68-75.
17. Girija M, Dhanavel D. Effect of gamma rays on quantitative traits of cowpea in M_1 generation. Inter. J Res. Biol. Sci, 2013;3(2):84-87.
18. Gnanamurthy S, Dhanavel D, Girija M. Effect of gamma irradiation on the morphological characters of cowpea (*Vigna unguiculata* (L.)Walp). Int. J Cur. Tr. Res. 2013;2(1):38-43.
19. Goyal S, Wani MR, Khan S. Comparative mutagenic analysis of gamma rays, ems and their combination treatments in black gram (*Vigna mungo* (L.) Hepper). Thai J Agric. Sci. 2019;52(1):20-33.
20. John GK. Induced mutagenesis for earliness in groundnut (*Arachis hypogaea* L.). M.Sc (Ag) thesis, Kerala Agricultural University, 1987, 139p.
21. KAU (Kerala Agricultural University). Package of

Practices Recommendations: Crops (15th Ed.). Kerala Agricultural University, Thrissur, 2016, 394p.
22. Khan S, Wani MR, Parveen K. Induced genetic variability for quantitative traits in *Vigna radiata* (L.) Wilczek. Pak. J Bot. 2004;36(4):845-850.
23. Khursheed S, Raina A, Parveen K, Khan S. Induced phenotypic diversity in the mutagenized populations of faba bean using physical and chemical mutagenesis. J Saudi Soc. Agric. Sci. 2017;18:113-119.
24. Kodym A, Afza R. Physical and chemical mutagenesis. In: Grotewold, E. (ed.). Methods in Molecular Biology, 2003;236:189-204. Doi: 10.1385/1-59259-413-1:189. PMID: 14501066.
25. Kozgar MI. Studies on the induction of mutation for quantitative traits in chickpea (*Cicer arietinum* L.) (Doctoral dissertation), Aligarh Muslim University, 2012, 277p.
26. Kumar VA, Kumari RU, Amutha R, Kumar TS, Hepziba SJ, Kumar CRA. Effect of chemical mutagen on expression of characters in arid legume pulse-cowpea (*Vigna unguiculata* (L.)Walp.). Res. J Agric. Biol. Sci. 2009;5(6):1115-1120.
27. Kumari R, Pal AK, Singh S. Induced chlorophyll mutation in cowpea (*Vigna unguiculata* L. Walp.). J. Pharmacogn. Phytochem. 2019;8(6):1246-1249.
28. Kurobane I, Yamaguchi H, Sander C, Nilan RA. The effects of gamma irradiation on the production and secretion of enzymes, and on enzyme activities in barley seeds. Environ. Exp. Bot. 1979;*19*(2):75-84.
29. Laskar RA, Khan S. Mutagenic effects of MH and MMS on induction of variability in broad bean (*Vicia faba* L.). Annu. Res. Rev. Biol. 2014;4(7):1129-1140.
30. Leskovar DI, Stoffella PJ. Vegetable seedling root systems: Morphology, development, and importance. HortScience, 1995;30(6):1153-1159.
31. Mahtab SG, Zaman MA, Valiollah R, Maryam H, Afshin E. Evaluation of agronomic traits of mutants induced by gamma irradiation in PF and RGS003 varieties of rapeseed (*Brassica napus* L.). J Crop Breed. 2015;7(15):135-144.
32. Micke A. Genetic improvement of grain legumes using induced mutations. In Improvement of grain legume production using induced mutations. FAO/IAEA division of isotope and radiation applications of atomic energy for food and agricultural development, Pullman, Washington, 1 to 5 July 1986-1988, 1-51.
33. Mini V. Induced mutations in cowpea (*Vigna unguiculata* cultivar Kuruthola-payar). M.Sc (Ag) thesis, Kerala Agricultural University, Thrissur. 1989, 99p
34. Nair R, Mehta AK. Induced mutagenesis in cowpea (*Vigna unguiculata*) var. Arka Garima. In. J Agr. Res. 2014;48(4):247-257.
35. Olasupo FO, Ilori CO, Forster BP, Bado S. Mutagenic effects of gamma radiation on eight accessions of cowpea (*Vigna unguiculata* [L.] Walp.). Am. J Plant Sci. 2016;7(2):339-351.
36. Shaikh MAQ, Majid MA, Begum S, Ahmed ZU, Bhuiya AD. Varietal improvement of pulse crops by the use of nuclear techniques. In: Induced mutations for the improvement of grain legume production, IAEA-TECDOC, 1980;234:69-72.
37. Sikora P, Chawade A, Larsson M, Olsson J, Olsson O. Mutagenesis as a tool in plant genetics, functional genomics, and breeding. Int. J Plant Genomics 2011, 13p. https://doi.org/10.1155/2011/314829
38. Singh BD. *Genetics* (3rd Ed.). Kalyani publishers, Ludhiana, 2003, 854p
39. Singh MK, Singh A, Rhods DS. Correlation, path analysis and genetic variability, of yield, and yield Components in chickpea (*Cicer arietinum* L.). Int. J Fauna Biol. Stud. 2018;5(3):131-135.
40. Umavathi S, Mullainathan L. Mutagenic effect of gamma rays and EMS on seed germination, seedling height reduction and survivability of chick pea (*Cicer arietinum* L.) var. Co-4. Int. Lett. Nat. Sci. 2014;11(1):38-43.
41. Waghmare VN, Mehra RB. Induced genetic variability for quantitative characters in grasspea (*Lathyrus sativus* L.). Ind. J Genet. 2000;60(1):81-87.
42. Wani AA, Anis M. Gamma rays induced bold seeded high yielding mutant in chickpea. In: Mutation breeding newsletter. 2001;45:20-21. INIS-XA-427
43. Yasmin K, Arulbalachandran D, Dilipan E, Vanmathi S. Characterization of 60CO γ-ray induced pod trait of blackgram-A promising yield mutants. Int. J Radiat. Biol. 2020;96(7):929-936.