International Journal of Allied Practice, Research and Review
Website: www.ijaprr.com (ISSN 2350-1294)

Gamma Radiation Induced Chlorophyll Mutations in Cluster bean *(Cyamopsis tetragonoloba (L.) Taub)* Var. NCB-12

Patil B. M.[1] and Rane G.M.[2]
[1]Department of Botany, J.A.T. College, Malegaon, M.S. India.
[2]Post Graduate Department of Botany, M.J. College, Jalgaon, M.S. India.

Abstract- In the present investigation, the frequency and spectrum of chlorophyll mutations was studied in the cluster bean Var. NCB-12 using a physical mutagen gamma rays. The seeds of cluster bean variety NCB-12 were irradiated with 10,20,30,40 and 50 kR doses of gamma rays using ^{60}CO as a source of radiation at Mutation Breeding Centre, Department of Biotechnology, B.A.R.C. Trombay, Mumbai. The treated seeds were grown immediately in the field along with control following RBD method to raise M_1 generation. The M_2 population was screened for the frequency and spectrum of chlorophyll mutations. In M_2 generation, lethal chlorophyll mutations and viable mutations were screened up for four weeks and through out the plant maturity duration. It was reported that frequency of chlorophyll mutations increased with increasing doses of gamma rays. The highest chlorophyll frequency 4.22% was recorded in 50kR treatment and lowest 1.87% was at 10 kR treatment. The frequency of chlorophyll mutants was higher at higher doses and lower at lower doses of gamma rays and 50 kR dose is found more efficient inducing chlorophyll mutations. The spectrum of chlorophyll mutation showed four types of chlorophyll mutants namely chlorina, xantha, albina and striata. Out of these mutants, chlorina mutant was 5.72%, showed maximum frequency whereas albina mutant 3.06% was least frequent. The spectrum of chlorophyll mutants was found dose dependent in this study.

Keywords - ***Cluster bean, chlorophyll mutations, mutagen, chlorophyll spectrum, gamma rays.***

I. Introduction

Cluster bean or guar *(Cyamopsis tetragonoloba (L.) Taub)* 2n=14, is a multipurpose legume crop, grown for feed, green fodder, vegetable, green mannuring etc. The importance of guar has been highly appreciated as a source of guar gum extracted from it's large endosperm of seeds. Guar gum is hydro colloidal polysaccharide known as galactomannan gum, which has diversified uses in industries and pharmaceuticals used as a medicine in digestive disorders, diabetes, reduction of cholesterols etc. Guar gum is also applied in the food industries and as a source of nitrogen in the soil improvement.

Genetic variability is essential for any crop improvement programme. creation and management of genetic variability is the basic need of crop breeding. Mutation breeding is an important alternative now a days employed in the crop breeding. Induction of mutations is an important source of genetic variability (Singh et al., 2000). In legumes, genetic variability has been exhausted due to natural selection and hence conventional plant breeding methods are not useful (Wani et al., 2001). Lack of sufficient genetic variability is one of the reasons for failure to active break through in the self pollinating crops like legumes as compared to the cereals hence the mutation breeding is the best method to induce genetic variability in the crops within a short time and played significant role in the development of many crop varieties (Micke, 1988).

Various physical mutagens like gamma radiations and chemical mutagens like EMS, MMS, NMU, SA, Colchicine could cause genetic changes in the plants through which promising progenies can be obtained which are useful in the improvement of crop plants. Gamma rays causes severe reshuffling of genetic material and induce different types of variations in the crops than any other radiation. EMS is potential chemical mutagen is responsible for changing metabolic pathways of the plants. The gamma rays and EMS are well known for their importance in mutation breeding and widely used by many workers in several crop plants for their genetic improvement (Kharakwal et al., 2004).

Chlorophyll Mutations are employed as a markers for the evaluation of gene action of mutagenic factors in inducing mutations studies (Gaul, 1964). The spectrum and frequency of chlorophyll mutants are observed in M_2 population easily and is being used as primary index of effectiveness of mutagens and mutability of the genotypes towards the mutagens. Induced chlorophyll mutations is one of the pre requisites to detect the efficiency of physical and chemical mutagens applied to create the spectrum of chlorophyll mutation in the production of genetic variability used for crop improvement (Sarwar and Haq, 2005). The chlorophyll mutations are used as a marker to know the effect of different doses of mutagens in the treated plants for viable mutations (Karunakaran and I.S. Kiss, 1971).

According to Chopra (2005), the high frequency of chlorophyll mutations obtained with mutagen, is due to selective action of chemical and physical mutagens on genes responsible for chlorophyll development or the effects on guanine in the G-C rich chloroplast genome. Generally the nuclear genes control the synthesis of chlorophyll in plants through the long chain of biochemical reactions which involves number of gene loci (Diana Svetleva, 2004). The nuclear genes also control the biogenesis of plastids and the synthesis of chlorophyll is under the control of nuclear genes by the products of regulatory genes, (Von Wettestein et al., 1971). According to some workers, the chlorophyll synthesis is under the control of nuclear and cytoplasmic genes. The chlorophyll mutations which are under nuclear control showed monogenic inherit. The mutation in nuclear gene can change genetic resistance of cytoplasmic DNA, present in chloroplasts or in mitochondrian (Van Herten A.M., 1998).

Several workers have been used physical mutagen like gamma rays and chemical mutagens such as EMS, MMS, NMU, SA to induce the spectrum and frequency of chlorophyll mutations in cluster bean Vig (1969), H.R. Mahala et al., (2010), Bhosle and Kothekar (2010), Velu S. Et al.,(2012). The frequency and spectrum of chlorophyll mutations was recorded by different investigator previously in several other crops such as Trigonella (Vasu and Hasan, 2011), Common bean (Svetleva 2004), Sesame (Sarwar and Haq, 2005, S. Nura et al., 2013, Ramaswamy M.M., 1973), Chick pea (Kharakwal, 1998), horse gram (Kulkarni and Mogle, 2013), rice (A. Vaseline and T. Sabesan, 2011), black gram (Jain S. K. et al., 2008, Gaibriyal et al., 2009), linseed (Rai and Das, 1975), mung bean (Khan, 1992, Yaqoob and Rasheed 2001, Singh and Singh, 2007, Sangiri et al., 2009), Soy bean (Khan and Tyagi, 2010), Jute (Paria and Basak 1997) and durum wheat (Bozzin Muncozza, 1970), induced chlorophyll mutations by using radioactive and chemical mutagens.

In the present investigation, the frequency and spectrum of chlorophyll mutations induced by a physical mutagen gamma rays studied in cluster bean variety NCB-12. Biosynthesis of photosynthetic pigments occurs in sequence of biochemical reactions. The gamma rays induce point mutations and chromosomal aberrations. Any alterations in the nucleotide components of the genes, that control the synthesis of enzymes involved in the biosynthesis of pigments, as a result of action of mutagen, would eventually lead to the observed chlorophyll mutations.

II. Materials and Method

The dry, genetically pure, non dormant viable and good quality seeds of cluster bean variety NCB-12 were irradiated with 10, 20, 30, 40 and 50 KR doses of gamma rays by using ^{60}CO as a source of Mutation Breeding Centre, Department of Biotechnology, B.A.R.C., Mumbai. Five batches of 500 seeds each were irradiated. 50 seeds from each treatment were dried between the folds of filter paper and germinated in Petri plates to record germination percentage. Remaining 450 seeds from each treatment were sown in the field immediately following randomized block design (R.B.D.), with three replications along with control to raise M_1 generation. The seeds were sown at the distance of 30 cm between the plants and 60 cm between the plants and 60 cm between the rows. All the cultural practices recommended by M.P.K.V., Rahuri including irrigation, mannuring, weeding and plant protection methods were followed during the growth period. At maturity all fertile M_1 plants were harvested separately and the seeds were sown in plant to row method to raise M_2 generation, in three replications. The treated and control M_2 plants were carefully screened for the frequency and spectrum of chlorophyll mutations. Frequency of chlorophyll mutations was recorded from the first day of emergence to four weeks on 100 M_2 plant basis. The spectrum of chlorophyll mutations was recorded as per the classification of Gustafsson (1940) and Blixt (1961) as follows -

i) Chlorina : The leaves of the seedlings were light yellowish green (pale green) in colour. They are viable and survived for a reasonably longer period.

ii) Xantha : The seedlings were completely yellowish, they are lethal and survived for only 7-8 days.

iii) Striata : The seedlings shows longitudinal strips of different colours and these are viable.

iv) Albina : The leaves are white in colour, these are lethal and the plant died after few days.

III. Results and Discussions

Frequency of chlorophyll mutations:

In the present investigation, mutation frequencies were calculated on M_2 plant basis and it was recorded that the mutation frequency increased with increasing dose of gamma rays. The chlorophyll mutations were found almost in all the doses of gamma rays. The frequency of chlorophyll mutants in the M_2 progeny increased from 1.87% to 4.22% in gamma rays treatments. The highest frequency of chlorophyll mutations 4.22% was observed in 50 kR while the lowest 1.87% in 10 kR doses, thus frequency of chlorophyll mutations was higher at higher doses and lower at lower doses of mutagen. In the present investigation 50 kR dose proved to be efficient in inducing the chlorophyll mutations.

The findings of this investigation are consistent with the earlier findings of H.R. Mahala et al., (2010) in the varieties of cluster bean RGC 936 and HGS 365. They studied induced chlorophyll mutations in these varieties through the gamma rays and EMS and found that the chlorophyll mutations induced with gamma rays had higher frequency than EMS induced mutations in both the varieties. The mutation frequency found increased with increasing doses of gamma rays and EMS. Similar results were also obtained by Bhosle and Kothekar (2010) in the cluster bean var. GE 36 and HR. The recorded increase in the mutation frequency with increased doses of gamma rays and chemical mutagens EMS and SA. Among the gamma rays treatments, the highest mutation frequency was recorded 2.6% in 15 kR and lowest 2.61% at in 5 kR treatment in the variety GE-36 and 3.33% in 15 kR and 1.16% in 5 kR treatments of gamma rays in the variety HR. These results are in accordance to the results of present studies.

The results of present investigation were supported by previous results recorded in various crops, such as S.K. Sharma and B. Sharma (1979) in Lentil, Mehta et al., (1998) in french bean, Sonone et al., (2008) in ground nut, Kothekar V.S. (1989) in moth bean, Kulkarni and Mogle (2013) in horse gram, Vasu and Hasan (2011) in Trigonella, Svetleva (2004) in common bean, Vaseline and Sabesan (2011) in rice and Gaibriyal et al., (2011) in black gram.

However, S. Velu et al., (2011), calculated mutation frequency in cluster bean variety Pusa-Navbahar on M_2 plant basis showed a dose dependency, where highest chlorophyll mutation frequency was recorded 1.69% at lower doses 20 kR of gamma rays and 0.2% EMS and lowest mutation frequency 1.0% at 0.89% at higher doses of gamma rays at 100 kR and 1.46% in EMS treatment. Thus they recorded decreased mutation frequency with increasing doses of gamma rays and EMS. These results are not confirmatory with the results of present studies but accordance to the results obtained in previous works by Gautam et al., (1998) in Rajmah, Kumar D. et al., (2003) in lima bean, Sarwar and Haq (2004) in sesame, They observed different frequencies of chlorophyll mutations at various doses of gamma rays at from 10,20,30,40,60,80 and 100 kR. The highest frequency (6.66%) was recorded at 100 kR followed by 2.37% at 10 kR, 1.47 % at 30 kR. The lowest frequency of chlorophyll mutations was noted 0.027% at 40 kR and 0.19% at 20 kR dose. These results are partially in accordance to results of present studies.

The frequency of chlorophyll mutations studied by Khan and Tyagi (2010) in the varieties of Soy bean PK-1042 and Pusa-16. It was observed that in cultivar PK-1042, the mutation frequency increased with increased dose of gamma rays while in case of EMS and combined treatments, the mutation frequencies were higher at intermediate doses and then showed a decline with further increase in the doses of mutagens. Whereas, in Pusa-16, the situation was some different, an increase in the doses of gamma rays showed increase in the mutation frequencies at intermediate doses and a graduate decrease at higher doses. Whereas EMS and combined treatment showed slight increase in the mutation frequency with the increased dose or concentration of mutagens.

High frequency of chlorophyll mutations in emmer wheat (Triticum durum var. Khapli., 2n=28) was observed by Swaminathan et al., (1962). Chopra and Swaminathan (1967), observed higher chlorophyll and viable mutation frequency in M_2 under EMS treatment during the comparative study by hydroxylamine and their combination.

According to Blixt et al., (1958), the high frequencies of chlorophyll mutations were obtained in the treatments with chemical mutagens than radiations.

Table 1 : Effect of Gamma rays on the frequency of the chlorophyll mutations in M_2 generation of cluster bean var. NCB-12.

Mutagen	**Dose**	**Total Mutation Frquency (%)**	**Spectrum of Mutations**			
			Albina	**Xantha**	**Chlorina**	**Striata**
Gamma rays	10 KR	1.87	0.40	--	0.87	0.60
	20 KR	2.45	--	0.77	1.17	0.51
	30 KR	3.20	0.64	0.95	0.83	0.78
	40 KR	3.89	0.80	0.90	1.45	0.57
	50 KR	4.22	1.22	1.15	1.40	0.45

Spectrum of chlorophyll Mutations:

In M_2 generation, spectrum of chlorophyll mutations indicates the presence of four chlorophyll mutants viz., albina, xantha, chlorina and striata. All the four types of mutants were recorded in 30,40 and 50 kR treatments, whereas 10 & 20 kR treatments induced only three type of mutants. Among the chlorophyll mutants, highest frequency 5.72% in chlorina and lowest 3.06% in albina chlorophyll mutants.

- **i)** **Albina :** The frequency of albina mutants increased with increasing doses of gamma rays in all the treatments. The highest frequency of albina mutant was recorded 1.22% at 40 kR and lowest 0.40% in 10 kR treatment.

- **ii)** **Xantha :** The frequency of xantha mutants also increased except 40 kR dose, where it was slightly declined. The maximum frequency of xantha mutant was recorded 1.15 % in 50 kR and minimum 0.77% in 20 kR treatment.

- **iii)** **Chlorina :** The highest frequency of chlorina mutant was observed 1.45% in 40 kR and lower 0.83% in 30 kR treatment. The frequency of chlorine mutant was lower at lower dose (0.87%) at 10 kR but alternately increased (1.17% at 20 kR) and decreased (0.83% at 30 kR) in the intermediate doses, again increased at higher doses of gamma rays (1.45% at 40 kR) and slightly decline at 50 kR (1.40%).

- **iv)** **Striata :** Striata mutants were found maximum 0.78% in 30 kR and minimum 0.45% in 50KR treatment. The frequency of striata mutants also showed variations, it was 0.60% at 10 kR, declines at 20 kR (0.51%) again increased (0.78% at 30 kR) and 0.74% at 40 kR but decreased at 50 kR (0.45%) i.e. Higher dose.

Fig.1 Spectrum of Chlorophyll Mutants in M2 generation of Gamma Rays

treated Cluster bean var. NCB-12

Xantha Mutant: 50 kR

Xantha Mutant: 40 kR

Striata Mutant: 30 kR Striata

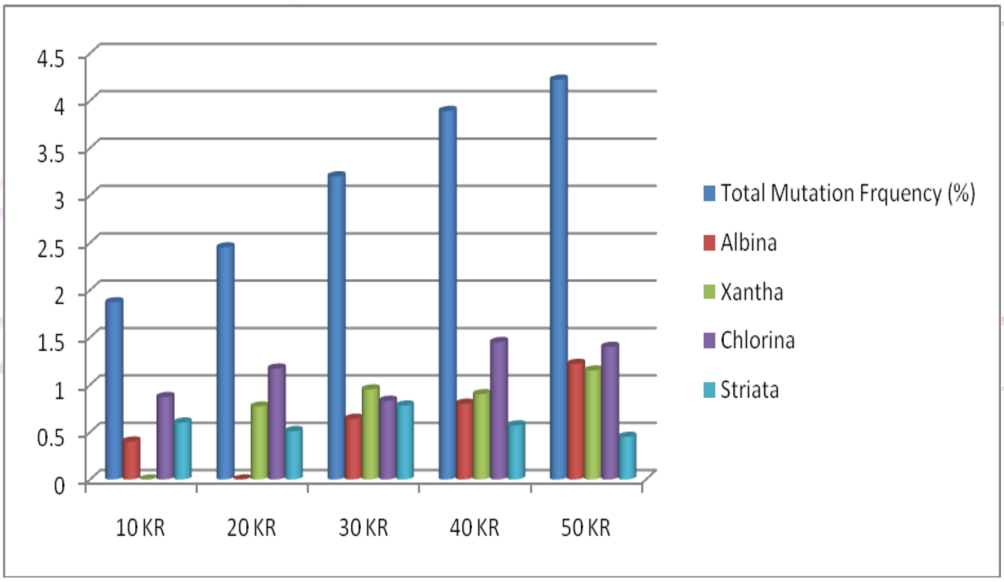
Striata Mutant: 40 kR

Chlorina Mutant: 20 kR

Chlorina Mutant: 30 kR

Albina Mutant: 40 kR

Fig.2 Effect of Gamma rays on the frequency of the chlorophyll mutations in M_2 generation of cluster bean var. NCB-



The induced spectrum of chlorophyll mutations showed irregular pattern in elationto the dose of gamma rays administered and were dose dependent. In the present investigation, all the four chlorophyll mutants were appeared in the following order according to their relative frequencies –

Chlorina (5.72%) > Xantha (3.77%) > Striata (3.08%) > Albina (3.06%)

Previously H. R. Mahala et al., (2010), reported only chlorina, xantha and albina mutants in cluster bean varieties HGS 365 and RGC 936. They observed that albina mutant showed highest frequency among all the treatments (EMS and gamma rays) as compared to EMS. The frequency of albina mutant was higher, in both the varieties however there was no specific pattern was observed for chlorina and xantha. These results supports the results of present investigations.

However Bhosle and Kothekar (2010), induced chlorophyll mutations in cluster bean varieties GE-36 and HR employing three mutagens Gamma rays, EMS and SA. The spectrum of chlorophyll mutants indicated three mutants viz., xantha, viridis and chlorina whereas striata mutant of present investigation, not appeared in these varieties instead viridis was present. Among them chlorina mutant in 0.8% EMS treatment was found higher, this result is analogous to present studies.

Similar results on spectrum of chlorophyll mutation have been reported by Velu S. et al., (2012) in cluster bean variety Pusa-Navbahar. Five types of chlorophyll mutants viz., xantha, chlorina, viridis, virescent and albina were appeared in M_2 population. Viridis and virescent mutants were absent in the present study. Among these mutants the frequency of chlorophyll mutant was highest 66.66% in EMS and 46.66% in gamma rays treatments respectively. The chlorina is more frequent than any other mutant from different doses of mutagens. These findings are in accordance to the present investigation.

Swaminathan et al., (1996) reported EMS was more superior than gamma rays producing higher mutation frequencies and wider spectrum of chlorophyll mutations in M_2 generation.

Khan M.F. and Tyagi S., (2010), induced spectrum of chlorophyll mutants in the cultivar Pusa-16 and PK-1042 of Soy bean through the mutagenic effect of gamma rays and EMS and observed viridis, chlorine, xantha and albina mutants in various treatments. The gamma rays were found more effective than EMS and their combined treatments to induce chlorophyll mutation in both the cultivars. The highest frequency of viridis mutant was reported than chlorina xantha and albina. In present study in cluster bean var, NCB-12, viridis not appeared but the order of mutants according to frequency of mutants like chlorina, xantha and albina in the descending order is in accordance to order of chlorophyll mutants of present investigation.

Gaul (1964) reported the appearance of greater number of viridis type of mutant which is attributed to the involvement of polygene in chlorophyll production.

Gaibriyal et al., (2009) reported six chlorophyll mutants viz., albina, xantha, dark xantha, chlorina, viridis and striata, in black gram var. IPU-982 induced through gamma rays and sodium azide treatment. Out of that frequency of chlorina and viridis were produced higher at lower doses or concentrations of mutagens whereas lethal mutants like albina, xantha and dark xantha were observed more frequent at relatively higher doses or concentration of mutagens. Viridis and dark xantha mutants were not appeared in the present investigation. Like wise chlorina mutant found more frequent at higher doses of gamma rays.

Similar results were obtained by Ignacimuthu and Babu (1988), in Vigna, species, Manju et al., (1983) in horse gram, they observed that spectrum of chlorophyll mutants was dose dependent, these results were in agreement with the results obtained in the present investigation.

Kulkarni and Mogle, (2013) applied three chemical mutagens like EMS, NMU and induced xantha, chlorina, chloroxantha, viridis mutants. Among these mutants chlorina and xantha were higher at higher doses of EMS as compared to SA and NMU. In the present investigation chlorina and xantha were more frequent but viridis and chloroxantha were absent.

In sesame, viridis, xantha and albina mutants were observed with maximum frequency of viridis (1.66%) at 40 kR, followed by xantha (0.43%) at 80 kR and lowest frequency of albina (0.38%) at 20 kR. All the mutants were found at 20 kR whereas at 40 kR only viridis and albina were observed and at 80 kR only xantha type were noted.

However Sheeba et al., (2003) recorded xantha, chlorina, striata and xantha viridis mutants in sesame. It was observed that xantha viridis is more frequent among all the mutants, which was not appeared in clusterbean var. NCB-12. Whereas increased frequency of xantha is in agreement with the xantha mutant and the present studies.

In Trigonella, induced chlorophyll mutations were studied by Vasu and Hasan (2011) and recorded albina, xanthalba, chlorina & striata, out of these xanthalba showed higher frequency and chlorina showed lower frequency. These results are comparable with the results of present investigation.

IV. Conclusions

In the present investigation, the frequency of chlorophyll mutations was increased according to increasing dose of gamma rays. It was higher at higher doses (50 kR) and lower at lower doses (10 kR) of gamma rays. Highest chlorophyll mutant spectrum was noticed in 50 kR treatment. Four types of chlorophyll mutants were recorded viz., albina, xantha, chlorina and striata. Out of that albina and xantha were lethal and chlorina and striata were viable mutants. Chlorina was most frequent whereas albina was less frequent. The spectrum of chlorophyll mutations was dose dependent and they have been appeared in irregular pattern in cluster bean var. NCB-12. These chlorophyll mutants induced by gamma radiation could be used in mutation breeding programmes for inducing viable mutations for improvement of cluster bean varieties.

V. Acknowledgement

The authors are thankful to Dr. S. G. Bhagwat, Head, Mutation Breeding Section, Department of Biotechnology, B.A.R.C., Trombay, Mumbai, for providing necessary facility of source of gamma radiation. Authors are also thankful to principal M. J. College, Jalgaon for providing necessary facilities for this research work.

VI. References

1. Anitha Vasline and T. Sabesan 2011. Assessment of chlorophyll and viable mutation in M_2 generation of rice *(oryzasativa l.)*. Electronic Journal of plant breeding. 2(1) : 107-111.

2. Bhosale Sunita S. and V. S. Kothekar. 2010. Mutagenic efficiency and effectiveness in clusterbean *(Cyamopsis tetragonoloba (L.) Taub).* Journal of Phytology 2010, 2(6): 21-27.

3. Blixt S. Ehrenberg, L. and Gelin, O. 1958. Quantitative Studies of induced mutations in peas I. Methodological investigations. Agri. Hort. Genet., 16 : 238-250.

4. Blixt S. L. 1968. Studies of induced mutations in peas XXIV. Genetically conditioned differences in radiation sensitivity 2. Hereditas, 59:303-325.

5. Bozzini, A. and Scarascia - Muncozza, G.T. 1970. Relative frequency of chlorophyll to morphological and sterility mutations induced in durum wheat by radiation and chemicals. Muta. Res., 9:589-597.

6. Chantira Sangsiri, Worawit Sorajjapinum, Peerasak Srinives. 2005. Gamma radiation induced mutations in Mung bean. Science Asia. 31: 251-255.

7. Chopra V. L. 2005. Mutagenesis : Investigating the processing the outcome for crop improvement. Current science. 89(2) : 353-359.

8. Dheeraj Vasu and Zia-Ul-Hasan 2011. Comparison of induced chlorophyll mutations and spectrum in two varieties of *Trigonella Foenum- Graceium L.* Journal of Phytology. 3(3) : 37-43.

9. Diana Lilova Svetleva. 2004. Induction of chlorophyll mutants in common bean under the action of chemical mutagens ENU and EMS. Journal of central European Agriculture. 5(2) : 85-90.

10. Gabriyal M.L., Bini Toms and Smith Sapha, 2009. Induced Chlorophyll mutations in Black gram. Asian Journal of Agricultural Sciences, 1(1): 1-3.

11. Gaul H. 1964. Mutation in Plant Breeding. Radiation Botany. 4:155-232.

12. Gautam A.S., Sood K.C. and Mitthal R. K. (1998). Mutagenic effectiveness and efficiency of gamma rays and ethyl methane sulphonate in Rajmah *(Phasealus vulgaris L.)* Legume Research 21 : 217-220.

13. Ghulam Sarwar and M.A. Haq. 2005. Radiation Induced chlorophyll mutations in Sesame, *Sesamum indicum L.* Journal of Agriculture Research. 43(4) : 317-322.

14. Gustafsson A. 1940. The mutation system of the chlorophyll apparatus, Lunda Guv. Asskr. M.F. Adv., 2(11) : 1-40.

15. H. R. Mahala, A. Shekhawat and D. Kumar. 2010. A study on EMS and Gamma mutagenesis of cluster bean *(Cyamopsis tetragonoloba (L.) Taub)*. Plant Mutation Reports. 2(2) : 28-32.

16. Ignacimuthu, S. and C.R. Babu 1998. Radio sensitivity of the wild and cultivated Urd and Mung beans. Indian Journal of Genetics and Plant Breeding. 48(3): 331-342.
17. Karunakaran, K. and I.S. Kiss. 1971. M_1 chlorophyll chimeras induced by different mutagens and their M_2 chlorophyll mutation yields in rice. Biologia plantarum (Praha) 13(3) : 207-208.

18. Khan S. and Siddiqui, B.A. 1992. Mutagenic effectiveness and efficiency of chemical mutagens in *Vigna radiata (L).* Wilezek. Thai Journal of Agricultural Sciences. 25: 291-297.

19. Kharakwal M.C., 1998. Induced mutations in chick pea *(Cicer arietinum L.)* A comparative mutagenic effectiveness and efficiency of physical and chemical mutagens. Indian Journal of Genetics. 58 : 159-167.

20. Kharakwal M.C., 2000. Induced mutations in chick pea *(Cicer arietinum L.)* IV. Types of macromutations induced. Indian Journal of Genetics. 60(3) : 305-320.

21. Kharakwal M.C. 2004. Plant breeding - Mendelian to molecular Approaches. (eds.) H.K. Jain and M.C. Kharkwal. 2004. Narosa Publishing House – New Delhi. India. 602-638.

22. Kothekhar V. S. 1989. Differential radio sensitivity in moth bean. Current Science, 58 : 758-760.

23. Kulkarni Ganesh B. and Umesh P. Mogle. 2013. Effects of mutagen on chlorophyll mutations in horse gram *(Macrotyloma uniflorum (Lam) Verdcourt).* Bioscience Discovery. 4(2) : 214-219.

24. Kumar, D., Sassi, Nepolean T., Gopalan A. 2003. Effectiveness and efficiency of the mutagens gamma rays and ethyl methane sulphonate on lima bean *(Phaseolus lunatus L.)*. Indian Journal of Agricultural Research. 37 (2) : 111-119.
25. Manju, P., S.T. Mercy and V. G. Nair, 1983. Induction of variability in horse gram *(Vigna unguiculata)* with EMS and Gamma rays. Legume Research., 6:21-28.

26. Mehta A. K., R. P. Pandey and Naidu A. K. (1998). Mutagenic efficiency and effectiveness of gamma rays and EMS in French bean *(Phaseolus vulgaris L).* Haryana Journal Horticultural Sciences. 27(20):191-121.
27. Micke A. 1988. Genetic improvement of grain legumes using induced mutation. In : Proc. FAO/IAEA workshop on Improvement of grain legume production using induced mutation, 1-5 July, 1986 Pullman, Washington (U.S.A.), IAEA, Vienna, 1988, 1-51.

28. Mudasir Hafiz Khan and Sunil Dutt Tyagi. 2010. Studies on effectiveness and efficiency of gamma rays, EMS and their combination in soy bean *(Glycine max (L.) Merill.)* Journal of Plant Breeding and crop Science: 2(3) : 55-58.

29. Paria P. and Basak S.L. 1997. Inheritance of some induced mutant characters in Jute *(Corchorus olitorius L.)* Indian Journal of Genetics. 57(1): 32-35.

30. Rai, M. and Das, K. 1975. Gamma ray induced chlorophyll mutation in linseed. Indian Journal of Genetics and Plant Breeding. 35: 462-466.

31. Ramasamy, M.M. 1973. Investigation on induced mutagenesis in black gram. Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbtore.

32. S. K. Jain, V.L. Mathur, V. Khandelwal. 2008. Comparative mutagenic efficiency and effectiveness of EMS and DMS in Black Gram *(Vigna mungo (L.) Hepper)*. Crop improvement 35(2) : 146-153.

33. S. Nura, A.K. Adamu, S. Mu Azu, D.B. Dangora and L.D. Fagwalawa. 2013. Morphological Characterization of colchicines induced mutants in Sesame *(Sesamum indicum)*. Journal of Bilogical Sciences. 13(4) : 277-282.

34. Sharma S.K., Sharma B. 1979. Mutagenic effectiveness and efficiency of Gamma Rays and N- Nitroso – N – Methyl Urea in Lentil Indian Journal of Genetics and Plant Breeding 39 (3) 516-520.

35. Sheeba, A., S. M. Ibrahim and P. Yogameenakshi. 2003. Induced chlorophyll mutant studies in sesame *(Sesamum indicum L.)*. Sesame and sunflower N.L. 18: 33-38.

36. Sheeba, A., Ibrahim, S.M.,. Yogameenakshi, P., Babu, S. 2004. Studies on induced chlorophyll mutation in sesame, *(Sesamum indicum L.)*. Madras Agricultural Journal 91(3): 75-78.

37. Singh A. K. and Singh R. M. 2007. Mutagenic effectiveness and efficiency of gamma rays, EMS and their synergetic effects in Mung bean *(Vigna radiata L. Wilczek)*. Crop Science. 34 : 198-202.

38. Singh V.P., Man Singh and J. P. Pal. 2000. Gamma ray and EMS induced genetic variability for quantitative traits in urd bean *(Vigna mungo L. Hepper)*. Indian Journal of Genetics., 60(1) : 89-96.

39. Sonone N.G., Deshmukh S. N., Patil S. P., Rathod S. T., More G. B. 2008. Comparision of mutagenic efficiency and effectiveness of EMS and gamma rays in groundnut *(Arachis hypogea L.)*. Crop Research. 36 (1,2&3) : 336-340.

40. Swaminathan, M.S., Chopra V.L. and Bhaskaran, S. 1962. Chromosome aberrations frequency and spectrum of mutations induced by EMS in barely and wheat. Indian Journal of Genetics. 22:192-207.

41. Swaminathan, M.S., Siddiq, E.A., Savin, V.N., Varughese, G. 1968. Studies on the enhancement of mutation frequency and identification of mutations of plant breeding and phylogenetic significance I n some cereals. Mutation in plant breeding. Proc. Panel. FAO/IAEA Symposium Vienna. 233-249.

42. Swaminathan M.S. 1996. Uses of induced mutations. Indian Farm. 16 : 34-35.

43. Van Harten A.M. 1998. Breeding. Theory and Practical Applications. Cambridge, University Press.

44. Velu S., Mullainathan and D. Arulbalachandran. 2012. Induced morphological variations in cluster bean *(Cyamopsis tetragonoloba (L.) Taub)*. Indian Journal of Current Trends In Research. 1(1) : 48-55.

45. Vig B.K. 1969. Studies with ^{60}CO – Radiated guar *(Cyamopsis tetragonoloba (L.) Taub)*. The Ohio Journal of Science. 69(1) : 18-30.

46. Von Wettstein D., K.W. Henningsen, J.E. Boynton, G.C. Kannangara, O.F. Nielsen. 1971. The Genetic Control of Chloroplast development in barely. In Autonomy and Biogenesis of Mitochondria and Chloroplasts, ed. N.K. Boardman, A.W. Linnae, R.M. Smillie, Amsterdam : North Holland Publishing Company, P. 205-223.

47. Wani A.A. and M. Anis. 2001. Gamma rays induced bold seeded high yielding mutants in chick pea. Mutation breeding newsletter. 45 : 20-21.

48. Yaqoob, M. and Rashid, A. 2001. Induced mutation studies in mung bean (*Vigna radiate (L.) Wilczek).* Cultivars. Online Journal of Biological Sciences. 1 : 805-808.