

Mutagenic effect on seed germination, seedling growth and seedling survival of Pigeon pea (*Cajanus cajan* (L.) Millsp)

M. Ariraman^a, S. Gnanamurthy, D. Dhanavel^b, T. Bharathi, S. Murugan

Division of Cytogenetics and Mutation Breeding, Department of Botany, Annamalai University, Annamalai Nagar - 608 002, Tamil Nadu, India

^{a,b}E-mail address: ariram15@yahoo.com , ddhana2005@yahoo.co.in

ABSTRACT

In the present investigation the seeds of Pigeon pea (*Cajanus cajan* (L.) Millsp) were treated with different doses of gamma radiation (05KR, 10KR, 15KR, 20KR, 25KR, 30KR, 35KR, 40KR, 45KR, and 50KR) and concentration of Ethyl Methane Sulphonate (05mM, 10mM, 15mM, 20mM, 25mM, 30mM, 35mM, 40mM, 45mM, and 50mM) for studying seed germination, seedling height, (shoot and root), seedling injury, seedling vigour index, and seedling survival of plants at 30th day. The seed germination percentage was decreased with increased in the concentration/doses when compared to control. The LD₅₀ (Lethal dose) value was determined based upon the seed germination percentage. The 50 percentage of seed germination and reduction was observed in 20KR of gamma rays and 25mM of EMS and it is considered as LD₅₀ value for both the treatments. The decrease in seed germination was more prominent with gamma rays than that of EMS treatments. The seedling parameters of gamma rays and EMS treated seedlings were progressively decreased with increase dose/concentration in all mutagenic treatments when compared to control. The maximum seedling parameters were observed in 05KR of gamma rays and 05mM of EMS. Minimum seedling parameters were observed in 50mM of EMS and 50KR of gamma rays respectively.

Keywords: Pigeon pea; Gamma rays; EMS; Lethal dose; seedling injury; mutagen and seedling survival

1. INTRODUCTION

Pigeon pea or red gram is an important crop in India, belongs to the family fabaceae, where it is next important pulse crop after chickpea. Pigeon pea seed protein content 21 % compares well with that of other important grain legumes. Pigeon pea contains high level of protein and the important amino acids methionine, lysine, and tryptophan. Pigeon pea is an important grain legume crop of rainfed agriculture in the semi-arid tropics. The genus *Cajanus* comprises 32 species most of which are found in India, Australia and one species is native to West Africa. Pigeon pea is cultivated in more than 25 tropical and sub-tropical countries, either as the sole crop or a mixed crop with sorghum, pearl millet, maize, or with short duration legumes, e.g., groundnut. It plays an important role in food security, balanced diet and alleviation of poverty because of its diverse usages as a food, fodder and fuel. India

is the largest producer of pigeon pea (2.30 mt) followed by Myanmar (0.54 mt) and Malawi (0.16 mt) (FAOSTAT 2007). The Indian sub continent alone contributes nearly 92 per cent of the total world production.

Mutation breeding is one of the conventional breeding methods in plant breeding. It is relevant with various fields like, morphology, cytogenetic, biotechnology and molecular biology etc. Induced mutations are highly effective in enhancing natural genetic resources and have been used in developing improved cultivars of cereals, fruits and other crops (Lee et al., 2002). These mutations provide beneficial variation for practical plant breeding purpose. During the past seven decades, more than 2252 mutant varieties have been officially released in the world (Maluszynski et al., 2000). Induced mutation is highly instrumental in plant biology to induce genetic variability in a great number of crops. The technology is simple, relatively cheap to perform and equally usable on a small and large scale (Siddiqui and Khan, 1999). By varying the mutagenic agent dose, the frequency and saturation of mutations can be regulated (Menda et al., 2004) and mutagenic agents can induce different extensions of genomic lesions, ranging from base mutations to larger fragment insertions or deletions (Kim et al., 2006). Mutagenesis has been widely used as a potent method of enhancing variability for crop improvement (Singh and Singh, 2001). Induced mutation, using physical and chemical mutagen, is a way to generate genetic variation, resulting in the creation of new varieties with better characteristic (Wongpiyasatid, 2000). Mutation has been successfully employed in breeding of several food crop varieties, ornamentals and export crops (Mohamad et al., 2005).

Gamma rays are the most energetic form of electromagnetic radiation, their energy level is from ten to several hundred kilo electron volts and they are considered as the most penetrating compared to other radiations (Kovacs and Keresztes 2002). Gamma radiation can be useful for the alteration of physiological characters (Chaudhuri, 2002), (Kiong et al., 2008). These radicals can damage or change important components of plant cells. They have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the radiation dose (Ashraf et al., 2003). Ethyl Methane Sulphonate (EMS) is mutagenic and carcinogenic organic compound, it produces random mutations in genetic material by nucleotide substitution; particularly by guanine alkylation and it is reported to be the most effective and powerful mutagen (Hajara, 1979) and typically produces only point mutations (Okagaki et al., 1991).

2. MATERIALS AND METHODS

The genetically pure seeds of Pigeon pea variety CO-7 (*Cajanus cajan* (L.) Millsp) received from Tamil Nadu agricultural university (TNAU) Coimbatore. The red gram seeds dried to reduce moisture content up to 10-12 %. Each dose/concentration comprised of 350 seeds. These seeds were irradiated with 5KR, 10KR, 15KR, 20KR, 25KR, 30KR, 35 KR, 40KR, 45KR, and 50KR of gamma rays from ⁶⁰Co source at Indira Gandhi centre for atomic research (IGCAR) Kalpakkam, a dose rate of 234KR/h. Another one mutagens Ethyl Methane Sulphonate (EMS), Solution of Mutagen was prepared in Phosphate buffer of pH 7.

The healthy seed were presoaked in distilled water for 4 hours at room temperature followed by six hours treated with various concentration such as 5mM, 10mM, 15mM, 20mM, 25mM, 30mM, 35mM, 40mM, 45mM, and 50mM, of EMS mutagen, followed by ten times thoroughly washing of seeds under running tap water. Out of 350 seeds in each

treatment, 50 seeds were kept in Petri-dishes for counting germination percentage was placed in the blotting paper.

The effect of gamma rays and EMS treatments was studied with respect to the germination percentage, seedling height, root length, shoot length, seedling vigour index, and seedling survival were analyzed in laboratory condition. Seed germination percentage was recorded at 15th day after sowing. Seedling height, root length, shoots length and seedling vigour index was measured 20th days after sowing. Seedling survival percentage was determined from 30th day after sowing. 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. Mean values of each parameter were recorded in the table.

3. RESULTS AND DISCUSSION

3. 1. Germination percentage

The seed germination in control was 91.00 % (Table 1). It decreased with an increase in the 05KR and the lowest (07.00%) in 50KR. A gradual decrease in germination percentage was observed with an increase in the concentration of EMS (Table 2). It was maximum (86.00 %) in 05mM EMS and minimum (12.00 %) in 50mM EMS concentration (Figure 2). The reduction in the germination percentage induced by EMS treatment was less as compared to that in the gamma rays. In gamma rays treatment induced the maximum inhibition in seed germination with the corresponding increase in its doses. The LD₅₀ (Lethal dose) value was determined based upon the seed germination percentage.

The 50 percentage of seed germination and reduction was observed in 20KR of gamma rays and 25mM of EMS and it is considered as LD₅₀ value for both the treatments. The results were supported by the earlier works of Anbarasan *et al.*, (2013) in sesamum, Bharathi *et al.*, (2013) in Ashwagantha. After the mutagenic treatments, an inhibitory effect on seed germination could be distinctly seen in pigeon pea. Mutagenic treatments revealed a gradual decreasing trend in germination from lower to higher doses (Sunil *et al.*, 2011). The results supported by the works done by Datir *et al.* (2007) in horsegram, Potdukhe and Narkhede (2002) in pigeon pea. The germination of the treated plants had shown a sharp dose rate relationship, which decreased with the increase in the doses / concentration of mutagenic treatments. Percentage reduction / stimulation in seed germination might have been due to the effect of mutagens on meristematic tissues of the seed.

The decrease in seed germination at higher doses / concentration of the mutagens may be attributed to disturbances at cellular level (caused either at physiological (or) physical level). Same results reported that Kumar and Mishra (2004) reported in okra (*Abelmoschus esculentus*) germination percentage generally decreased with increasing doses / concentrations of gamma rays and EMS. Reduced germination percentage with increasing doses of gamma radiation has also been reported in *Pinus* (Thapa, 2004), Rye (Akgun and Tosum, 2004) and Chickpea (Khan *et al.*, 2005 and Toker *et al.*, 2005).

3. 2. Seedling height

The seedling height in control plants was 31.30 cm (Table 1). It was reduced with the corresponding Increase in the doses of gamma rays (Fig. 1), being maximum (28.09 cm) in 05KR and minimum (13.20 cm) in 50KR. The gradual decrease in seedling height was recorded with an increase in the concentration of EMS (Table 2). The highest seedling height (25.12 cm) was observed in 05mM EMS while the lowest (11.53 cm) was noted in 50mM EMS (Fig. 2). The seedling height reduction induced by gamma rays was less as compared to that of EMS. However, the drastic reduction it was recorded in the gamma rays followed by EMS treatments (Tables 1-2, Figure 1-2). Maximum seedling vigour index was 2172.19 at 05 KR dose of gamma rays and 2160.32 at 05mM EMS. The minimum vigour index was 92.4 and 138.36 for 50 KR doses of gamma rays and 50mM EMS respectively as compared to control (2848.3). This indicates that 05KR dose of gamma rays and 05mM EMS treatments have a Stimulatory effect on germination rate and growth of seedling (length of root and shoot) as vigour index calculated by germination percentage multiplying with length of seedling.

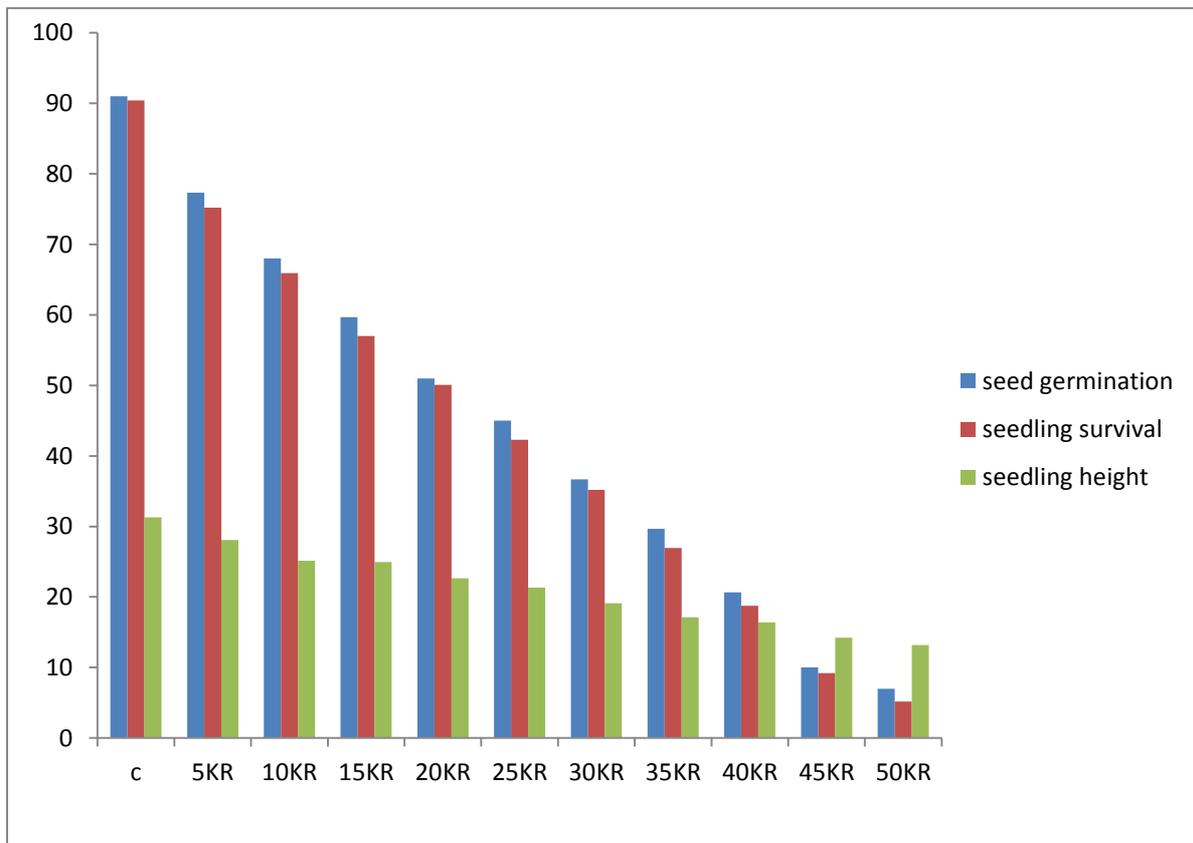
Gamma rays and EMS was drastically reduced the length of root, shoot, seedling and vigour index in Pigeon pea at higher doses / concentrations. Similar observations were made by several workers in sunflower (Jayakumar and Selvaraj 2003). The inhibitory effect of mutagens on the length of seedling was evident from the decrease in length of root and shoot with increasing dose / concentration of gamma rays and EMS. The reduction in length of root and shoot was attributed to the effects of mutagens on the physiological system (Gaul, 1977) such a reduction in length of root and shoot arising out of mutagenic treatments was previously reported in crop plants (Reddy and Gupta, 1989; Amarnath and Prasad 1998 and Uma and Salimath, 2001). The stimulatory effect was observed in lower doses / concentrations of gamma rays and EMS on the length of root, shoot and seedling. The hypothetic origin of these stimulations by irradiation and EMS treatments was due to in cell division rates as well as an activation of growth hormone, e.g., auxin (Zaka et al., 2004).

Table 1. Effect of gamma rays on seed germination and seedling characters of pigeon pea.

Gamma rays Doses	Number of seed sowing	Seed Germination %	Seedling Shoot length (cm)	Seedling Root Length (cm)	Seedling total length (cm)	Seedling vigour index	Seedling injury	Seedling survival %
control	50	91.00	18.66±0.55	10.2±0.30	31.30±0.93	2848.3	—	90.43
05KR	50	77.33	17.26±0.51	9.26±0.27	28.09±0.84	2172.19	10.25	75.21
10KR	50	68.00	16.77±0.50	7.4±0.22	25.14±0.75	1709.52	19.68	65.93
15KR	50	59.66	14.93±0.44	7.2±0.21	24.97±0.74	1489.71	20.22	57.00
20KR	50	51.00	13.93±0.41	6.2±0.18	22.64±0.67	1154.64	27.66	50.08
25KR	50	45.00	12.46±0.37	5.66±0.16	21.33±0.63	959.85	31.85	42.29
30KR	50	36.66	11.13±0.33	5.54±0.16	19.10±0.57	700.20	38.97	35.17
35KR	50	29.66	11.03±0.33	5.53±0.16	17.14±0.51	508.37	45.23	26.93
40KR	50	20.66	08.73±0.26	4.4±0.13	16.39±0.49	338.61	47.63	18.74
45KR	50	10.00	07.6±0.22	4.33±0.12	14.21±0.42	142.1	54.60	09.21
50KR	50	07.00	05.73±0.17	4.13±0.12	13.20±0.39	92.4	57.82	05.16

Table 2. Effect of EMS on seed germination and seedling characters of Pigeon pea.

EMS	Number of seed sowing	Seed Germination %	seedling Shoot length (cm)	seedling Root Length (cm)	Seedling total length (c)	Seedling vigour index	Seedling injury	eedling survival %
control	50	91.00	16.97±0.50	11.98±0.35	28.94±0.86	2633.54	_	90.43
05mM	50	86.00	16.53±0.49	10.05±0.30	25.12±0.75	2160.32	13.19	82.00
10mM	50	75.66	14.51±0.43	9.24±0.27	23.31±0.69	1753.63	19.45	70.33
15mM	50	70.33	14.32±0.42	8.12±0.24	20.78±0.62	1461.45	28.19	67.62
20mM	50	63.66	13.25±0.39	7.06±0.21	19.11±0.57	1216.54	33.96	60.92
25mM	50	50.66	12.18±0.36	6.99±0.20	18.67±0.56	945.82	35.48	49.74
30mM	50	44.66	11.58±0.34	6.45±0.19	16.86±0.50	752.96	41.74	42.09
35mM	50	30.66	9.78±0.29	6.44±0.19	15.00±0.45	459.9	48.16	28.19
40mM	50	23.00	9.38±0.28	5.78±0.17	14.03±0.42	322.69	51.52	20.00
45mM	50	18.33	8.79±0.26	5.05±0.15	13.40±0.40	245.62	53.69	17.02
50mM	50	12.00	6.91±0.20	4.59±0.13	11.53±0.34	138.36	60.15	10.36

**Figure 1.** Effect of gamma ray on seedling characters of pigeon pea.

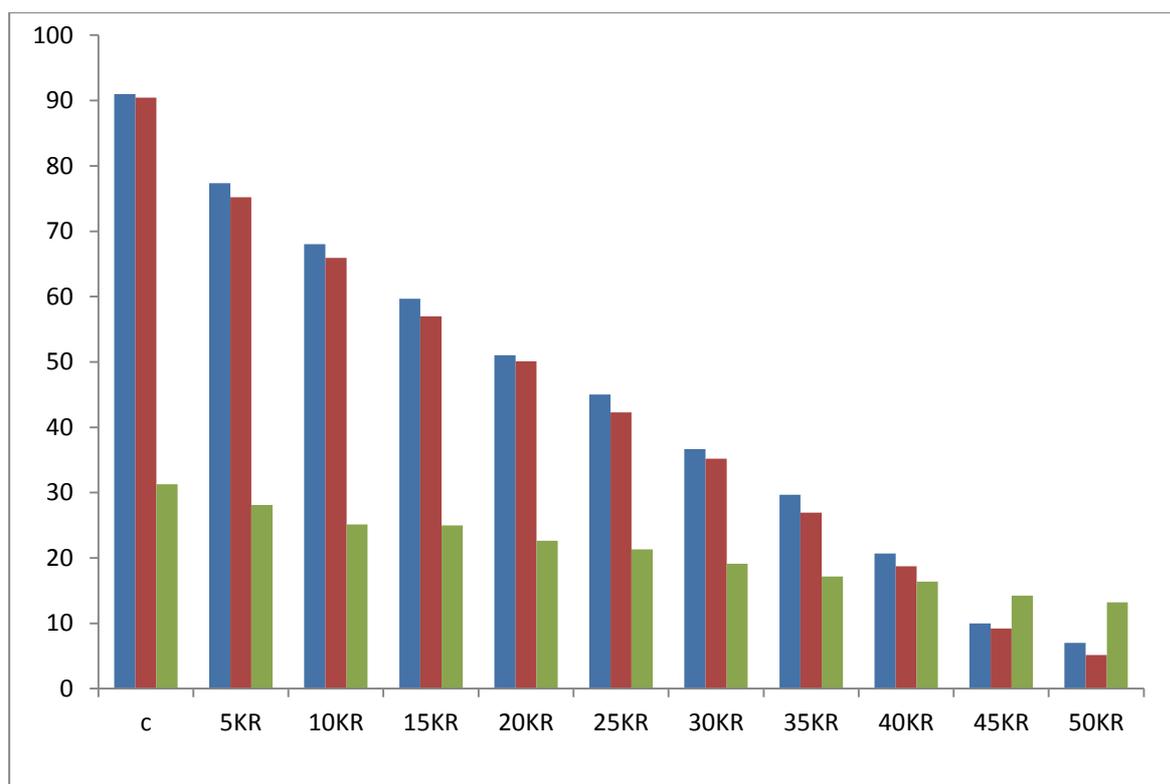


Figure 2. Effect of EMS on seedling characters of Pigeon pea.

3. 3. Seedling survival

The survival of plants in control was 90.43 % on 30th day, (Table 1). However, it was decreased with the increasing doses of gamma rays (Figure 1, 2). It was maximum 75.21 in 05KR and minimum 05.16 in 50KR. The gradual decrease in survival of plants was observed with an increase in the concentration of EMS. It was the highest 82.00 in 05mM EMS and the lowest 10.36 in 50mM EMS (Figure 2). The reduction in the survival percentage induced by gamma rays was less as compared to that by EMS treatments. Sree Ramulu (1970) observed more drastic reduction in the percentage of germination and survival in Sorghum in combination treatment than their alone treatments. Sayed et al. (1975) reported increased lethality with EMS treatment in *Hordeum*. Din et al (2003) decrease in survival percent due to mutagenic treatments was reported by Dalvi (1990) in horsegram, Auti (2005), Barshile (2006), Dhanavel et al., (2008), Kavithamni et al., (2008) and Potdukhe and Narkhede (2002) in various pulse crops.

4. CONCLUSION

Percent seed germination and seedling growth was inhibited due to increasing doses/ concentrations of mutagens. The survival rate was highly reduced with increasing dose/concentration of mutagens. Almost all the mutagenic treatments caused decrease in seedling height, (root and shoot length), seedling injury, and seedling vigour index in a laboratory condition.

References

- [1] Akgun I., Tosum M. (2004). Agricultural and cytological characteristics of M1 perennial rye (*Secale montanum* Guss.) as effected by the application of different doses of gamma rays. *Pakistan Journal of Biological Science*, 7(5); 827-833.
- [2] Amernath S. and A.B. Prasad, (1998), Induced variability in homozygous and heterozygous genotypes of tobacco. *Indian Journal of Genetics*, 58(1), 69-77.
- [3] Amernath S. and A.B. Prasad, (1998), Induced variability in homozygous and heterozygous genotypes of tobacco. *Indian Journal of Genetics* 58(1), 69-77.
- [4] Ashraf, M., Cheema, A. A., M.Rashid, Zia-ul-Qamar. (2003). Effect of gamma rays on M1 generation in basmati rice. *Pakistan Journal of Botany*, 35(5), 791-795.
- [5] Auti S. G. Mutational Studies in mung (*Vigna radiata* (L.) Wilczek). Ph.D. Thesis. (2005); University of Pune, Pune (MS), India.
- [6] Barshile JD. Studies on effect of mutagenesis employing EMS, SA and GR in Chickpea (*Cicer arietinum* L.). Ph.D. Thesis. (2006); University of Pune, Pune (MS), India.
- [7] Bharathi T., Gnanamurthy, S., D. Dhanavel, S. Murugan, M. Ariraman, (2013). Induced Physical mutagenesis on seed germination, lethal dosage and morphological mutants of Ashwagandha (*Withania somnifera* (L.) Dunal). *International Journal of Advanced Research* 1(5), 136-141.
- [8] Dalvi V.V. (1990). Gamma rays induced mutagenesis in horsegram (*Macrotyloma uniflorum* (Lam.) Database. *Mut. Breed. Rev.*, 12, 1-12.
- [9] Datir S.S., Dhumal K.N., Pandey R.N., Gamma radiation and EMS induced variation in seed germination and plant survival in horsegram (*Macrotyloma uniflorum* (Lam.)Verdc). *J. Arid Legumes*. 4(1) (2007) 15-17.
- [10] Dhanavel D, Pavadai P, Mullainathan L, Mohana D, Raju G, Girija M and Thilagavathi C. (2008). Effectiveness and efficiency of chemical mutagens in cowpea (*Vigna unguiculata* (L.) Walp). *African J. of Biotechnology* 7(22): 4116-4117.
- [11] Din R., M.M. Khan, M. Qasim, S. Jehan, M.I. Khan (2003). Induced Mutability Studies in three Wheat (*Triticum aestivum* L.) varieties for Some morphological and agronomic characteristics. *Asian Jour. of Plant Sciences*, 2(17): 1179-1182.
- [12] Gaul H. (1977). Mutagen effects observable in the first generation. I. plant injury and lethality, II. Cytological effects, II sterility In: Manual on Mutation Breeding (second edition). IAEA technical report series No. 119, IAEA, Vienna, Austria, pp. 85-99.
- [13] Hajara N.G. (1979). Induced of mutations by chemical mutagens in tall *indica rice*. *Indian Agric.*, 23: 67-72.
- [14] Jayakumar S., R. Selvaraj (2003). Mutagenic effectiveness and efficiency of gamma rays and ethyl methane sulphonate in sunflower. *Madras Agriculture Journal*, 90(7-9), 574-576.
- [15] Kavithamni D., Kalamani A., Vannirajan C., Uma D. (2008). Mutagenic effectiveness and efficiency of gamma rays and EMS in Soybean (*Glycine max* (L.) Merrill). *Agric. J.* 95(7-12), 448-451.

-
- [16] Khan M.R., A.S. Qureshi, A.H. Syed, M. Ibrahim (2005). Genetic variability induced by gamma irradiation and its modulation with gibberellic acid in M2 generation of Chickpea (*Cicer arietinum* L.). *Pakistan Journal of Botany*, 37(2), 285-292.
- [17] Kim Y., K.S. Schumaker, J.K. Zhu (2006). EMS mutagenesis of Arabidopsis. *Meth. Mol. Biol.* 323: 101-103.
- [18] Kovacs E., Keresztes A. (2002). Effect of gamma and UV-B/C radiation on plant cells. *Micron*, 33(2), 199-210.
- [19] Kumar A., M.N. Mishra (2004). Effect of gamma-rays, EMS and NMU on germination, seedling vigour, pollen viability and plant survival in M1 and M2 generation of Okra (*Abelmoschus esculentus* (L.) Moench). *Advances in Plant Science*, 17(1), 295-297.
- [20] Lee Y. I., I.S. Lee, Y.P. Lim (2002). Variation in sweet potato regenerates from gamma-rays irradiated embryogenic callus. *J Plant Biotech* 4, 163-170.
- [21] Maluszynski, K.N., L.V. Zanten, B.S. Ahloowalia (2000). Officially released mutant varieties. The FAO/IAEA.
- [22] Menda N., Y. Semel, D. Peled, Y. Eshed, D. Zamir (2004). In silico screening of a saturated mutation library of tomato. *Plant J.* 38:861-872.
- [23] Mohamad O., Herman, S., Nazir, B.M., Shamsudin, S., Takim, M. (2005). A dosimetry study using gamma irradiation on two accessions, PHR and PHI, in mutation breeding of roselle. (*Hibiscus sabdariffa* L.). In: 7th MSAB Symposium on Applied Biology, 3-4 June, Sri Kembangan, 1-10.
- [24] Okagaki R.J., M.G. Neffer, S.R. Wessler (1991). A deletion common to two independently derived waxy mutations of maize. *Genetics*, 127: 425-431.
- [25] Potdukhe N.R., Narkhede M.N. (2002). Induced mutation in pigeonpea (*Cajanus cajan* (L.) Millsp.). *J. Nuclear Agric. Biol.* 31 (1): 41-46.
- [26] Reddy V.R.K. and P.K. Gupta, (1989), Biological effects of gamma rays and EMS in hexaploid Triticale. *Acta Botanica*, 17, 76-86.
- [27] Sayed H.I. (1975). Effects of gamma rays and some chemical mutagens on induction of Telotrisomies in *Hordeum*. *Can J. Genetics and Cytology*. XV: 815-825.
- [28] Siddiqui B.A., S. Khan (1999). Breeding in Crop Plants: Mutations and in Vitro Mutation Breeding. 1st ed. Kalyani Publishers, Ludhiana.
- [29] Singh M., Singh V.P. (2001). Genetic analysis of certain mutant lines of urdbean for yield and quality traits in M4 generation. *Ind. J. Pulses Res.* 14(1): 60-62.
- [30] Sree Ramulu (1970). Sensitivity and induction of mutations in Sorghum. *Mutation research*. 10: 197-205.
- [31] Studies on the mutagenic effect of EMS on seed germination and seedling characters of Sesame (*Sesamum indicum* L.) Var. T MV3. *International Journal of Research in Biological Sciences* 3(1): 68-70.
- [32] Sunil M. Sangle, Swapnil E. Mahamune, Sopon N. Kharat, V. S. Kothekar. (2011). Effect of mutagenesis on germination and pollen sterility in pigeonpea. *Bioscience discovery* 102(1).

-
- [33] Thapa C.B. (2004). Effect of acute exposure of gamma rays on seed germination and seedling growth of *Pinus kesiya* Gord and *P. wallichiana* A.B. Jacks. *Our Nature*, 2, 13-17.
- [34] Toker C., B. Uzen, H. Canci, F.O. Ceylan (2005). Effects of gamma irradiation on the shoot length of *Cicer* seeds. *Radiation Physics and Chemistry*, 73, pp. 365-367.
- [35] Wongpiyasatid A., Chotechuen S., Hormchan P. (2000). Induced mutations in mungbean breeding Regional yield trail of mungbean mutant lines. *Kasetsart J. (Nat. Sci.)*. 34: 443-449.
- [36] Zaka R., C. Chenal, M.T. Misset (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.
- [37] M. E. Emefiene, A. B. Salaudeen, A. Y. Yaroson, *International Letters of Natural Sciences* 1 (2013) 6-16.
- [38] M. E. Emefiene, V. I. Joshua, C. Nwadike, A. Y. Yaroson, N. D. E. Zwalnan, *International Letters of Natural Sciences* 13(2) (2014) 73-88.

(Received 22 July 2014; accepted 29 July 2014)