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

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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 LD_{50} (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 LD_{50} 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 ^{60}Co 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 15thday after sowing. Seedling height, root length, shoots length and seedling vigour index was measured 20th days after showing. Seedling survival percentage was determined from 30thday 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 LD_{50} (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 LD_{50} 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

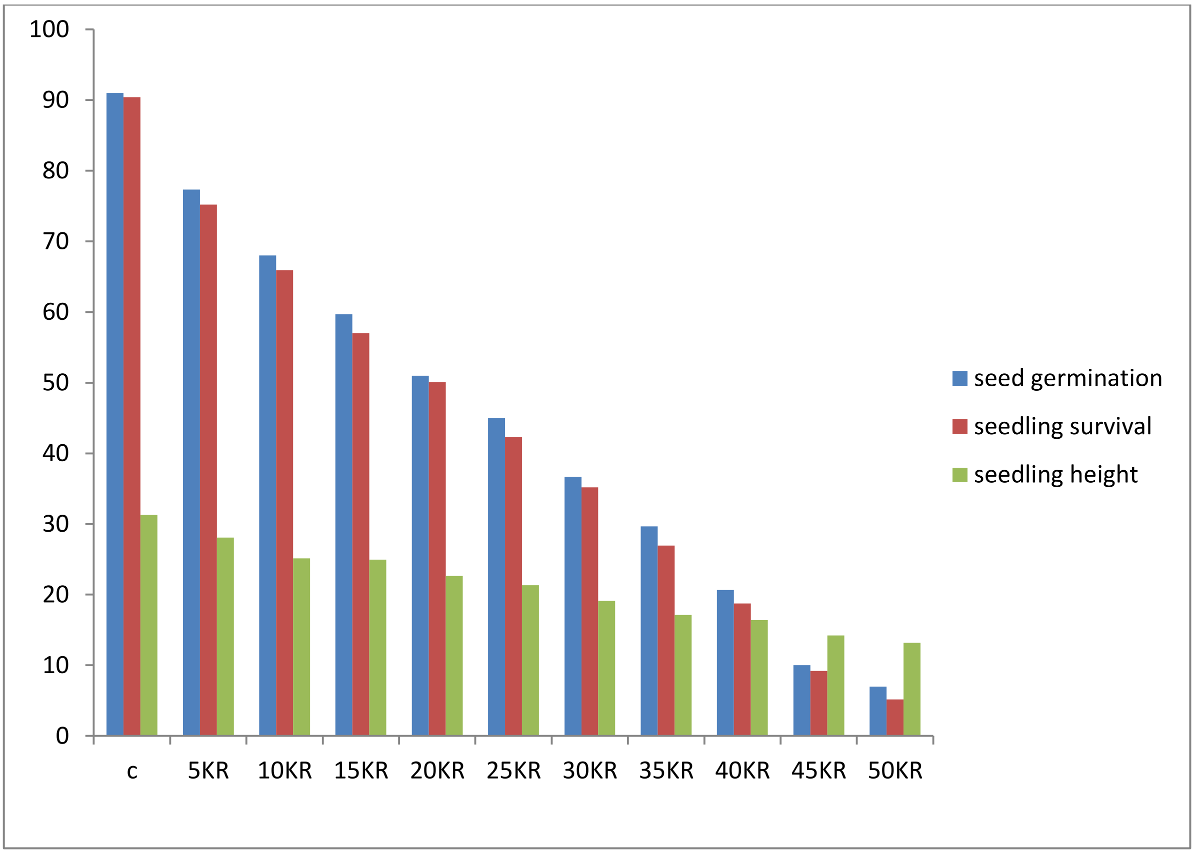

Figure 1. Effect of gamma ray on seedling characters of pigeon pea.

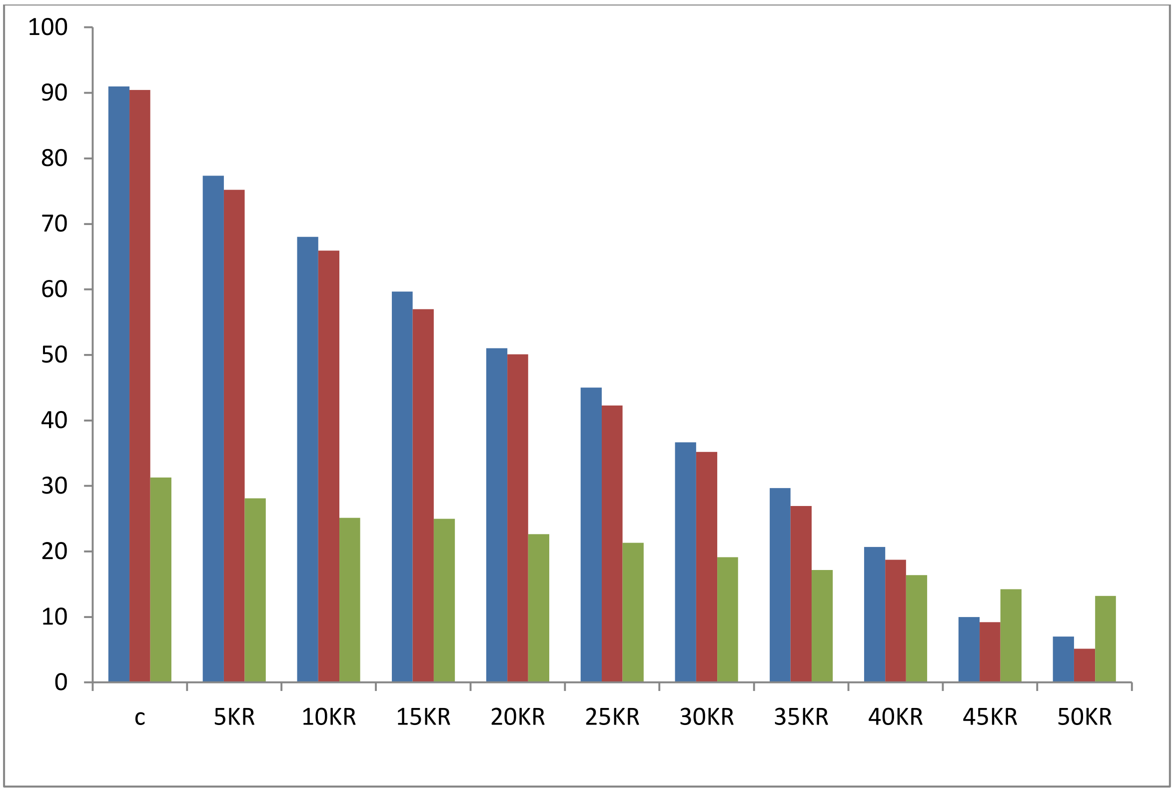

Figure 2. Effect of EMS on seedling characters of Pigeon pea.

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.

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