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Original Research Article

Mutagenic Effect on Seed Germination, Seedling Growth and Seedling Survival of Pea (*Pisum sativum* L.)

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Abstract

In the present investigation the seeds of pea (*Pisum sativum* L.) were treated with different doses of gamma radiation (5KR, 10KR, 15KR and 20 KR) and concentration of Ethyl Methane Sulphonate (0.05%, 0.10%, 0.15% and 0.20%) 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 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 an increase in dose/concentration in all mutagenic treatments when compared to control. Comparatively maximum seedling parameters were observed in EMS concentrations than doses of gamma ray treatment.

Keywords

Ethyl Methane Sulphonate
Gamma ray
Mutagen
Pisum sativum

Introduction

Pea (*Pisum sativum* L.) is one of the earliest known human foods that thrive well in places with cool climate; in India, it is grown in an area of 0.77million ha with an annual production of 0.71 million tonnes and average productivity of 915kg/ ha (www.faostat.fao.org). It is rich in protein, carbohydrate and digestible nutrient content but low in fibre content, which make it an excellent livestock feed. Most of the pea growing area in our country is occupied by traditional and high yielding varieties of field pea which suffers from some constraints like late maturity, lodging, susceptibility to rust *etc*. Induced mutations may play a vital role for the improvement of pea variety. Induced mutations offer possibility for the induction of desired changes in various attributes, which can be exploited as such or through recombination breeding (Akbar and Manzoor, 2003; Khin, 2006). A large range of chemical and physical mutagens have been investigated for their use in crop improvement. Physical mutagens, specially the ionizing radiation, have been widely and routinely used to generate genetic variability in various crop species including pulses (Tomlekova, 2010).

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. 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). 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, can be useful for the alteration of physiological characters. 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).

Materials and methods

The genetically pure seeds of pea variety (DDR-53) received from PDKV Akola. The 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, and 20 KR doses of gamma rays from 60Co source at Department of Biophysics, Government Institute of Science, Aurangabad (M.S.) 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 seeds were presoaked in distilled water for 4 hours at room temperature followed by six hours treated with various concentration such as 0.05%, 0.10%, 0.15% and 0.20% 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 on blotting paper for counting germination percentage. The effect of gamma rays and EMS treatments was studied with respect to the germination percentage, seedling height (root and shoot length), seedling vigour index, and seedling survival percentage were analyzed in laboratory condition.

Seed germination (%)

Seed germination percentage was recorded at 15thday after sowing in Petri-dishes for pilot experiment.

Seed germination (%) = $\frac{\text{No.of seeds germinated}}{\text{No.of seeds sown}} \times 100$

Seedling height (cm)

Seedling height (root and shoot length) was measured on 15th day after sowing in Petri-dishes for pilot experiment.

Seedling vigour index

Seedling vigour index was measured 20th days after sowing.

Seedling vigour index = Seed germination (%) × seedling height

Seedling survival (%)

Seedling survival percentage was determined from 30th day after sowing.

Seedling survival (%) = $\frac{\text{No.of seeds survived}}{\text{No.of seeds germinated}} \times 100$

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.

Results and discussion

Seed germination percentage

The seed germination in control was 96.00 %. It was decreased with an increase in the dose of gamma ray treatment.The germination was recorded maximum (80.00%) in 05KR and the minimum (57.00%) in 20KR dose of gamma ray treatment (Table 1). A gradual decrease in germination percentage was observed with an increase in the concentration of EMS (Table 2). It was maximum (86.00 %) in 0.05% EMS and minimum (32.00 %) in 0.20% EMS concentration (Fig. 1). 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. After the mutagenic treatments, an inhibitory effect on seed germination could be distinctly seen in 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. 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*. Reduced germination percentage with increasing doses of gamma radiation has also been reported in Rye (Akgun and Tosum, 2004) and Chickpea (Khan et al., 2005 and Toker et al., 2005).

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

Gamma rays Doses	Number of seeds sown	Seed germination (%)	Seedling shoot length (cm)	Seedling root Length (cm)	Seedling total length (cm)	Seedling vigour index	Seedling injury	Seedling survival (%)
Control	50	96.00	16.20±0.44	10.55±0.30	28.30±0.68	2716.80	-	94.13
05KR	50	80.00	15.43±0.51	08.14±0.35	24.89±0.50	1991.20	09.85	75.21
10KR	50	74.00	13.80±0.41	08.18±0.41	22.33±0.61	1652.42	17.22	68.67
15KR	50	60.00	11.92±0.33	06.00±0.67	18.05±0.32	1083.00	31.36	60.22
20KR	50	57.00	08.73±0.26	05.12±0.81	14.20±0.83	809.40	54.85	45.16

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

EMS Conc. (%)	Number of seeds sown	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	18.51±0.17	11.01±0.40	30.02±0.84	2731.82	-	92.20
0.05	50	86.00	16.01±0.05	10.11±0.67	28.92±0.78	2487.12	16.91	82.00
0.10	50	78.00	14.20±0.51	08.82±0.62	24.01±0.92	1872.78	21.19	73.27
0.15	50	68.00	12.11±0.32	07.50±0.22	20.32±0.64	1381.76	33.17	63.59
0.20	50	62.00	09.52±0.61	07.98±0.11	17.83±0.61	1105.46	41.47	50.36

Seedling height (cm)

The seedling height in control plants was 28.30 cm (Table 1). It was reduced with the corresponding increase in the doses of gamma rays (Fig. 2), being maximum (24.89 cm) in 05KR and minimum (14.20 cm) in 20KR. The gradual decrease in seedling height was recorded with an increase in the concentration of EMS (Table 2). The highest seedling height (28.92 cm) was observed in 0.05% EMS while the lowest (17.83 cm) was noted in 0.20% EMS (Figure 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 and 2, Figs. 1 and 2). This indicates that 20KR dose of gamma rays and 0.20% of EMS treatments have aninhibitory effect on seedling height (length of root and shoot). 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 by Reddy and Gupta (1989) in *Triticale* and Amernath and Prasad (1998) in tobacco.

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.

Seedling vigour index

Maximum seedling vigour index was 1991.20 at 05 KR dose of gamma rays and 2487.12 at 0.05% of EMS. The minimum vigour index was 809.40 and 1105.46 for 20 KR doses of gamma rays and 0.20% of EMS respectively as compared to control (2716.80 and 2731.82). The seedling vigour index was calculated by germination percentage multiplying with length of seedling. Gamma rays and EMS was drastically reduced the seedling vigour index in pea at higher doses / concentrations. Similar observations were made by several workers in sunflower (Jayakumar and Selvaraj, 2003). The stimulatory effect was observed in concentrations of EMS towards seedling vigour index. 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).

Fig. 1: Effect of EMS concentration on seed germination and seedling characters of pea.

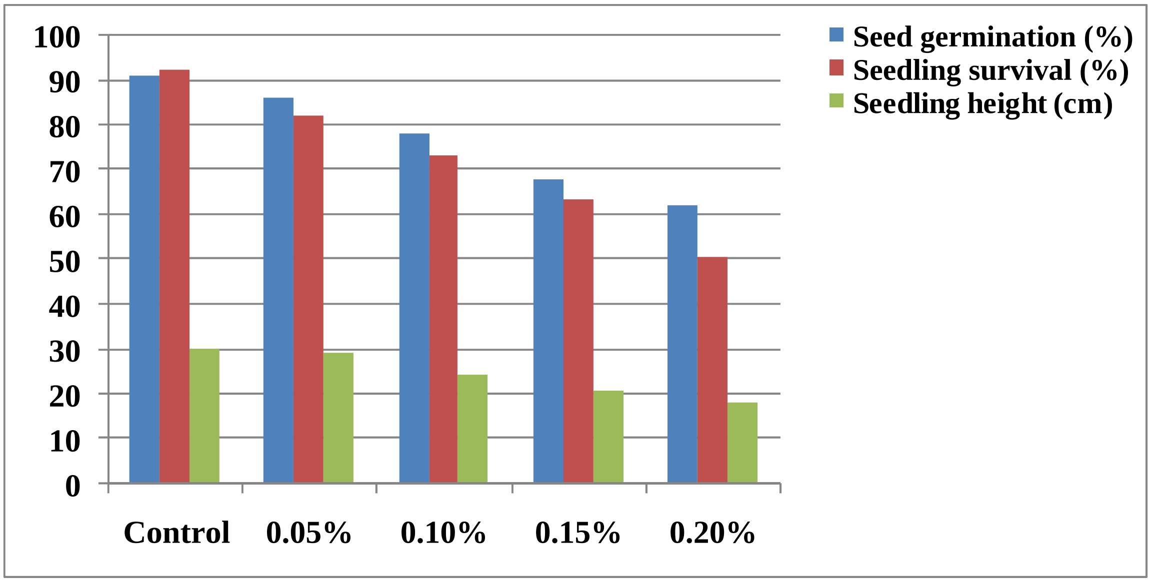
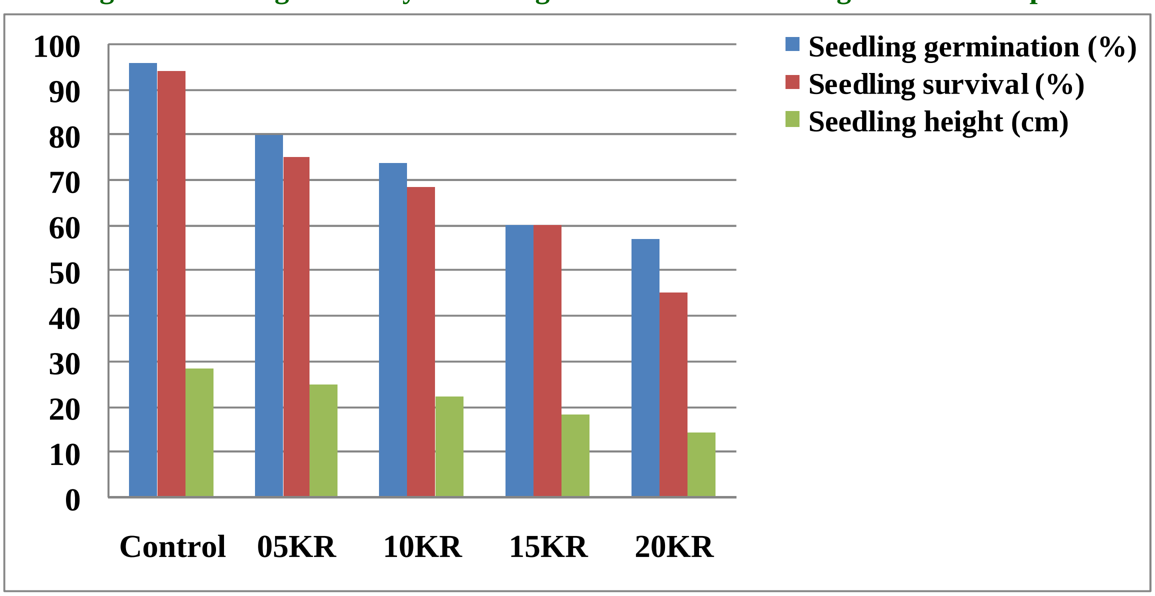


Fig. 2: Effect of gamma rays on seed germination and seedling characters of pea.



Seedling survival (%)

The seedling survival (%) in control was 94.13 % on 30th day, (Table 1). However, it was decreased with the increasing doses of gamma rays (Figs. 1 and 2). It was maximum 75.21 in 05KR and minimum 45.16 in 20KR. The gradual decrease in survival of plants was observed with an increase in the concentration of EMS. It was the highest 82.00 in 0.05% EMS and the lowest 50.36 in 0.20% EMS (Fig. 1). The reduction in the survival percentage induced by gamma rays was less as compared to that by EMS treatments. SreeRamulu (1970) observed more drastic reduction in the percentage of germination and survival in *Sorghum* in combination treatment than their alone treatments. Sayed (1975) reported increased lethality with EMS treatment in *Hordeum*. Decrease in survival percent due to mutagenic treatments was reported byAuti (2005), Barshile (2006), Dhanavel et al. (2008) and Kavithamni et al. (2008) in various pulse crops.

Conclusion

The percentage of seed germination and seedling growth was inhibited with an increasing dose/ concentration of mutagens. The survival rate was highly reduced with an increasing dose/ concentration of mutagens. Almost all the mutagenic treatments caused decrease in seedling height, seedling injury and seedling vigour index in laboratory condition. Comparatively the physical mutagen, gamma ray shows more inhibitory effect towards all parameters.

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