

Gamma Rays and EMS Induced Morphological Parameters in *Hibiscus Sabdariffa*

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Abstract: Dry seeds of *Hibiscus Sabdariffa* (Family - Malvaceae) were irradiated with Gamma rays at 10 to 60 KR. Presoaked seeds (6 to 18 hrs.) were treated with 0.1 to 0.5 % EMS & For combination treatments, irradiated seeds were immersed in 0.2 % EMS. Observations on the morphological parameters were recorded after One, Two & Three months. The lower exposures of gamma rays reduced plant height, number of leaves and area of leaf to smaller extent while drastic reduction was observed with higher doses. EMS in alone treatments induced reduction in all these parameters with the corresponding increase in the duration of presoaking & concentration of EMS. In case of combination treatments all these parameters showed gradual decrease with the corresponding increase in the dose of gamma rays followed by EMS concentration. The present paper deals with the results of these experiments.

Keywords: *Hibiscus sabdariffa*, Gamma rays, EMS, Combination treatments

1. Introduction

Roselle (*Hibiscus sabdariffa* L.) belonging to family Malvaceae is an annual plant cultivated for its stem, fibres, edible calyces, leaves & seeds. In some regions, it is grown for fibres & pulp obtained from stem (Rao, 1996). The calyx is widely used due to its high content of anthocyanin and antioxidant properties (Francis, 2000). *Hibiscus sabdariffa* is traditionally used to deal with several health problems, including hypertension, pyrexia and liver disorders, microorganism growth and also as a diuretic, sedative, or digestive (Faraji and Tarkhani, 1999; Chen *et al.*, 2003; Akindahunsi and Olaleye, 2003). Due to these utilities, now a days the demand for improved varieties of this plant is increasing. Ionizing radiations & chemical mutagens are the principal agents to increase mutation frequency in plants (Dabholkar *et al.*, 2006). In this plant, the work on mutational studies is very meagre (Shrivastava & Tiwari, 2008; Mohamad *et al.*, 2009). Considering the wide scope of mutagenesis in developing new varieties, the present investigators have made an attempt to induce variability in *Hibiscus sabdariffa*.

2. Materials and Methods

Genetically pure seeds of *Hibiscus sabdariffa* (2n=72) obtained from Centre of Science for Villages (Dattapur, Dist. Wardha) were used in these studies. Dry seeds were irradiated at Post Graduate Teaching Department of Chemistry, RTM Nagpur University, Nagpur for 10, 20, 30, 40, 50 and 60 KR.

Dry and presoaked seeds (6, 12, and 18 Hours) were treated with freshly prepared 0.1, 0.2, 0.3, 0.4, 0.5% solutions of EMS for 18 hours with post soaking of 2 hours in deionized water.

For combination treatments the gamma irradiated seeds (10, 20, 30, 40, 50 KR) were immersed in 0.2% EMS for 18 hours and handled in the same manner as those of alone EMS treatments. Control seeds were handled in the same manner as those of treated ones.

3. Results & Discussion

1) Plant height

The data related to the plant height induced by different mutagenic treatments have been given in Tables 1-3 & Figs. 1-3.

a) Effect of Gamma rays :

In control, the plant height after three months was 49.6 cm (Table 1). It decreased with an increase in the dose of gamma rays (Fig. 1). The lower doses of gamma rays reduced the height to smaller extent while drastic reduction was observed with higher doses. After three months the maximum height (47.2 cm.) was recorded in 10 KR while minimum (32.7 cm) was seen in 60 KR.

b) Effect of EMS :

After three months the plant height in control was 58.7 cm, 55.2 cm, 53.1 cm & 50.0 cm in dry, 6 hrs, 12 hrs & 18 hrs presoaked sets, respectively (Table 2). EMS in alone treatment induced reduction in height of plant with the corresponding increase in duration of presoaking & EMS concentration. After three months the maximum height (55.3 cm, 52.4 cm, 51.9 cm & 48.7 cm) was recorded in 0.1 % EMS while minimum (44.1 cm, 39.7 cm, 36.5 cm & 34.7 cm) was noted in 0.5 % EMS in dry, 6 hrs, 12 hrs & 18 hrs sets, respectively (Fig. 2).

c) Effect of Gamma rays followed by EMS :

In control, after three months the plant height was 46.5 cm. (Table 3). It was decreased with an increase in the dose of gamma rays followed by EMS concentration. After three months, it was the highest (43.3 cm) in 10 KR + 0.2 % EMS & the lowest (29.1 cm) in 50 KR + 0.2 EMS (Fig - 3).

The reduction in plant height induced by gamma rays & gamma rays followed by EMS was more as compared to that in the EMS alone treatments. (Tables 1-3, Figs. 1-3).

According to Sparrow & Gunckel, (1955) damaged chromosomes due to mutagenic treatments have an important correlation with the decline in growth. Konzak *et al.* (1972); Katoch *et al.* (1992) and Wang *et al.* (1995); Cheema and Atta (2003), reported seedling height reduction in rice. Sinha and Chowdhury (1991) in Pigeon pea

documented Seedling height reduction. Banerji & Datta,(1992) reported a reduction in plant height of *Chrysanthemum* when irradiated with gamma rays doses of 20 or 25 krad. In the opinion of other workers, the mutation frequency, might have been controlled by several factors, for instance the mutagen mechanism of action (Griffiths *et al.*, 1993), position of gene in genome (Swoboda *et al.*, 1993), the size of target gene and composition of nucleotide

(Bichara *et al.*, 1995).Harding & Mohamad (2009) in Roselle recorded reduction in plant height after treating with Gamma rays. Osman *et al* (2011), reported the reduced plant height in Roselles after treating with higher gamma rays. Higher doses of mutagens showed lower plant height because they restricted the somatic cell division, reduced viability & increased growth abnormalities.

Table 1: Effect of gamma rays on plant height, number of leaves & leaf area.

| Sr. No. | Doses | Plant height (cm) | | | Number of leaves | | | Leaf area (cm ²) | | |
|---------|---------|-------------------|------------|--------------|------------------|------------|--------------|------------------------------|------------|--------------|
| | | One month | Two months | Three months | One month | Two months | Three months | One months | Two months | Three months |
| 1 | Control | 28.3 | 46.8 | 49.6 | 8.8 | 12.6 | 13.7 | 12.4 | 12.8 | 13.8 |
| 2 | 10 KR | 26.2 | 44.3 | 47.2 | 8.6 | 12.2 | 13.3 | 12.3 | 12.4 | 13.4 |
| 3 | 20 KR | 25.3 | 42.1 | 46.5 | 8.2 | 11.8 | 12.4 | 11.5 | 12.2 | 13.2 |
| 4 | 30 KR | 23.2 | 39.8 | 44.7 | 7.6 | 11.6 | 12 | 10.5 | 11.8 | 12.8 |
| 5 | 40 KR | 22.3 | 36.2 | 40.0 | 7.4 | 10.0 | 10.9 | 9.9 | 11.0 | 12.0 |
| 6 | 50 KR | 20.1 | 32.1 | 35.8 | 6.8 | 8.8 | 9.8 | 9.1 | 10.0 | 11.3 |
| 7 | 60 KR | 18.2 | 29.3 | 32.7 | 5.6 | 6.2 | 7.2 | 8.4 | 9.8 | 10.8 |

Table 2: Effect of EMS treatment on plant height, number of leaves & leaf area.

| Sr. No. | Doses | Plant height (cm) | | | Number of leaves | | | Leaf area (cm ²) | | |
|---------|--------------------|-------------------|------------|--------------|------------------|------------|--------------|------------------------------|------------|--------------|
| | | One month | Two months | Three months | One month | Two months | Three months | One months | Two months | Three months |
| 1 | Control (DRY) | 31.7 | 45.9 | 58.7 | 12.8 | 14.2 | 22.4 | 22.1 | 22.7 | 23.0 |
| 2 | Dry 18h 0.1EMS | 28.6 | 43.7 | 55.3 | 12.6 | 13.6 | 21.6 | 20.0 | 20.5 | 21.1 |
| 3 | Dry 18h 0.2EMS | 26.5 | 42.6 | 51.7 | 11.8 | 12.6 | 20.8 | 15.5 | 16.1 | 16.7 |
| 4 | Dry 18h 0.3EMS | 24.6 | 40.5 | 49.5 | 11.2 | 12.2 | 19.6 | 14.8 | 15.3 | 15.9 |
| 5 | Dry 18h 0.4EMS | 22.1 | 38.5 | 46.7 | 10.2 | 11.2 | 12.8 | 12.8 | 13.9 | 14.5 |
| 6 | Dry 18h 0.5EMS | 21.9 | 34.6 | 44.1 | 8.8 | 9.8 | 11.6 | 11.9 | 12.8 | 13.9 |
| 1 | Control (6 h psw) | 28.6 | 44.1 | 55.2 | 12.6 | 13.6 | 21.0 | 20.1 | 21.0 | 22.2 |
| 2 | 6 h psw+18h0.1EMS | 25.5 | 42.9 | 52.4 | 12.0 | 13.0 | 20.8 | 18.7 | 19.3 | 20.8 |
| 3 | 6 h psw+18h0.2EMS | 24.7 | 41.1 | 49.3 | 11.6 | 12.0 | 18.3 | 13.4 | 15.8 | 16.5 |
| 4 | 6 h psw+18h0.3EMS | 22.6 | 39.7 | 44.7 | 10.6 | 11.6 | 16.2 | 12.6 | 14.8 | 15.7 |
| 5 | 6 h psw 18h 0.4EMS | 20.2 | 33.6 | 42.6 | 9.8 | 10.8 | 11.9 | 12.3 | 13.6 | 14.2 |
| 6 | 6h psw+18h0.5EMS | 19.8 | 30.3 | 39.7 | 8.2 | 9.2 | 10.0 | 11.2 | 12.3 | 13.3 |
| 1 | Control (12h psw) | 23.5 | 42.7 | 53.1 | 12.2 | 13.2 | 19.7 | 17.5 | 17.8 | 19.1 |
| 2 | 12h psw 18h0.1EMS | 21.7 | 41.1 | 51.9 | 11.8 | 12.8 | 18.3 | 16.5 | 17.4 | 18.7 |
| 3 | 12hpsw+18h0.2EMS | 20.1 | 38.9 | 46.7 | 10.8 | 11.8 | 17.4 | 13.9 | 15.5 | 16.2 |
| 4 | 12h psw+18h0.3EMS | 18.0 | 36.7 | 42.9 | 10.0 | 11.0 | 15.3 | 12.5 | 14.2 | 15.0 |
| 5 | 12h psw+18h0.4EMS | 16.7 | 30.1 | 40.8 | 9.4 | 10.4 | 10.9 | 12.2 | 13.3 | 13.9 |
| 6 | 12h psw+18h0.5EMS | 15.3 | 28.7 | 36.5 | 7.8 | 8.8 | 9.6 | 11.0 | 12.1 | 12.7 |
| 1 | Control (18h psw) | 21.0 | 40.9 | 50.0 | 12.0 | 13.0 | 17.9 | 17.0 | 17.5 | 17.9 |
| 2 | 18h psw+18h0.1EMS | 19.9 | 38.6 | 48.7 | 11.4 | 11.8 | 15.5 | 16.1 | 17.2 | 17.8 |
| 3 | 18h psw+18h0.2EMS | 18.1 | 36.7 | 44.8 | 10.2 | 11.2 | 13.6 | 13.2 | 15.3 | 15.6 |
| 4 | 18h psw+18h0.3EMS | 16.9 | 34.4 | 40.1 | 9.2 | 10.2 | 12.0 | 12.4 | 14.0 | 14.1 |
| 5 | 18h psw+18h0.4EMS | 15.1 | 28.1 | 37.6 | 9.0 | 10.0 | 11.2 | 11.3 | 13.1 | 13.2 |
| 6 | 18h psw+18h0.5EMS | 14.6 | 26.3 | 34.7 | 7.0 | 7.6 | 8.9 | 10.8 | 11.9 | 12 |

h = hours ; psw = pre-soaked in water.

Table 3: Effect of gamma rays followed by EMS treatment on height of plant, number of leaves & area of leaf

| Sr. No. | Doses | Plant height (cm) | | | Number of leaves | | | Leaf area (cm ²) | | |
|---------|--------------------|-------------------|------------|--------------|------------------|------------|--------------|------------------------------|------------|--------------|
| | | One month | Two months | Three months | One month | Two months | Three months | One months | Two months | Three months |
| 1 | Control | 26.1 | 43.1 | 46.5 | 8.6 | 12.6 | 13.6 | 11.5 | 12.6 | 13.9 |
| 2 | 10KR+18 h 0.2EMS | 24.7 | 41.9 | 43.3 | 8.4 | 12.2 | 13.2 | 9.3 | 12.2 | 13.5 |
| 3 | 20KR+18 h 0.2 EMS | 22.1 | 40.2 | 41.2 | 8.0 | 12.0 | 13.0 | 9.1 | 12.0 | 12.8 |
| 4 | 30 KR+18 h 0.2 EMS | 21.3 | 34.3 | 37.3 | 7.4 | 11.6 | 12.6 | 6.6 | 11.6 | 12.3 |
| 5 | 40 KR+18 h 0.2 EMS | 20.6 | 30.1 | 33.2 | 7.2 | 10.8 | 11.8 | 6.0 | 10.8 | 11.4 |
| 6 | 50 KR+18 h 0.2 EMS | 18.7 | 25.7 | 29.1 | 6.4 | 8.6 | 9.6 | 5.1 | 8.6 | 9.9 |

h = hours

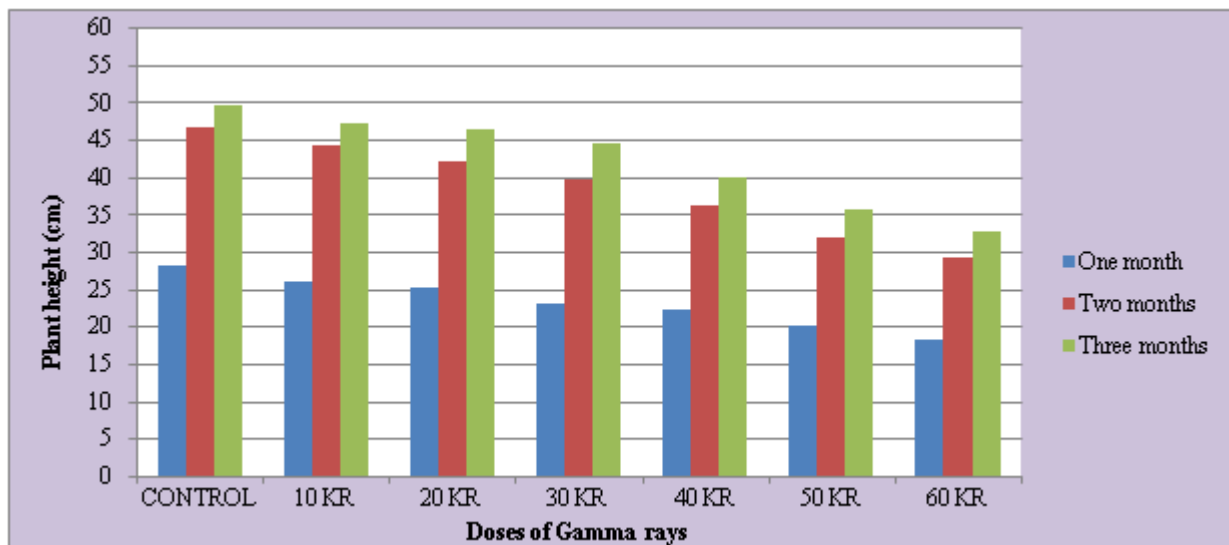


Figure 1: Effect of gamma rays on plant height (cm)

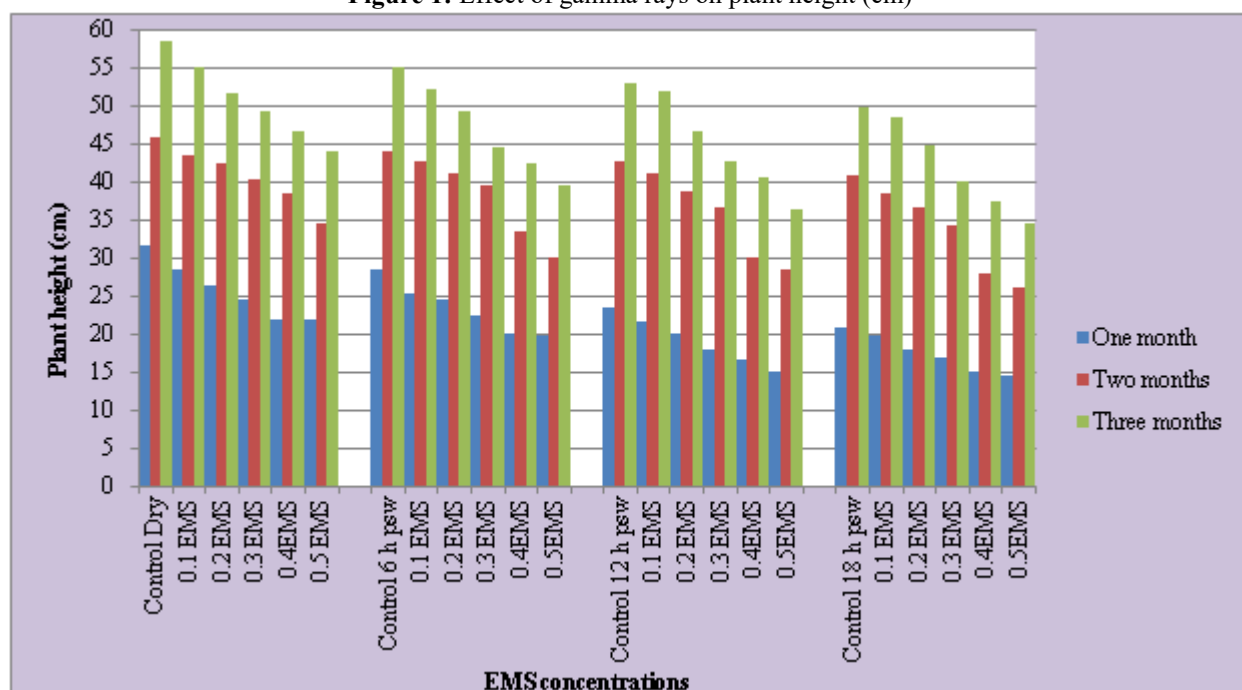


Figure 2: Effect of EMS on plant height (cm)

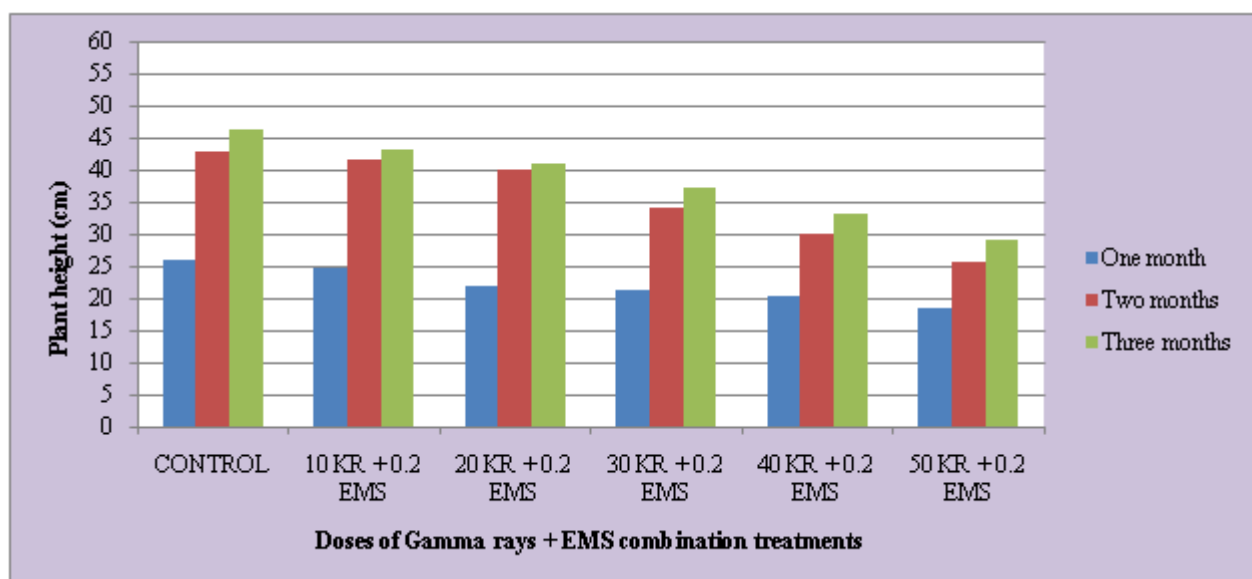


Figure 3: Effect of gamma rays followed by EMS on plant height (cm)

2) Number of leaves

Gamma irradiation ,EMS treatments and gamma rays followed by EMS treatments showed effect on average number of leaves as mentioned in Tables 1-3 & Figs. 4-6.

a)Effect of Gamma rays

In control, the number of leaves after three months was 13.7 (Table 1) .The average number of leaves gradually decreased with increasing doses of gamma rays (Fig.4) .It was the highest (13.3) in 10 KR & the lowest (7.2) in 60 KR.

b)Effect of EMS

In EMS treatment, after three months the number of leaves in controls were 22.4, 21.0, 19.7 ,17.9 in dry,6 hrs,12 hrs & 18 hrs presoaked sets, respectively (Table 2).EMS in alone treatment induced reduction in average number of leaves with the corresponding increase in duration of presoaking & EMS concentration. After three months it was maximum i.e.21.6 ,20.8, 18.3 & 15.5 in Dry, 6 hrs, 12 hrs & 18 hrs., sets,. respectively, treated with 0.1% EMS. It was minimum i.e.11.6, 10.0, 9.6 & 8.9 in dry , 6 hrs, 12 hrs & 18 hrs sets., respectively treated with 0.5% EMS. (Fig.5).

c) Effect of Gamma rays followed by EMS

After three months the number of leaves in control was 13.6 (Table 3).The number of leaves were decreased with an increase in the dose / concentration of gamma rays followed by EMS. It was highest (13.2) in 10 KR+ 0.2 % EMS & the lowest (9.6) in 50 KR + 0.2 EMS (Fig .6).

The reduction in the number of leaves induced by gamma rays & gamma rays followed by EMS was more as compared to the EMS alone treatments .(Tables 1-3, Figs 4-6).

Ramachandran & Goud (1983) in Safflower reported decreased number of leaves at higher doses of gamma irradiations. Thilagavathi and Mullainathan, (2009) in Black gram and Girija and Dhanavel, (2009) in Cowpea observed the decreased in all the morphological characters of M₁ generation with the increase in concentration of mutagenic treatments. The observed reduction was more in the case of EMS. which was depended on physiological characters for different crops. Yaqoob and Ahmad (2003) in Mung beans & Abdul Majeed (2010) in *Lepidium sativum* observed the reduction in number of leaves by radiation doses.

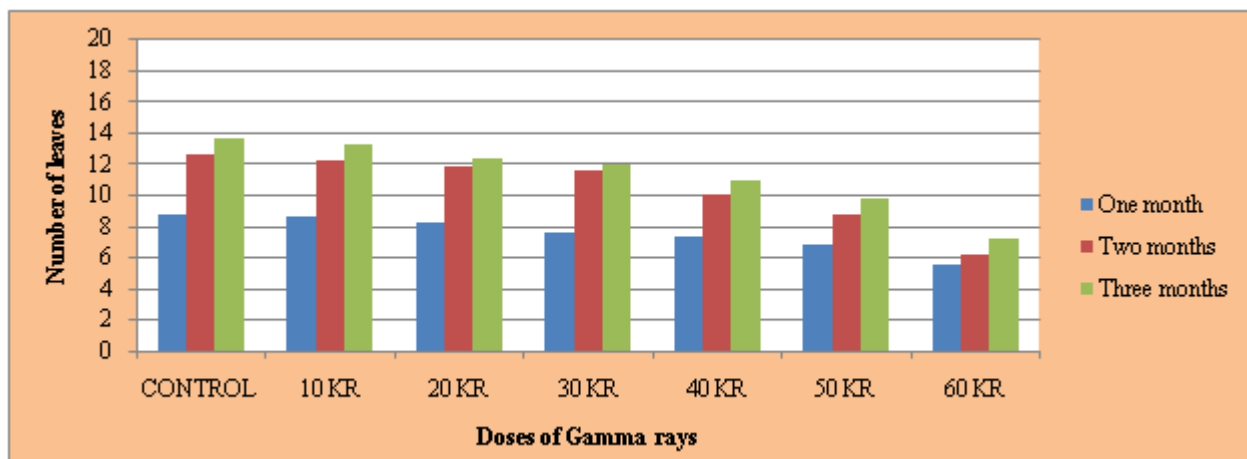


Figure 4 : Effect of gamma rays on number of leaves

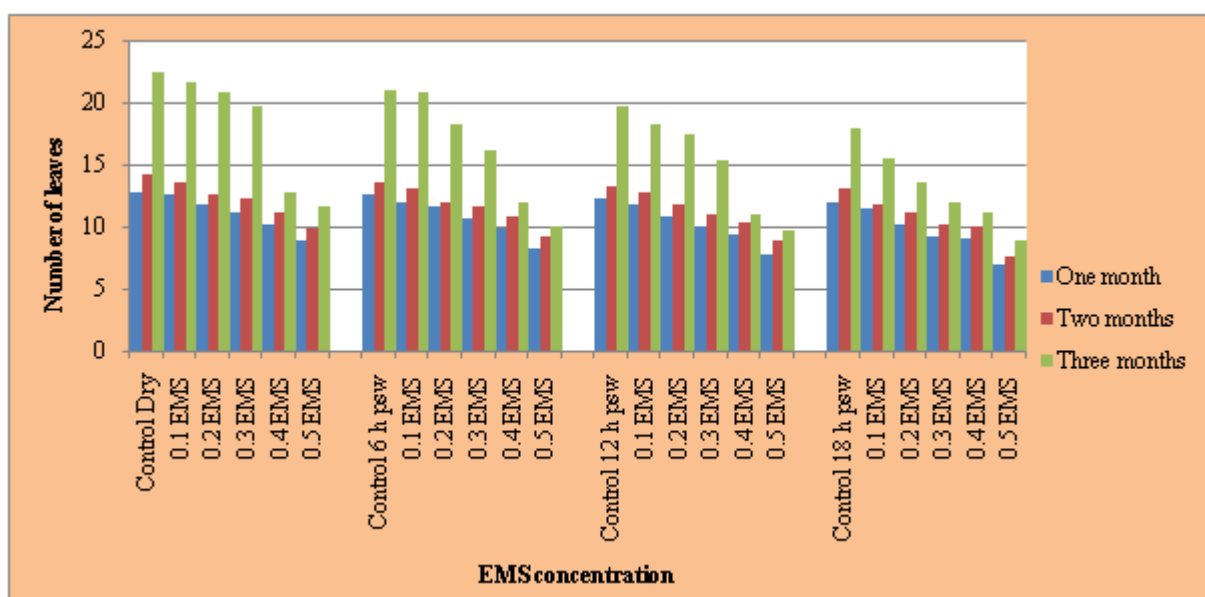


Figure 5: Effect of EMS on number of leaves.

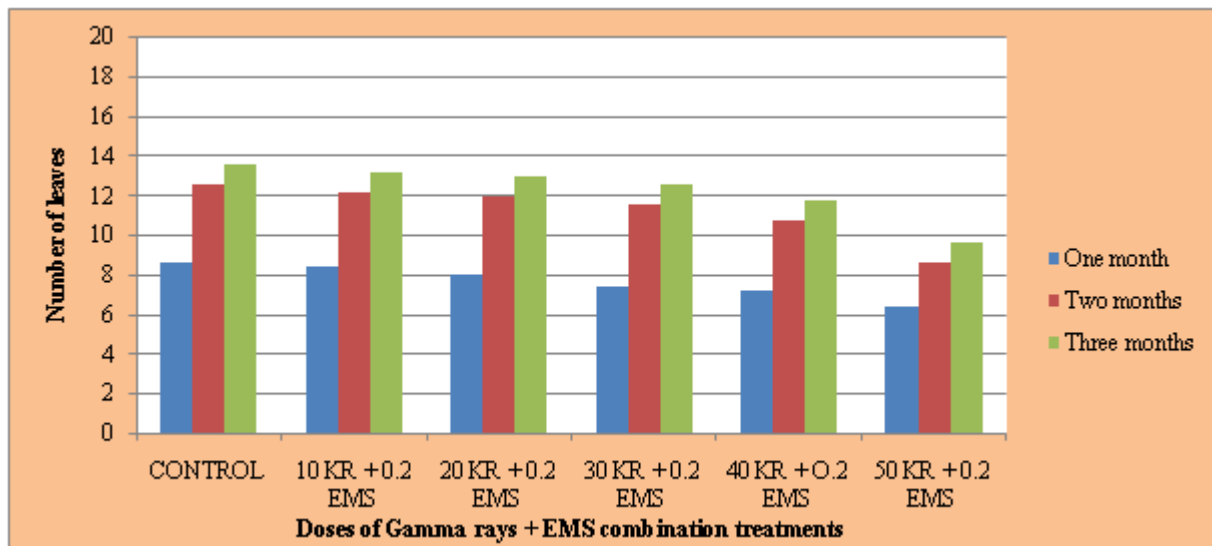


Figure 6: Effect of gamma rays followed by EMS on number of leaves

3) Area of leaves

The data related to the effect of mutagens on average area of leaves have been given in Tables 1-3 & Figs,7-9.

a) Effect of Gamma rays :

In control, the area of leaf after three months was 13.8 cm² (Table 1). It decreased with an increase in the dose of gamma rays (Fig. 7). The lower doses of gamma rays reduced the area of leaf to smaller extent while drastic reduction was observed with higher doses. After three months, the area of leaf recorded was the highest (13.4 cm²) in 10 KR & the lowest (10.8 cm²) in 60 KR.

b) Effect of EMS

After three months, the area of leaf in dry, 6 hrs, 12 hrs & 18 hrs presoaked controls was 23.0 cm², 22.2 cm², 19.1 cm², 17.9 cm², respectively (Table 2). The gradual reduction in area of leaf was recorded with the corresponding increase in duration of presoaking & EMS concentration. After three months the maximum area of leaf i.e. 21.1 cm², 20.8 cm², 18.7 cm², 17.8 cm² was noted in dry, 6 hrs, 12 hrs & 18 hrs sets, respectively in 0.1 % EMS, & minimum i.e. 13.9 cm², 13.3 cm², 12.7 cm² & 12 cm² was observed in respective same sets treated with 0.5 % EMS (Fig.8).

c) Effect of Gamma rays followed by EMS :

In control, the area of leaf after three months was 13.9 cm² (Table 3). It was decreased with an increase in the dose of

gamma rays followed by EMS concentration. It was highest (13.5 cm²) in 10 KR+ 0.2 % EMS & the lowest (9.9 cm²) in 50 KR + 0.2 EMS (Fig. 9).

The reduction in the area of leaf induced by gamma rays & gamma rays followed by EMS was more as compared to the EMS alone treatments (Table 1-3, Figs.7 - 9).

The results of leaf area were in agreement with the results of various workers. Kiong *et al.*, (2008) reported that plant sensitivity is increased after gamma irradiations due to reduced level of endogenous growth hormones, such as cytokinins, as a result of breakdown or lack of synthesis. Interaction of mutagens significantly results relating to leaf area. Pakorn *et al.* (2009) who demonstrated that leaf area in *Anubias congenis* decreased with the increasing intensity of irradiation due to destruction of genetic material and reduction of cell division and ultimately growth retarded. Shahin *et al.*, (2011) reported that a dose range of 5-10 gray produced highest leaf area in grape cultures and at 30 Gy gave lowest leaf area. Naheed Akhtar (2014) in *Lycopersicon esculentum* reported that generally, the leaf area reduced by increasing the doses of gamma radiations and EMS. might be due to the significant genetic variation created by mutagens.

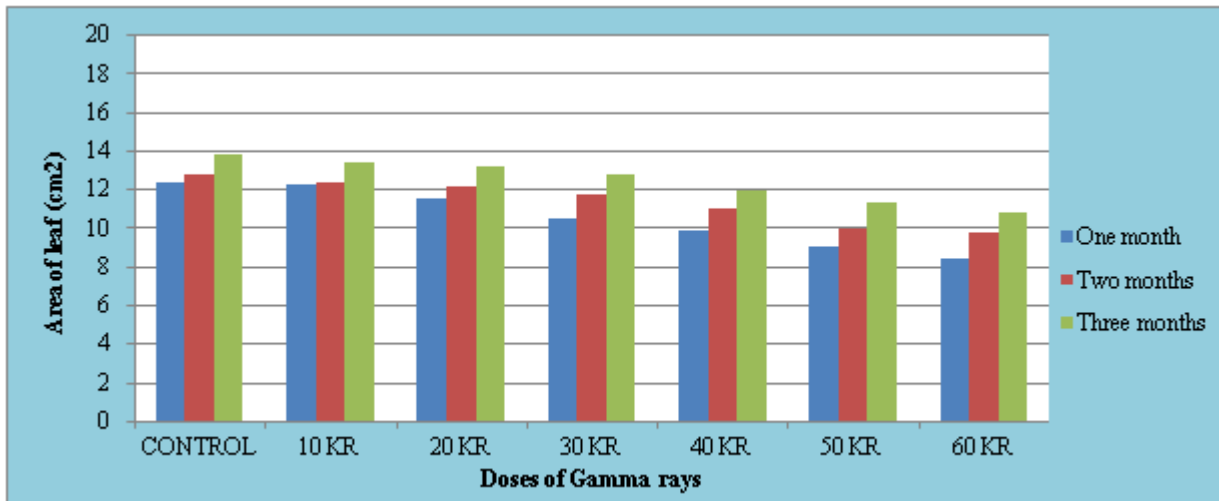


Figure 7: Effect of gamma rays on area of leaves (cm²)

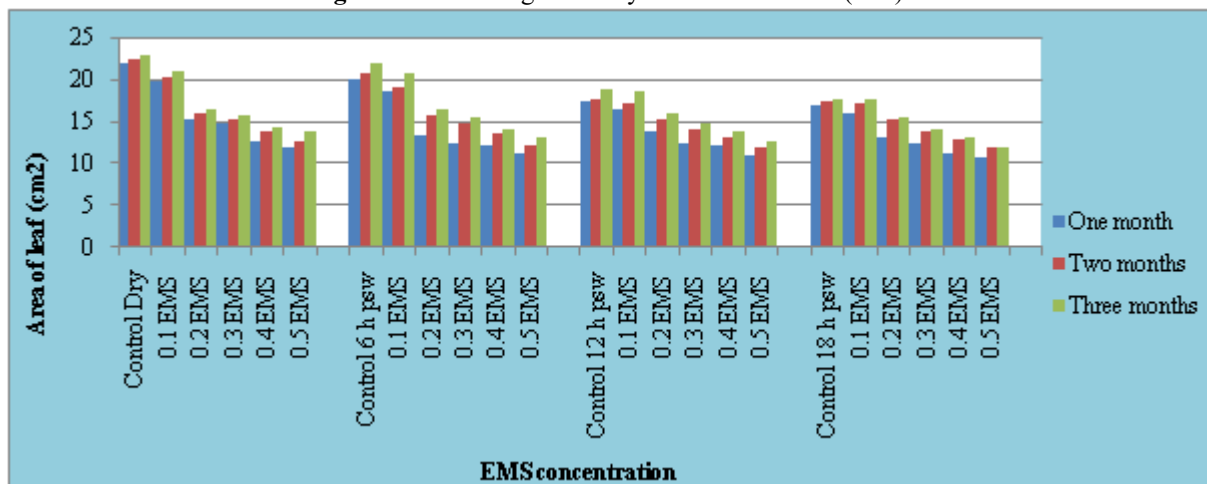


Figure 8: Effect of EMS on area of leaves (cm²)

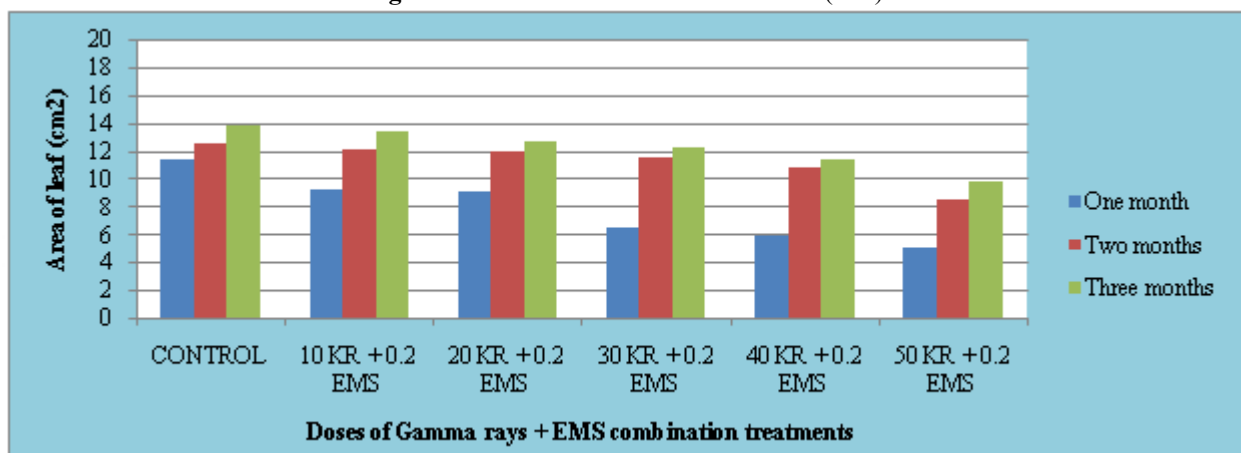


Figure 9: Effect of gamma rays followed by EMS on area of leaves (cm²)

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