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EFFECT OF PHYSICAL AND CHEMICAL MUTAGENESIS IN LITTLE MILLET (*PANICUM SUMATRENSE* ROTH EX ROEMER AND SCHULTZ) ON SEED GERMINATION, SEEDLING SURVIVAL THROUGH INDUCED MUTATION

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ABSTRACT

Little millet (*Panicum sumatrense* Roth ex Roemer & Schultz) belongs to Poaeceae family and it is Indian in origin. Milletsare mostly rain fed crops and used as food and fodder. Mutations can be induced in a variety of ways, such as by exposure to electromagnetic or ionizing radiation or chemical mutagens. The seed of little millet variety of Co (samai) 4 seeds were treated with different dose/concentration of physical mutagens (5,10,15,20,25,30,35,40,45 and 50KR) and chemical mutagens like EMS (Ethyl Methane Sulphonate) (5,10,15,20,25,30,35,40,45 and 50 mM). After that the treated seeds are sown in Petri dish under the laboratory conditions. It was found that the M_1 generation is effected in seed germination, seedling survival and morphology mutation.

Keywords: Little millet, Seed germination, EMS, Gamma Rays

INTRODUCTION

Little millet (*Panicum sumatrense* Roth ex Roemer and Schultz.) belongs to the grass family Poaecaey, Little millet belongs to the genus *Panicum* having a chromosome number of 2n = 36, with basic chromosome number of X=9. It was domesticated in India (De Wet *et al.,* 1983a). It is important minor millet in low and moderate-rainfall areas. In India, this millet is grown primarily in the drought-prone arid and semi-arid zones and used mostly for food purposes especially by people of economically weaker sectors. Besides India, it is cultivated in Nepal, Western Burma, and also in some African countries. It has a good nutritional profile and is comparable to staple cereals, namely, rice and wheat in terms of protein, minerals, and vitamins (Gopalan *et al.,* 1987). Little millet is also a good source of Nutraceuticals such as Phenolics, Gama-amino butyric acid (gaba), lignans, resistant starch, sterols, and phytates. The additive and synergistic effects of these bioactive Nutraceuticals in millet-based foods may offer several health benefits (Prathapan *et al.,* 2011).

Mutation breeding is one of the conventional breeding methods of plant breeding (Bhattacharjee. 1998). In Plant Breeding, mutation induction has become an effective way of supplementing existing germplasm and improving cultivars (Micke *et al.,* 1987).The main aim of these investigations was to calculate percentage of seed germination through induced mutation (Jelena S. *et al.,*). About 89 % of mutant variation have been established using physical mutagens such as X-rays, gamma rays, thermal and fast neutrons whereas with gamma rays alone accounting bringing about 60% of the mutant varieties (Kharkwal, 2000). Physical mutagens are an alternative breeding method to classical breeding methods and genetically modified organisms because of the feet that their applications are relatively safe and cost-effective (Jain, 2010).

According to international atomic energy (2016) data, there are at least 3233 mutant cultivars. In context of products, maximum mutant variations were obtained in grains with 48% and vegetable species grown with mutation breeding remained at 3% (Mba,2013).The ionizing radiation method, which began to be used in the early 20th century, plays an important role in the development of superior plants in more than 50 years of plant breeding studies (Kharkwal, 2012) as reported to physical mutagens such as gamma rays are less risky to health than chemical mutagens because they require no application to remove mutagen from the material (Khan, Iqbal, and Zafar, 2000). The LD_{50} dose should be determined to detect the most appropriate mutation dose (Predieri,2001).Mutations are randomly distributed in the genome. The high degree of mutation saturation can be achieved with a mutagen like gamma rays that does not cause a lot of collateral DNA damage (Bhosale and More, 2013).

Induced mutation is highly effective in enhancing natural genetic resources and have been used to develop improved cultivars of cereals, millet, fruits and other crops (Siddiqui and Khan, 1999). A huge range of chemical and physical mutagens have been investigated for their use in crop improvement. Induced mutation through using physical and chemical mutagens is a way to produce genetic variation, resulting in the making of fresh varieties with improved characters (Wongpiyasatid, 2000).

MATERIALS AND METHODS

Physical mutagen

In case of Little millet (*Panicum sumatrense* Roth Ex Roemers and schults) variety of CO (Samai) 4 seeds

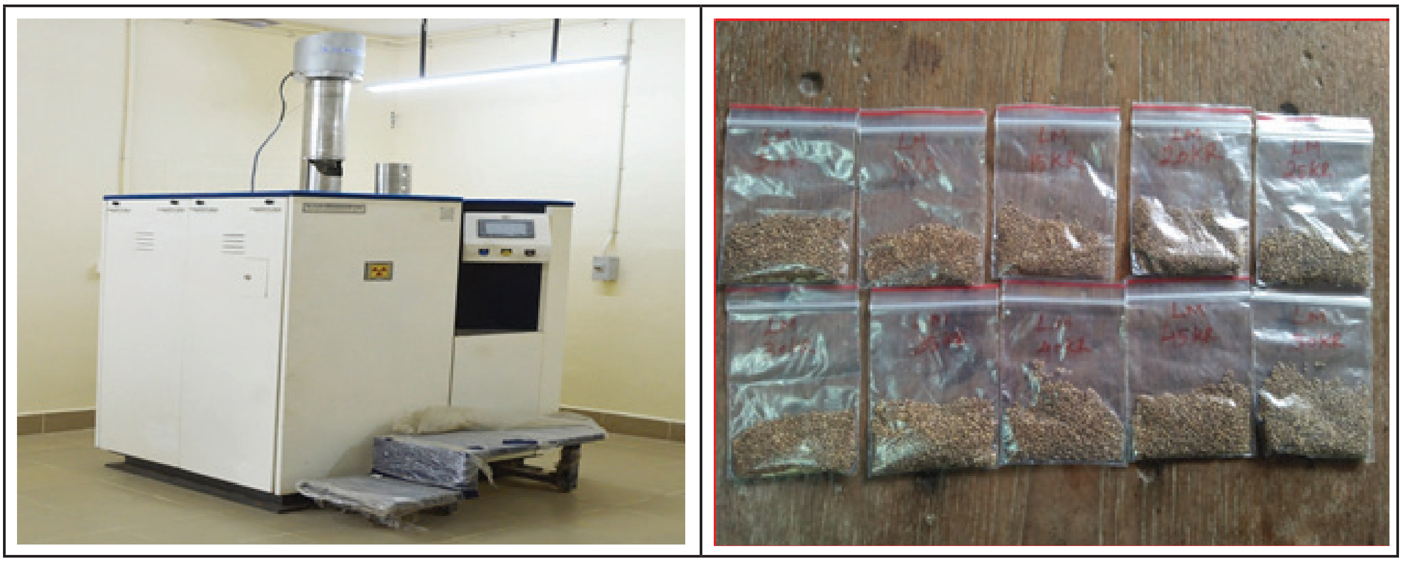
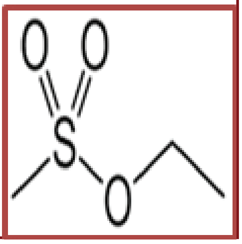


Figure1. Gamma chamber and little millet seeds

EMS

- Molar mass: 124.16 g/mol
- Chemical Formula: $CH_3\ SO_3\ C_2\ H_5$
- Density: 1.15 g/cm^3
- Melting point : <25ºC



were Collected from TNAU (Tamil Nadu Agricultural University) Coimbatore. 5gm of well matured seeds were taken from Zip Cover for irradiation. The mutagen of gamma Irradiation was given at Indira Gandhi Centre for Atomic Research (IGCAR) Kalpakkam, at a dose rate of 234KR/h. Ten sets of 5gm seed were taken as irradiated with different dosage of gamma rays. (5KR,10KR,15KR,20KR,25KR,30 KR,35KR,40KR,45KR and 50KR) were given inside the gamma chamber in source of cobalt 60. The gamma ray irradiated seeds were sown in pot culture in the Botanical garden, Department of Botany, Annamalai University. The assessment of seed germination percentage and seedling survival percentage of treated and control seed of *P. sumatrense* were recorded in laboratory at room temperature (24µ2). Growth of the radical following bursting of the seed coat was taken as an index for germination in petriplates. The extent of injury was assessed by determining the relative reduction in growth in the treated samples under controlled condition as compared to control. A gradual reduction of seed germination and seedling survival in Little Millet Seeds under similar condition were observed in moist filter paper kept on petri plates, because the gamma irradiation affects the seed germination and seedling survival as the consequence of mutagenic effect on growth phenomenon. Lethality was determined from reduction in germination with respect to control. Seed germination was recorded at 15th day after sowing and seedling survival percentage was determined from 30th day after sowing.

Chemical mutagen

The EMS (Ethyl methane sulphonate) was treated to 25gm of well matured seeds. Seeds were pre-soaked in distilled water for 6hours followed by EMS at 5, 10, 15,20,25,30,35,40,45 and 50mM concentrations. Non treated dry seeds were pre-soaked in distilled water for 8hours and then used as control. EMS were thoroughly washed in running tap water for 8 to 10 times and then transferred to Petri dishes containing two layers of moist filter paper for germination. The control and treated seeds were then subjected to germination test. The concentrations of EMS were fixed for further studies.

Seed germination %

Seed germination % was recorded at 7th day after sowing in petri dishes for under the laboratory.

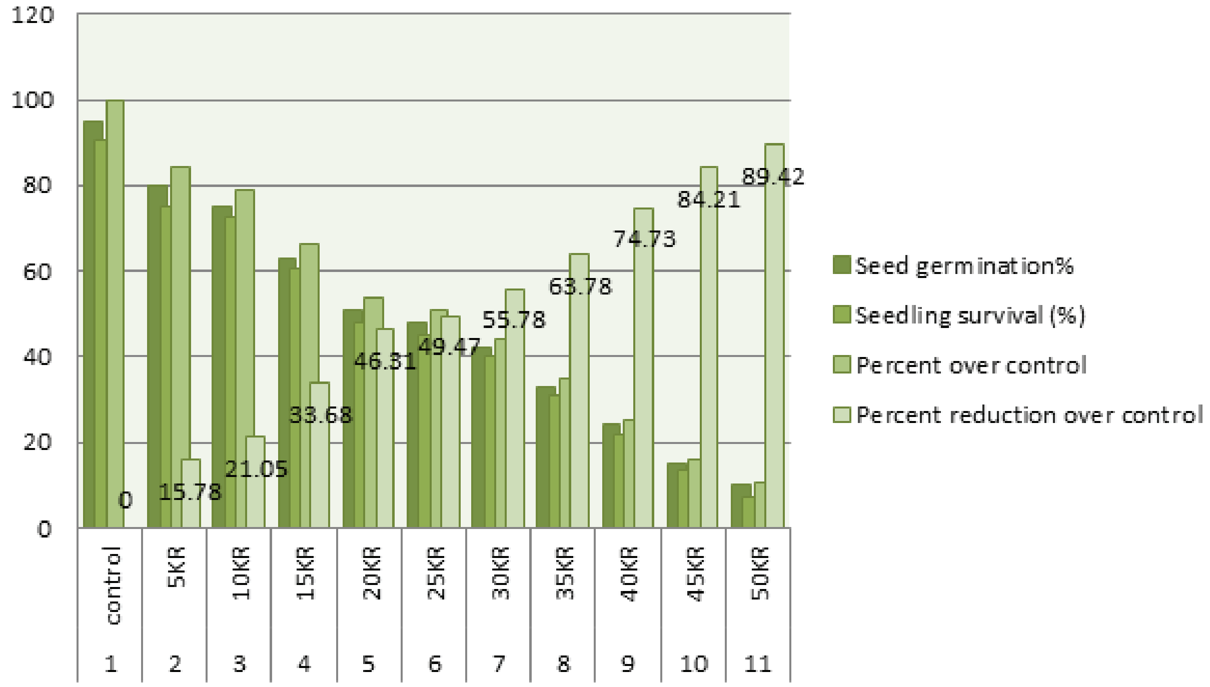
$$\text{Seed germination \%} = \frac{\text{No. of seed germination}}{\text{No. of seeds sown}} \times 100$$

$$\text{Seed survival \%} = \frac{\text{No. of seed survival}}{\text{No. of seed germination}} \times 100$$

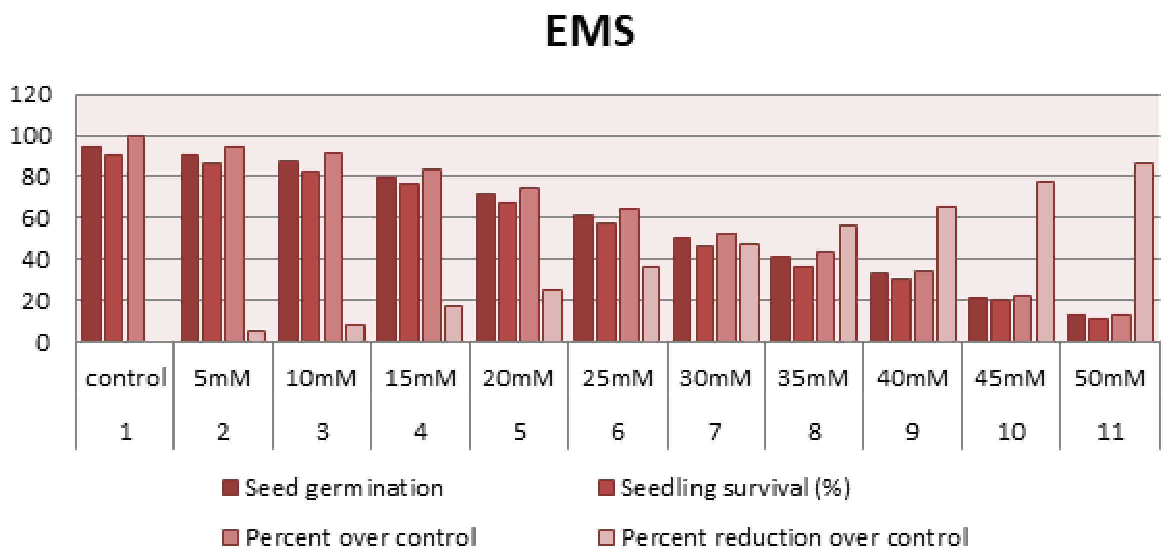
RESULTS AND DISCUSSION

Seed germination and seedling survival percentage

The reduction in the seed germination and seedling survival at different mutagenic treatments indicate that mutagen had an effect on these parameters. The biological effect was determined from the observation made on seed germination and seedling survival, which were decreased in all mutagens. In the present investigation germination and survival percentage decreased with increase in concentration and a field condition was observed in M_1 generation. Similar results were observed in little millet. The LD_{50} values were calculated on the basis of 50% reduction of germination counts on 10th day. The present investigation exhibited that the germination percentage of little millet decreased with increased concentration of the mutagens used. LD_{50}values has to be found out for further studies.



Graph 1: Determination of LD50 value for Gamma rays



Graph 2: Determination of LD50 value for EMS

Table-1: Determination of LD50 value for Gamma rays

S. No	Dose/ Gamma rays	Seed germination%	Seedling survival (%)	Percent over control	Percent reduction over control
1.	control	95.00	90.77	100	0.00
2.	5KR	80.00	75.18	84.21	15.78
3.	10KR	75.00	72.58	78.94	21.05
4.	15KR	63.00	60.58	66.31	33.68
5.	20KR	51.00	48.05	53.68	46.31
6.	25KR	48.00	45.03	50.92	49.47
7.	30KR	42.00	40.12	44.21	55.78
8.	35KR	33.00	31.03	34.73	63.78
9.	40KR	24.00	21.72	25.26	74.73
10.	45KR	15.00	13.66	15.78	84.21
11.	50KR	10.00	7.33	10.52	89.42

Table-2: Determination of LD_{50} value for EMS

S. No	Dose/ Gamma rays	Seed germination%	Seedling survival (%)	Percent over control	Percent reduction over control
1.	control	95	90.77	100.00	0.00
2.	5KR	90	86.66	94.73	5.26
3.	10KR	87	82.75	91.57	8,42
4.	15KR	79	75.94	83.15	16.84
5.	20KR	71	67.60	74.73	25.26
6.	25KR	61	57.37	64.21	35.78
7.	30KR	50	46.60	52.63	47.36
8.	35KR	41	36.58	43.15	56.84
9.	40KR	33	30.30	34.73	65.26
10.	45KR	21	20.47	22.10	77.89
11.	50KR	13	11.53	13.68	86.31

This genotype was subjected to study the effect of physical and chemical mutagens namely Gamma rays and EMS, through the biological changes in M_1 generation in corn (P. Rajapandian and Dhanam D, 2018). It is reported that as low doses of gamma irradiation promoted root development on hard wheat, but root development was inhibited with increasing doses. Depending on the increase in application dose, the leaf width and length of the cultivars have also decreased (Kayan and Eser, 2004). DNA Damage and mutation rates are higher, but most of them die or have sterility (Koornneef, 2002; Preuss and Britt, 2003). The control plants and the plants of the M_1generation are compared according to their growth and survival rates. To determine the appropriate mutation concentration, the dose of LD_{50} should be determined (Mba, 2013; Predieri, 2011). Gamma ray applications reveal differences in the morphological characteristics of plants. These differences may be occurring in the later stages of plant growth, such as changes in seed colour (Moh, 1971).

It is stated that the reduction in the percentage of germination caused by the high doses of radiation applied may have resulted in a reduction in the amount of internal growth regulators depending on the radiation (Kiong *et al.,* 2008).The stimulatory effect was observed in lower doses/ concentrations of Gamma rays and EMS on the length of root, shoot and seedling. (Zaka *et al.,* 2004) 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 the length of root and shoot was attributed to the effects of mutagens on the physiological system (Gaul, 1977). It is inferred that pre-soaking renders the seed vulnerable to the effect of physical and chemical mutagens and germination is inhibited due to biochemical interference of physical and chemical mutagens with the physiological activities involved in the process of seed germination. On this parameter, chemical mutagens have more effect in comparison to physical mutagens (Bhat *et al.,* 2016).

The reduction in plant survival percentage following with physical and chemical mutagens has been reported by many workers, and results of present investigation regarding survival are in agreement with earlier findings in different plants (Cheema *et al.,* 2003 Singh *et al.,* 2006). The seed germination and seedling survival were reduced with increasing in concentration of EMS, DES and sodium azide treatment than control. Similar results have been reported indifferent crops, Soybean (Pavadai and Dhanavel, 2004) cluster bean (Velu *et al.,* 2007), cowpea (Girija and Dhanavel, 2009) and little millet (R. Ramkumar and D. Dhanavel 2019).

Ashwagandha seed reduction in seed germination proportion and bit by bit increase within the production of active radicals liable for spermatophyte survival (lethality) and increasing dose of gamma rays was forthwith harm the physiological activities of the seed (Bharathi *et al.,* 2013).The seed germination, seedling survival, days to first flower, plant height, number of branches per plant, number of leaves per plant, number of berries per plant and seed yield per plant (gm) were gradually decreased with increase in various dosage of gamma ray. Similar results in morphological parameters were analysed in Cowpea (Gnanamurthy *et al.,* 2013).

CONCLUSION

Seed germination and seedling survival was decreased with increasing till the dosage of 20KR Gamma rays, as well as 30mM concentration of EMS. The seed germination LD_{50} (lethal dose) values were calculated at 50% of reduction seed germination. Induced the morphological mutation was found the under the stress condition so we have the further generation and work studies continue. This work will be helpful in future breeding use.

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