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Induced chemical mutagenesis on prosomillet (*Panicum miliceium*) L Var- Co_3 in seedling characters of m_1 generation

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Abstract
This study was performed by exposing the seed of prosomillet (*panicum miliceium (L.).Var co₃ Ethy* methane sulphonate and diethyl sulpate (DES) it is important food crop as food, forage and product. It is distributed in tropical and temperate region of the the world. Means performance the different quatitative traits were absorbed better in control when compared to treated and also observed that the LD-50 was found at 30mM of EMS and 40mM of DES. So it was found mutagens at lower treatment have influenced less biological damage and could be suitable for inducing desirable mutation in panicum miliceium.

Keywords: EMS, DES, LD-50 value *Panicum miliceium. L.* Seed germination, survivability

Introduction
Prosomillet (panicum miliceiumL.) popularly known as, varagu belongs to family poaceae this may be due to the crop cultivation in neglected and ill fertile soil under rained condition. Poor yield with an improper marketing chain divert the farmer to go for other major cereals crop cultivation which is not having the balanced nutrients. Hence development of high yielding varieties with climate resilient Capacity is a need of the hour to increase the area under varagu. The yield potential of this crop is low and with a number of diseases. Though the crop has been important over centuries, more concentrated research effort is geared in recent years to evolve improved varieties and develop production technology. Varagu is commonly called as, nutrition millet as the grains are nutritionally superior to many cereals providing fair amount of proteins, minerals, calcium and vitamins in abundance to the people. It is the cheapest and prefered food crop of economically suppressed but physically hard working people. It is appreciated by the people; because it can digest slowly there by furnish energy for hard work throughout the day. Prosomillet meets the firsts and most needs of mankind, the energy and hunger satisfaction. It leaves a sense of being well fed to any farmer. The protein of prosomillet has been reported to possess a fairly high biological value, which is needed for the maintance of nitrogen equilibrium of the body. The higher fibre content of prosomillet helps in many ways as it prevents constipation, high cholesterol formation and intestinal cancer. Hence, people suffering from diabetics are advised to eat prosomillet and other millets instead of rice according to the report given [1].

Mutation breeding is one of the most effective ways of inducing genetic variability available to the plant breeder [2, 3]. The main advantage of mutation breeding is the possibility of improving one or two character without changing the rest of the genotype [4] Aritifical induction of mutation provides raw materials for the genetic improvement of economic crops [5, 7] and also used to create genetic variability in quantitative traits of various crop plants. Induced mutation using physical and chemical mutagens is a method to create genetic varation resulting in new varieties with better characteristics. The chemical mutagens, EMS has been quite useful in inducing point mutation in the genomes of a diverse Range of plants largely because of its well established mode of action [8] Mutation is the ultimate source of variability in organism. It can be used for plant breeding in many different ways. The direct use of mutation is valuable supplementary approach to plant breeding, particularly when it is desired to improve one or two easily identifiable characters in an otherwise well adapted variety [9, 10]. Induced mutation is the eventual source of all the genetic variability in crop plants that may be difficult to bring through cross breeding and other breeding proceducers, since mutation gives rise to non-existing variations [11].

Materials and Methods
Plant Material
Dry and healthy seeds of prosomillet (Panicum miliceiumL.) varCo_3 were obtained from Tamil Nadu agricultural Research Institute coimboture.

Mutagens employed Chemical mutagens namely, Ethyl methane sulphonate and diethyl sulphate were used at various concentrations to induced mutagenesis.

Mutagenic Treatments
Ethylmethane sulphonate (EMS). (CH3SO2OC2H5), an alkylating agent having Molecular weight 124.16 was used in the present study. For the treatment of EMS, the seeds were pre-soaked in distilled water for 6 hours in order to make then relatively more sensitive to mutagenic action. Presoaked seeds were treated with different concentration of EMS (10, 20, 30, 40, and 50mM,) for 4 hours with repeated stirring. After the chemical treatment, the treated seeds were washed throughly in running tap water to remove the residues of the chemicals. Healthy, well matured and untreated seeds were used as control.

Diethyl Sulphate (DES)
Seeds of Prosomillet were subjected to different treatment levels (10, 20, 30, 40, 50,) of Diethyl sulphate for induced mutagenesis. Before treatment, seeds were pre-soked in

distilled water for 12hrs at room temperature. Later these seeds were dried on filter paper. All seeds were uniformly exposed to Diethyl sulphate solution by stirring with a glass rod. After treatment seeds were rinsed thoroughly with distilled water air- dried and stored for later studies.

Raising of M_2 Generation

The treatment were sown in seed beds and Watered at least once a day. After25-30 days the seedling were transplanted to experimental field in completely Randomized Block design with three replicants to raise M_1 population. The M_1 generation (produced directly from mutagen treated seeds) was grown in the field experiment at the Botanical garden, Department of Botany, Annamalai university. All the recommended culture practices were carried out during the plants growth period.

Seed Germination (%)

In the laboratory, the seeds of each treatment along with control were placed on absorbent cotton- wet petridishes. For each treatment three replicates were studied and the number of seeds germinated on the 7th day was counted and the germination percentage was calculated. Based on the reduction of 50% seed germination, the LD_{50}value were fixed and the three treatments of EMS and DES around LD_{50}value for further studies.

$$\text{Germination (\%)} = \frac{\text{No of seeds germinated}}{\text{No of seeds placed on petriplates}} \text{X } 100$$

Plant Survival on 30th Days

The number of plants survived on 30th day after sowing was counted from each treatment and the survival percentage was calculated by using the following formula.

$$\text{Plant survival (\%)} = \frac{\text{No of plants survived}}{\text{No of seeds germinated}} \text{X100}$$

Results and Discussion

Seed Germination (%)

The seed germination data on prosomillet (*panicum miliceium L.) VarCo3* are given in table. The seed germination percentage of various mutagenic Treatments under laboratory conditions revealed that, the germination percentage was decreased with increasing concentrations of EMS and DES. [10] The percentage of seed germination was higher in lower concentration of EMS (30mM 58%) and DES (40mM 50%). Based on the seed germination percentage on the 7thDay, the LD_{50} values were fixed at 30mM of EMS and 40mM of DES [11].

Reductoin in seed germination may be due to the effect of mutagen on meristematic tissues of the panicle [13]. One of the physiological effects caused by treatment of these mutagens particularly Chemical mutagens might be due to the disturbances in the formation of enzymes involved in the germination process [14]. Smiliar inhibitory effect on seeed germination by the various mutagenic treatments were reported earlier in Onion [15].

Table 1: Determination of LD_{50} (EMS)

Mutagens	Treatments conc. mm	No of seed Sown	No of seed germination	Percentage of seed germination
Control	Control	50	45	90
EMS	10mM	50	40	80
	20mM	50	35	70
	30mM	50	29	58
	40mM	50	25	50
	50mM	50	20	40
DES	10mM	50	35	70
	20mM	50	30	60
	30mM	50	29	58
	40mM	50	25	50
	50mM	50	19	38

Table 2: Effect of mutagens on Plant survival in M_1 generation

Mutagens	Treatments. conc. (mM)	Range	Mean±SE	Percent of reduction over control
Control		68-75	70.3±0.789	0.00-
EMS	10 mM	65-70	62.5±3.406	11.09
	20 mM	42-72	61.4±2.88	12.66
	30 mM	48-78	62.1±3.40	11.66
	40 mM	36-65	54.3±2.89	22.75
	50 mM	30-51	42.3±2.21	39.82
DES	10 mM	49-80	65.3±3.47	7.11
	20 Mm	48-86	62.2±3.068	11.52
	30 mM	34-70	56.3±3.30	19.9
	40 Mm	50-73	63.7±2.53	9.38
	50 Mm	29-50	37.6±2.22	46.5

Plant Survival on 30th Day

In general, a gradual reduction in the seedling survival in all mutagenic treatments is shown in the maximum plant survival was recorded at 30mM of (58%) of EMS and 40mM (50%) of DES. Increasing frequency of chromosomal harm with increasing radiation dose may be responsible for reduction in plant survival (Talebi *et al*, 2012) [9]. The reduction in plant survival due to the mutagenic treatments has also been reported in Dianthus [16] Horse grame [17] and Ashwagantha [18] Pearlmillet [19] Seame [20].

Conclusion

The prosomillet seed germination and survivability were decreased by increasing concentration of Ethyl Methane (EMS) and Diethyl sulphate (DES). It can be concluded that, EMS and DES could be utilized as a potential tool for inducing genetic variability. Among the different concentration of mutagens 30mM of EMS and 40mM of DES.

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