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Effect of Ethyl Methane Sulphonate on Growth and Development of Mulberry (*Morus* sp.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Ethyl methanesulfonate (EMS) is a genotoxic agent, it has been widely used as a model compound in experimental work to establish the receptiveness of the test system under examination. EMS induces damage to DNA by a direct mechanism, acting as a monofunctional ethylating agent. In the present study, mulberry genotypes V1 and MR2 were treated with EMS concentrations of 0.1%, and 0.3% with a duration of 3 hours and 6 hours respectively, and untreated control. Growth parameters observed were longest root length, dry root weight, seedling height, and number of leaves per seedling. The results showed that the treatment of EMS concentration 0.3% for 6 hours can increase the percentage of germination of V1 varieties (89.58%), longest root length (14.17cm), dry root weight (0.78g), height of seedling (15.08cm) and number of leaves per seedling (6.00) compared to other treatments. In general, survival percent and growth parameters were increased with an increase in the EMS dose and duration of treatment in V1 and MR2 varieties. Thus, it infers that the genetic variability of survivability and growth traits among V1 and MR2 varieties show strong positive or negative dose dependent co-relationship with EMS concentrations.

Keywords: Mutagen; ethyl methane sulphonate; Morus sp.; growth traits.

1. INTRODUCTION

"Mulberry (*Morus* sp.), a perennial tree or shrub, is an economically important plant, and its foliage is used to feed for the domesticated silkworm, *Bombyx mori*. Mulberry is cultivated in East, Central, and South Asia for silk production and is widely distributed in Asia, Europe, North America, South America, and Africa countries. Because it adapts easily to different ecological conditions and is easily hybridized, both naturally and artificially, abundant mulberry germplasm resources are available, making its genetic background rather complicated and highly heterozygous" [1]. "China and India are the major silk producing countries, have developed several mulberry varieties suitable for different agro climatic conditions" [2-4]. "Most of the mulberry varieties were developed from a few species such as *Morus alba* L., *M. atropupurea* Roxb, *M. bombycis* Koidz, *M. indica* L., *M. latifolia* Poir and *M. multicaulis* Perr" [2]. "The major reasons for this restricted utilization of species are lower leaf yield and the poor acceptability of silkworms as feed material. The reasons may be the coarseness of the leaf, lower moisture content, lower moisture retention capacity in the harvested leaves, and poor quality" [5].

"Reduction in the cost of production of silk cocoon is closely associated with qualitative and quantitative improvement of mulberry. Hence production of quality mulberry leaves is a pre requisite for the healthy growth and development of silkworms and silk production. Mulberry is a highly heterozygous, perennial plant so there is a great scope to induce mutations artificially" [6]. "It is also helpful to eliminate certain undesirable characteristics and to improve the mulberry plant qualitatively and quantitatively. Varieties of mulberry respond differently to mutation rate and mutation spectrum after gamma ray treatment" [6].

"Ethyl methanesulfonate (EMS) is considered a monofunctional ethylating agent. It has shown its mutagenic effect in a wide variety of genetic test systems, ranging from viruses to mammals" [7]. "Chemical mutagens such as sodium azide, ethyl methanesulphonate (EMS), and N-ethyl-N-nitrosourea (ENU) have varying effects on genetic structure in mutated populations" [8]. "EMS is a colorless liquid at room temperature and its molecular weight is 124.2. The boiling point of EMS is 213-213.5°C (761 mmHg) and its density is 1.1452 at 22°C relative to water at 4°C (IARC, 1974). EMS is a potent alkylating mutagen and is sometimes more effective than physical mutagens" [9]. "Mutation induction techniques such as radiation or chemical mutagens are good tools for increasing variability in crop species because spontaneous mutations occur with a very low frequency. Mutation techniques have significantly contributed to plant growth development to improve the productivity of the economic value of some crops. Mutation breeding has been widely used in recent times for improving vegetatively propagated crop plants and gamma rays have been proven to be highly potent in inducing variability in mulberry plants" [10].

Ramesh et al. [11] "reported that gamma ray is a potent physical mutagen that could induce variability in the mulberry variety M5. The higher yields in the mutants derived from M5 were found to be increased by 11.09 percent. Comparable results showed shortened internodes coupled with increased leaf area were recorded in radiation induced mutants".

Anil Kumar et al. [12] "reported that the concentrations of 0.1 percent and 0.3 percent of EMS treatment significantly altered the morpho-metric characters, biomass yield, and phyto chemical constituents. The significant variation in the morpho-metric characters such as plant height, number of branches, stem girth, number of leaves per plant, and increased biomass was recorded in the M1V2 clones of 0.1% EMS treatment and 0.3% EMS treatment. Further significant improvement was recorded in nutritive parameters such as proteins, reducing sugars, minerals, and moisture content. Moisture retention capacity and Chlorophyll contents were also high in mutant clones recovered from 0.1% and 0.3% EMS treatments".

The present investigation is to study the effect of the chemical mutagen of Ethyl Methane Sulphonate in mulberry using the existing mulberry cultivars.

2. MATERIALS AND METHODS

The experiment was conducted in the Department of Sericulture, Forest College and Research Institute, Mettupalayam.

2.1 Seed Treatment of Ethyl Methane Sulphonate (EMS)

Mulberry seeds of V1 and MR2 varieties were treated with 0.1 and 0.3 percent of EMS solution. Treated seeds were sown in seedling trays with 20 cavities each and dimensions of 35×35×25 cm for 3h and 6h. The seed trays were filled with fine soil, vermiculite, and Farm Yard Manure (1:1:1) mixture [13]. At regular intervals, watering and all intercultural operations were followed. Three replications each with five plants were maintained. Observations about mulberry growth and survivability were recorded after the 11th day of germination.

Treatment details:

T1 – V1 seeds treated with 0.1 % EMS for 3h
T2 – V1 seeds treated with 0.3 % EMS for 3h
T3 – V1 seeds treated with 0.1 % EMS for 6h
T4 – V1 seeds treated with 0.3 % EMS for 6h
T5 – MR2 seeds treated with 0.1% EMS for 3h
T6 – MR2 seeds treated with 0.3% EMS for 3h
T7 – MR2 seeds treated with 0.1% EMS for 6h
T8 – MR2 seeds treated with 0.3% EMS for 6h
T9 – Untreated Control – V1 seeds without treatment
T10 – Untreated Control – MR2 seeds without treatment

2.2 Germination per cent (%)

Germination started eleven days after sowing from the seeds. From the 11th day observations were taken regularly and germinated seeds were counted daily to calculate the germination percentage. It was calculated according to the formula given below:

Germination percentage = (No. of seeds germinated / No. of seeds sown) × 100

2.3 Survival per cent (%)

The survival percent of seedlings was estimated by counting the available seedlings on the 60th day after sowing in each treatment.

$$\text{Survival per cent} = \frac{\text{Number of survived seedlings on 60th DAS}}{\text{Total number of sowed seeds}} \times 100$$

2.4 Longest Root length (cm)

The root length was measured using a normal scale in centimeter from the base to the tip of the seedling.

2.5 Dry Root Weight (g)

To weigh the whole plant root, it was trimmed from the seedling in the origin and was dried between the folds of a blotting paper. The weight of the root portion of these seedlings was recorded in grams using a digital balance. After that, the average value of five seedlings was taken.

2.6 Height of Seedling (cm)

Seedling height was determined by measuring the branch length from the base of the plant to the tip of the largest glossy leaf. The measurements were expressed in centimeter.

2.7 Number of Leaves per Seedling

The number of leaves per plant and the leaves on each seedling were counted manually.

2.8 Statistical Analysis

Experiments were carried out in three replicates and analyzed through Analysis of Variance (ANOVA) using SPSS 21.0 statistical software.

3. RESULTS AND DISCUSSION

The results of the studies on the effect of ethyl methane sulphonate on the growth and development traits of mulberry are presented in Table 1.

3.1 Germination and Survival Percent (%)

Germination percent was observed significantly high in T_9 (92.17%), whereas, T1 (83.42%) was on par with T_7 (82.50%) and T_8 (84.58%). T_5 (79.17%) showed minimum germination percent. Significantly maximum survival per cent was recorded in T10 (87.33%) followed by T9 (83.00%). However, it was minimum in T1 (12.08%), whereas, T6 (34.33%) and T7 (30.42%) were on par with T8 (32.42%). "Sprouting is an inherent capacity of plant material to unfold the buds and produce a new flush of shoots. Successful establishment of the garden in vegetatively propagated plants mainly depends on sprouting ability. The EMS treated M5 mulberry cuttings show variations in response to different concentrations of the mutagen. Decrease in sprouting percentage was observed in plants treated with EMS compared to control. It is evident that at 0.4% concentration, higher sprouting of 75.71% was recorded and the least sprouting of 44.44% at 0.1% concentration was observed when compared to control (89.66%)" [14].

3.2 Longest Root Length (cm)

Significantly longest root length was recorded in T10 (16.42 cm) and T3 (14.08 cm) was on par with T4 (14.17 cm) and T9 (14.42 cm). "Variations in rooting behavior may be due to the nature of soil, conductivity, pH, etc. With an increase in chemical concentration, there was an increase in the mutation rate leading to variations in rooting" [15].

3.3 Dry Root Weight (g)

The highest dry root weight was registered in T10 (0.86 g) followed by T4 (0.78 g) which was statistically on par with T9 (0.75 g).

3.4 Height of Seedling (cm)

Significantly maximum height of seedling was recorded in T10 (16.42 cm) and it was the minimum in T8 (9.25 cm). However, T1 (12.17 cm) was on par with T3 (11.42 cm) and T7 (12.08 cm).

3.5 Number of Leaves per Seedling

The size and symmetry of leaves in the mulberry varieties due to EMS treatment has been altered to a considerable extent. A maximum number of leaves per seedling was registered in T10 (7.33) which was on par with T9 (7.00) and T4 (6.00). "A confirmation study revealed that the size and symmetry of leaves in the M5 mulberry variety due to EMS treatment have been altered to a considerable extent. Fusion of leaves, increase in thickness and changes in texture of leaves, and occurrence of xantha and albino were common in plants treated with 3% EMS" [14].

"Studies revealed that lower concentrations of EMS had a stimulatory effect on plant height and the higher concentrations showed an inhibitory effect as compared to control" [16]. "Variability in plant height in chickpea through gamma irradiation has revealed that the radiation doses of 5 and 10 Krad have slightly reduced plant height while other doses had no considerable effect on plant height" [17]. "A similar kind of variability was also observed through EMS treatments in *Capsicum annum"* [18]. "Mutations affecting the plant height, indicate that the mutagen doses would cause both positive and negative genetic variability in plant height" [19]. These workers have concluded that low doses of irradiations could be used as safe and effective for mutation studies in mulberry. Sastry et al. [20], "while studying the sprouting and survival ability in mulberry varieties S, S, and K showed that the injury was directly proportional to the concentration of mutagens".

Table 1. Effect of Ethyl methane sulphonate (EMS) on survivability and growth parameters of mulberry seedlings

Treatments	Germination per cent (%)	Survival per cent (%)	Longest Root length (cm)	Dry Root weight (g)	Height of Seedling (cm)	No. of Leaves per seedling
T1 – Treatment of V1 seeds with 0.1 % EMS for 3h	83.42	12.08	13.25	0.73	12.17	4.67
T2 – Treatment of V1 seeds with 0.3% EMS for 3h	85.33	20.50	13.42	0.66	10.08	4.00
T3 – Treatment of V1 seeds with 0.1% EMS for 6h	87.42	24.25	14.08	0.57	11.42	4.33
T4– Treatment of V1 seeds with 0.3% EMS for 6h	89.58	22.25	14.17	0.78	15.08	6.00
T5 – Treatment of MR2 seeds with 0.1% EMS for 3h	79.17	28.17	12.25	0.43	11.00	4.00
T6 – Treatment of MR2 seeds with 0.3% EMS for 3h	81.50	34.33	11.33	0.37	10.75	5.00
T7 – Treatment of MR2 seeds with 0.1% EMS for 6h	82.50	30.42	10.83	0.31	12.08	3.00
T8 – Treatment of MR2 seeds with 0.3% EMS for 6h	84.58	32.42	10.33	0.35	9.25	3.33
T9 – Untreated Control – V1 seeds without treatment	92.17	83.00	14.42	0.75	14.50	7.00
T10 – Untreated Control – MR2 seeds without treatment	90.17	87.33	16.42	0.86	16.42	7.33
S.Em ±	0.6791	1.0646	0.3005	0.0189	0.3873	0.8692
CD at 5%	1.4165	2.2207	0.6268	0.0393	0.8079	1.8132

4. CONCLUSION

Observations in the present study show that the V1 mulberry variety treated with 0.3 percent of EMS for 6 h recorded maximum germination percent, longest root length, dry root weight, height of seedling, and number of leaves per seedling. In general, survival percent and growth parameters were increased with an increase in the EMS dose in V1 and MR2 varieties. Thus it infers that the genetic variability of survival and growth traits of V1 and MR2 varieties show strong positive or negative dose dependent co-relationship with EMS concentrations, which is in conformities with the present findings.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during the writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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