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Effect of Ethyl Methane Sulfonate on Regermination and Survival of *Momordica cymbalaria* Fenzl. Implications for Conservation and Mutation Breeding of a Rare Medicinal Plant

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ABSTRACT

Momordica cymbalaria Fenzl.is a rare medicinal plant with notable pharmacological potential, but its conservation and propagation are challenging due to limited seed viability and low germination rates. This study investigates the impact of ethyl methane sulfonate (EMS), a chemical mutagen, on the regermination and survival of *M. cymbalaria*. Tubers were treated with varying concentrations of EMS (10 mM to 50 mM) for different durations (4hrs, 6hrs, and 8hrs). Germination rates, survival, and morphological abnormalities were systematically recorded. Results revealed a concentration-dependent response to EMS treatment. Low concentrations of EMS enhanced germination rates suggesting a stimulatory effect on viability. In variance, higher concentrations remarkably reduced survival rates and induced morphological abnormalities. These findings highlight the dual role of EMS as both a germination enhancer at lower concentrations and a potent mutagen at higher concentrations.

INTRODUCTION

Momordica cymbalaria Fenzl., a member of the family Cucurbitaceae, is a perennial herbaceous climber that can either spread along the ground or climb using tendrils for support (Chinthan et al. 2021). This plant is commonly found in tropical regions of Africa and India, where its mature fruits are used as a vegetable. Compared to bitter gourd, the fruits of *M. cymbalaria* are notably rich in crude fiber, calcium, potassium, sodium, and vitamin C (Mohammed et al. 2024). Additionally, the plant exhibits disease resistance, and its fruits are valued for their medicinal properties (Jha, Koneri, and Samaddar 2018; Nadkarni 2007). However, cultivating *M. cymbalaria* presents several challenges. A significant limitation is the lack of a standardized propagation method, coupled with low-yield (Jeyadevi et al. 2012). The plant relies on perennial tubers that survive in the soil, but only a small number produce a single plant in the following season. The population is further diminished by animals consuming the tubers and the deterioration or drying out of tubers under extreme conditions such as drought or waterlogging. Moreover, the fruit contains few seeds, which often remain dormant and exhibit low germination rates, hindering effective propagation through seeds (Nikam et al. 2009).

Ethyl methane sulfonate (EMS) is a widely used chemical mutagen in plant breeding and genetic studies (Ariraman et al. 2014). It induces point mutations, which can alter plant traits, including germination efficiency and stress tolerance (Kale and Kothekar 2006). While EMS has been extensively studied in crop plants, its effects on rare medicinal species like M. *cymbalaria* remain unexplored (Ahloowalia, Maluszynski, and Nichterlein 2004). This study aims to evaluate the impact of EMS on the regermination and survival of M. *cymbalaria*, to identify optimal mutagenesis conditions for improving germination and supporting conservation efforts. The optimal EMS concentration and treatment duration for *M. cymbalaria* would need to be determined empirically, perhaps through a dose-response experiment.

Material and Methods

Healthy Plant Material, and uniform tubers were selected for the experiment. The tubers of *Momordica cymbalaria* Fenzl. were collected from the agricultural sites of Pune and Solapur district of Maharashtra State. The plantlets were conserved at the shade

net house of the Department of Botany, Vidya Pratishthan's Supe Arts, Science, and Commerce College supe, and were used for further experiments. The tubers were manually inspected for uniformity and free from visible damage.

Treatment

Tubers were washed with normal water and distilled water then pre-soaked in distilled water. EMS solutions were prepared freshly before each treatment at concentrations of 10mM, 20mM, 30mM, 40mM, and 50mM. Each concentration of EMS was prepared in 100 ML of distilled water for treatment. tubers were soaked in the EMS solutions for three different durations 4hrs,6hrs, and 8hrs at room temperature. After treatment with the mutagen, the tubers were washed completely with distilled water for 30 minutes to remove the residual mutagens. A control group was treated with distilled water.

Regermination Assay

Treated and control tubers of *M. cymbalaria* were sown in sterile soil under controlled conditions germination was monitored daily for 20 days, and the regermination percentage was calculated. Survival rates were recorded 30 days after germination.

Experimental setup and statistical analysis

The experiment was performed in three replications and a total ten tubers were used for each treatment. the experimental design was completely randomized design (CRD). Treated and control tubers of *M. cymbalaria* were sown in sterile soil under controlled conditions The tuber regermination data were collected daily, and the other parameters including regermination percentages were performed after 28 days. The data were analyzed using Microsoft Excel and data were presented in mean ± standard deviation (SD).

Regermination of tubers

After the treatment, tuber regeneration ability was measured in terms of regermination rate, regermination percentage, and the survival of regerminated tubers. Survival percentages were checked after untreated tubers were 100 % regerminated. Along with the treated tubers, the parameters of control were also tested. Regermination percentages and survival percentages were calculated using the following formula

$$Regermination\ \% = \frac{Number\ of\ tubers\ regerminated}{Total\ number\ of\ Tubers} \times 100$$

$$Servival\ \ \% = \frac{Number\ of\ tubers\ servival}{Total\ number\ of\ Tubers} \times 100$$

Results and Discussion

Tuber regermination and survival

In general, a gradual reduction in the percentage of regermination and Survival percentage of tuber was observed as per corresponding increasing levels of mutagen concentrations of EMS. In this study regermination and survival percentage were checked for their, without treating it with EMS. The 100±0 % regermination was observed in the absence of EMS. However, the survival of the regerminated tubers varies. The maximum survival was recorded in the Control followed by other different concentrations of EMS (Table 1). Next, EMS was applied to the tubers to check the plant's response. Upon observation, the Regermination survivability was reduced after the application of EMS. Control tubers regerminate after 7th days of planting however treated tubers exhibited delayed regerminations with increasing concentration and time duration of treatments. Tubers treated with 10 mM showed a 90 ± 0, 83.33±5.7, and 80±0 regermination in three different time durations increase in duration and decreased regermination percentage compared to the control, indicating a stimulatory effect at low concentrations. Likewise, an increase in concentration and time duration decrease in the regermination percentage of tubers. The control group exhibited 100% regermination in all three different time durations of treatment while 10 showed 90 ± 0% 83.33±5.7% 80±0 percent regermination of tubers of *M. cymbalaria* in three different treatment durations. 20mM Show 80 ±0 %70±0 % 73.33±11.54% of regermination of tubers. 30 mM observed 70 ±0%, 4-hour time duration 6 hours and 8hrs time duration of treatment of EMS shows the same germination percentage 66.66±5.7%. in 40 mM observed 56.6±5.7, 53.33±5.7 43.3±5.77percent of germination of treated tubers. 50 mM EMS treatment resulted in only 43.3±5.7 % in 4 hours of exposure. 43.33±5.7 6 hours' time duration 26.66±5.77 percent in 8 hours' time duration (Table 1). It indicates that the increase in time exposure to mutagenic treatment decreases the regermination percentages.

Mutagenic concentration and time exposure also impact on survivability of *M. cymbalaria* in untreated tubers all germinated plants survive but different concentrations of mutagen adversely affect the survival of plants. In control 100 percent survival but in treated concentrations like 10mM 93±5.77%,86.66 ±5.7 %, and 93.33 ±5.77 percentage of survival in three different time exposures of mutagenic treatments.in 20mM 90±0 % survival of plants in 4-hour duration in 6hrs and 8hrs time exposure observed the same survival percentage of regerminated plants of *M. cymbalaria* likewise 30mM concentration showed 80±0% survival observed in 4-hour time duration of mutagenic treatment in 6hrs and 8hrs time duration of mutagenic treatment show same survivability 76.66 ±5.7 in 40 mM mutagenic concentration show variation in survival in three different time durations. in 40mM observed that 63.33±5.7% in 4 hrs, 56.66 ±5.7% in 6 hrs, and 53.33 ±5.77 % in 8hrs. In 50 mM observed 36.66±11.54 percent survival in 4 hours' time exposure to mutagenic treatments in 6hrs and 8 hrs of mutagenic treatment time duration observed 33.33 ±15.27 and 33.33 ±5.77 survivability regerminated plants of *M. cymbalaria* the result of regermination and survivability demonstrate that the low concentration of EMS and short duration of treatment enhancing the regermination and survivability of *M. cymbalaria* plants.

Table 1. Effect of different EMS Concentration on regermination of tubers and survival of *M. cymbalariya* in three different time durations.

Time Duration of Treatment	**4 Hrs treatment**		**6 Hrs treatment**		**8 Hrs treatment**	
Concentration	Reger. %	Survival %	Reger. %	Survival %	Reger. %	Survival %
Control	100± 0	100±0	100±0	100 ±0	100±0	100 ±0
10 mM	90 ± 0	93±5.77	83.33±5.7	86.66 ±5.7	80±0	93.33 ±5.77
20 mM	80 ±0	90±0	70±0	86.66 ±5.7	73.33±11.54	86.66 ±5.77
30 mM	70 ±0	80±0	66.66±5.7	76.66 ±5.7	66.66±5.7	76.66 ±5.77
40 mM	56.6±5.7	63.33±5.7	53.33±5.7	56.66 ±5.7	43.3±5.77	53.33 ±5.77
50 mM	43.3±5.7	36.66±11.54	43.33±5.7	33.33 ±15.27	26.66±5.77	33.33 ±5.77

Reger. = Regermination; Data represented as mean ± SD

Fig 2: Effect of Different EMS treatment on Survival of *M.cymbalaria* plant.

CONCLUSION

The dose-response analysis demonstrated that higher concentrations of EMS and prolonged treatment durations significantly reduced the regermination rates of *Momordica cymbalaria*, establishing a clear inverse relationship between increased mutagen exposure and plant viability. Additionally, the number of regerminated tubers declined markedly with elevated EMS concentrations and extended exposure times. Tubers treated with higher EMS concentrations for 8 hrs failed to survive, further emphasizing the detrimental effects of excessive mutagenic stress. furthermore, increased EMS concentrations and longer exposure durations delayed shoot regeneration and survivability of treated plants indicating that mutagenic stress adversely affects the plant's regenerative capacity.

Fig 1: Effect of Different EMS treatment on regermination of *M.cymbalaria* tubers

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