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Dose-Dependent Effects of Ethyl Methane Sulphonate on Tubers Regeneration and Morphological Traits in *Momordica cymbalaria* Fenzal. Approaches to Conservation and Mutation Breeding of the Rare Medicinal Plant.

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ABSTRACT:

Momordica cymbalaria Fenzal., a medicinally important Cucurbitaceae species, is traditionally used for its anti-diabetic, anti-inflammatory, and nutritional properties. despite its cultivation, it faces challenges such as low germination rates and limited genetic diversity. Induced mutagenesis using Ethyl Methane Sulphonate (EMS) offers a potential solution by generating genetic variability for improved traits. This study investigates the dose-dependent effects of EMS on tuber regeneration, morphological traits, and genetic diversity in *Momordica cymbalaria*. Tubers were treated with EMS concentrations ranging from 10 mM to 50 mM to assess their impact on regermination, survival, and growth parameters. Results indicate that moderate EMS doses enhance callus formation and shoot regeneration, whereas higher concentrations reduce survival rates. Significant variations in morphological traits, including plant height, leaf structure, and tuber morphology, were observed among mutants. Results revealed a dose-dependent decline in regermination and survival rates, with moderate concentrations (10–30 mM) inducing beneficial mutations while maintaining viability, whereas higher concentrations (40–50 mM) severely impaired plant development. The study identifies optimal EMS concentrations for balancing mutation induction and plant viability, offering a promising approach for conservation and mutation breeding of this rare medicinal plant. These findings contribute to the sustainable utilization of *Momordica cymbalaria* in agriculture and traditional medicine.

Keywords:- *Momordica cymbalaria*, EMS mutagenesis, Tuber regeneration, Morphological traits, Medicinal plant breeding, Conservation.

INTRODUCTION:

Momordica cymbalaria Fenzal. belonging to the Cucurbitaceae family, is a medicinally important plant native to India and South Asia. Traditionally utilized in Ayurveda, the tubers and fruits of *Momordica cymbalaria* exhibit anti-diabetic, anti-inflammatory, and anticancer properties, owing to the presence of bioactive compounds such as flavonoids and glycosides (Mohammed et al., 2024). Also, the plant holds nutritional value, serving as a source of vitamins, minerals, and antioxidants in certain regional diets. (Jeyadevi et al., 2012). The fruits and leaves were used as vegetables that contain vitamin C, maleic acid, and high crude fibers (Kirtikar, 1933; Parvathi et al.,2002). The plant part extracts exhibit antidiabetic, hypolipidemic, anti-diarrheal, antimicrobial, anti-ulcer, neuroprotective, cardioprotective, anti-ovulatory, abortifacient, hepatoprotective, nephroprotective, anticancer, and antioxidant activities (Chaitanya et al., 2020; Srinivasulu et al., 2017).

Due to its limited distribution and decreasing population, *Momordica cymbalaria* requires conservation efforts. The lack of awareness, limited cultivation efforts, and genetic erosion

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further highlight the importance of preserving its germplasm. Conservation strategies are essential not only to prevent its genetic diversity but also to facilitate its sustainable use in the pharmaceutical and agricultural sectors. Mutation breeding using chemical mutagens such as Ethyl Methane Sulphonate (EMS) presents a promising approach for developing desirable traits and enhancing regeneration capacity. This study explores the dose-dependent effects of EMS on tuber regeneration and morphological traits, aiming to contribute to both the conservation and improvement of this rare medicinal plant.

In spite of its medicinal and nutritional value, *Momordica cymbalaria* faces cultivation challenges, including poor seed germination and survival rates, susceptibility to pests and diseases, and limited genetic diversity (Nikam et al. 2009). These constraints hinder its large-scale production and genetic improvement, necessitating the development of effective breeding strategies to enhance desirable traits. This species is under threat. Ethyl Methane Sulphonate (EMS) is a widely used chemical mutagen in plant breeding, known for inducing genetic variations that can lead to improved agronomic traits (Ahloowalia et al., 2004; Jankowicz-Cieslak and Till, 2016). EMS functions by alkylating guanine bases in DNA, generating mutations that may enhance yield, disease resistance, and morphological characteristics (Puripunyavanich et al., 2023). While EMS mutagenesis is cost-effective and versatile, its success depends on optimizing concentration, exposure time, and plant genotype to balance mutation frequency with plant viability (Shamshad *et al.*, 2023).

The rationale for studying dose-dependent effects of EMS lies in its potential to improve tuber regeneration, alter morphological traits, and enhance genetic diversity in *Momordica cymbalaria*. Identifying the optimal EMS concentration is crucial to maximize beneficial mutations while minimizing detrimental effects, ultimately aiding in the development of high-yielding, disease-resistant, and nutritionally enriched varieties of this valuable medicinal plant (Talebi *et al.*, 2012).This study aims to investigate the impact of varying EMS doses on tuber regeneration efficiency, morphological modifications, and genetic variability in *Momordica cymbalaria*, providing insights for its sustainable cultivation and genetic enhancement.

MATERIAL & METHODS

Uniform and healthy tubers of *Momordica cymbalaria* Fenzl. were selected for the study. The tubers were collected from agricultural fields located in the Pune and Solapur districts of Maharashtra, India. Collected tubers were manually examined to ensure uniformity and the absence of visible damage. The resulting plantlets were maintained under shade net conditions at the Department of Botany, Vidya Pratishthan's Supe Arts, Science, and Commerce College, Supe, and utilized for subsequent experimental procedures.

Treatment

Selected tubers of *Momordica cymbalaria* were first thoroughly washed with tap water, then rinsed with distilled water, and subsequently pre-soaked in distilled water. Ethyl methane sulfonate (EMS) solutions were freshly prepared before each treatment at concentrations of 10 mM, 20 mM, 30 mM, 40 mM, and 50 mM using 100 mL of distilled water for each concentration. The pre-soaked tubers were then exposed to the EMS solutions for 6 hours at room temperature (~25 ± 2°C).

Experimental setup and Statistical analysis

The trial was laid out in a completely randomized design with each treatment replicated three times. For each EMS concentration, including the

control, ten tubers of *Momordica cymbalaria* were used per replication. The planting medium consisted of a mixture of black soil and cocopeat. EMS-treated and control tubers of *Momordica cymbalaria* were planted in individual pots containing the prepared medium. Moisture levels were consistently maintained, and ambient humidity was regulated by covering the pots with transparent plastic sheets. Natural sunlight was used as the primary source of light. Observations on tuber sprouting were conducted eight days post-treatment. Preliminary results indicated that all 10 control tubers (untreated) produced one shoot each, totalling 10 shoots. Regermination data were recorded daily, while cumulative parameters, including regermination percentage, were assessed 15 days after sowing. Data were analyzed using Microsoft Excel 2021 and results were expressed as mean ± standard deviation (SD).

RESULT & DISCUSSION:

Ethyl Methane Sulfonate (EMS) demonstrated a clear concentration-dependent inhibitory effect on plant regeneration, survival, and overall growth dynamics. At lower concentrations (10–30 mM), partial regeneration and development were observed, although significant reductions in key physiological and reproductive parameters were evident. In contrast, higher concentrations (≥40 mM) resulted in pronounced growth retardation and reproductive failure, reflecting strong cytotoxicity. These findings highlight the necessity of optimizing EMS dosage to ensure effective mutagenesis while minimizing adverse effects on plant viability. This is particularly critical for rare or threatened plant species such as *Momordica cymbalaria*, where conservation efforts must be balanced with genetic improvement strategies. When carefully applied, controlled mutagenesis can be a valuable tool for inducing novel traits and enhancing genetic diversity, all while preserving the survival of the species. Further investigation into the molecular mechanisms underlying EMS-induced stress responses may provide insights to support both conservation and sustainable utilization of vulnerable medicinal plants.

The study revealed a dose-dependent decline in both regermination (%) and survival (%) of tubers with increasing EMS concentrations. The control group (untreated tubers) exhibited 96.66 ± 5.77% regermination and 100 ± 0% survival, indicating optimal growth conditions in the absence of EMS (Kale and Kothekar, 2006). With increasing EMS concentration, both parameters showed a significant decline. At 10mM EMS, regermination decreased to 86.66 ± 5.77%, while survival was 93.33 ± 5.77%, suggesting that even low mutagen concentrations begin to impair tuber viability (Twumasi et al., 2023). At 20 mM EMS, regermination was 76.66 ± 5.77% and survival was 86.66 ± 5.77%, thereby confirming the EMS-induced effect.

A more noticeable reduction was observed at 30mM EMS, where regermination dropped to 73.33 ± 5.77%, and survival decreased to 80 ± 0%, indicating that moderate mutagen concentrations significantly hinder tuber development (Table 1). The most severe effects were seen at 40mM and 50mM EMS, where both regermination and survival plummeted to 43.33 ± 5.77% and 23.33 ± 15.27%, respectively (Twumasi et al., 2023). This drastic reduction suggests that high EMS concentrations are highly detrimental to tuber viability. These findings align with previous studies demonstrating that EMS-induced mutagenesis negatively affects plant regeneration and survival, with higher concentrations causing greater damage (Muqaddasi and Arif, 2012). The observed decline in regermination and survival percentages could be attributed to DNA damage, impaired cell division, and oxidative

stress caused by EMS, which disrupts normal physiological processes.

Apart from, the delayed regermination in treated tubers compared to the control suggests that EMS not only reduces viability but also slows down metabolic recovery. The fact that lower concentrations (10–20 mM) still permit partial regermination implies that mild mutagenic treatments could be used for inducing mutations without complete lethality, whereas higher doses (40–50 mM) may be too harsh for practical use (Talebi et al., 2012).

Fig.1 Effect of EMS on tuber regeneration of *M. cymbalaria*

Concentration EMS Dose	Reger. %	Survival %
Control	96.66±5.77	100±0
10 mM	86.66±5.77	93.33±5.77
20 mM	76.66±5.77	86.66±5.77
30 mM	73.33±5.77	80±0
40 mM	43.33±5.77	43.33±5.77
50 mM	23..33±15.27	23.33±15.27

Table1.Effect of different dose concentrations of EMS on Reger. % and Survival % Reger. = Regermination; Data represented as mean ± SD

The study evaluated the impact of increasing concentrations of a mutagenic treatment on various growth parameters, including plant height, number of leaves, number of flowers, and number of pods. A progressive decline in these parameters was observed with increasing mutagen concentrations, indicating a dose-dependent inhibitory effect on plant growth and reproductive development. In the control group (untreated plants), the maximum values for all measured parameters were recorded as plant height (181.3 ± 8.08 cm), number of leaves (111.3 ± 3.21), number of flowers (31.66 ± 2.8), and number of pods (26.3 ± 2.8) (Weldemichael et al.,

2023). However, as the mutagen concentration increased, a significant reduction in these parameters was observed (Islam et al., 2022). At 10mM, plant height decreased to 158.3 ± 2.88 cm, while the number of leaves, flowers, and pods was 104 ± 5.29, 26 ± 3.6, and 23 ± 3.6, respectively (Table 2). This trend continued with 20mM, where further reductions were recorded: plant height (145 ± 5 cm), leaves (86 ± 3.60), flowers (20.6 ± 4.04), and pods (17 ± 3.6) (Mageed et al., 2016).

Concentration Of EMS	Height in cm	Number of Leaves	Number of Flowers	Number of Pods
Control	181.3 ± 8.08	111.3 ± 3.21	31.66±2.8	26.3 ±2.8
10 mM	158.3 ± 2.88	104 ± 5.29	26±3.6	23 ±3.6
20 mM	145 ± 5	86 ± 3.60	20.6±4.04	17 ±3.6
30 mM	11.6 ± 2.88	57.3 ± 6.4	14±1.7	11.3 ±2.08
40 mM	73.3 ± 5.7	36 ± 5.2	10.3±1.5	10.3 ±6.5
50 mM	53.3 ± 5.7	17.6 ± 2.5	7.3±2.5	5.3 ±3.05

Table 2. Effect of different doses of Concentration of EMS on morphological traits of *M. cymbalaria*. Data represented as mean ± SD

A more drastic decline was observed at 30 mM, where plant height was severely stunted (11.6 ± 2.88 cm), along with a significant reduction in leaves (57.3 ± 6.4), flowers (14 ± 1.7), and pods (11.3 ± 2.08) (Gulzar et al., 2024). This trend persisted at higher concentrations (40 mM and 50 mM), where plant growth and reproductive output were significantly suppressed. Morphological abnormalities, such as early flowering, leaf curling, and dwarfism, were observed in the treated plants. At 50 mM, the lowest values were recorded: plant height (53.3 ± 5.7 cm), leaves (17.6 ± 2.5), flowers (7.3 ± 2.5), and pods (5.3 ± 3.05) (Samadi et al., 2022).

These findings suggest that the mutagen exerts a concentration-dependent inhibitory effect on plant growth and development. Lower concentrations (10–30 mM) resulted in moderate reductions, whereas higher concentrations (40–50 mM) caused severe growth retardation, likely due to cytotoxic and mutagenic effects disrupting normal physiological processes. The observed decline in reproductive structures (flowers and pods) further indicates that the mutagen may adversely affect reproductive fitness, potentially impairing seed production and overall plant viability. The results align with previous studies demonstrating that mutagenic agents at sub-lethal concentrations can induce growth inhibition, while higher concentrations may lead to severe developmental abnormalities or lethality (Bahar and Akkaya, 2009). The progressive reduction in plant height, leaf number, and reproductive output suggests that the mutagen disrupts cellular division, metabolic pathways, and hormonal regulation essential for normal plant growth.

CONCLUSIONS

The present study demonstrates the dose-dependent effects of Ethyl Methane Sulphonate (EMS) on *Momordica cymbalaria*, with emphasis on tuber regeneration and key morphological characteristics. The findings reveal that low to moderate EMS concentrations (10–30 mM) can

induce useful mutations while maintaining acceptable levels of regermination and plant survival, making them potentially valuable for mutation breeding. In contrast, higher EMS concentrations (40–50 mM) severely impaired tuber development, reduced survival rates, and negatively affected plant height, leaf number, flower production, and pod formation. These adverse effects are likely due to excessive DNA damage, disrupted cell division, and oxidative stress caused by high EMS levels. In addition to its applications in breeding, this research holds significance for the conservation of *M. cymbalaria*, a medicinally important and underutilized plant species threatened by habitat degradation, overharvesting, and poor natural regeneration. By identifying EMS doses that maintain plant viability while introducing genetic variation, the study offers a valuable approach for both ex-situ conservation and sustainable utilization, potentially aiding in the development of improved, resilient variants for future cultivation and medicinal use.

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REFREANCES :

Ahloowalia, B.S., Maluszynski, M., Nichterlein, K., 2004. Global impact of mutation-derived varieties. *Euphytica* 135, 187–204. https://doi.org/10.1023/B:EUPH.0000014914.85465.4f

Bahar, B., Akkaya, M.S., 2009. Effects of EMS treatment on the seed germination in wheat. *J. Appl. Biol. Sci.* 3, 59–64.

Chaitanya, G., Suvarchala, V., Ramakrishna, D., Sunitha, D., Shasthree, T., 2020. In vitro plant regeneration of *Momordica cymbalaria* Fenzl. and assessment of genetic fidelity using ISSR primers. *Res. J. Biotechnol.* Vol 15, 2.

Gulzar, A., Ali, A., Rana, R.M., n.d. Mutation breeding using chemical mutagen Ethyl Methane Sulphonate (EMS): An approach to morpho-physiological improvements in okra (*Abelmoschus esculentus* L.). 31(3),55-70.

Islam, S., Akter, S., Chowdhury, N., Jahin, S.A., Khatun, S.M., Hossain, M.A., 2022. Development of a high-yielding early mutant from fatemadhan through chemical mutagenesis. *Plant Arch.* 22 (2) 206-215.

Jankowicz-Cieslak, J., Till, B.J., 2016. Chemical Mutagenesis of Seed and Vegetatively Propagated Plants Using EMS. *Curr. Protoc. Plant Biol.* 1, 617–635. https://doi.org/10.1002/cppb.20040

Jeyadevi, R.A., Sivasudha, T., Rameshkumar, A., Sangeetha, B., Ananth, D.A., Aseervatham, G.S.B., 2012. Nutritional constituents and medicinal values of *Momordica cymbalaria* (Athalakkai)–A review. *Asian Pac. J. Trop. Biomed.* 2, S456–S461.

Kale, V.P., Kothekar, V.S., 2006. Mutagenic effects of ethyl methane sulphonate and sodium azide in potato (*Solanum tuberosum* L.). *Indian J. Genet. Plant Breed.* 66, 57–58.

Kirtikar, K., 1933. R. and Basu, BD: *Indian Medicinal Plants*, Vol. 1. Lalit Mohan Basu Allahabad 83.

Mageed, Y.A.A., Abdelkader, M.A.I., Awad, A.E., Khalil, B.M. Morphological and flowering variability of *Lathyrus odoratus* by using some physical and chemical mutagens. *Zagazig Journal of Horticultural Science* 43 (4) 1121-1135.

Mohammed, F.S., Babu, D., Irfan, Z., Fayed, M.A., 2024. A review on the traditional uses, nutritive importance, pharmacognostic features, phytochemicals, and pharmacology of *Momordica cymbalaria* Hook F. *PeerJ* 12, 16928.

Muqaddasi, Q., Arif, M., 2012. Ethyle methane sulphonate (EMS) induced mutagenic attempts to create genetic variability in Basmati rice. *J. Plant Breed. Crop Sci.* 4, 101–105.

Parvathi, S., Kumar, V.J.F., 2002. Studies on chemical composition and utilization of the wild edible vegetable athalakkai (*Momordica tuberosa*). *Plant Foods Hum. Nutr.* 57, 215–222. https://doi.org/10.1023/A:1021884406024

Puripunyavanich, V., Chanchula, N., Maikaeo, L., Limtiyayothin, M., Orpong, P., Tamman, A., Piriyaphattarakit, A., 2023. Effects of Ethyl Methanesulfonate on Mutation Induction in *Chrysanthemum* spp. *Trends Sci.* 20, 6904–6904.

Samadi, N., Naghavi, M.R., Moratalla-López, N., Alonso, G.L., Shokrpour, M., 2022. Morphological, molecular and phytochemical variations induced by colchicine and EMS chemical mutagens in *Crocus sativus* L. *Food Chem. Mol. Sci.* 4, 100086.

Shamshad, A., Rashid, M., Jankuloski, L., Ashraf, K., Sultan, K., Alamri, S., Siddiqui, M.H., Munir, T., uz Zaman, Q., 2023. Effect of ethyl methanesulfonate-mediated mutation for enhancing morpho-physio-biochemical and yield contributing traits of fragrant rice. P*eerJ* 11, 15821.

Shantakani Srinivasulu, S.S., Yarrama Pallavi, Y.P., Devi, B.G., Jyothi, H.K.P., 2017. Phytochemical and HPTLC studies on fruit extracts of *Momordica cymbalaria* Fenzl, a medicinally important plant.

Srinivasulu s., Pallavi, y., devi, b.g., jyothi, h.k.p., 2017. Phytochemical and HPTLC studies on fruit extracts of *Momordica cymbalaria* Fenzl, a medicinally important plant. *Not. Sci. Biol.* 9, 350–360.

Talebi, Ali Benjavad, Talebi, Amin Benjavad, Shahrokhifar, B., 2012. Ethyl methane sulphonate (EMS) induced mutagenesis in Malaysian rice (cv. MR219) for lethal dose determination. *Am. J. Plant Sci.* 3, 1661–1665.

Twumasi, P., Asare, P.A., Afutu, E., Amenorpe, G., Addy, S.N., 2023. Chemosensitivity analysis of tiger nuts (*Cyperus esculentus* L.) using ethyl methanesulfonate (EMS) and colchicine mutagens. *Heliyon* 9.

Weldemichael, M.Y., Bitima, T.D., Abrha, G.T., Tesfu, K., Gebremedhn, H.M., Kassa, A.B., Kindeya, Y.B., Mossa, M.M., 2023. Improving desirable agronomic traits of M2 lines on fourteen Ethiopian Sesame (S*esamum indicum* L.) genotypes using Ethyl Methane Sulphonate (EMS). Plos One 18, e0287246.

Yeddula Ezra, Y.E., Syed Muneer, S.M., Mohammad Mansoor, M.M., Rao, D.S., 2014. In vitro antioxidant and hypolipidemic activity of Momordica cymbalaria Fenzl in wistar rats.