*Original Research Article*

Determination of the Lethal Dose of Ethyl Methane Sulfonate (EMS) for Optimal *In Vitro* Mutagenesis in Banana (*Musa* spp. cv. Phee-gyan)

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ABSTRACT

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| The genetic variation in *Musa* (ABB group, Bluggoe subgroup) cv. ‘Phee-gyan’, a popular cultivar in Myanmar, was enhanced by *in vitro* application of ethyl methane sulphonate (EMS) mutagenesis to proliferated shoot tips. Shoot tips were treated with EMS at concentrations of 0%, 0.3%, 0.6%, 0.9%, 1.2%, and 1.5% across three incubation periods (30 min, 60 min, and 90 min) and cultured in five replicates using a randomized block design. The experiment was conducted at the laboratories of New Genetics (YAU) and Biotechnology Research Section (DAR) during 2023-2024. The results showed a dose-dependent decrease in survival percentage, shoot length, and fresh weight in the M1V1 generation compared to the control. The highest survival rate (91.48%) was recorded at 0.3% EMS (30 min), while the lowest survival (31.29%) occurred at 1.5% EMS (90 min). A 50% survival rate was recorded at 1.2% EMS (90 min), indicating a balance between mutation induction and plant viability. Shoot length was reduced to ~ 50% of the control at 1.2% EMS across all incubation times, while fresh weight decreased to ~ 50% of the control at 1.5% EMS (60 min and 90 min). Determining the lethal dose (LD₅₀) is essential to identify the optimum EMS concentration that maximizes mutagenesis efficiency while minimizing plant damage. Probit analysis determined LD₅₀ values based on survival percentage as 1.9% EMS (30 min and 60 min), and 1.2% EMS (90 min). For shoot length, LD₅₀ values were 1.3% EMS (30 min), and 1.1% EMS (60 min and 90 min), while for fresh weight, they were 1.9% EMS (30 min), 1.7% EMS (60 min), and 1.5% EMS (90 min). Based on these findings, 1.2% EMS (90 min) was determined to be the optimal mutagenesis condition for inducing genetic variability in banana cv. ‘Phee-gyan’. This optimized treatment could facilitate the improvement of key traits in banana breeding programs. |

*Keywords: Banana, Chemical mutagenesis, Ethyl methane sulphonate (EMS), LD50, Survival percentage.*

1. INTRODUCTION

Banana (*Musa* spp.) is considered as world’s most important food crops, widely cultivated across tropical and subtropical regions. In developing countries, they rank as the fourth most vital crop after rice, wheat, and maize, playing a significant role in national economies. Banana is a staple food and an essential part of daily nutrition for people living in most of the tropical countries. They are rich in fiber, carbohydrates, protein, potassium, phosphorus, calcium, manganese, magnesium, copper, vitamin B complex, vitamin A and vitamin C, as well as various antioxidants and phytochemicals (Li et al. 2013, Ranjha et al. 2020, Wan et al. 2005). Cultivated bananas are generally believed to have originated in the warm, moist regions of Southeast Asia, including India, Myanmar, Thailand, and Indonesia, as well as in west Oceania (Li et al. 2013, Wang et al. 2019).

In Myanmar, banana is one of the most important and commonly consumed fruits, valued not only as a food source but also for their role in religious and traditional ceremonies. They can be cultivated throughout the country and are available year-round. The total area under banana cultivation was approximately 111,000 hectares (Department of Planning, 2023). Myanmar is also recognized as a rich source of both cultivated bananas and their wild relatives. A total of thirteen *Musa* species, including wild types, are widely grown, with *Musa acuminata*, *M. cavendishii*, and four varieties under *M. sapientum* being the most prevalent varieties (Aye Tun, 2004).

A local banana cultivar *Musa* (ABB group, Bluggoe subgroup), ‘Phee-gyan’ is widely cultivated in Myanmar due to its adaptability and popularity. It plays a significant role in supporting the livelihoods of many smallholder farmers. Valued for its high yield, good taste, rich nutrient content, and strong market demand, Phee-gyan provides a reliable source of income and contributes to the local economy. However, banana production in Myanmar faces several challenges, including leaf spot diseases, leaf streak, bunchy top, fusarium wilt, other viral infections, and stem borer infestations (International Plant Protection Convention, 2015). These problems negatively affect both yield and postharvest quality, posing constraints for farmers throughout the country.

Mutation breeding is considered as a highly efficient tool, increasing possibility of inducing desired traits which are rare in nature. Mutations can be induced using physical and chemical mutagens, with chemical mutagens generally having a larger ratio of mutational to undesired alterations compared to physical mutagens (Oladosu et al. 2016). As a result, chemical mutagens have become an essential component of mutation breeding. One of the commonly used chemical mutagens is ethyl methane sulfonate (EMS), an alkylating agent that induces chemical modification in nucleotides by introducing an active alkyl group. This leads to base changes and nucleotide mutations (Arisha et al. 2014, Gillmor and Lukowitz, 2020, Sabetta et al. 2011). EMS has been used to create new germplasm in several crops, such as barley (Sharamo et al. 2021), rice (Serrat et al. 2014), potato (Moon et al. 2018), wheat (Wang et al. 2018), and maize (Zhang et al. 2020a).

The genetic characteristics of banana, such as parthenocarpy, polyploidy, irregular meiotic behavior, low fertility, and seed viability, among others make conventional breeding through crossing difficult in dessert banana varieties (Wang et al. 2021). EMS-induced mutagenesis is a valuable tool in banana research and breeding programs for producing somaclonal diversity (Omar et al. 1989). It enables the development of new banana varieties with improved traits, addressing major challenges faced by banana growers, such as disease susceptibility and yield limitations. As a result, EMS has been applied in banana breeding to improve agronomic traits, enhance cold tolerance, and disease resistance (Liu et al. 2024, Wang et al. 2021).

There is limited R&D for banana in the country, which has reduced the genetic diversity available for banana improvement programs. The present study aims to enhance genetic variation in Myanmar's local banana cv. Phee-gyan, through *in vitro*-induced mutations using chemical mutagen EMS. The objectives of this study are to determine the optimum dosage of EMS and to evaluate its effects on the growth and survival of mutants.

2. materialS and methods

**2.1 Experimental Site**

The experiment was conducted at the laboratory of the Biotechnology Research Section, Department of Agricultural Research, and Department of New Genetics, Advanced Center for Agricultural Research and Education, Yezin Agricultural University, Yezin, Nay Pyi Taw, during 2023-2024.

**2.2 Plant Materials and Surface Sterilization**

The banana cultivar Phee-gyan was used for current study. The suckers with an estimated size (30-45 cm) were collected from pest and disease free, healthy mother plants grown at germplasm maintenance block of the Biotechnology Research Section, Department of Agricultural Research (DAR), Yezin.

The suckers were thoroughly washed with tap water to remove adherent soil particles. The leaf sheaths and outer tissues of suckers were then trimmed and prepared to initiate explants. The explants were first sterilized in a fungicide solution (Homai) at a concentration of 1 gram per liter for 30 min under the laminar flow cabinet, then washed three to five times with sterilized distilled water. They were subsequently treated with 20% sodium hypochlorite (NaOCl) for 5 min, followed by three to five washes with sterilized distilled water. The explants were then sterilized with 70% ethanol for 2 min, washed again three to five times with sterilized distilled water, and air-dried for 5-10 minutes. The explants (approximately 2-3 cm in length) were vertically cut and cultured on MS medium (Murashige and Skoog, 1962) supplemented with 6-benzylaminopurine (BAP) to initiate shoot formation. The shoot tips were then sub-cultured (three times) at a 30-day interval in the same medium to produce multiple shoots.

**2.3 Mutagen Treatment and Data Collection**

A total of 30 excised shoot tips (about 1 cm in length) were prepared for each treatment, with three shoot tips per vessel across 10 culture vessels. Five different concentrations of EMS were prepared: Control (0.0 %, soaking in sterile water), 0.3 %, 0.6 %, 0.9 %, 1.2 %, and 1.5 %. The required concentrations of EMS solution (300 μl, 600 μl, 900 μl, 1200 μl, and 1500 μl) were added separately to 100 ml of sterile water in different vessels. The shoot tips were treated with freshly prepared EMS solutions and incubated for three different durations: 30 min, 60 min, and 90 min. The treatments were conducted at room temperature with an orbital shaker set at 80 rpm.

The EMS-treated shoot tips were rinsed three times with sterile distilled water to remove any chemical residues and then blotted dry on sterile filter paper. The shoot tips (M1V0) were subsequently cultured on solid MS medium supplemented with BAP at pH (5.8). The cultures were maintained at 25 ± 1°C with a 16-hour light and 8-hour dark photoperiod for 30 days. Finally, survival (%), the fresh weight (g) and shoot length (cm) of the explants were measured after 30 days.

**2.4 Experimental Design and Data Analysis**

The experiment was set up as a factorial Randomized Complete Block Design using a series of EMS concentrations and incubation times, resulting in 18 treatment combinations with five replications. The data were analyzed using Analysis of Variance (ANOVA) with Statistix (version 8) software. To compare treatment means, the least significant difference (LSD) was applied at the 0.05 % significant level. Linear regression analysis was performed using Microsoft Excel.

The lethal dose (LD50) was determined using Probit analysis (Finney. 1978) based on survival percentage, shoot length, and fresh weight. The Probit analysis was performed in Microsoft Excel using the method described below. The percentage of explants that survived after EMS treatment was calculated using the following formula.

Consequently, the percentage of shoots tips that died was calculated and rounded to the nearest whole number. The corrected mortality percentage is then calculated using Abbott’s formula, as shown below: Corrected mortality (%) = [(M observed – M control / (100 – M control)] x 100.

3. results and discussion

**3.1 Effect of EMS Treatment on Survival Rate**

The two-way ANOVA results showed a significant effects of EMS concentrations and treatment duration on mutagen-induced plant responses (Table 1). A significant reduction in the survival rate was observed with increasing EMS concentrations and longer treatment duration (*P* < 0.01). The survival percentage was highest in the control (0 % EMS) and gradually decreased with EMS treatment, ranging from 91.48 % (0.3 % EMS) to 64.04 % (1.5 % EMS) for 30 min; 87.07 % (0.3 % EMS) to 60.95 % (1.5 % EMS) for 60 min; and 85.11 % (0.3 % EMS) to 31.29 % (1.5 % EMS) for 90 min.

Also significant interaction effect was observed between the treatments and incubation time (*P* < 0.05). The highest survival percentage (91.48 %) was recorded with 0.3 % EMS treatment for 30 min, followed by 0.3 % EMS treatment for 60 min (87.07 %) and the lowest survival percentage (31.29 %) was observed with 1.5 % EMS treatment for 90 min. Approximately 50 % survival rate was observed at 1.2 % EMS treatment for 90 min and survival of shoot tips declined as EMS concentrations increased (Table 1 and Fig. 1). These results are consistent with previous findings in soybean, banana, and papaya (Anusha et al. 2024, Hofmann et al. 2004, Liu et al. 2024, Ravi et al. 2023, Shirani et al. 2016), which confirmed that survival percentage decreased with increasing EMS levels.

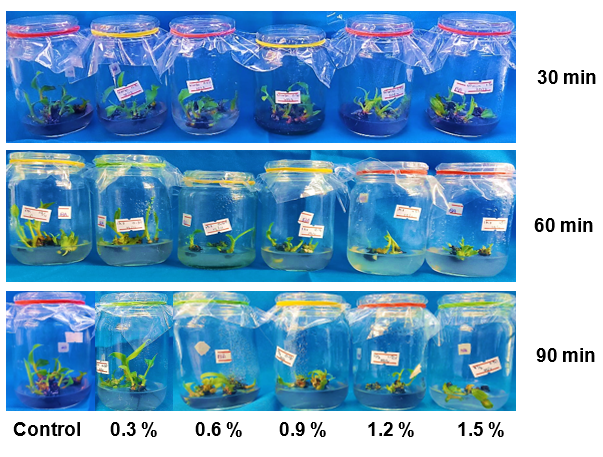
Biological damage induced by mutagens in the M1 generation such as reduced seed germination and plant survival can serve as an indicator of mutagenic effects (Gual. 1970). Alkylating agents such as EMS introduce mutations by adding methyl or ethyl groups to DNA bases, resulting in base mis-paring. The incorporation of toxic alkylated bases (e.g., 3-alkyl adenine) into the DNA template disrupts replication, leading to replication fork stalling. This inhibition of DNA synthesis suppresses cell division and differentiation, ultimately reducing survival rate.

**Table 1. Survival percentage of banana cv. ‘Phee-gyan’ induced by EMS mutagenesis**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **EMS**  **dosages**  **(%)** | **30 min** | | **60 min** | | **90 min** | |
| **Survival**  **(%)** | **Percent over control** | **Survival**  **(%)** | **Percent over control** | **Survival**  **(%)** | **Percent over control** |
| 0 | 78.89 a | 100.00 | 76.67 a | 100.00 | 74.44 a | 100.00 |
| 0.3 | 72.22 b | 91.48 | 66.67 b | 87.07 | 63.33 b | 85.11 |
| 0.6 | 70.00 b | 88.31 | 60.00 c | 78.36 | 52.22 c | 70.09 |
| 0.9 | 58.89 c | 74.04 | 56.67 c | 74.13 | 47.78 c | 64.16 |
| 1.2 | 56.67 c | 72.56 | 52.22 d | 68.14 | 40.00 d | 53.69 |
| 1.5 | 51.11 d | 64.04 | 46.67 e | 60.95 | 23.33 e | 31.29 |
| LSD (0.05) | 2.18 |  | 1.89 |  | 3.03 |  |
| CV (%) | 4.13 |  | 3.88 |  | 7.39 |  |
| F test | EMS Dosage (D) | | | \*\* | | |
| Incubation Time (T) | | | \*\* | | |
| D x T | | | \* | | |

*Note: \*\* P<0.01; \* P<0.05*

*Numbers with different letters are significantly different at P < 0.05 based on an LSD test.*



**Fig. 1. Effect of EMS treatment on survival of mutants in banana cv. ‘Phee-gyan’**

**3.2 Effect of EMS Treatment on Shoot Length and Fresh Weight**

Shoot length significantly decreased with increasing EMS concentrations across all treatments (*P* < 0.01) compared to their respective controls (0 % EMS: 3.76 cm, 3.53 cm, and 3.16 cm for 30 min, 60 min, and 90 min incubations, respectively). For the 30 min treatment, shoot length decreased from 2.97 cm (0.3 % EMS) to 1.46 cm (1.5 % EMS). Similarly, in the 60 min treatment, it declined from 2.69 cm (0.3 % EMS) to 1.27 cm (1.5 % EMS); while the 90 min treatment showed a reduction from 2.39 cm (0.3 % EMS) to 1.11 cm (1.5 % EMS) (Table 2). At 1.2 % EMS, shoot length was reduced by approximately 50 % across all three incubation periods. No significant interaction effect was observed between EMS concentration and incubation time on shoot length.

Similar results were reported in banana (Anusha et al. 2024, Shirani et al. 2016), acid lime (Davi et al. 2021), and papaya (Ravi et al. 2023), where shoot length progressively declined with increasing EMS concentrations. Mutants also exhibited delayed and weak growth compared to control, along with morphological abnormalities such as white leaf stripes, xantha, chlorosis, and dwarfism (Fig. 2).

The fresh weight of shoot tips significantly decreased across all EMS treatments (P < 0.01) compared to the control (0 % EMS: 1.69 g, 1.63 g, and 1.57 g for 30 min, 60 min, and 90 min incubations, respectively). After 30 min of EMS exposure, fresh weight declined from 1.57 g (0.3 % EMS) to 1.11 g (1.5 % EMS). Similarly, the 60 min treatment showed a reduction from 1.44 g (0.3 % EMS) to 0.89 g (1.5 % EMS), while the 90 min treatment decreased from 1.30 g (0.3 % EMS) to 0.73 g (1.5 % EMS) (Table 3). The highest fresh weight (1.57 g) was recorded at 0.3 % EMS (30 min), whereas the lowest (0.73 g) occurred at 1.5 % EMS (90 min). A nearly 50 % reduction in fresh weight was observed at 1.5 % EMS (60 min) and 1.2 % EMS (90 min). No significant interaction effect was observed between EMS concentration and incubation time on shoot length.

These findings align with previous studies on banana cultivars (Arachchige et al. 2016, Bidabadi et al. 2012, Jankowicz-Cieslak et al. 2012), where increasing EMS doses similarly reduced fresh weight. The reduction in both shoot length and fresh weight of explants is a commonly used indicator of the biological effects of chemical mutagens in M1 generation, likely due to disruptions in metabolic processes at the embryonic level.

**Table 2. Shoot length of banana cv. ‘Phee-gyan’ induced by EMS mutagenesis**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **EMS dosages (%)** | **30 min** | | **60 min** | | **90 min** | |
| **Shoot**  **length(cm)** | **Percent Over control** | **Shoot**  **length(cm)** | **Percent Over control** | **Shoot**  **length (cm)** | **Percent Over control** |
| 0 | 3.76a | 100.00 | 3.53a | 100.00 | 3.16a | 100.00 |
| 0.3 | 2.97b | 78.99 | 2.69b | 76.20 | 2.39b | 75.63 |
| 0.6 | 2.73b | 72.61 | 2.29bc | 64.87 | 1.89c | 59.81 |
| 0.9 | 2.59b | 68.88 | 2.20c | 62.32 | 1.86c | 58.86 |
| 1.2 | 1.89c | 50.27 | 1.63d | 46.18 | 1.43d | 45.25 |
| 1.5 | 1.46d | 38.83 | 1.27d | 35.98 | 1.11d | 35.13 |
| LSD (0.05) | 0.18 |  | 0.21 |  | 0.19 |  |
| C.V% | 11.13 |  | 14.69 |  | 14.73 |  |
| F test | EMS Dosage (D) | | | \*\* | | |
| Incubation Time (T) | | | \*\* | | |
| D x T | | | n.s | | |

*Note: \*\* P<0.01; \* P<0.05; n.s., non-significant*

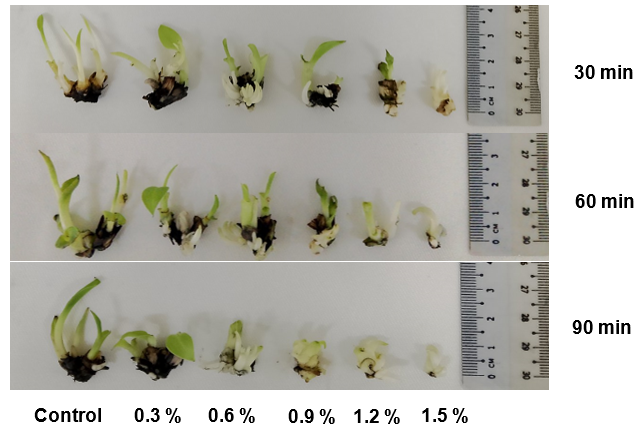
*Numbers with different letters are significantly different at P < 0.05 based on an LSD test.*

**Table 3. Fresh weight of banana cv. ‘Phee-gyan’ induced by EMS mutagenesis**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **EMS dosages (%)** | **30 min** | | **60 min** | | **90 min** | |
| **Fresh weight (g)** | **Percent Over control** | **Fresh weight (g)** | **Percent Over control** | **Fresh weight (g)** | **Percent Over control** |
| 0 | 1.69a | 100.00 | 1.63a | 100.00 | 1.57a | 100.00 |
| 0.3 | 1.57ab | 92.90 | 1.44b | 88.34 | 1.30a | 82.80 |
| 0.6 | 1.56ab | 92.31 | 1.42b | 87.12 | 1.22b | 77.71 |
| 0.9 | 1.45b | 85.80 | 1.31c | 80.37 | 1.18b | 75.16 |
| 1.2 | 1.26c | 74.56 | 1.14d | 69.94 | 0.90c | 57.32 |
| 1.5 | 1.11c | 65.68 | 0.89d | 54.60 | 0.73c | 46.50 |
| LSD (0.05) | 0.07 |  | 0.05 |  | 0.09 |  |
| C.V% | 8.12 |  | 6.19 |  | 12.47 |  |
| F test | EMS Dosage (D) | | | \*\* | | |
| Incubation Time (T) | | | \*\* | | |
| D x T | | | n.s | | |

*Note: \*\* P<0.01; \* P<0.05; n.s., non-significant*

*Numbers with different letters are significantly different at P < 0.05 based on an LSD test.*



**Fig. 2. Morphological variations in proliferated shoot tips of banana cv. ‘Phee-gyan’ induced by EMS mutagenesis**

**3.3 Determination of LD50 Value**

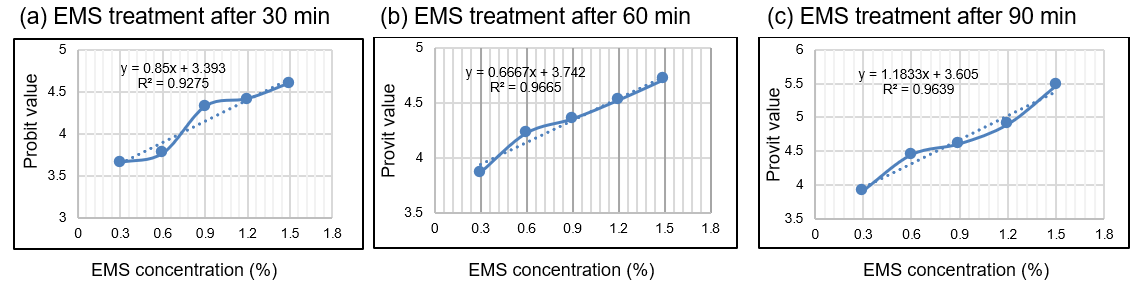
Determination of the lethal dose is essential for any mutation breeding program in crops. The lethal dose 50 (LD50) is defined as the EMS concentrations and treatment duration that results in 50 % mortality or 50 % survival of mutants (Arisha et al. 2015). LD50 generally indicates the concentration that induces a high mutation rate with minimal plant damage (Hohmann et al. 2005). This value is also influenced by factors such as explant type, crop species, variety, season, chemical properties, maturity level, moisture content and other (Parthasarathi et al. 2020).

In the present study, the banana variety ‘Phee-gyan’ was treated with the chemical mutagen EMS, and the LD50 value for EMS was calculated using Probit analysis based on survival percentage, shoot length and fresh weight (Table 4 and Appendix Table 1 and 2). The dose response curves based on probit values were drawn and presented in Fig. 3, 4 and 5 for 30 min, 60 min and 90 min respectively.

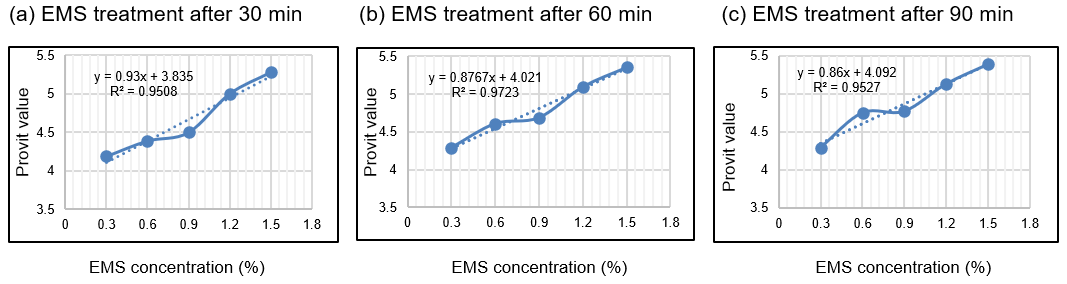
The results demonstrated LD50 values of 1.9 %, 1.9 %, and 1.2 % for survival rate; 1.3 %, 1.1 % and 1.1 % for shoot length; and 1.9 %, 1.7 % and 1.5 % for fresh weight across the three incubation periods. These finding align with previous reports of optimum EMS mutagenesis conditions: 1.4 % for 60 min and 0.8 % for 120 min in banana cv. ‘Tella Chakkerakeli’ (Anusha et al. 2024), 0.8 % for 4 h in banana cv. ‘FenJiao’ (Wang et al. 2021), 0.33 % for 6 h in acid line cv. ‘PKM1’ (Devi et al. 2021), 0.55 % for 3 h in papaya cv. ‘CO7’ (Ravi et al. 2023), and 1 % for 24 h in cucumber (Wang et al. 2014).

**Table 4. Effect of EMS mutagenesis on LD50 for survival percentage in banana cv. ‘Phee-gyan’**

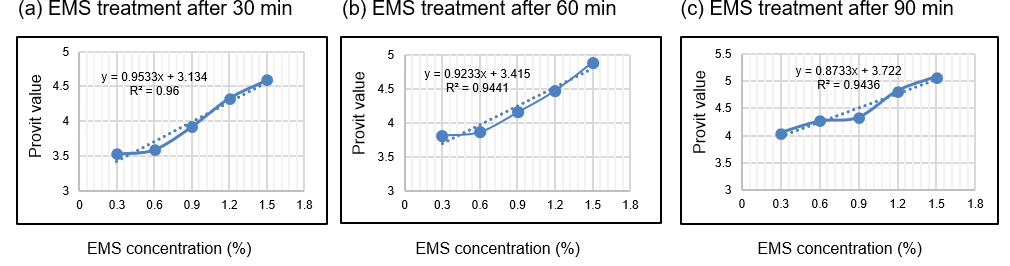
|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **EMS dosages (%)** | **Survival (%)** | | **Survival**  **(%) over control** | **Mortality (%) over control** | **Observed mortality (%)** | **Corrected mortality (%)** | **Probit value** | **LD50** |
| 30 min incubation time | | | | | | | | |
| 0 | | 78.89 | 100.00 | - | 21.11 | - | - | 1.9 % |
| 0.3 | | 72.22 | 91.48 | 8.52 | 27.78 | 8.45 | 3.66 |  |
| 0.6 | | 70.00 | 88.31 | 11.69 | 30.00 | 11.27 | 3.77 |  |
| 0.9 | | 58.89 | 74.04 | 25.96 | 41.11 | 25.35 | 4.33 |  |
| 1.2 | | 56.67 | 72.56 | 28.72 | 43.33 | 28.18 | 4.42 |  |
| 1.5 | | 51.11 | 64.04 | 35.96 | 48.89 | 35.21 | 4.61 |  |
| 60 min incubation time | | | | | | | | |
| 0 | | 76.67 | 100.00 | - | 23.33 | - | - | 1.9 % |
| 0.3 | | 66.67 | 87.07 | 12.93 | 33.33 | 13.05 | 3.87 |  |
| 0.6 | | 60.00 | 78.36 | 21.64 | 40.00 | 21.74 | 4.23 |  |
| 0.9 | | 56.67 | 74.13 | 25.87 | 43.33 | 26.09 | 4.36 |  |
| 1.2 | | 52.22 | 68.14 | 31.86 | 47.78 | 31.89 | 4.53 |  |
| 1.5 | | 46.67 | 60.95 | 39.05 | 53.33 | 39.13 | 4.72 |  |
| 90 min incubation time | | | | | | | | |
| 0 | | 74.44 | 100.00 | - | 25.56 | - | - | 1.2 % |
| 0.3 | | 63.33 | 85.11 | 14.89 | 36.67 | 14.92 | 3.96 |  |
| 0.6 | | 52.22 | 70.09 | 29.91 | 47.78 | 29.85 | 4.48 |  |
| 0.9 | | 47.78 | 64.16 | 35.84 | 52.22 | 35.82 | 4.64 |  |
| 1.2 | | 40.00 | 53.69 | 46.31 | 60.00 | 46.27 | 4.90 |  |
| 1.5 | | 23.33 | 31.29 | 68.71 | 76.67 | 68.65 | 5.50 |  |



**Fig. 3. LD50 for survival percentage in banana cv. ‘Phee-gyan’ across three different incubation times**



**Fig. 4. LD50 for shoot length in banana cv. ‘Phee-gyan’ across three different incubation times**



**Fig. 5. LD50 for fresh weight in banana cv. ‘Phee-gyan’ across three different incubation times**

**4. CONCLUSION**

The successful induction of desirable mutations depends critically on optimization of both EMS concentration and mutagenesis duration. This study demonstrated that varying EMS concentrations and incubation times significantly influenced explant fresh weight, shoot length, and survival rates (*p* < 0.05). A clear dose-dependent response was observed, where increasing EMS concentrations progressively reduced fresh weight, shoot length and survival percentage. For the banana cv. ‘Phee-gyan’, the optimal mutagenesis condition was identified as 1.2 % EMS treatment for 90 min, yielding a 53.69 % survival rate with favorable growth retention (45 % shoot length and 57 % fresh weight). While M1V1 mutants in bananas provide valuable initial phenotypic characterization, their use in breeding programs requires additional validation for long-term stability due to biological constraints including clonal propagation, polyploid genome complexity, and genotype-by-environment interactions. The EMS-induced ‘Phee-gyan’ mutants developed in this study will facilitate efficient screening for enhanced tolerance to climate change-associated stresses, providing a foundation for developing more resilient banana cultivars.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declares 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 this manuscript.

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APPENDIX

**Table 1. Effect of EMS mutagenesis on LD50 for shoot length in banana cv. ‘Phee-gyan’**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **EMS**  **Dosages (%)** | **Shoot length (cm)** | **(%) Over control** | **(%) Reduction over control** | **Probit unit** | **LD50** |
| 30 min incubation time | | | | | |
| 0 | 3.76 | 100.00 | - | - | 1.3 % |
| 0.3 | 2.97 | 78.99 | 21.01 | 4.19 |  |
| 0.6 | 2.73 | 72.61 | 27.39 | 4.39 |  |
| 0.9 | 2.59 | 68.88 | 31.12 | 4.48 |  |
| 1.2 | 1.89 | 50.27 | 49.73 | 5.00 |  |
| 1.5 | 1.46 | 38.83 | 61.17 | 5.25 |  |
| 60 min incubation time | | | | | |
| 0 | 3.53 | 100.00 | - | - | 1.1 % |
| 0.3 | 2.69 | 76.20 | 23.80 | 4.29 |  |
| 0.6 | 2.29 | 64.87 | 35.13 | 4.61 |  |
| 0.9 | 2.20 | 62.32 | 37.68 | 4.69 |  |
| 1.2 | 1.63 | 46.18 | 53.82 | 5.10 |  |
| 1.5 | 1.27 | 35.98 | 64.02 | 5.36 |  |
| 90 min incubation time | | | | | |
| 0 | 3.16 | 100.00 | - | - | 1.1 % |
| 0.3 | 2.39 | 75.63 | 24.37 | 4.29 |  |
| 0.6 | 1.89 | 59.81 | 40.19 | 4.75 |  |
| 0.9 | 1.86 | 58.86 | 41.14 | 4.77 |  |
| 1.2 | 1.43 | 45.25 | 54.75 | 5.13 |  |
| 1.5 | 1.11 | 35.13 | 64.87 | 5.39 |  |

**Table 2. Effect of EMS mutagenesis on LD50 for fresh weight in banana cv. ‘Phee-gyan’**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **EMS**  **dosages (%)** | **Fresh weight (g)** | **(%) Over control** | **(%) Reduction over control** | **Probit unit** | **LD50** |
| 30 min incubation time | | | | | |
| 0 | 1.69 | 100.00 | - | - | 1.9 % |
| 0.3 | 1.57 | 92.90 | 7.10 | 3.52 |  |
| 0.6 | 1.56 | 92.31 | 7.69 | 3.59 |  |
| 0.9 | 1.45 | 85.80 | 14.20 | 3.92 |  |
| 1.2 | 1.26 | 74.56 | 25.44 | 4.33 |  |
| 1.5 | 1.11 | 65.68 | 34.32 | 4.59 |  |
| 60 min incubation time | | | | | |
| 0 | 1.63 | 100.00 | - | - | 1.7 % |
| 0.3 | 1.44 | 88.34 | 11.66 | 3.82 |  |
| 0.6 | 1.42 | 87.12 | 12.88 | 3.87 |  |
| 0.9 | 1.31 | 80.37 | 19.63 | 4.16 |  |
| 1.2 | 1.14 | 69.94 | 30.06 | 4.48 |  |
| 1.5 | 0.89 | 54.60 | 45.40 | 4.9 |  |
| 90 min incubation time | | | | | |
| 0 | 1.57 | 100.00 | - | - | 1.5 % |
| 0.3 | 1.30 | 82.80 | 17.20 | 4.05 |  |
| 0.6 | 1.22 | 77.71 | 22.29 | 4.23 |  |
| 0.9 | 1.18 | 75.16 | 24.84 | 4.33 |  |
| 1.2 | 0.90 | 57.32 | 42.68 | 4.82 |  |
| 1.5 | 0.73 | 46.50 | 53.50 | 5.10 |  |