Current Applied Science and Technology e-ISSN: 2586-9396

Curr. Appl. Sci. Technol. 2026, Vol. 26 (No. 2), e0264707

Research article

EMS-Induced Genetic Variation and Morphological Changes in *Musa laterita*

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Received: 16 September 2024, Revised: 7 March 2025, Accepted: 25 March 2025, Published: 7 July 2025

Abstract

Musa laterita (orange torch banana) holds ornamental and cultural value, yet its natural breeding potential is limited by sterility and low seed germination. Induced mutation through ethyl methanesulfonate (EMS) is a promising method to generate genetic diversity for plant improvement. This study aimed to evaluate the effects of EMS on *M. laterita*, focusing on tissue culture propagation, mutation induction, and genetic variation analysis. Tissue-cultured plants were exposed to varying EMS concentrations (100-500 mM) for 1, 6, and 12 h. Survival rates decreased with increasing EMS concentration and exposure time, with no survival at concentrations above 400 mM for prolonged duration. The optimal EMS concentration for mutation was found at 300 mM with 6 h of exposure, yielding significant morphological changes, including an increase in root length (5.99 cm), while pseudostem length showed an upward trend but was not significantly different from the control. Genetic variation was assessed using ISSR markers, with 47.24% polymorphism detected. The greatest genetic distance (51.02%) was observed between control and the treatment at 300 mM for 6 h, confirming the effectiveness of EMS in inducing genetic mutations. These findings demonstrate that EMS can induce useful genetic diversity in *M. laterita*, thereby offering a valuable resource for future breeding efforts aimed at developing improved ornamental cultivars.

Keywords: *Musa laterita*; ethyl methanesulfonate (EMS); induced mutation; genetic variation; ISSR markers

1. Introduction

Musa laterita, a member of the Rhodochlamys section, is a dwarf ornamental banana native to northeastern India and the Indochina region. Its horticultural value lies in its upright leaves with dark midribs, orange-yellow bi-seriate male flowers, and vibrant brick-red bracts (National Parks, 2024). The plant thrives in full sunlight and is widely cultivated for

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https://doi.org/10.55003/cast.2025.264707

ornamental landscaping (Natarajan et al., 2020). Despite its commercial appeal, *M. laterita* exhibits high sterility, parthenocarpy, and low seed germination, which are common reproductive barriers in wild bananas. These limitations make conventional breeding methods largely ineffective (Waniale et al., 2024).

To address these challenges, *in vitro* tissue culture has emerged as an essential tool for propagating genetically uniform and healthy plantlets. Previous studies on bananas and related ornamentals, such as *Cymbidium finlaysonianum* and *Hedychium longicornutum*, have demonstrated the effectiveness of tissue culture techniques in shoot regeneration and genetic fidelity (Chuengpanya et al., 2022; Sorn et al., 2024). However, tissue culture alone does not introduce new genetic variation. Mutation breeding using chemical mutagens such as ethyl methanesulfonate (EMS) has become an increasingly important strategy to expand the genetic base and generate novel traits, particularly in sterile or clonally propagated species.

EMS is a widely used mutagen known to induce point mutations by alkylating guanine bases, leading to base mispairing during DNA replication (Ke et al., 2019; Guo et al., 2024). Its efficacy has been confirmed across various plant species, including economically important ornamentals such as chrysanthemum, *Erycina pusilla*, and *Etlingera elatior*, where EMS-induced mutations have led to desirable traits like leaf variegation, altered plant form, and enhanced flowering (Purente et al., 2020; Maynard & Ruter, 2023). The detection of such mutations is often facilitated by Inter Simple Sequence Repeat (ISSR) markers, which are efficient, reproducible, and do not require prior genomic knowledge (Costa et al., 2016; Joshi et al., 2023).

Despite the successful use of EMS in mutation breeding for other ornamental crops, no published studies have yet investigated its application in *M. laterita*. This represents a significant gap in the field, especially considering the species' high ornamental potential and propagation constraints. Furthermore, the integration of EMS mutagenesis with ISSR molecular analysis offers a powerful strategy for both inducing and identifying genetic variation.

Therefore, the objective of this study was to evaluate the effects of EMS on the morphological and genetic variation of *M. laterita* using a tissue culture-based approach. The study aimed to determine optimal EMS concentrations and exposure durations that induce genetic mutations while preserving plant viability. ISSR markers were employed to assess the extent of induced polymorphism and genetic divergence among treatments. As the first report on EMS-induced mutagenesis in *M. laterita*, this research contributes new insights into mutation breeding strategies for sterile ornamental banana species and lays the groundwork for future cultivar development.

2. Materials and Methods

2.1 Plant material and tissue culture propagation

The research on *M. laterita* commenced with tissue culture propagation to ensure uniform and healthy plant material for further experimentation. The propagation process involved taking samples from plant suckers or runners, which were initially washed thoroughly with tap water. Following the cleaning process, the samples underwent surface sterilization to eliminate any microbial contaminants. The sterilization process involved two critical steps to ensure the plant samples were free from contamination. Initially, the samples were immersed in a 70% alcohol solution for one minute. This was followed by a treatment with a 20% Clorox solution for 20 min. After discarding the Clorox solution, the samples were

thoroughly rinsed three times using autoclaved distilled water to remove any remaining chemicals. The sterilized plant material was then cut into four sections and cultured on Murashige and Skoog (MS) medium supplemented with 5 mg/L benzylaminopurine (BA), adhering to the established protocol (Abdel-Motagaly et al., 2018). The culture process, lasting between 8 and 16 weeks, promoted shoot formation. Once the shoots had sufficiently developed, they were transferred to a fresh MS medium containing 2 mg/L BA, a concentration proven to be effective in inducing bud formation, as noted by Reddy et al. (2014). This method enabled the proliferation of plant material, yielding an adequate number of shoots for further experimental treatments.

2.2 EMS treatment

To study the effects of EMS on *M. laterita*, the developed shoots from the tissue culture were separated into individual units and subjected to EMS treatments at varying concentrations. EMS treatment was applied at five concentrations (100 mM, 200 mM, 300 mM, 400 mM, and 500 mM) and three exposure durations (1, 6, and 12 h), resulting in a total of 16 treatment groups, including a control group. After treatment, the shoots were rinsed thoroughly with autoclaved distilled water and cultured on MS medium containing 3 mg/L BA. The treated samples were incubated in a controlled environment at 25±2°C with a 12-h photoperiod. Morphological changes and survival rates were recorded after 16 weeks of incubation, providing insights into the effects of different EMS concentrations and exposure durations on the growth and development of *M. laterita*.

2.3 Morphological assessments

Parameters such as survival rate, shoot proliferation, fresh weight, number of leaves, pseudostem length, root number, and root length were recorded for each treatment group. These traits were carefully monitored to determine the impact of EMS-induced mutations on plant growth. The assessments were carried out after 16 weeks of incubation. For each treatment, one randomly selected plant from each replicate was used to measure the various morphological traits, ensuring a comprehensive analysis of the effects of EMS on plant morphology.

2.4 Genetic variation analysis

To investigate genetic changes induced by EMS treatment, plant samples from each treatment group were selected after 16 weeks of culture. Selection was based on observable morphological variations such as leaf variegation, altered shoot growth, or root development, compared to the untreated control group. From each treatment, three randomly selected plants (one per replicate) were chosen for genetic analysis, ensuring representation across biological replicates. The genomic DNA was extracted from the leaf tissue using a modified protocol based on Gawel and Jarret (1991). The quality and quantity of the DNA were verified using agarose gel electrophoresis and spectrophotometry. The ISSR primers were employed to amplify the extracted DNA via polymerase chain reaction (PCR), following Bornet and Branchard (2001) method. The resulting PCR products were analyzed on a 1.5% agarose gel, and the banding patterns were compared with a DNA ladder to assess genetic diversity among the treated plants.

2.5 Data collection and statistical analysis

Data from morphological and genetic variation analyses were subjected to statistical analysis to determine the significance of the observed differences. Each treatment group consisted of three replicates, with three plants per replicate (n=9 plants per treatment). An analysis of variance (ANOVA) was conducted to assess the impact of different EMS concentrations and treatment durations on the morphological traits of *M. laterita*. Duncan's Multiple Range Test (DMRT) was used for pairwise comparisons of means at a 95% confidence level ($p < 0.05$). Genetic analysis involved scoring the presence or absence of bands in the ISSR banding patterns. A genetic similarity matrix was constructed using the unweighted pair group method with arithmetic mean (UPGMA) analyzed using the DendroUPGMA program (Garcia-Vallvé et al., 1999) and visualized as a dendrogram generated with TreeView 3.0 (Page, 1996). This dendrogram illustrates the genetic relationships between the EMS-treated plants and the control group, clearly representing the genetic diversity induced by EMS treatment.

3. Results and Discussion

This study aimed to investigate the impact of varying concentrations of EMS on the morphological and genetic characteristics of *M. laterita*, focusing on survival rates, growth traits, and genetic variability. The research was divided into three major components: the effects of EMS on plant survival, growth characteristics, and genetic variation assessed through ISSR marker analysis and UPGMA genetic distance analysis.

3.1 Survival rates of *M. laterita* after EMS treatment

The first part of the study focused on the survival rates of *M. laterita* following exposure to varying concentrations of EMS and different immersion times. The results indicated a clear negative correlation between EMS concentration, immersion time, and plant survival. The control group exhibited a 100% survival rate, while survival rates dropped significantly as EMS concentration and immersion duration increased. For example, at 100 mM EMS for 1 h, the survival rate was 77.78%, but as exposure time increased to 6 and 12 h, survival decreased to 66.67% and 55.55%, respectively. Higher EMS concentrations further exacerbated the decline in survival rates, with 300 mM EMS reducing survival to 44.44% after 1 h and no survival observed at 12 h of exposure to 500 mM EMS. Statistical analysis confirmed significant differences between treatments, highlighting the substantial impact of EMS concentration and immersion time on survival rates (Table 1). These findings align with previous works on *in vitro* propagation and mutation breeding. Sorn et al. (2024) showed that shoot regeneration in Cavendish banana was highly influenced by cytokinin levels, similar to the use of BA in this study, which was essential for the successful tissue culture propagation of *M. laterita*.

The decline in survival rates observed in this study is likely due to the mutagenic effects of EMS, which induce extensive DNA damage. EMS alkylates guanine bases in DNA, leading to mutations that disrupt cellular processes and cause cell death. Higher EMS concentrations increase the mutational load beyond the plant's ability to repair DNA, leading to reduced viability (Manova & Gruszka, 2015; Lu et al., 2021). Plants have DNA repair mechanisms such as base excision repair (BER) and nucleotide excision repair (NER), but when the level of DNA damage surpasses repair capacity, it results in cellular dysfunction and reduced survival (Torgovnick & Schumacher, 2015).

Table 1. The survival percentage of *M. laterita* after being immersed in different concentrations of EMS solutions for 1, 6 and 12 h and subsequently cultured on solid MS medium for 8 weeks

Treatment	EMS Concentration (mM)	Immersion Time (h)	Survival Percentage	SD.
Control	-	-	100.00^{a}	0.00
E01	100	1	77.78^{ab}	19.24
E02	200	1	55.56^{bcd}	19.25
E03	300	1	44.44^{bcd}	19.25
E04	400	1	22.22^{de}	38.49
E05	500	1	0.00^{e}	0.00
E06	100	6	66.67^{abc}	33.34
E07	200	6	55.55^{bcd}	38.49
E08	300	6	33.33^{cde}	0.00
E09	400	6	0.00^{e}	0.00
E10	500	6	0.00^{e}	0.00
E11	100	12	55.55^{bcd}	38.49
E12	200	12	44.44^{bcd}	19.25
E13	300	12	0.00^{e}	0.00
E14	400	12	0.00^{e}	0.00
E15	500	12	0.00^{e}	0.00

Note: Different letters in the same column indicate statistically significant differences at the 95% confidence level, based on Duncan's new multiple range test (DMRT).

The findings from this study reveal that increasing EMS concentrations and exposure durations resulted in a marked decline in the survival rates of *M. laterita*. Lower concentrations, such as 100 mM EMS for 1 h, resulted in relatively high survival rates (77.78%), while higher concentrations like 300 mM EMS led to a drastic reduction in plant survival, particularly after prolonged exposure, where no survival was observed at 12 h (500 mM). The decline in survival rates with increasing EMS exposure is largely due to the mutagenic effects of EMS, which induces extensive DNA damage, particularly by alkylating guanine bases in DNA. This leads to the formation of mutations that can disrupt normal cellular processes and eventually result in cell death (Greene & Jinks-Robertson, 1997; Saini et al., 2020; Ovejero et al., 2021; Türkoğlu et al., 2023). High EMS concentrations or prolonged exposure times increase the mutational load beyond the plant's ability to repair DNA, overwhelming its repair mechanisms and leading to reduced viability. Similar findings have been reported in other studies where prolonged EMS exposure compromised plant survival due to excessive DNA damage and impaired DNA repair mechanisms (Manova & Gruszka, 2015; Lu et al., 2021).

Plants possess various DNA repair mechanisms such as base excision repair (BER) and nucleotide excision repair (NER), which work to rectify DNA damage caused by environmental stressors, including mutagenic agents like EMS. However, when the level of DNA damage surpasses the repair capacity, it leads to cellular dysfunction and a decline in survival, as observed in *M. laterita* and other plant species exposed to high concentrations of EMS (Torgovnick & Schumacher, 2015).

3.2 Effects of EMS on growth characteristics of *M. laterita*

The second part of the study examined how EMS treatment affected various growth characteristics of *M. laterita*, grown on solid MS medium for 16 weeks. EMS exposure resulted in significant changes in growth traits, including shoot number, fresh weight, leaf production, pseudostem length, root number, and root length (Table 2).

The highest shoot number was observed in treatment E01, which produced 6.06 shoots, a result that was not significantly different from the control group (5.56 shoots). In contrast, treatment E04 yielded the lowest shoot number (0.83 shoots). Similarly, fresh weight was highest in treatments E11 (17.39 g) and E08 (17.07 g). While these values were numerically higher than the control (15.68 g), statistical analysis indicated no significant differences between these treatments and most other EMS-treated groups, except for E04, which had significantly lower fresh weight (5.47 g). On the other hand, treatment E04 again showed the lowest fresh weight at 5.466 g. Leaf production showed slight numerical variation across treatments. Treatment E06 had the highest number of leaves per plant (5.17), while the control produced an average of 4.33 leaves per plant. However, statistical analysis indicated that these differences were not significant among most treatments. In contrast, treatment E04 exhibited markedly reduced leaf production (0.83 leaves per plant), which was significantly lower than other treatments.

Pseudostem length in treatment E08 was 13.544 cm, showing a numerical increase compared to the control (11.365 cm); however, statistical analysis indicated no significant difference between E08 and most other treatments. Treatment E04 had the shortest pseudostem at 3.970 cm, which was significantly lower than most treatments. Root production was numerically higher in treatments E12 and E02 (6.83 roots) compared to the control group (6.56 roots); however, statistical analysis showed no significant difference among most treatments, except for E04, which had significantly lower root production. Root length was maximized in treatment E08, where plants reached 5.997 cm, significantly longer than the control's 4.169 cm.

In conclusion, treatments E08 and E11 showed the greatest potential for promoting growth in *M. laterita*, particularly for fresh weight, pseudostem length, and root length. Conversely, treatment E04 consistently inhibited growth across all parameters. These findings highlight the importance of selecting the appropriate EMS concentrations to optimize tissue culture outcomes, as excessive or insufficient concentrations can drastically affect plant growth.

For the statement about the impact of EMS on morphological traits and the optimal concentration, multiple studies support the findings that moderate EMS exposure can induce beneficial mutations while maintaining plant viability. For instance, EMS treatments around 300 mM for 6 h have been reported to enhance growth characteristics such as pseudostem and root length in *M. laterita*, aligning with similar results observed in *Chrysanthemum indicum* where optimal EMS concentrations induced favorable morphological changes without significantly compromising plant health (Ke et al., 2019; Purente et al., 2020). Conversely, higher EMS concentrations (e.g., 500 mM) have been shown to severely inhibit plant growth and overall development, as seen in both *Musa* and other plant species such as cauliflower, where higher doses resulted in drastic reductions in plant viability and growth (Xu et al., 2012; Ke et al., 2019).

These findings underscore the importance of carefully calibrating EMS concentration to balance mutation induction with survival, suggesting that 300 mM EMS for 6 h is an optimal condition for inducing mutations in *M. laterita* to achieve desirable morphological improvements.

Table 2. The total number of shoots, fresh weight, number of leaves per plant, pseudostem length, number of roots per plant, and root length of *M. laterita* after being immersed in different concentrations of EMS solutions for 1, 6 and 12 h, and subsequently cultured on solid MS medium for 16 weeks

Treatment	Total Shoots (shoots)	Fresh Weight (g)	Leaves per Plant (leaves)	Pseudostem Length (cm)	Roots per Plant (roots)	Root Length (cm)
Control	5.56^{ab}	15.680^{a}	4.33^{a}	11.365^{ab}	6.56^{a}	4.169^{bc}
E01	6.06^{a}	16.136^{a}	4.83^{a}	10.563^{ab}	6.56^{a}	3.129^{cd}
E02	5.50^{ab}	16.540^{a}	4.67^{a}	11.325^{ab}	6.83^{a}	5.239^{ab}
E03	4.50^{abc}	15.689^{a}	3.83^{a}	11.805^{ab}	6.17^{a}	4.807^{abc}
E04	0.83^{d}	5.466^{b}	0.83^{b}	3.970^{c}	2.17^{b}	1.591^{de}
E05	NA	NA	NA	NA	NA	NA
E06	5.50^{ab}	16.009^{a}	5.17^{a}	9.776^{b}	6.72^{a}	4.425^{abc}
E07	3.56^{bc}	16.543^{a}	4.44^{a}	11.130^{ab}	5.89^{a}	4.233^{bc}
E08	3.67^{bc}	17.066^{a}	4.00^{a}	13.544^{a}	5.33^{a}	5.997^{a}
E09	NA	NA	NA	NA	NA	NA
E10	NA	NA	NA	NA	NA	NA
E11	4.22^{abc}	17.388^{a}	5.44^{a}	9.858^{ab}	5.67^{a}	3.461^{bc}
E12	3.17^{c}	14.892^{a}	3.83^{a}	11.645^{ab}	6.83^{a}	4.740^{abc}
E13	NA	NA	NA	NA	NA	NA
E14	NA	NA	NA	NA	NA	NA
E15	NA	NA	NA	NA	NA	NA

Note: Different letters in the same column indicate statistically significant differences at the 95% confidence level, based on Duncan's new multiple range test (DMRT). 'NA' denotes treatments where plant survival was not observed, and therefore, no data could be recorded for these parameters.

3.3 ISSR marker analysis of mutated *M. laterita* lines

To assess the genetic variability induced by EMS treatment, leaf samples from each treatment group were collected for DNA extraction, and ISSR primers were employed to amplify DNA through polymerase chain reaction (PCR). Ten ISSR primers successfully amplified *M. laterita* DNA across all treatments: P2, P3, P4, P5, P6, P7, P10, P13, P19, and P20 (Table 3). The results indicate that the polymorphism levels varied depending on

Table 3. The total number of bands (TB), polymorphic bands (PB), and percentage of polymorphism (PP) for each *M. laterita* treatment from PCR analysis using ISSR primers

Treatment	Values	Primers										
		P02	**P03**	**P04**	**P05**	**P06**	**P07**	**P10**	**P13**	**P19**	**P20**	**AVG**
Control	TB	2	6	5	6	5	2	13	4	5	7	**5.50**
	PB	0	1	1	3	1	0	1	1	0	2	**1.00**
	PP	0.00	16.67	20.00	50.00	20.00	0.00	7.69	25.00	0.00	28.57	**16.79**
E01	TB	4	6	5	6	5	2	13	4	5	7	**5.70**
	PB	2	1	1	3	1	0	1	1	0	2	**1.20**
	PP	50.00	16.67	20.00	50.00	20.00	0.00	7.69	25.00	0.00	28.57	**21.79**
E02	TB	5	6	5	5	5	2	13	4	5	7	**5.70**
	PB	3	1	1	2	1	0	1	1	0	2	**1.20**
	PP	60.00	16.67	20.00	40.00	20.00	0.00	7.69	25.00	0.00	28.57	**21.79**
E03	TB	5	6	6	5	4	2	13	4	5	7	**5.70**
	PB	3	1	2	2	0	0	1	1	0	2	**1.20**
	PP	60.00	16.67	33.33	40.00	0.00	0.00	7.69	25.00	0.00	28.57	**21.13**
E04	TB	2	6	5	7	6	4	13	5	5	5	**5.80**
	PB	0	1	1	4	2	2	1	2	0	0	**1.30**
	PP	0.00	16.67	20.00	57.14	33.33	50.00	7.69	40.00	0.00	0.00	**22.48**
E06	TB	2	6	5	6	5	4	13	5	5	5	**5.60**
	PB	0	1	1	3	1	2	1	2	0	0	**1.10**
	PP	0.00	16.67	20.00	50.00	20.00	50.00	7.69	40.00	0.00	0.00	**20.44**
E07	TB	3	7	5	6	6	5	13	4	5	7	**6.10**
	PB	1	2	1	3	2	3	1	1	0	2	**1.60**
	PP	33.33	28.57	20.00	50.00	33.33	60.00	7.69	25.00	0.00	28.57	**28.65**
E08	TB	4	6	5	6	7	6	13	4	5	8	**6.40**
	PB	2	1	1	3	3	4	1	1	0	3	**1.90**
	PP	50.00	16.67	20.00	50.00	42.86	66.67	7.69	25.00	0.00	37.50	**31.64**
E11	TB	5	7	4	6	6	5	13	4	5	7	**6.20**
	PB	3	2	0	3	2	3	1	1	0	2	**1.70**
	PP	60.00	28.57	0.00	50.00	33.33	60.00	7.69	25.00	0.00	28.57	**29.32**
E12	TB	5	6	5	5	4	3	12	4	5	7	**5.60**
	PB	3	1	1	2	0	1	0	1	0	2	**1.10**
	PP	60.00	16.67	20.00	40.00	0.00	33.33	0.00	25.00	0.00	28.57	**22.36**

the EMS treatment. The control group showed an average of 5.50 total bands (TB) and 1.00 polymorphic bands (PB), resulting in a mean polymorphism percentage (PP) of 16.79%. Among the treated groups, E08 demonstrated the highest average PP at 31.64%, indicating a substantial increase in genetic variability compared to the control. Treatments E07 (28.65%) and E11 (29.32%) also exhibited elevated polymorphism levels. Conversely, treatments E06 (20.44%) and E16 (20.42%) showed relatively lower polymorphism. The numbers of TB and PB were consistent across treatments, with primers such as P07 and P06 contributing the most significant number of polymorphic bands. Primer P07 demonstrated its effectiveness by producing a high number of polymorphic bands and achieving the highest PP (66.67%) in treatment E08, making it a key indicator of genetic variability (Figure 1). These results suggest that intermediate EMS concentrations and durations, particularly in E08, were most effective in inducing genetic variability. This genetic diversity, as revealed by ISSR markers, underscores the potential of mutagenesis for ornamental plant breeding in *M. laterita*.

EMS has emerged as a powerful tool in mutation breeding, particularly for enhancing genetic diversity in plants. This chemical mutagen has proven effective in creating genetic variations that are vital for developing new plant varieties with desirable traits. ISSR markers have been widely adopted to identify and analyze the polymorphisms induced by EMS across various plant species, including ornamentals. The polymorphic bands revealed by ISSR analysis indicate successful genetic modifications, which can potentially lead to valuable phenotypic characteristics beneficial for ornamental breeding programs. Recent studies have demonstrated the efficacy of EMS mutagenesis in significantly boosting genetic diversity. For example, research on *Chrysanthemum* and *Hyoscyamus* species has shown that ISSR markers can detect distinct genetic variations associated with improved traits following EMS treatment (Puripunyavanich et al., 2022; Chen et al., 2023). In the context of tissue culture and plant propagation, the importance of assessing genetic fidelity in *in vitro* propagated plants has been highlighted by researchers such as Chuengpanya et al. (2022). This principle underscores the relevance of using ISSR markers to detect genetic variations induced by EMS treatment, as demonstrated in the current study.

The successful identification of polymorphisms using ISSR techniques further emphasizes the potential of EMS as an effective method for generating genetic diversity. This diversity is crucial for the selection and development of novel ornamental varieties(Penna et al., 2023). These findings collectively underscore the significant role of EMS induced mutations in driving phenotypic diversity, which is a primary objective in ornamental plant breeding programs.

3.4 Genetic variation analysis using UPGMA

The UPGMA cluster analysis revealed genetic variation among the control and EMS-treated groups, with an average genetic distance of 33.93%. Treatment E08, where plants were exposed to 300 mM EMS for 6 h, exhibited the highest genetic variation from the control group at 51.02% (Table 4), indicating significant genetic divergence. Conversely, treatment E06 displayed the lowest genetic variation at 25.72%. These findings align with previous studies demonstrating that optimal EMS concentrations induce substantial genetic and phenotypic variability (Talebi et al., 2012; Chen et al., 2023).

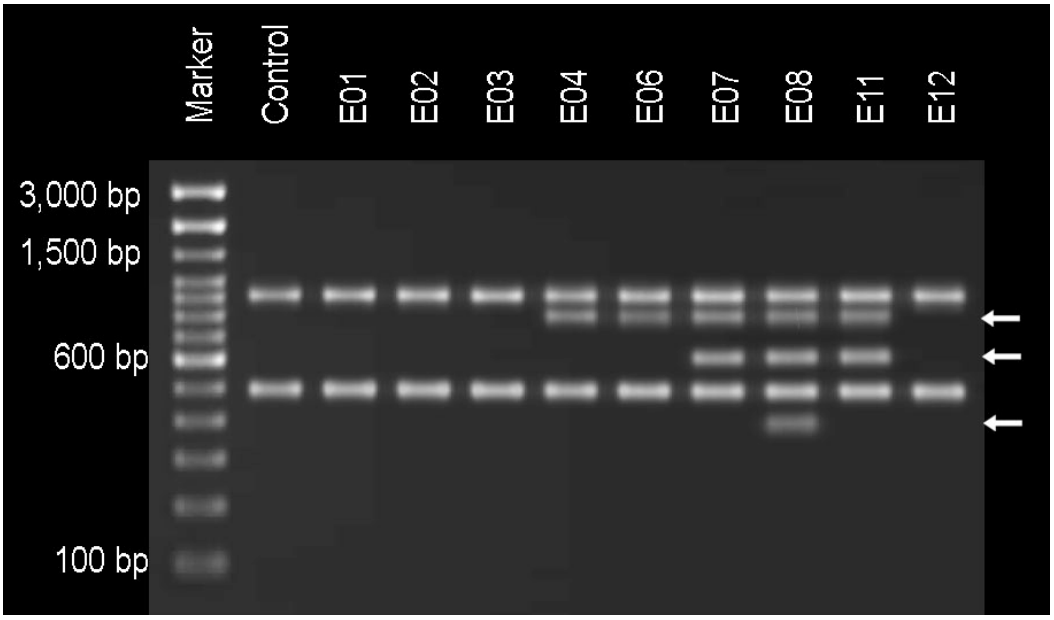

Figure 1. ISSR fingerprinting of *M. laterita* in each treatment using ISSR primer P07, compared to the 100 bp DNA ladder marker. Treatment numbers correspond to those in Table 1. The arrows indicate polymorphic bands.

Table 4. Genetic distance index values of *M. laterita* in each treatment, analyzed using the DendroUPGMA program (Garcia-Vallvé et al., 1999)

	Control	**E01**	**E02**	**E03**	**E04**	**E06**	**E07**	**E08**	**E11**	**E12**
Control	0.00	28.789	28.789	28.789	26.005	25.72	35.117	51.015	38.208	42.894
E01		0.00	17.763	23.684	44.925	43.855	43.106	47.131	40.21	32.149
E02			0.00	17.763	44.925	43.855	36.836	40.491	33.83	26.296
E03				0.00	44.925	43.855	36.836	53.77	33.83	32.149
E04					0.00	11.679	28.119	45.05	37.938	47.241
E06						0.00	20.499	42.553	36.07	52.071
E07							0.00	38.114	23.591	45.291
E08								0.00	42.673	55.679
E11									0.00	29.762
E12										0.00

The clustering pattern in the UPGMA dendrogram aligned with observed morphological variations among treatments. For instance, E08, which exhibited the highest genetic variation, also displayed a numerical increase in pseudostem length (13.54 cm) and root length (5.99 cm) compared to the control. However, statistical analysis indicated that these differences were not significant when compared to most other treatments. These results suggest that the genetic mutations induced by EMS directly influenced phenotypic variability, supporting findings from similar research on mutation-induced trait variation in ornamental plants like *Chrysanthemum* and *Hedychium* (Purente et al., 2020; Chuengpanya et al., 2022). Similarly, treatments E07 and E11, which displayed moderate genetic distances (36.84% and 40.21%, respectively) and elevated polymorphism levels, also demonstrated comparable increases in root and pseudostem lengths. In contrast, E06, which had lower genetic variation and clustered closer to the control group, exhibited fewer morphological changes (Figure 2). This reinforces the link between genetic diversity and phenotypic expression observed in other EMS studies, where intermediate treatments often yield the most favorable balance between variability and viability (Türkoğlu et al., 2023).

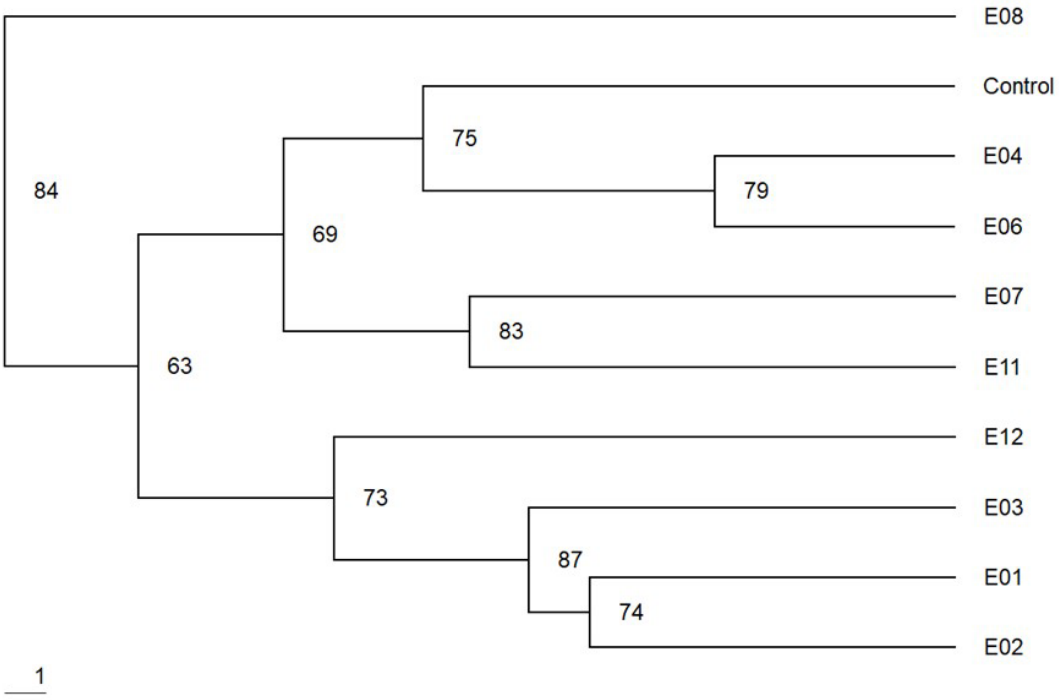

Figure 2. Dendrogram illustrating the genetic variation of *Musa laterita* in each treatment using the unweighted pair group method with arithmetic mean (UPGMA). The numbers shown on each branch represent bootstrap values, indicating the probability of occurrence from 100 resamples. The genetic distance scale is located at the bottom-left of the dendrogram, providing a reference for interpreting clustering relationships.

The relationship between genetic clustering and morphological changes provides a framework for selecting treatments that maximize both genetic and phenotypic variability. Treatments like E08, which showed significant divergence in genetic markers and desirable morphological traits, represent promising candidates for breeding programs aimed at developing ornamental *M. laterita* varieties with enhanced commercial value. These findings align with broader research advocating for the integration of genetic marker analysis and phenotypic assessments in mutation breeding (Penna et al., 2023).

The findings of this study align with previous research on EMS-induced mutations in various plants, where higher EMS concentrations and longer exposure times were associated with reduced survival and greater mutagenic effects (Talebi et al., 2012). In studies on orchids and other ornamental species, EMS has been shown to induce beneficial mutations that enhance morphological traits such as leaf variegation, plant height, and root development (Tütüncü et al., 2023). In a similar way to the current research, these studies also identified optimal EMS concentrations that balanced mutation efficiency with plant viability. However, some differences were noted in the extent of genetic variation observed, which could be attributed to species-specific responses to EMS. This study contributes to the growing body of knowledge on EMS-induced mutations by confirming its effectiveness in generating genetic variation in *M. laterita*, consistent with findings from other plant species.

4. Conclusions

The study comprehensively demonstrated that EMS treatment affects both the morphological characteristics and genetic of *M. laterita*. Survival rates, growth traits, and genetic variation were all influenced by EMS concentration and exposure time. Higher EMS concentrations and longer immersion times severely reduced survival rates and inhibited growth. While genetic variation was confirmed in the treatments E08 and E11, their growth traits showed numerical differences but were not significantly different from the control. These findings suggest that EMS can induce genetic changes without necessarily resulting in pronounced morphological alterations. The ISSR marker analysis further revealed significant genetic changes, particularly in treatment E08, indicating that EMS can induce useful genetic variation when applied at optimal concentrations. These findings contribute to a better understanding of the use of EMS in mutation breeding, helping researchers balance between beneficial mutations and adverse effects on plant development.

5. Acknowledgements

This research was supported by the Science, Research and Innovation Promotion Fund (Fundamental Fund; FF66), Fiscal Year 2023, Kalasin University.

6. Authors' Contributions

Ladda Sonsan: Conducted tissue culture propagation, EMS treatment, and morphological data collection. Assisted in molecular analysis, including PCR amplification and ISSR marker evaluation. Participated in data interpretation and minor manuscript revisions. Piyachat Wiriyaampaiwong: Supported statistical analysis, figure development, and technical formatting of the manuscript. Anupong Tankrathok: Supported statistical analysis, figure development, and technical formatting of the manuscript. Nattapong Srisamoot: Conceived and designed the study, supervised all research activities, performed critical

data interpretation, revised the manuscript extensively, and handled correspondence with the journal.
All authors have read and approved the final version of the manuscript.

7. Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper. All research activities, including experimental design, data collection, and analysis, were conducted independently and impartially.

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