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Detection of Polymorphism in Ethyl Methyl Sulfonate Treated *Cordyline fruticosa* (L.) *A.Chev*. mutants using RAPD markers

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Abstract

Cordyline fruticosa (L.) A. Chev. is an ornamental plant with significant horticultural value but limited genetic diversity, which constrains breeding for improved traits. This study aimed to generate and evaluate genetic and morphological variation in *C. fruticosa* using Ethyl Methyl Sulfonate (EMS) mutagenesis. *In-vitro* plantlets were exposed to EMS at concentrations of 1%, 2%, 3%, and 4%, for 10 minutes and their growth was monitored over nine weeks. Key morphological parameters, including plant height, leaf number, and leaf pigmentation, were recorded to assess the impact of EMS treatment. Genomic DNA was extracted from treated and control plants, and genetic variation was evaluated using Random Amplified Polymorphic DNA (RAPD) markers. The results demonstrated that EMS induced concentration-dependent changes in plant morphology, with 1% EMS producing the greatest reduction in plant height and leaf number, as well as lighter leaf pigmentation. RAPD analysis revealed increased genetic polymorphism in EMS-treated groups compared to the control, with the highest level observed in the 1% EMS treatment. Cluster analysis further confirmed the genetic divergence between treated and control plants. These findings indicate that EMS mutagenesis, particularly at lower concentrations, is effective in generating both morphological and genetic diversity in *C. fruticosa*. The study provides a foundation for future breeding programs aiming to enhance the ornamental and adaptive traits of this species through targeted mutation breeding.

Keywords: *Cordyline fruticose*, EMS mutagenesis, genetic diversity, RAPD markers, mutation breeding

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Introduction

Cordyline fruticosa (L.) A. Chev., commonly known as the ti plant or good luck plant, is widely cultivated for its vibrant foliage and cultural significance across tropical and subtropical regions (Aziz et al., 2019a; Raslan et al., 2021). The limited genetic variability currently available in commercial *C. fruticosa* cultivars severely restricts breeding efforts aimed at developing varieties with improved traits such as enhanced stress tolerance, novel coloration patterns, or modified growth habits. This narrow genetic base represents a significant bottleneck for both the immediate commercial potential of the species and its long-term adaptability to changing environmental conditions (Nandwani, 2019). Chemical mutagens such as Ethyl Methanesulfonate (EMS) have proven particularly effective for inducing heritable genetic variation in ornamental plants (Chen et al., 2023). EMS induces point mutations that can result in new alleles and phenotypes of horticultural and scientific interest. Previous studies in related species have demonstrated that EMS can successfully generate morphological and genetic diversity detectable through molecular markers, particularly RAPD analysis (Hromadová et al., 2023). However, critical gaps remain in our understanding of the optimal parameters for EMS mutagenesis in *C. fruticosa*, the relationship between mutagenic dose and resulting phenotypic variation, and the most effective molecular approaches for characterizing induced genetic diversity. Additionally, while previous work has explored the effects of various chemical mutagens on *C. fruticosa* (Tawfik & Fathy, 2022), comprehensive studies specifically examining EMS-induced variation using both morphological and molecular approaches remain limited.
This study addresses these knowledge gaps by systematically evaluating the effects of EMS mutagenesis on *C. fruticosa* through integrated morphological and molecular analysis. The research aims to evaluate the effects of varying EMS concentrations on the morphological traits of C. fruticosa, to analyse the degree of genetic variation induced by EMS treatment using RAPD markers, and to determine the optimal EMS concentration for producing beneficial genetic diversity in this ornamental species.

Materials and Methods

Plant Material and EMS Treatment

C. fruticosa plants,were propagated *in vitro* at the Plant Tissue Culture Laboratory of the Sri Lanka Institute of Information Technology (SLIIT). Shoot tips of 5 to 10 mm in size were excised from *in vitro* cultures established on Murashige and Skoog (MS) medium supplemented with 3% sucrose (Murashige & Skoog, 1962). After about one week,these shoot tips were fully submerged in solutions of Ethyl Methanesulfonate (EMS) at concentrations of 1%, 2%, 3%, and 4% for 10 minutes. Following treatment, shoot tips were exposed to 1 M sodium thiosulfate to stop the reaction and thoroughly rinsed with sterile distilled water and transferred to fresh MS medium with 3% sucrose. Untreated shoot tips, cultured under identical conditions, served as the control group. As EMS is a toxic compound with genotoxic effects upon prolonged exposure, additional safety precautions were applied during its handling (Müller et al., 2009) All activities involving EMS were carried out in a fume hood following all biosafety standards.

DNA Extraction and Quality measurement

Genomic DNA was extracted from fresh leaf tissues using the CTAB method described by Doyle and Doyle (1990), with specific modifications. Leaf samples were ground in CTAB buffer containing β-mercaptoethanol and PVP. The homogenate was incubated at 65 °C with intermittent vortexing to facilitate cell lysis, followed by chloroform:isoamyl alcohol extraction and centrifugation at 4 °C. RNase treatment was included to remove RNA contamination. DNA was precipitated overnight at –20 °C using ice-cold

isopropanol, pelleted by centrifugation at 4 °C, washed with 70% ethanol, air-dried, and resuspended in TE buffer. DNA quality and quantity were evaluated via 0.8% agarose gel electrophoresis and spectrophotometry.

RAPD-PCR Amplification

RAPD analysis was conducted using four primers (RFu-25, Deca-11, OPB-17, and OPZ-07). Duplex PCR reactions were performed for efficiency. Each 25 μL reaction mixture contained: 10.3 μL nuclease-free water, 2.5 μL of 10× PCR buffer (without $MgCl_2$), 3.2 μL of 50 mM $MgCl_2$, 2 μL dNTP mix (2.5 mM), 2.5 μL of primer mix (10 μM each), 2.5 μL Taq DNA polymerase (5 U/μL), and 2.0 μL of template DNA. Thermal cycling conditions included an initial denaturation at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 1 minute, annealing at 36 °C for 1 minute, and extension at 72 °C for 2 minutes, with a final extension at 72 °C for 7 minutes.

Table 1: *List of primers used in the RAPD-PCR of C. fruticosa*

Number	Primer Name	Primer sequence	GC %	Melting Temperature (Tm)
1	OPB-17	5′-AGGGAACGAG-3′	60	33.1
2	OPZ-07	5′-CCAGGAGGAC-3′	70	34.6
3	RFu-25	5′-CCGGCTGGAA-3′	70	39.8
4	Deca-11	5′-ATCGGCTGGG-3′	70	39.3

Electrophoretic Analysis

PCR products were resolved on 1.8% agarose gels in 1× TAE buffer. Each well was loaded with 10 μL of product and 2 μL loading dye and a 5 μL DNA ladder. Gels were stained with ethidium bromide, run at 60 V for 2.5 hours, and visualized under UV light using a gel documentation system.

Data Analysis

Morphological data (plant height and leaf number) were analyzed using two-factor ANOVA to assess the effects of EMS concentration and time ($p \leq 0.05$). For genetic analysis, RAPD banding profiles were scored as binary data (1 = presence, 0 = absence) to calculate polymorphism percentages. Genetic similarity and distance were estimated using Jaccard's and Nei's coefficients, respectively (Hromadová et al., 2023). Cluster analysis was performed using UPGMA, and results were visualized via dendrograms.

Results

Morphological Variation in Response to EMS Treatment

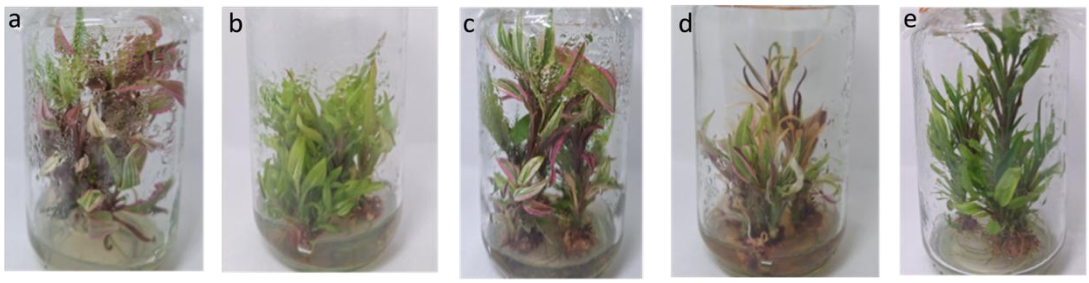

Figure 1: *Effect of different EMS concentrations on plantlet morphology. Image (a) shows untreated control plantlets maintained under standard culture conditions. Images (b–e) represent plantlets exposed to increasing EMS concentrations: (b) 1%, (c) 2%, (d) 3%, and (e) 4%.*

Morphological assessment revealed a dose-dependent response to EMS treatment in *Cordyline fruticosa* plantlets. As shown by Figure 1 (a) and Table 2, at 1% EMS, plantlets exhibited the most pronounced phenotypic changes, including significant stunting and pale green leaves, indicating reduced chlorophyll content. The 2% EMS group showed minimal morphological deviation from the control. Plants treated with 3% EMS displayed moderate stunting but no distinct pigmentation changes. In contrast, the 4% EMS treatment led to dark green pigmentation and reduced overall vigor, likely due to physiological stress or impaired metabolic activity.

Table 2: *Morphological parameters of plant height and leaf number in C. fruticosa at Week 1 and Week 9 under different EMS concentrations. "0" indicates the in vitro control.*

	Week 1		Week 9	
EMS Concentration (%)	Height of the plant (cm)	No. of leaves	Height of the plant (cm)	No. of leaves
4	6.0	8	6.3	12
4	5.2	11	5.5	15
4	4.8	6	5.0	11
4	4.8	8	4.9	9
4	4.8	10	5.2	13

3	6.5	8	6.55	11
3	6.4	8	6.5	10
3	5.8	6	8.0	8
3	6.5	8	6.7	11
3	5.6	7	6.0	9
2	6.0	9	6.7	11
2	5.2	8	5.5	11
2	4.3	17	4.5	19
2	5.4	13	5.7	14
2	4.6	15	4.8	17
1	4.5	8	4.7	12
1	5.3	9	5.8	11
1	3.1	11	3.3	15
1	3.6	7	3.9	12
1	4.5	9	4.7	11
0	7.0	23	7.6	25
0	6.7	25	6.9	28
0	6.5	23	6.6	24
0	6.3	23	6.7	24
0	7.0	22	7.5	23

ANOVA-Based Growth Parameter Analysis

Two-way ANOVA showed that EMS concentration significantly affected plant height and leaf number ($F = 85.99$, $p < 0.0001$). Control plants showed superior growth across nine weeks in comparison to the treated plants. Among treatments, 1% EMS caused the greatest reduction in both parameters, followed by 3% and 4%, while 2% EMS showed growth metrics comparable to the control, where the number of leaves and height of the plant is comparable to the control plant, as shown by Figure 1(a) and (c) above.

PCR Amplification and Band Scoring

After DNA extraction and quantification, PCR amplification with four RAPD primers using duplex reactions produced clear, high-resolution profiles (Fig. 3.3-2 and 3.3-3), improving polymorphic locus detection across samples. Amplified fragments ranged from approximately 200 to 1500 bp. Bands were scored as "1" (present) or "0" (absent) to create a binary matrix for calculating polymorphism and genetic similarity.

Table 3: *Binary matrix based on the PCR gel imaging*

Sample ID	OPB-17	OPZ-07		RFu-25	Deca-11		
		B1	B2		B1	B2	B3
Control – *in-vitro*	0	0	1	0	0	0	0
JC1	0	1	1	1	1	1	1
JC2	1	1	1	1	0	1	0
JC3	1	1	0	1	0	1	0
JC4	1	1	0	1	1	1	1

Note: JC1 represents 1% EMS sample. JC2 represents 2% EMS sample. JC3 represents 3% EMS sample. JC4 represents 4% EMS sample

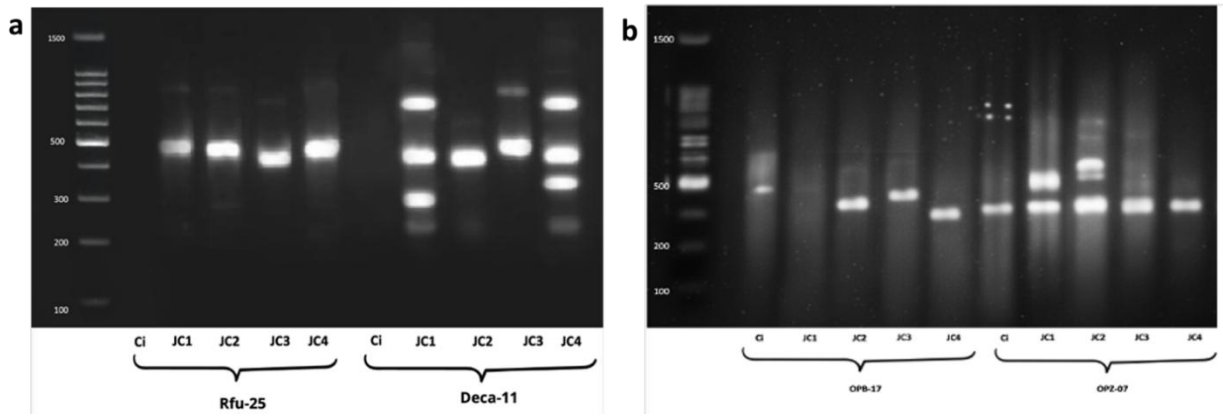

Figure 2: *1.8% Agarose gel image showing the products from duplex PCR for the five samples. Image (a) with Rfu-25 and Deca-11 primers. Image (b) with OPB-17 and OPZ-07 primers.*

As illustrated in Table 3, a concentration-dependent response to EMS mutagenesis was evident in the banding profiles. JC1 (1%) showed the highest number of unique bands, followed by JC4 (4%). JC2 and JC3 had fewer polymorphic bands, while the control showed minimal variation, reflecting its unmutagenized state.

Percentage of Polymorphism

Percentage polymorphism was calculated using the binary matrix derived from the presence and absence of RAPD bands. These values represent the proportion of polymorphic bands relative to the total number of bands per group. Based on these calculations, a graph was drawn. The highest polymorphism was observed in the 1% EMS group (31.8%), followed by 4% (27.2%), 2% (18.18%), and 3% (13.6%), while the control showed the lowest (9.09%), confirming a dose-dependent genetic variation induced by EMS.

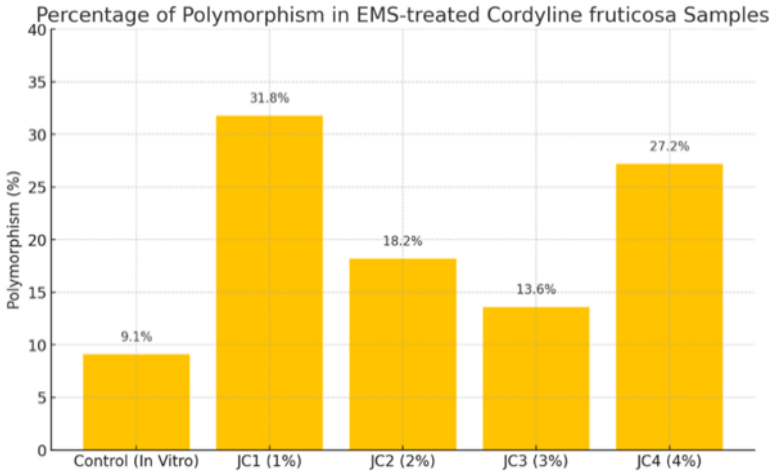

Figure 3: *Bar graph illustrating the percentage of polymorphism observed in EMS-treated and control Cordyline fruticosa samples, highlighting the dose-dependent effect of EMS on genetic variability.*

Genetic Similarity and Divergence

Table 4: *Genetic similarity index relative to Control – in-vitro sample*

Sample	Jaccard's Coefficient (J)
JC1	0.285
JC2	0.5
JC3	0.666
JC4	0.333

Table 5: *Nei's Genetic Distance Relative to Control – in-vitro*

Sample	Nei's Genetic Distance (D)
JC1	0.8109
JC2	0.4055
JC3	0.2231
JC4	0.6931

Cluster Analysis and Dendrogram

Jaccard's Coefficient revealed the lowest similarity values in JC1 (0.285) and JC4 (0.333), indicating strong genetic divergence from the control group. JC2 and JC3 exhibited moderate similarity coefficients of 0.5 and 0.666, respectively. These findings were further supported by Nei's Genetic Distance analysis, where JC1 (D = 0.8109) and JC4 (D = 0.6931) showed the highest divergence, while JC2 (D = 0.4055) and JC3 (D =

0.2231) reflected relatively milder genomic alterations. Together, these results confirm that 1% and 4% EMS treatments induced substantial genetic variation, consistent with polymorphism patterns observed in RAPD profiling.

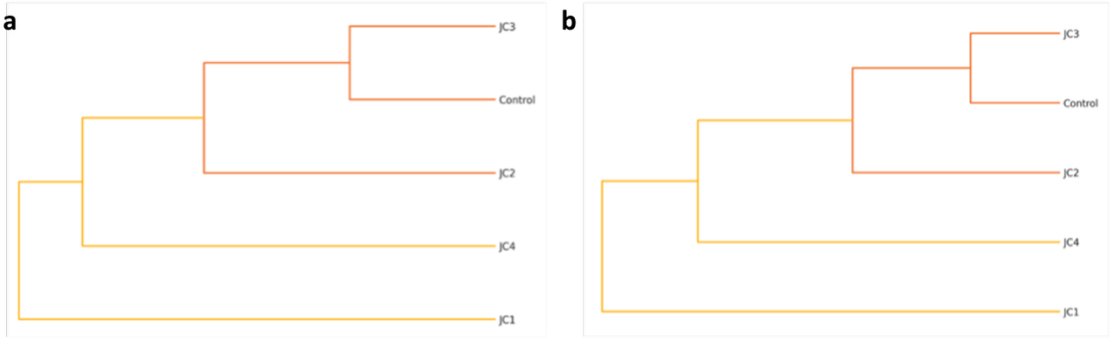

Figure 4: *Dendrogram constructed, (a) using Jaccard dissimilarity (1 - similarity) to evaluate presence/absence-based variation in EMS-treated samples compared to Control – in-vitro. (b) showing the genetic relationships between EMS-treated C. fruticosa samples (JC1–JC4) and Control – In-vitro based on Nei's Genetic Distance*

Dendrograms based on Jaccard's and Nei's coefficients showed JC1 as the most genetically divergent, followed by JC4, while JC3 clustered closest to the control. Figure 4 (a) and (b) visualize the hierarchical relationships among the EMS-treated and control samples.

Discussion

This study confirms that EMS induces both morphological alterations and genetic polymorphism in *C. fruticosa*, with 1% EMS showing the most pronounced effects. The reductions in plant height and leaf number, along with pigmentation shifts in the treated plants, are likely due to EMS's ability to alkylate guanine and induce Guanine to Adenine transitions. These point mutations can disrupt genes involved in cell division and hormone regulation pathways, as similarly observed in crop species (Predieri, 2001; Chen et al., 2023).

At the molecular level, 1% EMS produced the highest polymorphism (31.8%), suggesting that this dosage offers an optimal balance between introducing mutations and maintaining cellular viability. The slightly lower polymorphism in the 4% group (27.2%) may reflect increased cytotoxicity leading to selective amplification from less-affected cells. These findings are consistent with studies in rice and peas where moderate EMS doses maximize genetic diversity while avoiding lethal effects (Talebi et al., 2012).

Genetic divergence assessed via Jaccard's similarity and Nei's distance further supports the dose-dependent mutagenic effect, with EMS-treated groups clearly differentiated from the control. This supports the conclusion that induced point mutations accumulate across the genome, altering RAPD primer binding sites. However, the reliance on RAPD markers, despite their utility for preliminary screening, limits reproducibility and locus-specific insights, underscoring the need for more robust markers (e.g., ISSR/SSR).

Future work should also evaluate the heritability and field stability of these variants to support their practical use in ornamental breeding programs.

Conclusion

This study demonstrates that EMS is an effective mutagen for inducing heritable genetic and phenotypic variation in *C. fruticosa*. The 1% EMS concentration yielded the greatest diversity, with the best results confirmed by RAPD analysis using the OPZ-07 primer, which produced clear, well-separated bands. The resulting traits, such as altered plant height and leaf coloration offer valuable potential for ornamental breeding. These findings support EMS mutagenesis as a practical tool for developing resilient, market-oriented cultivars and advancing sustainable plant improvement in vegetatively propagated species.

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