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In Vitro Mutation Induction of Chrysanthemum (*Chrysanthemum morifolium*) Maruta Variety for Lowlands Using EMS (Ethyl Methane Sulfonate)

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Abstract
Chrysanthemums (*Chrysanthemum morifolium*) are among the most popular ornamental plants due to their diverse types, shapes, and colors, making them highly valued in the horticultural market. In Indonesia, chrysanthemums are typically grown in highland regions, where production and flower quality are declining due to global temperature changes. To sustain production, new varieties adapted to lowland conditions are required. This study aimed to induce mutations in the Maruta chrysanthemum variety using Ethyl Methane Sulfonate (EMS) to enhance adaptability and biodiversity. Explants were treated in vitro with EMS concentrations of 1%, 1.5%, and 2%. Quantitative traits—including survival percentage, explant height, number of shoots, leaves, and roots—were analyzed using ANOVA and Tukey's test at the 5% level. Qualitative traits, such as stem and leaf color, were assessed using the Munsell Color Chart. Results showed that more than 50% of Maruta explants survived under all treatments. However, explant height, number of leaves, and number of roots decreased as EMS concentration increased, compared with the control.

Keywords: Biodiversity, Chrysanthemum Explants, Concentration, Soaking Treatment, Survival Percentage

1. Introduction

Chrysanthemums are renowned as popular ornamental plants. Their wide variety of forms, shapes, and colors is a key attraction, making them highly sought after in the horticultural market. The aesthetic appeal of chrysanthemums contributes to their high market value. Demand for chrysanthemums originates not only from the domestic market but also from international markets. Data released by the Central Statistics Agency (BPS) in 2020 indicates that chrysanthemum production ranks highest among ornamental plants. Over three consecutive years, harvests totaled 466,056,093 stalks in 2018, 459,188,329 stalks in 2019, and 378,910,135 stalks in 2020 (Central Statistics Agency, 2020). This finding, which sustains high demand, presents significant opportunities for commodity development.

Currently, chrysanthemum cultivation is concentrated in highland areas, which are reaching production limits. To address this challenge, production must be expanded to lowland areas where chrysanthemum diversity remains limited. Mutation induction provides an effective means of generating genetic variability, resulting in new varieties that can adapt to higher temperatures (Indrawanis, 2025).

Mutation induction can be performed using the chemical mutagen Ethyl Methane Sulfonate (EMS). EMS induces point mutations by altering nitrogenous base pairs, particularly through transitions of adenine/guanine (A/G) to cytosine/thymine (C/T). These random and non-directional changes generate broad genetic diversity, which may be expressed through variations in physiological traits, developmental processes, and plant morphology (Viana et al., 2019). As an alkylating agent, EMS interacts with DNA by modifying guanine bases, leading to the formation of 7-ethylguanine. During replication, this altered base often mispairs with thymine, thereby increasing the frequency of mutations(Andriyani & Muslihatin, 2017). The mutagenic efficiency of EMS is influenced by several technical factors, including the pH of the phosphate buffer solution,

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treatment temperature, exposure duration, timing of explant immersion, and the application technique (Alfikri *et al.*, 2022). For example, Krupa-Małkiewicz et al. (2017) demonstrated that EMS treatment at 0.5% concentration for one to two hours enhanced the environmental stress tolerance of petunia plants.

An effective approach to increasing genetic variation and expanding chrysanthemum diversity is the in vitro induction of mutations. This tissue culture technique propagates plants under sterile, aseptic conditions, allowing for continuous seedling production independent of the growing season. Moreover, in vitro propagation produces seedlings that are genetically uniform and largely disease-free. The controlled environment also enables rapid multiplication of seedlings within relatively limited space (Pratama *et al.*, 2024).

Given these advantages, it is essential to investigate the optimal EMS concentration for chrysanthemum varieties to generate mutants suitable for lowland cultivation. Comprehensive research is necessary to evaluate the effects and interactions of various EMS concentrations on chrysanthemum development and to determine treatments that optimize genetic improvement while preserving plant viability.

2. Material and Methods

This research was conducted from September to December 2024 at the Biotechnology Laboratory, Faculty of Agriculture, National Development University "Veteran" in East Java. The research location is situated at coordinates 7° 20' 2.93" South Latitude and 112° 47' 28.17" East Longitude, with a room temperature of 23˚C and a relative humidity of 70%, at an altitude of 4 meters above sea level.

Various tools used in the research include analytical scales, paper, beaker glass, oven, petri dish, measuring cup, Erlenmeyer flask, dropper pipette, hot plate, magnetic stirrer, pH meter, funnel, pan, stove, spatula, culture bottle, heat-resistant plastic, aluminum foil, rubber band, refrigerator, autoclave, sprayer, Laminar Air Flow, tissue, tweezers, scissors, scalpel, Bunsen burner, matches, culture rack, plastic wrap, label, mobile phone, and stationery.

The materials used in the research include Murashige and Skoog (MS) media, Ethyl Methane Sulfonate (EMS), 6-Benzyl Amino Purine (BAP), liquid soap, sterile distilled water, NaOH, HCl, 70% alcohol, spirits, betadine, and 1-month-old chrysanthemum plantlets of the Maruta Agrihorti, Erika Agrihorti, and Asmarini Agrihorti varieties obtained from the BALITHI Cipanas Laboratory, Cianjur, West Java.

This research began with the chrysanthemum explants being cleaned of leaves and roots. Then, the stems were cut between the nodules approximately 1 cm apart. Next, they were soaked in each EMS concentration for 90 minutes. Then, they were rinsed with sterile distilled water three times. Then, they were dipped in 70% alcohol and betadine for 1 second. Finally, the explants were planted in MS media, covered with plastic and rubber, and labeled with the planting date.

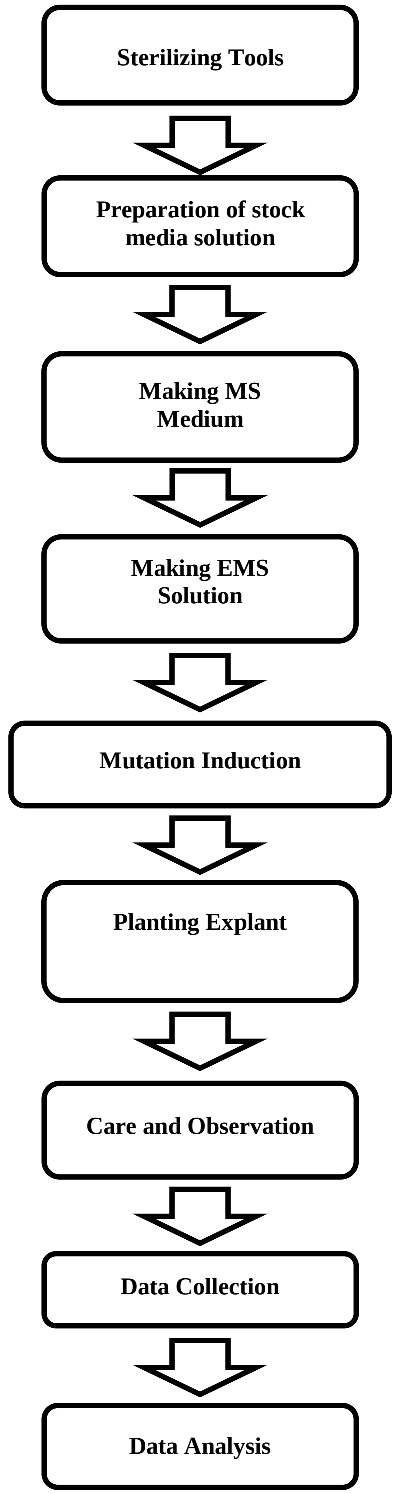

Figure 1. Research flow diagram

This study employed a Completely Randomized Design (CRD) with two factors and three replications. The first factor was the Maruta chrysanthemum variety explant, while the second factor consisted of four levels of EMS (Ethyl Methane Sulfonate) concentration treatments, namely E0 (Without EMS), E1 (EMS 1%), E2 (EMS 1.5%), and E3 (EMS 2%). Each combination of treatments from the two factors was repeated three times for each research unit. Each bottle contained three explants, resulting in 45 explants in each treatment unit.

The data from the observations were then analyzed using a non-factorial RAL. Further testing with a 5% BNJ was conducted and is described in the discussion, following the identification of significant differences in the ANOVA. The parameters observed are:

a) Percentage of live explants (%)

Calculate the number of surviving chrysanthemum explants in each treatment using the following formula:

$$\%\text{Living Explanation} = \frac{\sum Living\ Explant}{\sum Entire\ Explant} \times 100\%$$

b) Explant height (cm)

The height of the chrysanthemum explants was measured from the top of the explant to the root tip in each treatment. These observations were made in weeks 2 and 8.

c) Number of shoots

Counting the number of chrysanthemum shoots in each treatment. This observation was conducted in weeks 2 and 8.

d) Number of leaves

Counting the number of chrysanthemum explant leaves in each treatment. Observations were made in weeks 2 and 8.

e) Number of roots

Counting the number of roots formed in each treatment of Maruta variety chrysanthemum explants. Observations were made at the end of the observation period.

3. Results and Discussion

3.1. Percentage of Living Explants (%)

Table 1. Percentage of Living Explants of Maruta Variety Chrysanthemum at Various EMS Concentrations.

Treatment	Percentage of Living Explants
Without EMS	100%
EMS 1%	100%
EMS 1.5%	93%
EMS 2%	86%

Table 1 shows the difference in the percentage of live explants of the Maruta variety of chrysanthemum between the control and those treated with EMS. The treatment without EMS showed a high rate of live explants, namely 100% or approximately 45 live explants. Furthermore, for explants that experienced immersion in a 1% EMS concentration, a high percentage of live explants was observed, specifically 100% or approximately 45 live explants. Then, for explants that experienced immersion in a 1.5% EMS concentration, the percentage of live explants decreased, specifically to approximately 93%, or approximately 42 live explants. Explants that underwent immersion in a high concentration of 2% EMS exhibited a more significant decrease in the percentage of live explants, specifically approximately 86%, or approximately 39 live explants.

Increasing the concentration of the Ethyl Methane Sulfonate (EMS) solution has a direct impact on decreasing the survival rate of plantlets. The higher the EMS concentration given, the greater the damage experienced by plant tissue, making it difficult for explants to survive. This decrease in viability is related to the disruption of cell totipotency, namely the ability of cells to grow and develop into whole individuals, which is caused by changes in the structure of nitrogen bases in chromosomes. These damaging mutations disrupt genetic stability in the meristem area, which is the center of plant growth, resulting in suboptimal cell regeneration (Tantasawat et al., 2017). With increasing EMS concentration, the plant growth process can be affected, which may cause stunted growth or even lead to the death of the plant (Putra & Purwani, 2017).

3.2. Explant Height

The results of the ANOVA analysis on explant height with various EMS concentrations showed an interaction. Each treatment, between varieties and EMS concentrations, showed an influence or interaction on the difference in explant height.

Table 2. Average Height of Maruta Variety Chrysanthemum Explants at Various EMS Concentrations

Height of Explant		
Treatment	2 Weeks	8 Weeks
Without EMS	0.06±1.89a	0.39±11.41a
EMS 1%	0.17± 0.64b	0.44±10.35ab
EMS 1.5%	0.00±0.50b	1.62±6.99b
EMS 2%	0.02±0.69b	0.79±7.63b
BNJ 5%	0.21	2.18

Description: Numbers that have the same letter for each chrysanthemum explant treatment indicate that the difference is not significant based on the Honestly Significant Difference Test (HSD) at the 5% level.

Table 2 shows that the height of Maruta variety chrysanthemum explants at week 2 exhibited a different height difference between the control explants and those treated with EMS at varying concentrations. At 2 weeks of age, all treatments had an average explant height of more than 0.5 cm. However, Maruta explants at 8 weeks of age had a different average explant height value between the control (not using EMS) and those treated with EMS. In the EMS treatment, there was an effect; the average value of the explant height with an EMS concentration of 1% had a significant height of approximately 10.35 cm, compared to explants at concentrations of 1.5% and 2%, which had average heights of 6.99 cm and 7.63 cm, respectively. According to research by Atikabudi (2022), the height of chrysanthemum plants treated with EMS was shorter, and the difference was highly significant. Compared to chrysanthemums without EMS at all observation ages. At the end of the observation, the height of the chrysanthemums given EMS was 51.96 cm (shortest), while the height of the chrysanthemums without EMS was 60.90 cm (highest).

Plant height is closely related to the number of leaves formed. This finding is due to the cell division process that occurs during photosynthesis in leaves (Hidayat *et al.*, 2020). The taller a plant grows, the more leaves it will form. According to Irawan (2023), a higher concentration of EMS will result in greater absorption of EMS into the plant. This can increase EMS toxicity, leading to a decrease in plant height.

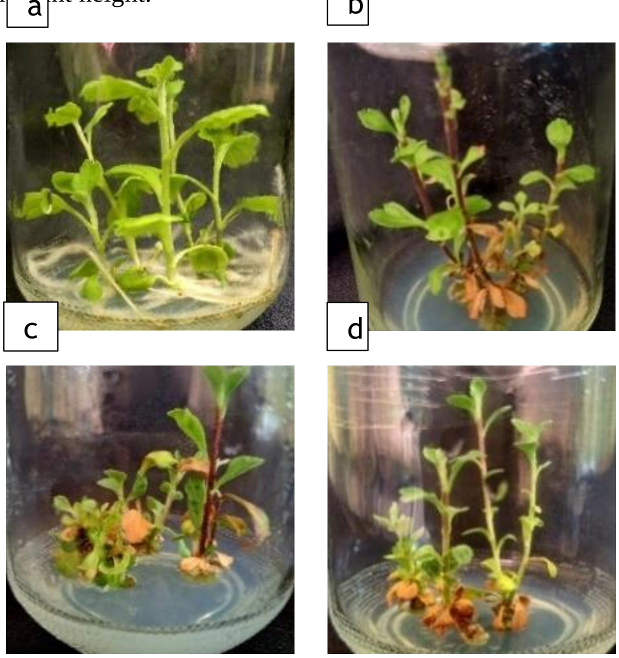

Figure 1. Height display of Maruta variety chrysanthemum explants at various EMS concentrations (a) E0 control, (b) 1% EMS, (c) 1.5% EMS, (d) 2% EMS

3.3. Number of Shoots

The results of the ANOVA analysis on the number of shoots with various EMS concentrations showed no interaction. Each treatment, between varieties and EMS concentrations, showed no effect or interaction on the difference in shoot number.

Table 3. Average Number of Shoots of Maruta Variety Chrysanthemum at Various EMS Concentrations

	Number of Shoots	
Treatment	**2 Weeks**	**8 Weeks**
Without EMS	0.00±1.00a	0.58±2.33a
EMS 1%	0.00±1.00a	0.58±1.67a
EMS 1.5%	0.06± 0.96a	0.56±2.26a
EMS 2%	0.78±1.08a	0.45±1.63a
BNJ 5%	Mr.	Mr.

Description: Numbers that have the same letter for each chrysanthemum explant treatment indicate that the difference is not significant based on the Honestly Significant Difference Test (HSD) at the 5% level.

Table 3 shows that the Maruta chrysanthemum variety exhibits a different average number of shoots at various EMS concentrations. At 2 weeks old, the 1% EMS treatment has an average of around 1 shoot, and when compared to other concentrations or the control, it still does not show the emergence of shoots because the average value is less than 1 shoot. However, at 8 weeks old, the average number of shoots in the treatments without EMS, 1% EMS, and 1.5% EMS is approximately 2 shoots, and when compared to the 2% EMS treatment, it has an average number of shoots below 2 shoots.

According to the research results from Kamila (2022), the increase in shoots that experienced inhibition was observed in the higher EMS treatment, namely EMS 0.8% at 60 minutes (P4Q2), which produced the lowest number of shoots, specifically 1 shoot, and was significantly different from the treatment without EMS (P0Q0). This finding is suspected because EMS at a low dose can stimulate shoot growth, but EMS at a higher concentration can inhibit shoot growth of Macodes petola.

Shoots represent the beginning of new growth. The resulting shoots play a crucial role in the propagation of chrysanthemums and seed production. As the number of shoots increases, the number of seedlings to be developed also increases. EMS treatment has been shown to reduce the number of shoots formed. The higher the mutagen dose used, the fewer shoots formed. This result indicates that the number of explants producing shoots tends to decrease with increasing mutagen dose (Qosim *et al.*, 2015).

3.4. Number of Leaves

The results of the ANOVA analysis on leaf number with various EMS concentrations showed a significant effect or interaction. Each treatment, between varieties and EMS concentrations, showed an interaction in leaf number.

Table 4. Average Number of Leaves of Maruta Variety Chrysanthemum at Various EMS Concentrations

	Number of Leaves	
Treatment	**2 Weeks**	**8 Weeks**
Without EMS	0.00±4.89a	0.64±10.78ab
EMS 1%	0.55±3.04ab	0.76±13.56a
EMS 1.5%	1.24±2.89ab	1.86±8.89b
EMS 2%	0.52±1.89b	1.15±9.33b
BNJ 5%	1.91	2.18

Description: Numbers that have the same letter for each chrysanthemum explant treatment indicate that the difference is not significant based on the Honestly Significant Difference Test (HSD) at the 5% level.

Table 4 shows that chrysanthemum explants at various EMS concentrations had different average leaf number values. At 2 weeks old, control chrysanthemum explants had an average leaf number of approximately 4.89 leaves, which is large in number compared to using the EMS concentration. However, at 8 weeks of age, a significant difference was seen in the average number of leaves. The 1% EMS treatment had a higher average number of leaves, approximately 13.48, compared to the control, which had a lower average number of shoots, 10.78. However, the 1.5% and 2% EMS concentrations had lower average values compared to the control explants, namely approximately 8.19 and 8.89 leaves. EMS affects the growth and formation of leaves in explants. According to the research results by Kamila (2022), the 0% (P0), 0.2% (P1), and 0.4% (P2) EMS treatments resulted in a higher increase in the number of leaves. Meanwhile, at 0.6% EMS, the lowest number of leaves was at 90 minutes in orchid plants.

Leaves play a crucial role in plant growth through photosynthesis, a process that produces carbohydrates, the primary energy source for plants, and supports growth. The higher the EMS concentration, the fewer leaves will form. Qosim (2015) stated that the inhibition of leaf growth due to EMS mutagen administration is a result of both physiological and genetic influences on the M1 generation.

3.5. Number of Roots

The results of the ANOVA analysis on root number with various EMS concentrations showed a significant effect or interaction. Each treatment, between varieties and EMS concentrations, showed an interaction in the difference in root number.

Table 5. Average Number of Maruta Variety Chrysanthemum Roots at Various EMS Concentrations

Number of Roots	
Treatment	**8 Weeks**
Without EMS	0.39±10.33a
EMS 1%	0.19± 6.22b
EMS 1.5%	0.50±1.22c
EMS 2%	0.19±3.56d
BNJ 5%	0.9

Description: Numbers that have the same letter in each chrysanthemum explant treatment indicate that the difference is not significant based on the Honestly Significant Difference Test (HSD) at the 5% level.

Table 5 shows the differences in the number of roots in the Maruta chrysanthemum variety with various EMS concentrations. Explants that did not undergo EMS treatment had a higher average value than those that did. 1% EMS explants had an average of around 6.22 roots, which was significantly lower than that of the control explants. 1.5% EMS explants had an average number of roots of approximately 1.22, which was the lowest number of roots among all concentrations and the control. The 2% EMS explants had an average number of roots of roughly 3.56, which was significantly lower than that of the control explants. This finding aligns with research (Romiyadi et al., 2018) that soaking explants in a 0.05% EMS solution can increase the number of orchid plantlet roots; however, increasing the concentration continuously causes a decrease in the number of roots.

Roots are the most crucial factor in plant growth, reflecting the extent to which a plant can reach and absorb essential nutrients for its development and growth. According to Romiyadi (2018), the use of low concentrations of EMS increases the number of roots, while increasing the concentration of EMS can reduce the number of roots.

4. Conclusion

The Maruta chrysanthemum variety exhibited tolerance to EMS treatment, with survival rates exceeding 50% across all concentrations. However, explant height, number of leaves, and root formation decreased as EMS concentration increased. The 1% EMS treatment provided the most favorable balance, maintaining high survival rates and promoting moderate growth, making it the optimal concentration for in vitro mutation induction.

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