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Induction of Genetic Diversity in Keladi (*Alocasia alba* Schott) using Streptomycin Sulfate Antibiotic

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Abstract
Alocasia alba Schott (keladi) is a popular ornamental plant valued for its unique morphology. The growing demand for this species presents opportunities for diversification through genetic improvements. This study aimed to investigate the most effective application technique to enhance the genetic diversity of *Alocasia alba* Schott, thereby improving its commercial value. The method used was a Randomized Complete Block Design. The first factor was streptomycin sulfate concentration (0, 500, 1000, and 1500 ppm). The second factor was soaking duration (3, 4, and 5 d). The observed parameters included the ability of the corms to produce shoots, the number of shoots and leaves, and leaf morphological traits such as shape, lamina color, and petiole color. Data were analyzed qualitatively and quantitatively using two-way ANOVA, followed by Duncan's Multiple Range Test (DMRT). The results showed a significant decrease in the ability of corms to produce shoots, the number of shoots, and the number of leaves, along with observable variations in leaf lamina color among the treatments.

Keywords: diversification; horticulture; ornamental plants; solution concentration; variation

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INTRODUCTION

Indonesia is home to the second-largest genetic mega-biodiversity in the world, owing to its high potential for biological diversity (Medrilzam *et al*., 2024). This encourages efforts to diversify plant variation, such as in Araceae, especially in the genus *Alocasia*, which has a high probability of discovering new varieties (Asharo *et al*., 2022; Ilhamullah *et al*., 2015; Irsyam *et al*., 2021; Marega *et al*., 2016; Widiyanti *et al*., 2017; Zuraida *et al*., 2018). In Indonesia, half of the *Alocasia* species can be found in lowland areas and extend to the lower slopes of mountains (Asih, 2024).

Alocasia is one of the most popular ornamental plants in Indonesia, widely sought after, and competitive with other ornamentals such as Monstera, Orchids, Anthurium, Aglaonema, Caladium, Philodendron, and cacti (Direktorat Jenderal Hortikultura, 2021). The ornamental plant *Alocasia alba* Schott has potential for diversification (Asih, 2024). However, ornamental plant breeding often faces challenges, including limited genetic resources and a narrow range of variations in plant form and color (Noman *et al*., 2017).

Many studies have investigated plant breeding and trait improvement using conventional methods, including mutation induction techniques, to generate genetic variation in plants (Damanik *et al*., 2018; Damayanti and A'ini, 2021; Ermayanti *et al*., 2018; Haswin *et al*., 2022; Listiani *et al*., 2021; Surtinah *et al*., 2022). One approach to increasing genetic diversity through mutation induction is the use of antibiotics, such as streptomycin (Haswin *et al*., 2022; Suhaimi *et al*., 2023; Surtinah *et al*., 2022; Susilowati *et al*., 2024). Previous studies have shown that streptomycin sulfate application can alter leaf shape and color, thereby enhancing genetic variation in plants (Haswin *et al*., 2022; Suhaimi *et al*., 2023). However, studies using streptomycin sulfate are still limited, as commonly used agents include colchicine, ethyl methanesulfonate (EMS), and gibberellins (Damanik *et al*., 2018; Damayanti and A'ini, 2021; Ermayanti *et al*., 2018; Listiani *et al*., 2021; Surtinah *et al*., 2022).

This study aimed to investigate the variation in *Alocasia alba* Schott induced by streptomycin sulfate, as affected by differences in concentration, soaking duration, and their combination. The observed parameters included the ability of the corms to produce shoots, the number of shoots and leaves, and leaf morphological characteristics.

MATERIALS AND METHODS

This study was an experimental research conducted from October 2023 to April 2024 at the Sukomulyo Residence in Lamongan. The experiment used a two-factor Randomized Complete Block Design (RCBD). The first factor was solution concentration, with levels of 0, 500, 1000, and 1500 ppm. The second factor was the soaking duration in the solution, which was 3, 4, and 5 days. The two factors were tested in all combinations. Data were analyzed qualitatively and quantitatively using two-way ANOVA, followed by Duncan's Multiple Range Test (DMRT).

In this study, *Alocasia alba* Schott corms were used, which were obtained from ornamental plant sellers in Bandung, West Java. The corms had diameters of 2—5 cm, heights of 7—10 cm, and weights of 80—150 g. The corms were soaked in streptomycin sulfate powder dissolved in distilled water to obtain various concentrations, with three replicates for each treatment. After soaking, the corms were planted in pots measuring 100 × 50 × 25 cm with 12 corms per pot. The plants were placed in an area protected from direct sunlight and watered daily.

Observations in this study were conducted over eight weeks after planting. Data were collected in the fourth and eighth weeks to obtain significant differences between the control plants and those treated with streptomycin sulfate (Damayanti and A'ini, 2021; Sandra, 2020; Suhaimi *et al*., 2023). The morphological traits observed in this study included the ability of the corms to produce shoots, the number of shoots and leaves, and leaf morphological traits such as shape, lamina color, and petiole color (Damayanti and A'ini, 2021).

RESULTS

The effects of various concentrations of streptomycin sulfate and soaking durations on the ability of corms to produce shoots are presented in Table 1. The results of Duncan's Multiple Range Test (Table 1) showed that corms treated with 0 ppm (control) produced shoots the fastest, with an average of 9–10 days after planting (DAP). In contrast, corms treated with 1500 ppm and a 5-day soaking duration had the longest sprouting time, averaging 36 DAP.

Table 1. Effect of various concentrations of streptomycin sulfate and soaking duration on the ability of Alocasia alba Schott corms to produce shoots after planting, based on the emergence of shoots (days).

Soaking Duration (Days)	Streptomycin Sulfate Concentration (ppm)			
	0	500	1000	1500
3	9.3 ± 1.2^{a}	12.7 ± 1.2^{ab}	24.7 ± 5.0^{c}	28.0 ± 3.5^{cd}
4	10.0 ± 2.0^{a}	15.3 ± 1.2^{b}	28.0 ± 3.5^{cd}	32.0 ± 3.5^{de}
5	9.3 ± 2.3^{a}	26.7 ± 3.1^{c}	33.3 ± 1.2^{e}	36.0 ± 2.0^{e}

Notes: Values followed by the same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at the 5% significance level.

The induction of streptomycin sulfate at various concentrations and soaking durations resulted in a decrease in the number of shoots and leaves (Tables 2 and 3). As a single agent, the concentration of streptomycin sulfate solution and soaking duration showed significant effects. However, their combination did not exhibit any interaction effects.

Table 2. Effect of various concentrations of streptomycin sulfate and soaking duration on the number of shoots of *Alocasia alba* Schott plants at 4 and 8 weeks after planting.

Age (Weeks)	Soaking Duration (Days)	Streptomycin Sulfate Concentration (ppm)			
		0	500	1000	1500
4	3	2.3 ± 0.6^{e}	1.7 ± 0.6^{cde}	1.0 ± 1.0^{abcd}	0.7 ± 0.6^{abc}
	4	2.0 ± 1.0^{de}	1.3 ± 0.6^{bcde}	0.7 ± 0.6^{ab}	0.3 ± 0.6^{ab}
	5	1.7 ± 0.6^{cde}	1.0 ± 1.0^{abcd}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
8	3	3.3 ± 5.8^{e}	2.7 ± 0.6^{cde}	2.0 ± 1.0^{abcd}	1.7 ± 0.6^{abc}
	4	3.0 ± 1.0^{de}	2.3 ± 0.6^{bcde}	1.7 ± 0.6^{abc}	1.3 ± 0.6^{ab}
	5	2.7 ± 0.6^{cde}	2.0 ± 1.0^{abcd}	1.0 ± 0.6^{a}	1.0 ± 0.0^{a}

Notes: Values followed by the same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at the 5% significance level.

In the fourth week of observation, plants treated with 0 ppm (control) had the highest number of shoots, averaging 1.7–2.3. This number continued to increase until the eighth week, reaching an average of 2.7–3.3 shoots per day. In contrast, plants treated with 1000 ppm and 1500 ppm concentrations with a soaking duration of five days had not produced shoots by the 36th day after planting (Table 1). Treatments at 1000 ppm and 1500 ppm for 5 d produced the fewest shoots among all treatments, averaging only one shoot per treatment.

Table 3. Effect of various concentrations of streptomycin sulfate and soaking duration on the number of leaves of *Alocasia alba* Schott plants at 4 and 8 weeks after planting.

Age (Weeks)	Soaking Duration (Days)	Streptomycin Sulfate Concentration (ppm)			
		0	500	1000	1500
4	3	2.3 ± 0.6^{d}	1.7 ± 0.6^{bcd}	1.3 ± 1.5^{abcde}	1.0 ± 1.0^{abcde}
	4	2.0 ± 1.0^{cd}	1.0 ± 1.0^{abcde}	0.7 ± 0.6^{abcd}	0.3 ± 0.6^{ab}
	5	2.3 ± 0.6^{d}	0.7 ± 0.6^{abcd}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
8	3	4.0 ± 1.0^{d}	3.3 ± 0.6^{bcd}	3.3 ± 1.5^{bcd}	2.7 ± 1.2^{abcd}
	4	3.3 ± 0.6^{bcd}	2.7 ± 0.6^{abcd}	2.0 ± 1.0^{ab}	1.7 ± 0.6^{a}
	5	3.7 ± 0.6^{cd}	2.3 ± 0.6^{abc}	1.7 ± 0.6^{a}	1.3 ± 0.6^{a}

Notes: Values followed by the same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at the 5% significance level.

At week 4, plants treated with 0 ppm (control) had the highest number of leaves, averaging 2—2.3. This number increased until week 8, with an average of more than three leaves per plant compared to the other treated plants. In contrast, plants treated with 1000 and 1500 ppm did not produce leaves by the fourth week. By the eighth week, the lowest leaf number was observed in plants treated with 1500 ppm for five days of soaking, averaging 1.3.

Treatments with different streptomycin sulfate concentrations and soaking durations also resulted in variations in the leaf color of *Alocasia alba* Schott (Tables 4 and Figure 1).

Table 4. Effect of various concentrations of streptomycin sulfate and soaking duration on the morphology of Alocasia alba Schott leaves at 8 weeks after planting.

Streptomycin Sulfate Treatment		Morphology
Concentration (ppm)	Duration (Days)	
0	3, 4, and 5	The upper surface of the leaves is dark green, The lower surface of the leaves and the petioles is light green
500	3	The upper surface of the leaves is green with light green spots (30%), The lower surface of the leaves and petioles is light green
	4	The upper surface of the leaves is green (70%), yellow-white (30%), The lower surface of the leaves and petioles is light green
	5	The upper surface of the leaves is green (50%), yellow-white (50%), The lower surface of the leaves is light green, and the petioles are light green
1000	3	The upper surface of the leaves is light green (80%), yellow-white (20%), The lower surface of the leaves and petioles is light green
	4	The upper surface of the leaves is light green (30%), yellow-white (70%), The lower surface of the leaves and petioles is light green
	5	The upper surface of the leaves, the lower surface of the leaves, and the petioles are yellow-white
1500	3	The upper surface of the leaves is light green (10%), yellow-white (90%), The lower surface of the leaves is light green- yellow-white, and the petioles are light green
	4	The upper surface of the leaves is light green (5%), yellow-white (95%), The lower surface of the leaves and petioles is yellow-white
	5	The upper and lower surfaces of the leaves and their petioles were white.

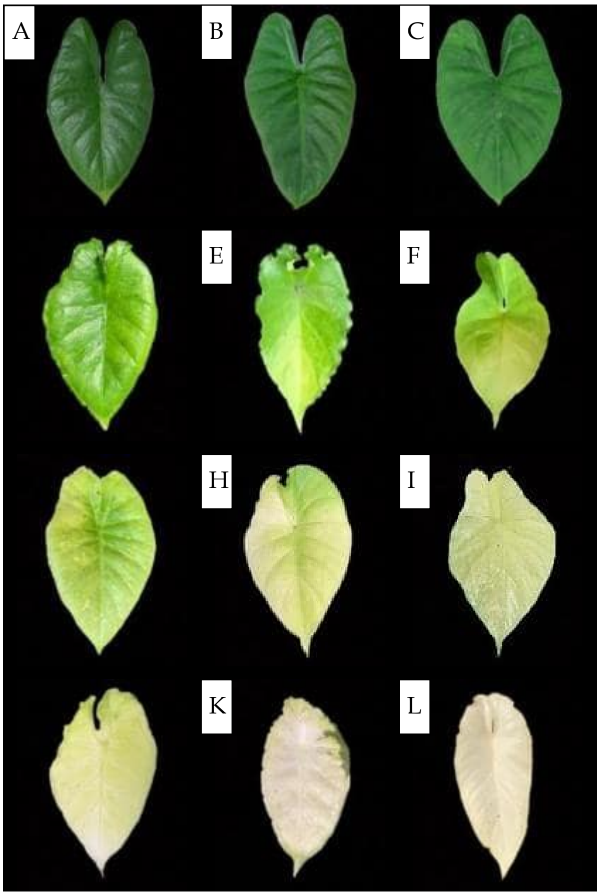

Figure 1. Observation of the leaf lamina of *Alocasia alba* Schott plants treated with various concentrations of streptomycin sulfate and soaking duration at 8 weeks after planting. A: 0 ppm (3 days), B: 0 ppm (4 days), C: 0 ppm (5 days), D: 500 ppm (3 days), E: 500 ppm (4 days), F: 500 ppm (5 days), G: 1000 ppm (3 days), H: 1000 ppm (4 days), I: 1000 ppm (5 days), J: 1500 ppm (3 days), K: 1500 ppm (4 days), and L: 1500 ppm (5 days).

Compared to the original plants, all treated plants exhibited the same leaf shape, characterized by elongated, heart-shaped leaves with wavy margins, cordate bases, and pointed tips (Figure 1). However, the application of streptomycin sulfate resulted in differences in leaf color (Table 4 and Figure 1). Plants exposed to streptomycin sulfate produced lighter leaf colors than the original or control plants. At a concentration of 1500 ppm with a soaking duration of five days, the treatment resulted in albino plants, characterized by the absence of leaf pigments.

DISCUSSION

The application of streptomycin sulfate influences the growth and development of shoots and leaves in *Alocasia alba* Schott. Interestingly, exposure to this compound alters the timing of shoot emergence, which may be attributed to differences in tissue responses (Asadi, 2013; Khoury, 2010; Suhaimi *et al*., 2023). This effect occurs because streptomycin inhibits the transcription of genes involved in meristematic cell division and differentiation, thereby reducing the cell division rate (Mensah *et al*., 2012; Sandra, 2020; Tasho *et al*., 2020; Valenzuela *et al*., 2019). The results of this study showed that lower streptomycin sulfate concentrations and shorter soaking durations accelerated shoot production in corms, whereas higher concentrations and longer durations delayed this process.

The ability of corms to produce shoots is closely related to the number of shoots and leaves formed (Damayanti and A'ini, 2021; Suhaimi *et al*., 2023). As meristematic cell division and differentiation are suppressed by streptomycin sulfate, the production of shoots and leaves correspondingly decreases, leading to overall growth inhibition (Sandra, 2020; Suhaimi *et al*., 2023). Earlier reports have shown similar effects, where plants treated with streptomycin sulfate exhibited impaired shoot and leaf development compared to that in control plants (Damayanti and A'ini, 2021;

Suhaimi *et al*., 2023). The results of this study showed that higher streptomycin concentrations and longer soaking times further hindered shoot and leaf growth.

Streptomycin sulfate affects shoot and leaf production and alters leaf color (Asadi, 2013; Edhi, 2020; EPA, 2013; Suhaimi *et al*., 2023; Sandra, 2020; Susilowati *et al*., 2024). This phenomenon is likely due to chlorophyll synthesis inhibition resulting from the reduced number of grana, as streptomycin disrupts the genes controlling leaf pigmentation (Damayanti and A'ini, 2021; Mensah *et al*., 2012; Sandra, 2020; Qian *et al*., 2012; Tasho *et al*., 2020). The results of this study showed that prolonged exposure to streptomycin led to its accumulation in leaves, resulting in a lighter leaf coloration. However, the treatments did not alter the shape of the leaf laminae (Figure 1).

The creation of variegated plants using streptomycin sulfate necessitates meticulous selection of the solution concentration and soaking duration. Low concentrations or brief exposure reduce the chance of generating differences from the original plant, whereas excessively high concentrations or prolonged soaking can cause plant death (Arisha, 2014; Sandra, 2020). This observation is consistent with previous studies that reported diverse plant responses to varying mutagen concentrations and soaking durations (Damanik *et al*., 2018; Ermayanti *et al*., 2018; Damayanti and A'ini, 2021; Listiani *et al*., 2021; Surtinah *et al*., 2022; Susilowati *et al*., 2024).

Sandra (2020) reported that streptomycin concentrations commonly used to induce variegation range from 1000 to 10000 ppm. In this study, exposure to 1500 ppm for 5 days resulted in the development of albino plants. However, this concentration did not reach the Lethal Dose 50 (LD_{50}), as all treated plants survived until week 8 (Sutapa and Kasmawan, 2016). Further investigation is needed to examine higher concentrations and extended observation periods to determine the optimal dose and LD_{50}, thereby ensuring long-term survival of plants.

Observations up to week 12 indicated that the leaf color changes in *Alocasia alba* Schott induced by streptomycin sulfate were unstable, as the leaves gradually reverted to green over time. Plants that retained their green color, including variegated types, often produced new green leaves similar to the original plants. In contrast, albino plants completely lacked green pigmentation in both petioles and laminae, and any new leaves remained white. In this study, albino plants did not survive until week 12, highlighting the need for further research on maintaining albino plants, which are known to have limited longevity (Ke *et al*., 2023; Li *et al*., 2020; Silva *et al*., 2020; Wang *et al*., 2020).

Leaf color instability in variegated plants is attributed to disrupted nutrient uptake and inter-plant competition, which may limit the resources available for growth. When nutrient levels are restored, variegated plants may revert to their original green color (Surtinah *et al*., 2022). Additionally, artificially induced mutations may prompt plants to retain the genetic traits of the original plant, allowing green leaves to reappear in variegated individuals (Suhaimi *et al*., 2023). The exact cause of this color reversion remains unknown, underscoring the need for further investigation in future studies to elucidate this phenomenon.

CONCLUSION

The application of various concentrations, soaking durations, and their combinations significantly reduced the ability of the corms to produce shoots, the number of shoots, and the number of leaves. The results also revealed variations in leaf and petiole color, but did not change the leaf shape. All treated plants exhibited leaf lamina colors that differed from those of the original plants. A concentration of 1500 ppm and a soaking duration of five days produced plants with the lightest leaf color.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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