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Ascorbic Acid Enhances In Vitro Growth of Raja Bulu Banana (*Musa* × *paradisiaca* L.) under Saline Stress

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Abstract

Raja Bulu banana (*Musa × paradisiaca* L.) is known for its exceptionally sweet flavor and can be efficiently propagated through tissue culture to produce large numbers of plants within a short time. Tissue culture involves isolating vegetative parts of the plant and cultivating them under aseptic conditions in a controlled environment. The most commonly used medium is a modified Murashige and Skoog (MS) medium, consisting of inorganic salts and organic compounds, and supplemented with sucrose, plant growth regulators, hormones, and specific vitamins. Exogenous application of ascorbic acid has been reported to enhance growth and development in various plant species. This study employed a Completely Randomized Design (CRD) with a single-factor, five-level ascorbic acid treatment: P0 (0 mg/L), P1 (2 mg/L), P2 (4 mg/L), P3 (6 mg/L), and P4 (8 mg/L). Each treatment was replicated three times. Data were analyzed using ANOVA, and significant differences among treatments were further evaluated using Tukey's HSD test at a 5% significance level. Qualitative observations were recorded through photographs on day 21. The results demonstrated that the application of 2 mg/L ascorbic acid significantly improved plantlet growth under saline In Vitro conditions, particularly in terms of growth percentage, plantlet height, root length, and carbohydrate content.

Keywords: *Musa × Paradisiaca* L; Ascorbic Acid; Tissue Culture; Salinity Stress; In Vitro Propagation

1. Introduction

Indonesia, recognized as a megabiodiversity country, harbors an exceptionally rich diversity of flora, including tropical plants such as bananas. One effective approach to conserving plant genetic resources is through tissue culture techniques, which enable rapid and large-scale propagation of plants [1].

The *Raja Bulu* banana (*Musa × paradisiaca* L.) is a local Indonesian variety with high economic value, both as a consumable fruit and as a raw material for various industries [2]. To sustainably meet increasing production demands, efficient propagation methods such as tissue culture offer a viable solution, allowing for the mass propagation of plants through the culture of cells, tissues, or organs under controlled environmental conditions [3].

Ascorbic acid (vitamin C) is a vital compound in plant metabolism, serving as an antioxidant, enzyme cofactor, and regulatory molecule in the plant's response to abiotic stress. In tissue culture, the application of ascorbic acid has been shown to enhance microspore embryogenesis and improve plantlet survival rates [4].

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However, salinity can adversely affect the performance of Murashige and Skoog (MS) medium. Elevated salt concentrations in the medium may inhibit shoot development and regeneration due to osmotic stress. Therefore, understanding the effects of salinity on tissue culture performance in MS medium is essential for optimizing plant growth under stress conditions [5].

2. Material and Methods

2.1. Materials and Equipment

The materials and equipment used in this study included a Laminar Air Flow (ESCO), autoclave, bunsen burner, analytical balance, Erlenmeyer flasks, beakers, measuring cylinders, culture bottles, stir rods, funnels, test tubes, test tube racks, plastic trays, tweezers, scalpels, scissors, mortar and pestle, dropper, micropipette, tips, cuvettes, pH meter, UV spectrophotometer, *Raja Bulu* banana plantlets, 70% and 96% alcohol, sterile distilled water, MS medium, sucrosa, agar, potassium hydroxide (KOH), hydrochloric acid (HCl), Ascorbic acid, fenol, sulfuric acid, label paper, Whatman No. 1 filter paper, tissues, aluminum foil and plastic wrap.

2.2. Experimental Design

This study was conducted over a period of 21 days in the Botany Laboratory, specifically in the In Vitro Culture Room, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung, Bandarlampung, Indonesia. Raja Bulu banana plantlets were cultured In Vitro on saline medium supplemented with varying concentrations of ascorbic acid. The experiment employed a Completely Randomized Design (CRD) with five ascorbic acid treatments: 0 mg/L (control), 2 mg/L, 4 mg/L, 6 mg/L, and 8 mg/L. Each treatment was replicated three times.

Data analysis included normality and homogeneity tests, followed by one-way ANOVA. Significant differences among treatments were further analyzed using Tukey's HSD test at a 5% significance level.

2.3. Percentage of live plantlets (%)

The percentage of live plantlets was recorded every four days over a 20-day observation period. The percentage was calculated using the following formula:

$$\text{Percentage of live plantlets} = \left[\frac{\text{Number of surviving plantlets}}{\text{Total number of plantlets}}\right] \text{X } 100$$

2.4. Plantlet Height (cm)

Plantlet height was measured using a ruler every four days over the 20-day observation period. Height data were recorded and documented in an observation table for further analysis.

2.5. Root Length (cm)

Root length was measured with a ruler every four days throughout the 20-day observation period. All measurements were recorded in an observation table for further analysis.

2.6. Carbohydrate Content (g)

Carbohydrate content was measured on day 21 using the phenol-sulfuric acid method [6]. A total of 0.1 grams of Raja Bulu banana plantlet leaves was weighed and ground with 10 mL of distilled water. The homogenate was filtered using Whatman No. 1 filter paper. From the resulting filtrate, 1 mL was mixed with 1 mL of concentrated H_2SO_4 and 2 mL of phenol. The mixture was transferred to a cuvette and the absorbance was measured at 490 nm using a spectrophotometer. The absorbance values were used to construct a standard curve and calculate carbohydrate concentration based on the linear regression equation in the form $Y = ax + b$.

3. Result and Discussion

3.1. Plantlet Survival Percentage

Observations of the growth and development of Raja Bulu banana plantlets were carried out every four days over a 20-day period. The effects of varying ascorbic acid concentrations on plantlet growth in saline medium are summarized in Table 1.

Table 1 Percentage of Living Banana Plantlets Through the Application of Various Doses of Ascorbic Acid in Saline Medium In Vitro

Ascorbic Acid Dosage (mg/L)	Percentage of Number of Living Plantlets on Day (%)				
	4	8	12	16	20
0	100	100	100	100	100
2	100	100	100	100	100
4	100	100	100	100	100
6	100	100	100	100	100
8	100	100	100	75	75

All plantlets exhibited 100% survival up to day 12. However, in the 8 mg/L ascorbic acid treatment, survival decreased to 75% between days 16 and 20, accompanied by visible browning of the tissue. This browning is indicative of oxidative stress (Figure 1).

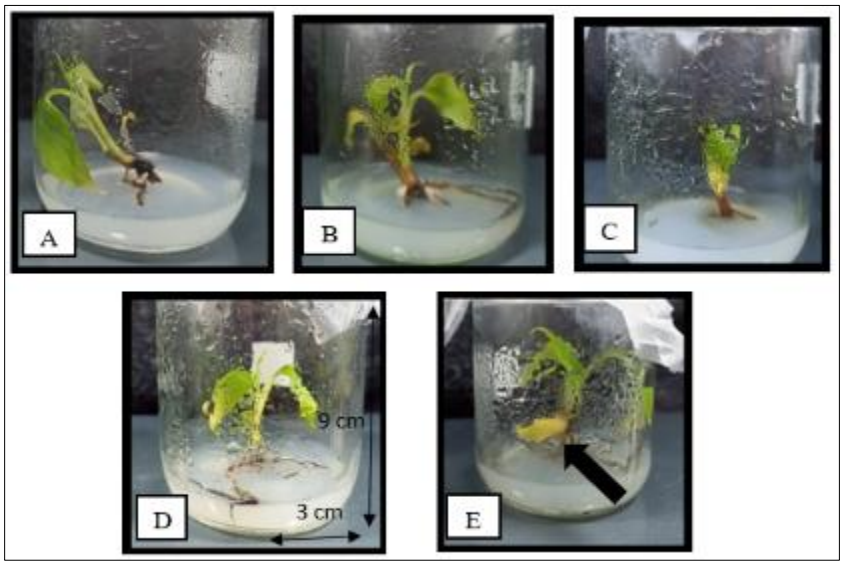

Figure 1 Raja Bulu Banana Plantlets After Ascorbic Acid Application on Day 20: A. 0 mg/L, B. 2 mg/L, C. 4 mg/L, D. 6 mg/L, and E. 8 mg/L

The high level of tissue browning and its relationship to the oxidation that occurs and is caused by physical damage to the tissue where excessive oxidation will be toxic to plant tissue and some cases cause plant death [7].

3.2. Plantlet Height

The results of the ANOVA test showed that the application of ascorbic acid had a significant effect on plantlet height. The highest average height was observed at the 8 mg/L dose (6.63 cm); however, the most stable and optimal growth was achieved at the 2 mg/L dose (5.40 cm). Plantlet growth at this dose was likely supported by efficient glucose absorption and enhanced metabolic activity. Detailed data are presented in Table 2.

Tabel 2 Average Height of Raja Bulu Banana Plantlets Through Ascorbic Acid Application on Saline Medium In Vitro

Ascorbic Acid Dosage (mg/L)	Average Plantlet Height on Day 20 (cm) $\bar{Y} \pm SE$
0	4.97 ± 0.088^{a}
2	5.40 ± 0.058^{b}
4	5.77 ± 0.088^{c}
6	6.20 ± 0.058^{d}
8	6.63 ± 0.088^{e}

Notes: $\bar{Y}$: Average height of plantlets; SE: Standard Error; Values followed by different letters indicate significant differences between treatments

Optimal plantlet height was influenced by several key factors, including the use of MS medium without added growth regulators, which provides a more natural environment for shoot development. The inclusion of sucrose serves as a critical energy source that supports shoot elongation. Additionally, the absence of exogenous hormones helps prevent hormonal imbalances, enabling more uniform growth among plantlets. The application of ascorbic acid further contributes to enhanced plantlet height by maintaining stable physiological conditions within the tissues [8].

3.3. Root Length

The highest root length was achieved at the 2 mg/L treatment (5.13 cm), indicating the effectiveness of a low dose in stimulating root growth. Auxin in the medium is influenced by the activity of ascorbic acid, which lowers the pH and enhances the activity of the expansin enzyme, as presented in Table 3.

Table 3 Average Root Length of Raja Bulu Banana Plantlets Through Ascorbic Acid Application on Saline Medium In Vitro

Ascorbic Acid Dosage (mg/L)	Average Plantlet Height on Day 20 (cm) $\bar{Y} \pm SE$
0	4.50 ± 0.115^{a}
2	5.13 ± 0.088^{ab}
4	4.30 ± 0.058^{bc}
6	4.50 ± 0.115^{bc}
8	4.90 ± 0.115^{c}

Notes: $\bar{Y}$: Average height of plantlets; SE: Standard Error; Values followed by different letters indicate significant differences between treatments.

The stimulatory effect on root growth is associated with the role of ascorbic acid in regulating calcium (Ca^{2+}) signaling and reactive oxygen species (ROS), both of which contribute to cell wall loosening and cell expansion. Ascorbic acid acts as a reducing agent that facilitates the production of hydroxyl radicals via the Haber–Weiss cycle, a process essential for cell wall loosening during root elongation [9].

3.4. Carbohydrate Content

Plantlets treated with 8 mg/L of ascorbic acid showed the highest carbohydrate content (0.614 g), but this did not correlate with other growth parameters. The 2 mg/L dose remained the most efficient option, as it did not induce excessive stress while still enhancing carbohydrate content, as presented in Table 4.

Table 4 Post-Hoc Test of Carbohydrate Content Through Ascorbic Acid Application on Saline Medium in Raja Bulu Banana Plantlets

Ascorbic Acid Dosage (mg/L)	Carbohydrate Content (g) $\bar{Y} \pm SE$
0	0.568 ± 0.072^{a}
2	0.244 ± 0.001^{ab}
4	0.423 ± 0.030^{abc}
6	0.394 ± 0.019^{bc}
8	0.614 ± 0.054^{c}

Notes: $\bar{Y}$: Average height of plantlets; SE: Standard Error; Values followed by different letters indicate significant differences between treatments.

Based on the data presented above, the plantlets appear to have reached an optimal stage in their photosynthetic activity, indicating that they no longer rely on cotyledonary food reserves for growth. While carbohydrate metabolism in the leaves may not directly determine leaf coloration, it can influence color changes by affecting anthocyanin synthesis within the tissues [10].

4. Conclusion

The application of ascorbic acid had a significant effect on the growth of Raja Bulu banana plantlets cultured on saline medium In Vitro. The optimal concentration was 2 mg/L, which resulted in the highest performance in terms of plantlet survival, height, root length, and carbohydrate content.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors have no conflicts of interest.

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