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Effect of Varying NAA Hormone Concentrations on In Vitro Subculture of Raja Bulu Banana (*Musa* sp.)

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Abstract: Tissue culture, a method involving the sterilized in vitro propagation of plant parts, offers potential for producing disease-free seedlings. This study aimed to (1) evaluate the effect of NAA (1-naphthaleneacetic acid) concentrations on root induction in Raja Bulu banana (*Musa* sp.) plantlets; (2) assess rooting percentage, root count, leaf number, root length, root mass, leaf color, plant height, and stem diameter; and (3) identify the optimal NAA concentration. Conducted from January to March 2025 at Salaman, Magelang (270 m a.s.l.), the experiment employed a Completely Randomized Design (CRD) with four NAA treatments (0, 2, 4, and 6 ppm) and six replications. Each bottle culture contained two plantlets. After 35 days, plantlets treated with 2 ppm NAA exhibited the highest root count (5.83 roots), with superior performance across other parameters. Elevated NAA concentrations (4–6 ppm) resulted in decreased rooting and chlorophyll content, indicating potential hormonal imbalance and phytotoxicity. The study concludes that 2 ppm NAA is the most effective concentration for rooting Raja Bulu banana in vitro. Future research should investigate the combined effects of auxins and cytokinins, natural biostimulants, and varying culture conditions to further enhance propagation efficiency.

Keywords: NAA, in vitro rooting, Musa sp., tissue culture, root induction

1. Introduction

Banana (*Musa paradisiaca* L.) is a major tropical fruit crop belonging to the Musaceae family, originating from Southeast Asia. It is widely cultivated across tropical and subtropical regions due to its adaptability to various agroecological zones, from lowland to upland areas. Beyond its role as a staple fruit, banana is considered a multi-purpose plant; various parts of the plant, including the roots, pseudostems, leaves, and fruits, have significant economic, nutritional, and medicinal value. These parts are used for food, animal feed, traditional medicine, handicrafts, and even in the cosmetic and pharmaceutical industries (Luh et al., 2023).

In the context of agricultural production, the demand for high-quality, disease-free banana planting materials has increased, particularly for cultivars with commercial importance such as Raja Bulu banana. Conventional propagation methods, such as sucker division, are often constrained by slow multiplication rates, disease transmission, and non-uniform plantlets. Therefore, in vitro propagation through tissue culture has become a preferred alternative to meet the growing demand for uniform, high-quality banana plantlets.

Tissue culture is a micropropagation technique that involves the aseptic culture of plant cells, tissues, or organs under controlled environmental conditions. It enables the mass production of genetically uniform and pathogen-free seedlings within a short period. The success of tissue culture depends on several factors, including the type of explant, culture media, and the use of plant growth regulators (PGRs) such as auxins and cytokinins. Auxins are particularly important for root induction and elongation during the rooting phase of micropropagation (Nurhanis et al., 2019; Kurnianingsih et al., 2020).

One of the most commonly used synthetic auxins in plant tissue culture is 1-naphthaleneacetic acid (NAA), which has been shown to effectively stimulate adventitious root formation in various crops. However, the response to NAA can vary depending on species, cultivar, and concentration. While low concentrations of NAA may stimulate root initiation, excessive concentrations can lead to hormonal imbalance, ethylene overproduction, and toxicity symptoms

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such as leaf necrosis or inhibited growth (Ali et al., 2021). Hence, determining the optimal NAA concentration is crucial for enhancing rooting efficiency without compromising plantlet health.

Several studies have investigated the role of NAA in root development. Hartati et al. (2016) found that NAA at moderate concentrations significantly improved root number and root length in Dendrobium orchid subcultures. Similarly, Putri et al. (2018) reported enhanced root induction in Raja Kinalun banana plantlets using 2 ppm NAA. However, studies focusing specifically on Raja Bulu banana remain limited, particularly in identifying a precise NAA dosage that supports both optimal rooting and overall plantlet vigor.

Therefore, the objectives of this study were to (1) assess the effects of different NAA concentrations on root formation in Raja Bulu banana plantlets cultured in vitro; (2) evaluate physiological parameters such as root number, root length, leaf number, and leaf color; and (3) identify the most effective NAA concentration that supports optimal rooting and healthy plantlet development. This research is expected to contribute to the refinement of micropropagation protocols for banana and support large-scale production of elite planting materials for commercial use.

2. Materials and Methods

2.1. Study Site and Duration

The experiment was conducted from January to March 2025 at the Food and Horticultural Crop Seed Garden, Salaman District, Magelang Regency, Central Java, Indonesia. The experimental site is located at an altitude of 270 meters above sea level, characterized by a tropical monsoon climate with stable indoor conditions, making it suitable for in vitro culture practices.

2.2. Plant Material

The plant material consisted of *Musa* sp. cv. Raja Bulu plantlets previously established through in vitro culture. Each plantlet was approximately 3 cm in height, bore at least three functional leaves, and had undergone subculturing to ensure uniform physiological status before being transferred to the rooting phase.

2.3. Chemicals and Culture Media

Murashige and Skoog (MS) basal medium was used as the foundation for culture media preparation, providing essential macronutrients and micronutrients required for plant growth. The medium was supplemented with vitamins, including thiamine hydrochloride (0.1 mg/L), nicotinic acid (0.5 mg/L), and pyridoxine hydrochloride (0.5 mg/L), as well as 100 mg/L myo-inositol to support cellular metabolism. Sucrose was added at a concentration of 30 g/L as the primary carbohydrate source, while activated charcoal (0.5 g/L) was included to absorb phenolic compounds and prevent browning of the medium. Agar was incorporated at 6 g/L to solidify the medium.

To evaluate the effect of auxin on root induction, the synthetic plant growth regulator 1-naphthaleneacetic acid (NAA), obtained from Merck-Schuchardt (Art. 806862), was added at four different concentrations 0 ppm (control), 2 ppm, 4 ppm, and 6 ppm according to treatment requirements. The pH of all media was adjusted to 5.8 using 0.1 N NaOH or HCl before autoclaving at 121°C and 15 psi for 30 minutes. Media were prepared under aseptic conditions and dispensed into sterile 250 mL culture bottles, with approximately 30 mL of solidified medium per bottle.

2.4. Culture Inoculation and Maintenance

2.4.1. Aseptic Preparation

All instruments (forceps, scalpels, Petri dishes) were sterilized in an autoclave. The Laminar Air Flow (LAF) cabinet was cleaned with 70% ethanol and exposed to UV light for 60 minutes before use. Tools were further flamed over a Bunsen burner before each transfer operation.

2.4.2. Plantlet Inoculation

Raja Bulu banana plantlets were trimmed uniformly by cutting the leaves to one-third of their length to reduce transpiration. The basal end of each plantlet was cleaned of residual medium and trimmed to expose meristematic regions. Using sterile forceps, two plantlets were inoculated into each bottle containing one of the four treatment media. The culture bottles were tightly sealed with aluminum foil and secured with plastic wrap to prevent contamination. Each bottle was clearly labeled with the treatment code and date.

2.4.3. Incubation Conditions

All cultures were placed on sterilized racks inside a culture room under a continuous photoperiod (24 hours light) at a constant room temperature of approximately 22 ± 2°C. Light intensity was provided using cool white fluorescent lamps (~40 µmol m^{-2} s^{-1}). Contaminated cultures (e.g., with fungal or bacterial growth) were immediately removed and excluded from analysis.

2.5. Parameters Measured

Observations were conducted 35 days after the plantlets were transferred to the rooting media. Several morphological and physiological parameters were measured to assess the influence of NAA concentrations on the growth performance of *Musa* sp. cv. Raja Bulu plantlets. The number of roots per plantlet was recorded by counting all adventitious roots that emerged from the basal region. The number of leaves per plantlet was determined by counting fully expanded leaves with visible lamina, excluding any developing leaf primordia. Root length was measured using a ruler, with the measurement taken from the base of the plantlet to the tip of the longest root to assess the extent of root elongation.

Root mass was evaluated by carefully extracting the roots from the medium, gently rinsing them with sterile distilled water to remove any agar residue, and weighing them using a precision digital balance. Leaf color was assessed using a qualitative rating scale ranging from 1 (pale yellow) to 5 (dark green), providing an estimate of chlorophyll content and general plantlet vigor. Plantlet height was measured from the base of the stem at the medium surface to the tip of the tallest leaf, giving an indication of shoot elongation. Stem diameter was measured at the midpoint of the pseudostem using a digital caliper to reflect overall structural robustness. These parameters were selected to provide a comprehensive evaluation of root development and overall plantlet health in response to the applied NAA treatments.

2.6. Experimental Design

The experiment was arranged using a Completely Randomized Design (CRD) with a single treatment factor, namely the concentration of NAA (1-naphthaleneacetic acid), applied at four levels: 0 ppm (P0) as the control, 2 ppm (P1), 4 ppm (P2), and 6 ppm (P3). Each treatment was replicated six times, and two plantlets were placed in each culture bottle, resulting in a total of 48 experimental units. The assignment of plantlets to treatment groups was done randomly to minimize the influence of external variables and ensure the validity of the experimental results. This design was chosen for its simplicity and suitability for evaluating the effects of a single factor on multiple response variables under controlled conditions.

2.7. Data Analysis

Quantitative data were subjected to one-way Analysis of Variance (ANOVA) to detect significant differences among treatments. If significant, comparisons between treatment means were performed using Bonferroni's post-hoc test at a 5% significance level ($\alpha = 0.05$). Statistical analyses were conducted using SPSS software version 25.0.

3. Results and Discussion

3.1. Number of Roots

Root development is a critical determinant of plantlet viability and adaptation during in vitro culture. In this study, the number of roots per plantlet was significantly influenced by the application of NAA (Figure 1). The 2 ppm NAA treatment produced the highest average number of roots (5.83), followed by the 4 ppm treatment (5.00), the 6 ppm treatment (4.83), and the control (2.83), which showed the lowest rooting response. These results suggest that a moderate concentration of NAA effectively stimulates adventitious root formation in *Musa* sp. cv. Raja Bulu plantlets.

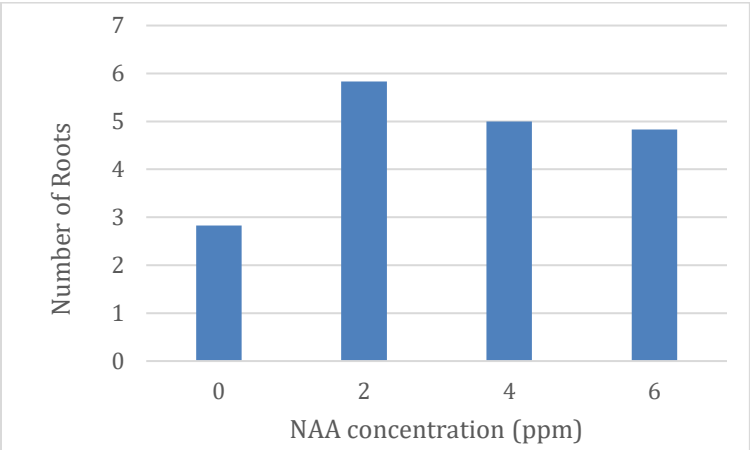

Fig. 1: Average Number of Roots per Plantlet under Different NAA Treatments

The enhanced rooting at 2 ppm is consistent with findings by Putri et al. (2018), who observed optimal root induction in banana plantlets at similar auxin levels. NAA, as a synthetic auxin, mimics the role of natural indole-3-acetic acid (IAA) by promoting cell elongation, root initiation, and differentiation. However, at higher concentrations (4–6 ppm), a decline in root number was observed, possibly due to hormonal imbalances or the accumulation of ethylene,

which may inhibit further root primordia development or induce tissue senescence (Ali et al., 2021). This trend underscores the need for precise auxin concentration control in micropropagation protocols.

3.2. Number of Leaves

The number of leaves per plantlet is an important indicator of shoot health and potential for photosynthetic capacity. In the present study, the highest number of leaves was recorded in the control treatment (2.50 leaves), with a gradual decline observed in plantlets exposed to increasing concentrations of NAA: 2 ppm (2.33), 4 ppm (1.83), and 6 ppm (1.67) as shown in Figure 2. This pattern indicates that exogenous auxin application may negatively affect leaf development, particularly at elevated concentrations.

These findings align with reports from Jannah et al. (2023), who found that high levels of plant growth regulators can disrupt endogenous hormonal equilibrium, thereby inhibiting shoot proliferation. Auxins in excessive amounts are known to stimulate callus formation and root development at the expense of shoot growth, which may explain the reduction in leaf number observed in this study.

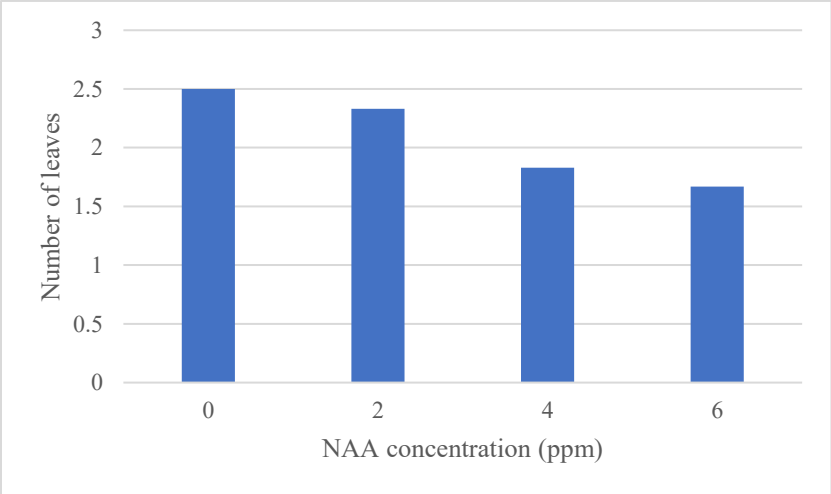

Fig. 2: Average Number of Leaves per Plantlet under Different NAA Concentrations

3.3. Root Length

Root elongation is vital for effective nutrient absorption and is influenced by the hormonal environment of the culture medium. In this study, the longest average root length was observed in the control treatment (4.17 cm), while the shortest roots were produced in the 4 ppm NAA treatment (2.48 cm) (Figure 3). Root length values for the 2 ppm and 6 ppm treatments were intermediate (3.85 cm and 2.67 cm, respectively).

Interestingly, while moderate NAA concentrations promoted root formation, they appeared to slightly suppress elongation, potentially due to excessive cell division at the root initiation sites that limited longitudinal expansion. Excessive auxin may also induce the production of ethylene, which has been shown to inhibit root elongation by affecting microtubule stability and cellular expansion (Khozin et al., 2024). Thus, while NAA is essential for initiating root primordia, optimal concentrations must be maintained to avoid adverse effects on elongation.

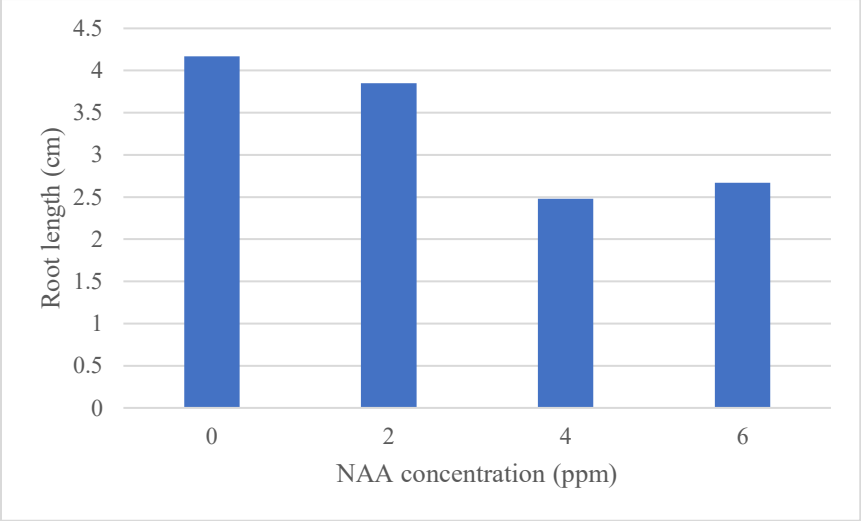

Fig. 3: Average Root Length (cm) per Plantlet under Different NAA Concentrations

3.4. Leaf Color

Leaf color serves as a proxy for chlorophyll content and overall plantlet vigor. The control treatment exhibited the most intense green coloration (average score of 3.33), while increasing NAA concentrations led to a reduction in leaf pigmentation, with the lowest score (2.17) observed at 6 ppm (Figure 4). This progressive decline suggests that high auxin levels may impair chlorophyll synthesis or promote senescence-like symptoms.

These observations are consistent with the findings of Fadhila et al. (2023) and Hasidah et al. (2017), who reported that hormonal imbalances, particularly elevated auxin levels, can negatively affect chlorophyll biosynthesis and result in yellowing or chlorosis of the leaves. The potential for phytotoxicity at higher auxin concentrations highlights the importance of balancing hormonal inputs to maintain healthy photosynthetic tissue during in vitro propagation.

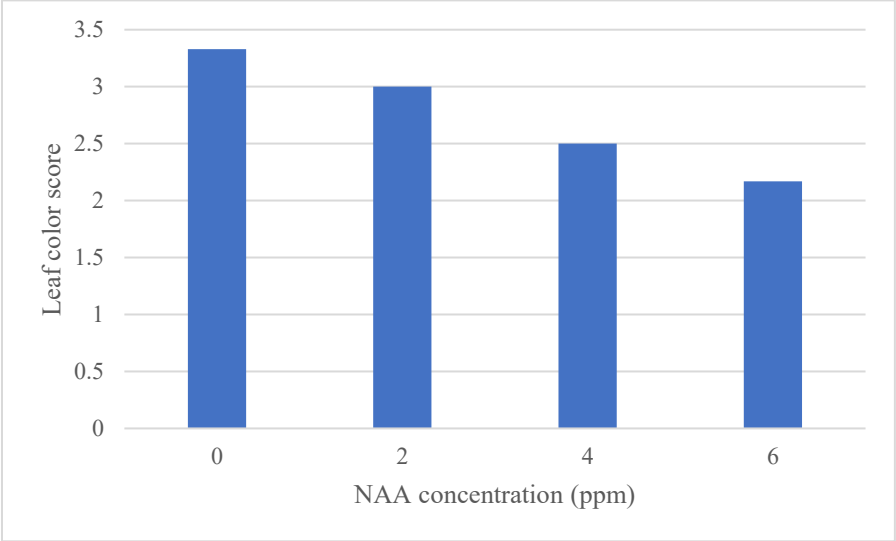

Fig. 4: Average Leaf Color Rating under Different NAA Concentrations

4. Conclusion

The present study demonstrated that the application of NAA at varying concentrations significantly influenced root formation and overall plantlet development in *Musa* sp. cv. Raja Bulu under in vitro conditions. Among the treatments tested, a concentration of 2 ppm NAA was found to be the most effective, resulting in the highest number of roots while maintaining satisfactory root length, leaf number, and healthy leaf coloration. In contrast, higher concentrations (4 and 6 ppm) tended to reduce growth performance and caused signs of hormonal stress, such as pale leaves and reduced elongation. These findings highlight the importance of precise auxin dosing in tissue culture systems to optimize rooting without inducing phytotoxic effects. It is recommended that future studies explore the synergistic interaction of NAA with cytokinins such as BAP, as well as test alternative organic biostimulants under varying environmental conditions to further enhance the efficiency and scalability of banana micropropagation.

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Conflict of Interest

The authors declare no conflicts of interest.

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