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In Vitro Growth Response of Raja Bulu Banana (*Musa × paradisiaca* L.) Plantlets to Varying Atonik Concentrations and Soaking Durations

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Abstract

Raja Bulu banana (*Musa × paradisiaca* L.) is a high-value commodity with significant economic potential; however, the availability of high-quality seedlings remains a major challenge in its cultivation. In vitro culture offers a promising solution for the rapid and large-scale production of uniform plantlets. This study aimed to determine the optimal concentration and soaking duration of the plant growth regulator Atonik for the in vitro growth of Raja Bulu banana plantlets. A completely randomized design (CRD) was employed, using two factors: Atonik concentration (0, 3, and 6 mL/L) and soaking duration (0, 10, and 20 minutes), resulting in nine treatment combinations with three replications each. The observed parameters included plantlet height, root length, and chlorophyll content. Data were analyzed using analysis of variance (ANOVA) followed by the least significant difference (LSD) test at the 5% significance level. The results showed that Atonik application significantly influenced the in vitro growth of Raja Bulu banana plantlets, particularly in terms of plantlet height and root length. The most effective treatment was observed at a concentration of 3 mL/L with a soaking duration of 10 minutes. Higher concentrations tended to inhibit growth. Chlorophyll content did not show a significant response to the treatments, indicating that Atonik primarily affected morphological rather than physiological aspects of plantlet development.

Keywords: Raja Bulu banana; *Musa × paradisiaca* L.; In vitro culture; Atonik; plantlet growth; Chlorophyll content

1. Introduction

Banana (*Musa spp*.) is a high-value tropical fruit that plays a crucial role in food security and the global economy [1]. Among the various cultivars, Raja Bulu banana is notable for its high economic value and strong market demand, making it a preferred choice for large-scale cultivation by farmers. However, conventional propagation methods are constrained by slow growth rates and susceptibility to diseases, which limit seedling availability and plantation success [2].

To address these challenges, in vitro culture has emerged as a rapid and efficient technique for producing disease-free and genetically uniform banana plantlets [3]. Micropropagation has been proven to enhance both the growth rate and the supply of high-quality seedlings [4]. The success of in vitro culture depends on several factors, including the composition of the growth medium, the type and concentration of plant growth regulators (PGRs), and environmental conditions such as temperature and light intensity [5].

In vitro culture techniques utilize nutrient media enriched with plant growth regulators (PGRs) to support plant development. PGRs are a group of organic compounds that regulate various plant developmental pathways and are essential for the success of both in vitro and in vivo propagation [6]. One commonly used PGR is Atonik, a biostimulant

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containing nitrophenol compounds that can enhance plant growth by stimulating cell division, root elongation, and overall plant metabolism [7]. Studies have demonstrated that Atonik can improve stress resistance and accelerate growth in various crops, including vegetables and fruit plants [8]. However, its effectiveness is influenced by concentration and soaking time, as excessive doses may lead to toxicity and inhibit growth [9].

Although Atonik shows great potential in plant tissue culture, the optimal dosage and soaking duration for Raja Bulu banana remain insufficiently explored. Therefore, this study aims to determine the most effective Atonik concentration and soaking duration to enhance the growth of Raja Bulu banana plantlets through in vitro culture. The findings are expected to contribute to the optimization of banana micropropagation techniques and improve mass propagation strategies for commercial applications.

2. Tools and Materials

The tools and materials 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, granulated sugar, agar, potassium hydroxide (KOH), hydrochloric acid (HCl), Atonik, methanol, label paper, Whatman No. 1 filter paper, tissues, aluminum foil, plastic wrap, rubber bands, detergent, and bleach (Bayclin).

A Completely Randomized Design (CRD) was employed, using two factors: Atonik concentration (0 mL/L, 3 mL/L, 6 mL/L) and soaking duration (0, 10, 20 minutes). The combination of these factors resulted in 9 treatment combinations, with 3 replications, totaling 27 experimental units. The following parameters were observed:

2.1. Plantlet Height

Plantlet height was measured in the third week after planting using a ruler, from the base of the plantlet to the highest growing point, carefully through the culture bottle without damaging the plantlet or the medium.

2.2. Root Length

Root length was measured after removing the plantlet from the culture bottle and cleaning it of any media residues. The measurement was taken using a ruler, from the base of the root to the tip of the longest root.

2.3. Chlorophyll Content

Chlorophyll content was analyzed using a UV spectrophotometer, following the method described by [10]. A 0.1 g leaf sample was ground in 10 mL of 80% acetone (v/v), filtered through Whatman No. 1 filter paper, and then transferred to a sealed vial. One milliliter of the solution and one milliliter of 80% acetone were placed in cuvettes for analysis at wavelengths of 663 nm and 646 nm. Each sample was tested in four replicates.

Chlorophyll content was calculated using the following formulas:

- $Chla=(12.25\times\lambda663)-(2.79\times\lambda646)$ (mg/L)
- $Chlb=(21.50\times\lambda646)-(5.10\times\lambda663)$ (mg/L)
- $\text{Total chlorophyll}=(7.15\times\lambda663)+(18.71\times\lambda646)$ (mg/L)

Where:

- **Chla** = Chlorophyll a
- **Chlb** = Chlorophyll b
- λ646 = Absorbance at wavelength 646 nm
- λ663 = Absorbance at wavelength 663 nm

3. Results and discussion

The application of plant growth regulators (PGRs) plays a crucial role in enhancing plant growth, particularly under in vitro conditions, as reflected in measurable changes in parameters such as plantlet height, root length, and chlorophyll content. Atonik, a widely used PGR containing nitrophenol compounds, has been shown to stimulate cell division and enhance plant metabolism [4]. However, the effectiveness of Atonik in promoting plant growth is highly dependent on both its concentration and soaking duration. Excessive doses or prolonged exposure may exert toxic effects, ultimately inhibiting plant development [9].

3.1. Plantlet Height

The effect of Atonik concentration and soaking duration on the height of Raja Bulu banana plantlets was evaluated based on the observed increase in plantlet height. This parameter was assessed by comparing plantlets that received no Atonik treatment with those treated at concentrations of 0 mL/L, 3 mL/L, and 6 mL/L, combined with soaking durations of 0, 10, and 20 minutes. The resulting plantlet height data after treatment are summarized in **Table 1.**

Table 1 Average height (cm) of Raja Bulu banana (*Musa × paradisiaca* L.) plantlets in the third week after planting under different Atonik concentrations and soaking durations

Atonik (mL/L)	Soaking durations (minutes)		
	0	**10**	**20**
0	5,23±0,14^{bc}	4,43±0,23^{cd}	4,50±0,05^{cd}
3	5,66±0,27^{ab}	6,30±0,17^{a}	5,26±0,32^{bc}
6	4,63±0,08^{cd}	4,23±0,145^{d}	4,10±0,05^{d}

Explanation

- $\mu = \bar{Y} \pm SE$
- $\bar{Y}$ = mean root length
- SE = standard error of the mean
- Values followed by the same letter are not significantly different at the 5% significance level.

An increase in plantlet height is a key indicator of successful in vitro culture, particularly in efforts to achieve rapid and efficient plant propagation. As shown in Table 1, the application of Atonik at a concentration of 3 mL/L combined with a soaking duration of 10 minutes resulted in the highest plantlet height, which was significantly greater than that observed in other treatments ($p < 0.05$). The interaction between Atonik concentration and soaking duration had a significant effect on plantlet height, suggesting that this specific combination provided optimal stimulation for shoot growth compared to other treatment variations.

Physiologically, Atonik contains active compounds that enhance metabolic processes, thereby supporting both root formation and overall plantlet development. Previous studies have demonstrated that the application of plant growth regulators (PGRs) can significantly improve the growth performance of various crops, including cucumber [4]. The effectiveness of Atonik at a concentration of 3 mL/L in this study is consistent with the findings of [12], who reported that this dosage significantly promoted vegetative growth in treated plants. Compared to untreated plantlets, those exposed to Atonik exhibited improved shoot elongation and root development, highlighting the potential of this biostimulant in optimizing in vitro propagation.

The use of PGRs at appropriate concentrations can stimulate the synthesis of auxin and cytokinin—two key hormones involved in stem elongation and cell division—thereby promoting increased plantlet height [12]. However, when applied at excessively high concentrations, such as 6 mL/L, Atonik tends to inhibit shoot growth. This inhibitory effect is likely due to toxicity resulting from the accumulation of active compounds, which may disrupt hormonal balance within the plant tissues [9]. Such an imbalance can interfere with the processes of cell division and elongation, ultimately leading to reduced plantlet development under in vitro conditions.

The growth performance of *Raja Bulu* banana plantlets following treatment with various Atonik concentrations and soaking durations is shown in **Figure 1**. The figure visually demonstrates the differences in plantlet development among

treatments, highlighting the influence of the plant growth regulator during the in vitro growth phase. These visual comparisons support the quantitative data, reinforcing the impact of Atonik on plantlet morphology.

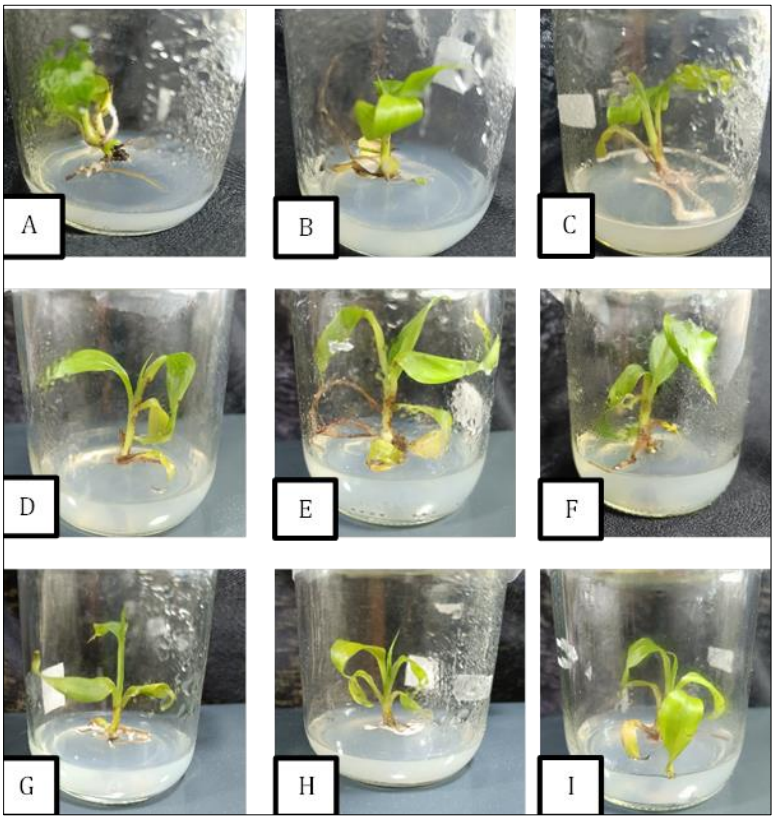

Figure 1 Raja Bulu banana (*Musa × paradisiaca* L.) plantlets in the third week after planting under different Atonik concentrations and soaking durations. The figure illustrates the visual differences in plantlet growth, showing the influence of Atonik on plantlet development during the in vitro phase

Explanation

- A: K1P1 (Atonik concentration 0 mL/L, soaking duration 0 minutes)
- B: K1P2 (Atonik concentration 0 mL/L, soaking duration 10 minutes)
- C: K1P3 (Atonik concentration 0 mL/L, soaking duration 20 minutes)
- D: K2P1 (Atonik concentration 3 mL/L, soaking duration 0 minutes)
- E: K2P2 (Atonik concentration 3 mL/L, soaking duration 10 minutes)
- F: K2P3 (Atonik concentration 3 mL/L, soaking duration 20 minutes)
- G: K3P1 (Atonik concentration 6 mL/L, soaking duration 0 minutes)
- H: K3P2 (Atonik concentration 6 mL/L, soaking duration 10 minutes)
- I: K3P3 (Atonik concentration 6 mL/L, soaking duration 20 minutes)

3.2. Root Length

Root length is a crucial parameter for assessing the success of plantlet growth in in vitro culture [13]. Roots are essential for water and nutrient absorption and provide structural stability for the plantlets during their development in the culture medium [14]. The application of Atonik has been shown to stimulate root growth by enhancing physiological processes such as cell division and root elongation [8].

In this study, the effect of Atonik concentration and soaking duration on the root length of *Raja Bulu* banana plantlets was evaluated by comparing root development under different treatments (0 mL/L, 3 mL/L, 6 mL/L) and immersion times (0, 10, 20 minutes). The root length data after treatment are presented in **Table 2.**

Table 2 Root Length (cm) ± SE of *Raja Bulu* Banana Plantlets in the 3rd Week After Planting under Different Atonik Concentrations and Soaking Durations

Atonik (mL/L)	**Soaking Duration (minutes)**		
	0 minutes	**10 minutes**	**20 minutes**
0 ml/L	3,13±0,14^{bc}	2,33±0,23^{bcd}	2,06±1,04^{cd}
3 ml/L	4,56±0,27^{ab}	6,06±0,21^{a}	2,83±0,14^{bc}
6 ml/L	1,40±0,72^{cd}	0,90±0,45^{cd}	0,36±0,36^{d}

Explanation

- $\mu = \bar{Y} \pm SE$
- $\bar{Y}$ = mean root length
- SE = standard error of the mean
- Values followed by the same letter are not significantly different at the 5% significance level.

Based on Table 2, the application of Atonik at different concentrations and soaking durations significantly influenced root length. The treatment with 3 mL/L Atonik combined with a 10-minute soaking produced the longest roots, which were statistically different from those of the other treatments. This finding indicates that the treatment effectively stimulates root development during the in vitro phase. An appropriate Atonik concentration can enhance physiological processes in plantlets, particularly by promoting cell division and elongation in root tissues, which are essential for the formation of a robust root system. Similarly, selecting the proper soaking duration is crucial to optimizing the effectiveness of Atonik in supporting root development and overall plantlet growth [15].

In contrast, a higher dose of 6 mL/L tended to inhibit root growth, possibly due to toxic effects resulting from the accumulation of active compounds in the Atonik-treated plantlets. This finding is consistent with previous studies, which suggest that the effectiveness of biostimulants such as Atonik depends on the applied concentration, as excessive doses may lead to physiological imbalances that inhibit plant growth [9]. Therefore, determining the optimal concentration and soaking duration is essential for maximizing plantlet development.

3.3. Chlorophyll Content

Chlorophyll is a green pigment essential for photosynthesis and serves as an indicator of a plant's physiological status. In this study, chlorophyll content in Raja Bulu banana plantlets was measured to evaluate the effect of Atonik, a plant growth regulator, applied at different concentrations and soaking durations. The average chlorophyll content of the plantlets is presented in **Table 3.**

Table 3 Average Chlorophyll Content of Raja Bulu Banana Plantlets Under Different Atonik Concentrations and Soaking Durations

Treatments	**Chlorophyll (Mean±Standard Error)**		
	Chlorophyll a	**Chlorophylll b**	**Total Chlorophyll**
K1P1	6,82±0,55	3,78±0,31	10,61±0,87
K1P2	5,65±1,13	3,19±0,54	8,85±1,67
K1P3	4,35±1,05	2,60±0,54	6,96±1,60
K2P1	5,82±0,69	3,24±0,30	9,07±0,99
K2P2	7,32±0,71	3,98±0,38	11,30±1,09
K2P3	6,47±0,21	3,52±0,05	9,99±0,25
K3P1	6,42±0,44	3,55±0,25	9,97±0,70
K3P2	4,69±0,35	2,73±0,18	7,42±0,54
K3P3	4,80±0,10	2,86±0,05	7,66±0,16

Based on the observations presented in Table 3, the soaking treatment with the plant growth regulator Atonik did not result in a statistically significant effect on the chlorophyll content of Raja Bulu banana plantlets. However, the K2P2 treatment (3 mL/L for 10 minutes) demonstrated the highest chlorophyll content among all treatments. This suggests that soaking plantlets in Atonik at an appropriate concentration and duration may enhance photosynthetic activity by increasing chlorophyll accumulation.

Chlorophyll content serves as a key indicator of photosynthetic efficiency, which directly affects biomass accumulation and overall plant growth. Soaking plantlets in an Atonik solution may enhance nutrient uptake, particularly of magnesium (Mg) and nitrogen (N), by increasing cell wall permeability and enzymatic activity. These nutrients play critical roles in chlorophyll biosynthesis, as Mg is the central atom in the chlorophyll molecule, while N constitutes an essential part of the chlorophyll structure and the proteins involved in photosynthesis [4].

According to previous studies, the effect of Atonik on chlorophyll content in plantlets varies depending on plant species, environmental conditions, and additional treatments. While some research indicates that Atonik can enhance chlorophyll content, other studies have reported no significant effect or even a decrease. For instance, [15] found no positive impact of Atonik on chlorophyll content in lettuce, and [16] observed a reduction in chlorophyll levels in hosta and bergenia plants treated with Atonik.

These varying results are likely attributed to the complex interactions between Atonik and other factors, including plant species, in vitro culture conditions, as well as the concentration and duration of Atonik soaking. Consequently, the lack of significant differences in chlorophyll content observed in this study may be due to these interacting variables influencing the plantlets' physiological response to Atonik treatment.

4. Conclusion

The results showed that the application of Atonik influenced the in vitro growth of Raja Bulu banana (*Musa × paradisiaca* L.) plantlets, particularly in terms of plantlet height and root length. The most effective treatment was observed at a concentration of 3 mL/L with a soaking duration of 10 minutes. Higher concentrations tended to inhibit growth. Chlorophyll content did not exhibit a significant response to the treatments, suggesting that Atonik primarily affected the morphological rather than the physiological aspects of plantlet development.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors have no conflicts of interest.

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