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Growth Response of Raja Bulu Banana (*Musa* × *paradisiaca* L.) Plantlets Induced by Atonik Solution Under *In Vitro* Drought Stress

Tantri Pratiwi, Endang Nurcahyani *, Yulianty and Sri Wahyuningsih

Applied Biology Study Program, Faculty of Mathematics and Natural Sciences, University of Lampung, Bandar Lampung, Lampung, Indonesia.

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Abstract

Raja Bulu banana (*Musa × paradisiaca* L.) is one of the popular table bananas that can be consumed fresh or processed into various food products. However, its production often declines under dry land conditions. This study aimed to determine the interaction between Atonik solution and PEG 6000 on the in vitro growth of Raja Bulu banana plantlets. The experiment was conducted using a Completely Randomized Design (CRD) with two factors: (A) Atonik concentration at three levels (0 mL L^{-1}, 3 mL L^{-1}, and 6 mL L^{-1}) and (B) PEG 6000 concentration at three levels (0%, 10%, and 20%). Each treatment was replicated three times, with one plantlet per culture bottle. Parameters observed included plantlet survival, growth visualization, stomatal density, and carbohydrate content. Quantitative data were analyzed using one-way ANOVA followed by the Honestly Significant Difference (HSD) test at the 5% significance level. The results showed that the optimal concentration of Atonik solution for selecting drought-tolerant Raja Bulu banana plantlets was 3 mL L^{-1}, while the tolerant concentration of PEG 6000 was 20%. A significant interaction between 3 mL L^{-1} Atonik and 20% PEG 6000 was observed in enhancing plantlet growth and stomatal density, but no significant effect was found on total solute content.

Keywords: Atonik; Drought Stress; In Vitro Culture; PEG 6000; Raja Bulu Banana

1. Introduction

Bananas are among the most widely cultivated horticultural fruit crops in tropical and subtropical regions, serving as an important source of food, income, and nutrition for millions of people. In Indonesia, bananas are grown almost year-round and represent one of the country's most economically significant fruit commodities. The genetic diversity of Indonesian bananas is remarkable, encompassing numerous cultivars with unique characteristics and adaptive potentials. One notable cultivar is the Raja Bulu banana (*Musa × paradisiaca* L.), which is highly appreciated for its sweet flavor, soft pulp texture, and rich antioxidant content. This cultivar is not only consumed fresh as a dessert fruit but is also processed into a wide variety of traditional and commercial food products, thereby contributing to both household economies and the local agroindustry (1,2).

Despite its economic and nutritional potential, banana cultivation faces serious limitations due to various abiotic stresses, with drought being one of the most critical factors affecting growth, yield, and physiological performance. Drought stress adversely influences several physiological and biochemical processes in plants, such as photosynthesis, leaf expansion, and stomatal conductance, ultimately leading to reduced biomass accumulation and productivity. Moreover, water deficit conditions often trigger oxidative stress, which damages cell membranes and inhibits enzyme activities essential for plant metabolism.

* Corresponding author: Endang Nurcahyani

To better understand and mitigate the effects of drought at an early developmental stage, in vitro culture techniques have been widely adopted as a controlled model system. Among various osmotic agents, Polyethylene Glycol (PEG) is commonly employed to simulate drought stress under sterile and reproducible laboratory conditions (3). PEG 6000, a non-ionic and high-molecular-weight polymer, reduces the osmotic potential of the culture medium by binding to water molecules through hydrogen bonding, thereby limiting water availability to plant tissues. Importantly, PEG 6000 is inert and non-toxic to cells, as it does not penetrate plant membranes. Due to these properties, PEG 6000 has become a preferred agent for inducing osmotic stress in tissue culture systems, enabling the selection and evaluation of plant responses to drought stress in a controlled environment (4).

In addition to osmotic agents, the use of plant growth regulators (PGRs) such as Atonik has attracted considerable attention in tissue culture studies. Atonik is a synthetic plant biostimulant containing compounds similar to auxins and phenolic derivatives that promote cell division, elongation, and differentiation. Its application has been reported to enhance root and shoot growth, increase chlorophyll synthesis, and stimulate the activity of antioxidant enzymes, thereby improving plant vigor even under stress conditions. The combined application of Atonik and PEG in vitro may therefore provide valuable insights into how exogenous biostimulants influence plantlet growth, physiological adaptation, and metabolic balance during osmotic stress.

Given this background, this study aimed to evaluate the effect of Atonik and PEG 6000 on the growth performance, carbohydrate content, and stomatal characteristics of Raja Bulu banana plantlets (*Musa × paradisiaca* L.) cultured in vitro. The results are expected to contribute to the development of drought-tolerant banana plantlets through physiological and biochemical screening approaches under controlled conditions.

2. Material and methods

This study was conducted from December 2024 to January 2025 in the In Vitro Culture Room, Botany Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung, Indonesia. The experiment was arranged in a factorial Completely Randomized Design (CRD) with two factors. Factor A was Atonik, consisting of three concentration levels: 0 mL L^{-1} (A0), 3 mL L^{-1} (A1), and 6 mL L^{-1} (A2). Factor B was PEG 6000, consisting of three concentration levels: 0% (B0), 10% (B1), and 20% (B2). These factors were combined into nine treatment combinations, each replicated three times, resulting in a total of 27 experimental units.

The materials used in this study included Raja Bulu banana (*Musa × paradisiaca* L.) plantlets, ethanol (70% and 96%), potassium hydroxide (KOH), polyethylene glycol (PEG 6000), hydrochloric acid (HCl), agar, acetone (80%), sodium hypochlorite (commercial bleach, Bayclin), distilled water, sucrose, Atonik, Murashige and Skoog (MS) medium, heat-resistant plastic, aluminum foil, tissue paper, label paper, and protective masks. All materials and tools were prepared and sterilized under aseptic conditions before use.

2.1. Media Preparation

A ready-to-use Murashige and Skoog (MS) medium was prepared by dissolving 4.43 g L^{-1} MS powder, 30 g L^{-1} sucrose, and 7 g L^{-1} agar in distilled water to a final volume of 1 L. The solution was heated until boiling and poured into culture bottles at a volume of 20 mL per bottle. The medium was sterilized in an autoclave at 121 °C for 15 minutes. After cooling to lukewarm temperature, PEG 6000 was added at concentrations of 0%, 10%, and 20%. The media were incubated for 2–3 days at room temperature (approximately 25 °C) before use.

2.2. Plantlet Sterilization

Raja Bulu banana (*Musa × paradisiaca* L.) plantlets were first soaked in sterile distilled water for 15 minutes, then immersed in a commercial bleach solution (Bayclin) for 30–60 seconds. The plantlets were rinsed twice with sterile distilled water and placed on sterile Petri dishes. All sterilization procedures were performed aseptically inside a laminar airflow (LAF) cabinet.

2.3. Plantlet Planting

Prior to planting, plantlets were induced with Atonik solution by soaking them in Atonik diluted with distilled water at concentrations of 0 mL L^{-1}, 3 mL L^{-1}, and 6 mL L^{-1} for 30 minutes. The plantlets were then transferred into their respective treatment media using sterile forceps under aseptic conditions.

2.4. Observation

Observations were carried out for four weeks after planting to determine the optimum concentration of Atonik under drought stress conditions. The observed parameters were as follows:

2.4.1. Number of Living Plantlets

The number of living plantlets was recorded and expressed as a percentage using the following formula (5):

$$\text{Persentage of living plantlets} = \frac{Number\ of\ Living\ Plantlets}{Total\ number\ of\ plantlets} x100\%$$

2.4.2. Plantlet Visualization

The visual appearance of plantlets was categorized into three color classes: Green (G), Yellowish-green (YG), and Brown (B). The data were presented in percentage form, calculated using the following formula (5):

$$\text{Persentage of plantlets color} = \frac{Number\ of\ planlets\ 0f\ each\ color\ (\text{G,YG,or B})}{Total\ number\ of\ plantlets} x100\%$$

2.4.3. Carbohydrate Content Analysis

The carbohydrate content was analyzed using the phenol–sulfuric acid method as described by (6). Approximately 0.1 g of leaf tissue was weighed and finely ground, followed by the addition of 10 mL of distilled water. The homogenate was then filtered through Whatman No. 1 filter paper, and the filtrate was collected in a test tube.

A 1 mL aliquot of the filtrate was mixed with 1 mL of concentrated H_2SO_4 and 2 mL of phenol solution. The mixture was allowed to react, and the resulting solution was transferred into a cuvette. The absorbance was measured using a UV–Visible spectrophotometer at a wavelength of 490 nm. The carbohydrate concentration was determined based on a standard glucose calibration curve.

2.4.4. Stomatal Density

Stomatal density was determined by collecting leaf samples from the plantlets and fixing them in 70% ethanol. A thin layer of clear nail polish was applied to the abaxial (lower) surface of the leaf to obtain a transparent impression. After air-drying, a piece of transparent adhesive tape was carefully pressed onto the polished area, gently peeled off, and mounted on a microscope slide.

The samples were observed under a compound light microscope at 10 × 10 magnification, connected to an Optilab imaging system. The number of stomata was counted in a defined microscopic field, and stomatal density was calculated using the following formula (7):

$$\text{Stomatal density} = \frac{\text{Number of Stomata}}{Area\ \text{of the field of view}\ (\text{mm}^2)}$$

3. Results and discussion

3.1. Number of Living Plantlets and Plantlet Visualization

Observations of the survival percentage of *Raja Bulu* banana (*Musa × paradisiaca* L.) plantlets over four weeks under various Atonik and PEG 6000 treatments showed clear differences in plantlet viability and morphological appearance. Atonik was applied at concentrations of 0 mL L^{-1}, 3 mL L^{-1}, and 6 mL L^{-1}, while PEG 6000 was applied at concentrations of 0%, 10%, and 20%.

The results indicated variation in the percentage of surviving plantlets and in their visual characteristics across the different treatment combinations. The data on plantlet survival are presented in Table 1, while plantlet visualization percentages (categorized as green, yellowish-green, and brown) are presented in Table 2.

Table 1 Percentage of living Raja Bulu banana plantlets (*Musa* × *paradisiaca* L.) under different combinations of Atonik and PEG 6000 concentrations over four weeks

Atonik concentration (mL L^{-1})	PEG 6000 Concentration (%)	Week I	Week II	Week III	Week IV
0	0	100	100	100	100
	10	100	100	100	100
	20	100	100	100	100
3	0	100	100	100	100
	10	100	100	100	100
	20	100	100	100	100
6	0	100	100	100	100
	10	100	100	100	100
	20	100	100	100	100

Table 2 Visualization and percentage of Raja Bulu banana plantlets (*Musa* × *paradisiaca* L.) per week under different Atonik and PEG 6000 treatments

Atonik (mL L^{-1})	PEG 6000 (%)	Week I	Week II	Week III	Week IV
0	0	G: 100	G: 100	G: 100	G: 100
	10	G: 100	G: 80; YG: 20	G: 100	YG: 100
	20	G: 100	G: 80; YG: 20	G: 80; YG: 20	G: 70; YG: 30
3	0	G: 100	G: 100	G: 100	YG: 100
	10	G: 100	G: 80; YG: 20	YG: 100	YG: 100
	20	G: 100	G: 100	G: 100	G: 80; YG: 20
6	0	G: 100	G: 30; YG: 70	YG: 100	YG: 100
	10	G: 100	G: 30; YG: 70	YG: 100	YG: 100
	20	G: 100	G: 30; YG: 70	YG: 100	YG: 100

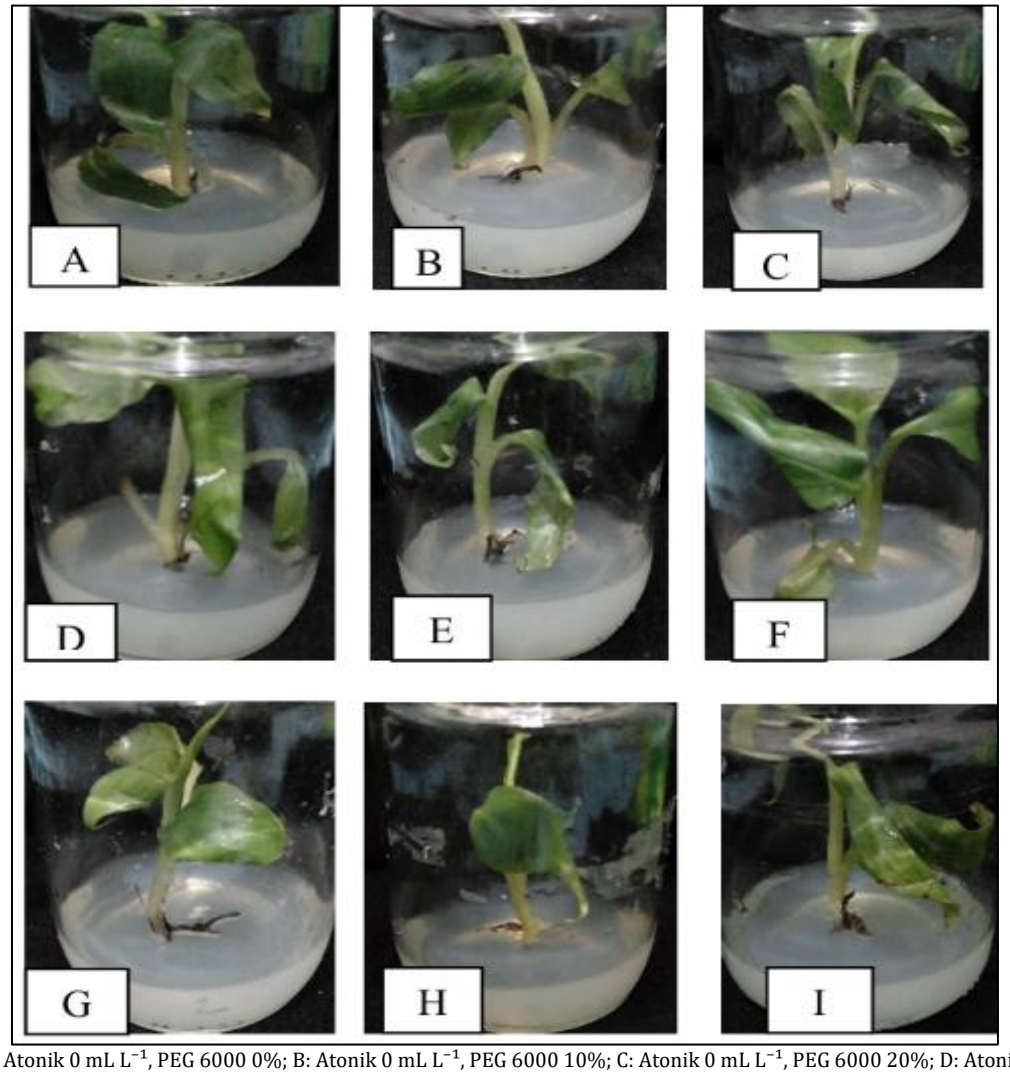
Notes:G = Green; YG = Yellowish-green; B = Brown (not observed in this study).

Table 2 shows that plantlet visualization gradually shifted from green (G) to yellowish-green (YG) with increasing PEG 6000 concentrations, indicating physiological stress under osmotic conditions. Treatments with higher Atonik concentrations (6 mL L^{-1}) combined with PEG 6000 at ≥ 10% tended to maintain plantlet viability but induced chlorophyll degradation, as reflected by the higher percentage of yellowish-green plantlets after the third and fourth weeks.

Based on Table 1 and Table 2, during the first week, the treatments with Atonik and PEG 6000 showed no noticeable effects on the plantlets, as indicated by the 100% survival rate and the uniformly green color of the plantlets. In the second week, however, the combined treatments of Atonik and PEG 6000 began to show visible effects, as indicated by the gradual color change of the plantlets from green to yellowish-green. This response reflects the physiological reaction of Raja Bulu banana plantlets to osmotic stress induced by PEG and Atonik treatments. According to [8], plants that are sensitive to stress typically exhibit visible changes, such as leaf discoloration from green to yellowish-green or brown.

Atonik is commonly used in plant tissue culture to promote root initiation and enhance shoot elongation. In addition, it functions as an antioxidant that helps reduce the accumulation of free radicals and lipid peroxidation, thereby minimizing the risk of tissue browning (9). Meanwhile, the use of PEG 6000 in selection processes can induce the development of plant lines with higher antioxidant activity, which tend to exhibit less browning under stress conditions

(10). The morphological appearance of Raja Bulu banana plantlets after four weeks of growth under different treatment combinations is presented in Figure 1.



Description: A: Atonik 0 mL L^{-1}, PEG 6000 0%; B: Atonik 0 mL L^{-1}, PEG 6000 10%; C: Atonik 0 mL L^{-1}, PEG 6000 20%; D: Atonik 3 mL L^{-1}, PEG 6000 0%; E: Atonik 3 mL L^{-1}, PEG 6000 10%; F: Atonik 3 mL L^{-1}, PEG 6000 20%; G: Atonik 6 mL L^{-1}, PEG 6000 0%; H: Atonik 6 mL L^{-1}, PEG 6000 10%; I: Atonik 6 mL L^{-1}, PEG 6000 20%

Figure 1 Raja Bulu banana (*Musa* × *paradisiaca* L.) plantlets after four weeks of growth under different Atonik and PEG 6000 treatments.

The visual observation presented in Figure 1 shows that the combination of Atonik and PEG 6000 treatments produced clear morphological differences among Raja Bulu banana plantlets. Plantlets grown in media without PEG (0%) generally exhibited vigorous growth with bright green leaves and normal morphology (Figure 1A, 1D, 1G). In contrast, plantlets treated with higher PEG concentrations (10–20%) showed gradual reductions in leaf greenness and vigor, accompanied by yellowish-green coloration as an early indication of osmotic stress (Figure 1B, 1C, 1E, 1F, 1H, 1I). The application of Atonik at 3 mL L^{-1} appeared to mitigate some of the negative effects of PEG, maintaining greener leaf color and more robust plantlet growth, particularly under 20% PEG treatment (Figure 1F). Meanwhile, the highest concentration of Atonik (6 mL L^{-1}) tended to result in excessive tissue elongation and slight chlorosis, suggesting that this concentration may exceed the optimal level for stress tolerance induction. Overall, the morphological responses indicate that moderate Atonik supplementation (3 mL L^{-1}) combined with PEG 6000 at 20% concentration produced the most balanced growth response under in vitro drought stress conditions.

3.2. Carbohydrate Content Analysis

The total soluble carbohydrate content of Raja Bulu banana (*Musa* × *paradisiaca* L.) plantlets cultured under different combinations of Atonik concentrations and PEG 6000 levels in MS medium is presented in Table 3.

Table 3 Average total soluble carbohydrate content (mg g^{-1} FW) of Raja Bulu banana (*Musa* × *paradisiaca* L.) plantlets after four weeks of in vitro cultivation under different Atonik and PEG 6000 treatments.

Atonik (mL L^{-1})	**PEG 6000 (%)**			**Mean**
	0	10	20	
0	0.87 ± 0.31	1.04 ± 0.34	0.95 ± 0.42	0.95
3	1.71 ± 0.41	1.94 ± 0.22	1.53 ± 0.21	1.72
6	1.46 ± 0.67	1.38 ± 0.28	1.28 ± 0.60	1.37
Mean	1.34	1.45	1.25	

Notes: μ = Ȳ ± SE; Ȳ = Mean carbohydrate content; SE = Standard Error; FW = Fresh Weight

Based on Table 3, the total soluble carbohydrate content of Raja Bulu banana plantlets varied under different combinations of Atonik and PEG 6000 treatments. In general, plantlets treated with 3 mL L^{-1} Atonik showed a higher average carbohydrate content (1.72 mg g^{-1} FW) compared to those treated with 0 mL L^{-1} and 6 mL L^{-1} Atonik. This indicates that an intermediate concentration of Atonik could enhance metabolic activity and carbohydrate synthesis, possibly due to its stimulatory effect on physiological processes such as photosynthesis and respiration.

Meanwhile, increasing PEG 6000 concentration from 0% to 20% tended to reduce carbohydrate accumulation, indicating that osmotic stress induced by PEG limited photosynthetic activity and sugar metabolism. Under drought-like conditions simulated by PEG, plantlets experience reduced water uptake and stomatal closure, which leads to a decline in carbon assimilation and consequently lowers carbohydrate synthesis. These results align with the concept that osmotic stress restricts carbohydrate production as an adaptive mechanism to maintain osmotic balance and cell turgor.

Based on Table 3, the analysis of variance showed that the interaction between Atonik and PEG 6000 did not have a significant effect on the total soluble carbohydrate content in Raja Bulu banana plantlets. This result suggests that Atonik, which belongs to the auxin group of plant growth regulators, primarily functions to promote root and shoot growth by enhancing the activity of respiration and photosynthetic enzymes rather than directly influencing carbohydrate accumulation (11). The present findings corroborate the report by (12), indicating that Atonik application had no significant effect on the total soluble carbohydrate content in *Citrus nobilis* (Siam Pontianak orange) plantlets under *in vitro* conditions.

Overall, the data indicate that while Atonik promotes general plant vigor and growth, PEG-induced osmotic stress tends to suppress carbohydrate accumulation due to limited photosynthetic efficiency. However, moderate Atonik application (3 mL L^{-1}) may partially mitigate this stress effect by enhancing enzymatic activities related to energy metabolism.

3.3. Stomatal Density

Table 4 Average stomatal density of Raja Bulu banana (*Musa × paradisiaca* L.) plantlets after Atonik and PEG treatments.

Atonik (mL L^{-1})	PEG 6000 (%)			Mean
	0 %	10 %	20 %	
0	5.51±0.225^{b}	6.17±0.760^{b}	6.65±0.780^{bc}	6.11
3	7.61±0.182^{cd}	8.64±0.176^{d}	9.98±0.408^{e}	8.74
6	6.62±0.417^{bc}	3.89±0.168^{a}	3.27±0.165^{a}	4.59
Mean	6.58	6.23	6.63	

Note: μ = Ȳ ± SE; Ȳ = Mean stomatal density; SE = Standard error

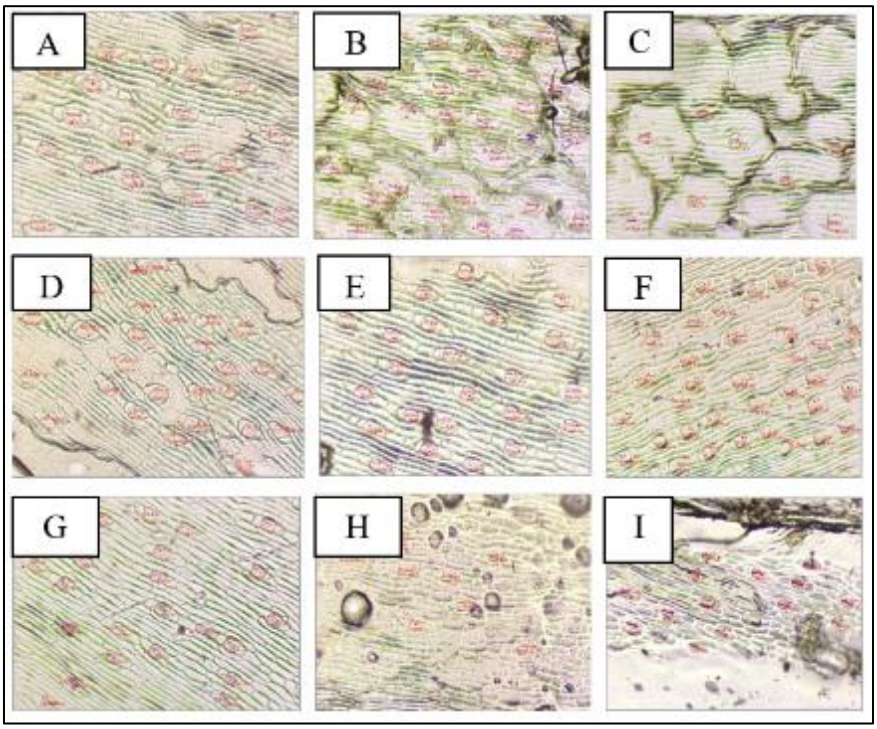
Based on Table 4, the analysis of variance showed that the combination treatment of Atonik and PEG 6000 produced significantly different effects on the stomatal density of *Raja Bulu* banana plantlets. The treatment with 3 mL L^{-1} Atonik combined with 20% PEG 6000 resulted in the highest stomatal density, reaching 9.98 mm^{-2}, which was significantly higher than most other treatments. This indicates that Atonik at a moderate concentration (3 mL L^{-1}) can stimulate stomatal formation, particularly under moderate osmotic stress induced by PEG.

Atonik contains plant growth regulators (PGRs) such as naphthalene acetic acid (NAA) and nitrophenolic compounds, which enhance cellular metabolism and differentiation, including the development of guard cells in the stomatal apparatus (13). According to (14), Atonik can improve the anatomical structure of leaf tissues in plants under stress; therefore, even though PEG reduces the water potential of the medium, Atonik continues to promote the formation of new stomata. This demonstrates that the metabolic stimulation induced by Atonik may mitigate the negative physiological effects of osmotic stress caused by PEG. Plants with higher stomatal density generally exhibit a greater transpiration capacity and enhanced gas exchange compared to those with lower stomatal density (15).

Similar findings were reported by (16), who found that auxin analogs can increase stomatal density by regulating guard cell development under stress conditions. Conversely, at higher Atonik concentrations (6 mL L^{-1}), stomatal density decreased significantly. This decline may be attributed to hormonal imbalance or oxidative stress, which can inhibit cell proliferation in leaf epidermal tissue. Meanwhile, increasing PEG concentration (up to 20%) tended to elevate stomatal density in the absence of excessive Atonik, suggesting that moderate drought stress induced by PEG can trigger adaptive anatomical adjustments.

These results align with (17), who reported that osmotic stress stimulates plants to increase stomatal density as a compensatory mechanism to maintain gas exchange and photosynthetic efficiency. However, excessive hormonal stimulation or stress intensity can suppress this adaptive mechanism, leading to a reduction in stomatal number.

Overall, the combination of 3 mL L^{-1} Atonik and 20% PEG 6000 appears to provide the optimal balance between stress induction and physiological adaptation in *Musa × paradisiaca* L. plantlets. The stomata on the lower epidermal surface of *Raja Bulu* banana leaves are shown in Figure 2.



Description: A : Atonik 0 mL L^{-1}, PEG 6000 0%; B : Atonik 0 mL L^{-1}, PEG 6000 10%; C : Atonik 0 mL L^{-1}, PEG 6000 20%; D : Atonik 3 mL L^{-1}, PEG 6000 0%; E : Atonik 3 mL L^{-1}, PEG 6000 10%; F : Atonik 3 mL L^{-1}, PEG 6000 20%; G : Atonik 6 mL L^{-1}, PEG 6000 0%; H : Atonik 6 mL L^{-1}, PEG 6000 10%; I : Atonik 6 mL L^{-1}, PEG 6000 20%

Figure 2 Stomata on the lower surface of Raja Bulu banana (*Musa × paradisiaca* L.) leaf at 100× magnification.

4. Conclusion

Based on the results and discussion of this study, it can be concluded that the combination treatment of Atonik at a concentration of 3 mL L^{-1} and PEG 6000 at 20% had a notable interaction effect on the growth performance and stomatal characteristics of Raja Bulu banana (*Musa × paradisiaca* L.) plantlets. The moderate concentration of Atonik effectively stimulated cell metabolism and stomatal formation, helping the plantlets maintain physiological activity under osmotic stress induced by PEG. Although the interaction did not significantly increase the total soluble

carbohydrate content, Atonik application was able to mitigate the negative effects of PEG-induced drought stress by maintaining plantlet vigor and promoting adaptive responses at the tissue level.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors have no conflicts of interest.

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