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December 2021

DOI: <https://doi.org/10.15294/biosaintifika.v13i3>

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Effects of BAP and Lighting Duration on Banana (*Musa paradisiaca* cv. Raja Bulu) Micropropagation

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Submitted: 2020-07-01. Revised: 2021-09-24. Accepted: 2021-11-10

Abstract. This study aims to determine the best concentration of 6-Benzilaminopurine (BAP) and lighting duration for Raja Bulu banana cultivar micropropagation. The explant was taken from Pagubugan Village, Cilacap Regency, Central Java. The experiment was arranged as a Randomized Completely Block Design (RCBD) with two factors. The first factor was BAP, with concentration used were 0; 2.5; 5; 7.5 mg/L and the addition of Indole-3-acetic acid (IAA) 0.5 mg/L for all treatment. The second factor was lighting treatment. All explants were put in dark incubation for two weeks followed by light duration treatment: 12 hours of light/day and 16 hours of light/day for the next two weeks. The addition of BAP increased the number of shoots, but did not affect time for shoot initiation, shoot height, number of leaves and number of roots. The best BAP concentration was 2.5 mg/L with an average number of 3.2 shoots/explant. Scalps were formed in the treatment of BAP 5 mg/L and 7 mg/L after four weeks of incubation and has the potential to become new shoots. Nevertheless, the lighting duration did not affect all observed variables.

Key words: BAP, light duration, Raja Bulu, scalp, shoot

How to Cite: Manurung, B. Y., Dewi, P. S., & Dwiati, M. (2021). Effects of BAP and Lighting Duration on Banana (*Musa paradisiaca* cv. Raja Bulu) Micropropagation. *Biosaintifika: Journal of Biology & Biology Education*, 13(3), 284-289.

DOI: <http://dx.doi.org/10.15294/biosaintifika.v13i3.25173>

INTRODUCTION

Banana is one of the most important fruits in the world because they have many health benefits and can supply human nutritional needs (Falcomer et al., 2019). Banana is the most widely cultivated tropical fruit plants. Global banana demand continues to increase throughout the year, this can be seen an increase in global banana production. Based on FAO data, global banana production reached 115.7 million tons in 2018. Lim (2012) reported that Indonesia has an indigenous banana cultivar, Raja Bulu. It has 6-9 hands and 14-17 fruits per hand. Various Raja Bulu banana cultivars are found in Indonesia, such as Raja Bulu Kuning released by the Ministry of Agriculture Indonesia with Decree Number 388/Kpts/SR.120/1/2009 which has 5-7 hands and 13-17 fruits per hand. However, both cultivars are different when compared to the local Raja Bulu banana cultivar from the Pagubugan Village Cilacap Regency Central Java, which has 7-10 hands and 20-22 fruits per hand. This banana is usually used at the wedding ceremony in Javanese culture. The fruit can be consumed directly or processed, while other parts can be used as traditional medicine, animal feed and organic fertilizer (Kumar et al., 2012; Suseno, 2017).

Raja Bulu cultivar is very susceptible to Panama (*Fusarium oxysporum*) and Cordana leaf spot (*Cordana musae*) disease. These diseases can inhibit

banana growth and reduce banana production and quality (Sari et al. 2018; Soesanto, Mugiastuti, and Ahmad 2013). Banana is usually propagated conventionally by division of suckers arising from the main stem or the underground corm. In addition, conventional propagation has low growth capacity, few shoots and time to obtain shoots is relatively long. Moreover, disease transmission from the mother plant to the new suckers are also the problems of this conventional propagation. Banana plant propagation through tissue culture offers clonal propagation with pathogen free, faster time and larger quantities.

Plant growth regulators (PGR) are usually added to growing media to speed up the multiplication of shoots and roots. The most appropriate PGR used for banana multiplication are from cytokinin and auxin group. BAP and IAA are the most effective combination of cytokinin and auxin for banana *in vitro* propagation. Deo & Pradhan (2017) reported the use of 4 mg/L BAP on *M. paradisiaca* multiplication becomes more effective when combined with 0.5 mg/L IAA which produced seven shoots. Srivastava et al. (2012) and Suseno (2017) found that BAP 6 mg/L was the best treatment with an average of 6.83 and 8.2 shoots per explant, respectively. External factors such as light duration also have important effects on *in vitro* propagation of banana plants. Abdel-Aal et al. (2016) reported that *in vitro* banana incubation with a

dark combination of 2 weeks followed by lighting 16 hours of light/day resulted in an average of 13.39 shoots/explant. Banana cultivar is a tropical plant which has 12 hours lighting duration/day. Experiments on the light duration effect on Raja Bulu banana cultivar by in vitro propagation have never been done, therefore further research is needed.

This study aims to observe the best BAP concentration and light duration treatment on micropropagation of Raja Bulu banana cultivar. It is expected to obtain the right concentration and duration of efficient light duration for growth and multiplication of shoots.

METHODS

Explant preparation: The plant material used was the local Raja Bulu banana cultivar from the farmer's garden in the Pagubugan Village Cilacap Regency Central Java. The part that was taken as the explant was a sword sucker with a healthy broodstock. Sword sucker was separated from the parent weevil and then washed thoroughly in running water. Leaves, midribs, roots and parts of the tuber that were not used were cut using a knife to a size of 3 x 3 x 3 cm. Finally, it was soaked with commercial detergent for two hours and washed thoroughly using running water for 20 minutes.

Sterilization of explants: The sterilization process is divided into three stages. Explant was prepared by removing the outer tissue layer using a knife. The explant was soaked in 96% ethanol for 1 minute, followed by 10% hydrogen peroxide for 10 minutes to remove dirt from the layer gap. The explant was soaked in 20% sodium hypochlorite for 20 minutes followed with 30% sodium hypochlorite for 10 minutes, respectively. The explant was then washed once, using sterile distilled water and then soaked in a mixture of carbendazyme and cefotaxime for 30 minutes and finally rinsed with sterile water for three times.

Inoculation stage: The explant was inoculated on Murashige and Skoog media (1962) supplemented with BAP 4 mg/L. The lighting equipment was cool white fluorescence lamp Phillips 18 watts with 16 hours/day and 8 hours light/day duration as recommended by Singh et al. (2011). Light intensity was 1500 lux at 26°C and incubated for 4 weeks. Furthermore, the explant was cut longitudinally and subcultured on the same media for 4 weeks.

Multiplication stage: The expanded shoot cluster was cut transversely 2 mm above the growing point. The medium used was MS medium (Murashige and Skoog, 1962) modified with the addition of 10 mg/L ascorbic acid, 0.5 mg/L IAA in all

treatment and 0 (P₀), 2.5 (P₁), 5 (P₂), or 7.5 (P₃) mg/L BAP. Furthermore, explants were planted one each aseptic culture vessel. Explants that had been planted were incubated dark for 2 weeks, followed with lighting duration treatment. Light was given at a light intensity of 3000 lux with a temperature of 28°C. The light duration treatment consisted of 12 hours light/day (C₁) and 16 hours light/day (C₂) until the fourth week. The following was a combination of treatments:

C₁P₀: 0 mg/L BAP + 12 hours of light/day
 C₂P₀: 0 mg/L BAP + 16 hours of light/day
 C₁P₁: 2.5 mg/L BAP + 12 hours of light/day
 C₂P₁: 2.5 mg/L BAP + 16 hours of light/day
 C₁P₂: 5 mg/L BAP + 12 hours of light/day
 C₂P₂: 5 mg/L BAP + 16 hours of light/day
 C₁P₃: 7.5 mg/L BAP + 12 hours of light/day
 C₂P₃: 7.5 mg/L BAP + 16 hours of light/day

Observed Variables: The observed variables were explant growth, with parameters such as time for shoot initiation (day after planting/dap), number of shoot and shoot height (cm), number of leaves and number of roots. Time for shoot initiation was observed every day after 2 mm shoots appeared. The number of shoots was calculated based on the number of shoots that appeared every day in each explant. The shoot height was measured with a caliper. The number of leaves was calculated based on the number of leaves formed. The number of roots was calculated according to the number of roots formed until the end of the observation. After all data was obtained, data analysis was performed.

Data Analysis: This study used a factorial Randomized Complete Block Design (RCBD) with two factors at the multiplication stage. The first factor was the concentration of BAP and the second factor was light duration treatment as described in the multiplication stage. Quantitative data were analyzed using analysis of variance with two factors and continued with the Least Significant Difference (LSD) test using *Statistical Research for Agricultural Research* (STAR) program. The result was analyzed with *CurveExpert* 1.3. Qualitative data were analyzed descriptively.

RESULTS AND DISCUSSION

Time for shoot initiation

The result of the recent experiment showed that shoot initiation was formed in the first week of multiplication stage (Figure 1a). The shoots were whitish under dark incubation (Figure 1a-b) and turned green in color having transferred to incubation with proper light intensity (Figure 1c-d). As many as 89% of the total explants with BAP treatment showed

shoot multiplication. However, there was no significant effect of BAP treatment and lighting duration on the shoot initiation. The average time of shoot initiation was 5.42 day after planting (dap) with the fastest and the longest initiation time was three and 14 dap, respectively. Ratnasari et al. (2016) reported that *M. paradisiaca* cv. Kepok Kuning had an average shoot time of 5.69 dap and Triharyanto et al. (2018) an average of 9.63 dap on *M. paradisiaca* cv. Raja Bulu. Explants ability to form shoot was affected by phytohormones, nutrients, metabolites, and interaction between various factors (Shahnawaz et al. 2014).



Figure 1. Explant growth response; (a) first week, (b) second week, (c) third week, (d) fourth week, (e) shoot growth of the explant supplemented with BAP 0 mg/L in the fourth week, shoot multiplication of explant supplemented with BAP 2.5 mg/L (f), BAP 5 mg/L (g), and BAP 7.5 mg/L (h) in the fourth week, (i) rooted explants, (j) occurrence of scalps at BAP 5 mg/L in the fourth week and (k) the scalp appearance at BAP 7.5 mg/L in the fourth week of light treatment.

Number of Shoot and Shoot Height

BAP increased the shoot multiplication of Raja Bulu banana cultivar, but did not affect the shoot height (Figure 1e-h). Hapsoro et al. (2017) also reported that number of shoots were increased with the addition of 0-8 mg/L BAP but no the shoot height on Raja Bulu banana cultivar. In addition Shahnawaz et al. (2014) also found that the height of the shoot was influenced by the type and concentration of auxin on Grand Nine cultivar. The results show that BAP stimulates cell division for shoot multiplication and inhibits shoot height (Elma et al. 2017; Ratnasari. et al. 2016). Further test results showed that 2.5 mg/L BAP gave efficient results on the multiplication of

this local Raja Bulu banana cultivar with an average of 3.2 shoots (Table 1). The result was higher than that reported by Yusnita et al. (2015) and Elma et al. (2017) that 2.5 mg/L BAP showed the best response on multiplication of Raja Bulu banana cultivar compared to other concentrations, with an average of 1.1 and 1.33 shoots per explant, respectively. The Quadratic Fit curve analysis showed that the BAP concentration was positively correlated with the addition of shoots. The R value obtained was 0.9995875. This value was near perfect so that the correlation was very strong with an S value of 0.0559017. The regression equation was obtained $Y = 1.45 + 8.675x - 7.5x^2$, therefore the maximum BAP concentration was 5.8 mg/L with a total of 3.9585 shoots (Figure 2). This result was close with Suseno (2017) who reported that 6 mg/L BAP treatment was the maximum concentration for multiplication of Raja Bulu banana cultivar with 8.2 shoots per explant.

Figure 3 shows that the control treatment entering the stationary phase after the third week (light incubation) but conversely the other treatments were still in the exponential phase. This shows there was an interaction between BAP concentration and light intensity to spur the growth of shoots. However light duration treatment did not affect the shoot multiplication and shoot height. Dobisova et al. (2017) reported that *CKII* gene plays an important role in BAP and light interaction, but until now the mechanism of action was unclear. Physiologically the BAP concentration was responsible for cell totipotency, thus affecting the rate of shoot formation (Pereira et al., 2018).

High BAP concentration can induce meristem clump (scalp) on bananas tissue culture. In this study scalp was found in 5 mg/L and 7.5 mg/L BAP treatment (Figure 1j-k). Scalp was characterized by white nodules that appear in groups. Sipe & Davey (2012) reported that 7 mg/L BAP also induced scalp formation in banana cultivars of Mas, Nangka, Berangan and Awak. Occurrence of scalp formation was influenced by the type of cytokinin and genotype used (Annisa, Setiaji, and Sasongko 2020). The appropriate sub-culture treatment can bring up new shoots through these nodules (Sukmadjaja, Purnamaningsih, and Priyatno 2016).

Table 1. Effect of BAP concentration on number of shoots of banana cultivar Raja Bulu

BAP concentration	Number of Shoots
0 mg/L	1.4±0.26b
2.5 mg/L	3.2±0.71a
5.0 mg/L	3.9±0.81a
7.5 mg/L	3.8±0.66a
LSD 0.05	1.541
CV	18.76%

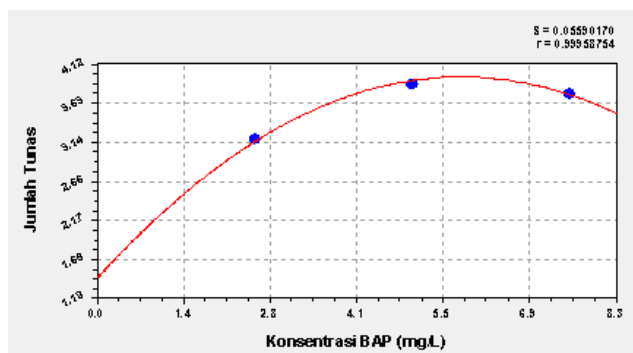


Figure 2. Relation between BAP concentration (X) and number of shoots (Y) in Quadratic Fit analysis with Curve Expert.

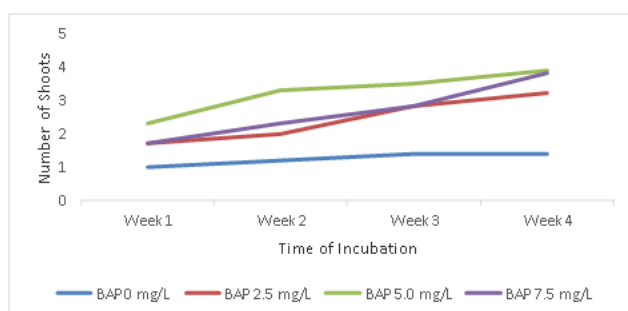


Figure 3. Effect of various BAP concentrations on the number of shoots in four weeks of incubation.

Number of leaves

There was no significant difference between the lighting duration and BAP treatment for the number of leaves formed. Some leaves were formed under no light condition in the first and second week, but were increased dramatically in the third and fourth week with the light treatment (Figure 4). This shows that leaf formation was induced by the presence of light as reported by Bhaya & Al-RazzaqSalim (2019) and Trivedi & Sengar (2017). The main regulator of leaf initiation is the hormonal response and then activated by the presence of light. Light directly regulates the transcription factor E2F associated with the photorespiratory cycle. Furthermore, the stimulus for cell proliferation and cytoplasmic growth occurred between 6-24 hours after exposure to light (Mohammed et al. 2018).

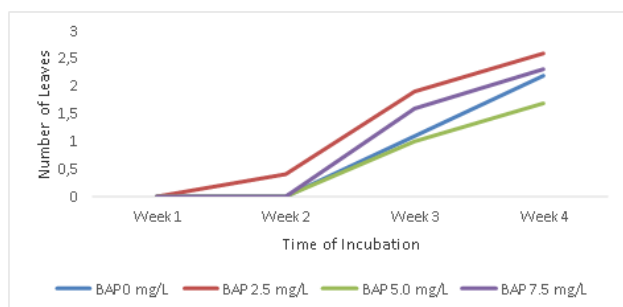


Figure 4. Effect of various BAP concentrations on the number of leaves for four weeks.

Number of Roots

The number of roots was strongly influenced by the presence of light but was not influenced by light duration and BAP treatment. Figure 5 shows a better increase in the number of roots during dark incubation compared to light incubation in the BAP 0 mg/L treatment, but lower in 2.5; 5 and 7.5 mg/L BAP treatment. In control treatment, the length of the main root and the number of lateral roots increased but the length of the root hair decreased when the explants were incubated under the dark condition. The dark condition also reduced the sensitivity of major roots to plant hormones, such as abscisic acid, brassinolide, and BAP, while sensitivity to IAA increases (Van-Gelderens, Kang, and Pierik 2018). In addition, 2.5; 5 and 7.5 mg/L BAP treatment inhibited root formation during the dark incubation but showed a better response to the lighting treatment. Street et al. (2016) explained that cytokines would affect the formation of ethylene in plants thereby inhibiting the formation of main roots. Cytokinins causes anti-gravitropic growth so that root growth was inhibited downward, a balance of auxin and cytokinin hormones was needed to stimulate good root and shoot formation (Waidmann et al. 2019). The presence of light in the third week activated the rate of photosynthesis. Photosynthesis is used by plants to produce energy to run metabolism and indirectly helps the formation of auxin. Furthermore auxin was transplanted from apical to basal meristem of banana shoots to initiate the formation of new roots (Napier 2017; Peer et al. 2011).

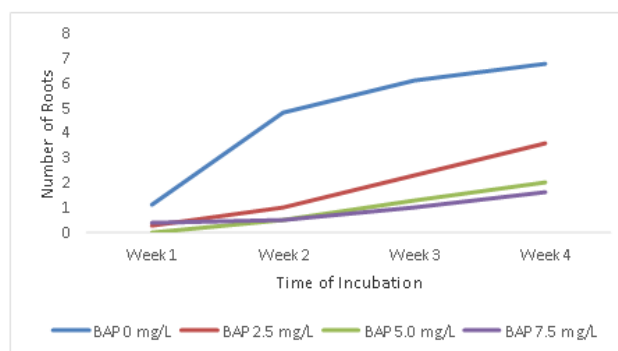


Figure 5. Graph of the effect of various BAP concentrations on the number of roots in four weeks

This study revealed that Raja Bulu banana cultivar formed more shoots when treated with 2.5 mg/L BAP and 12 hours of light/day incubation. The use of concentration and duration of lighting can be a reference for researchers and the Raja Bulu banana tissue culture industry in the future to save energy and production costs.

CONCLUSION

This study found that 2.5 mg/L with 12 hours of light/day was the best treatment for shoot multiplication of the local Raja Bulu banana cultivar. Giving light more than 12 hours/day has no effect on the addition of shoots. In addition, higher concentrations of BAP can induce scalp formation in local Raja Bulu explants.

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