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Research Article

The Growth of *Alocasia macrorrhiza* (L) Schott Variegata Roots on Murashige and Skoog Medium Supplemented 6-Benzyl Amino Purine (BAP) and Kinetin

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ABSTRACT
The sente variegata plant (Alocasia macrorrhiza variegata) is a species of ornamental taro. The unique leaf color of sente variegata, which is a combination of white and green, attracts ornamental plant lovers for hunting or collecting. There has been no cultivation effort from the ornamental plant suppliers and traders for multiplication purposes until now. Sente variegata is obtained directly from the forest. This activity can cause extinction. Micropropagation efforts using plant tissue culture techniques are needed for commercial and sustainability issues. This study aimed to determine the effect of 6-Benzyl Amino Purine (BAP) and kinetin on the root formation and growth as one step in the shoot multiplication of sente variegata bulb explants. Variations in BAP and kinetin concentrations used separately in this study were 0, 2, 5, and 10 mg. L^{-1}. The results showed that the application of BAP and Kinetin significantly affected the root growth of sente Variegata. Finally, kinetin 10 mg L^{-1} is the concentration and type of cytokinins recommended for the formation and root growth of sente variegate.

Key words: *Alocasia;BAP; Kinetin; Root; Variegata.*

Introduction

Sente variegata (*Alocasia macrorrhiza* (L) Schott variegata) is a type of taro that has the sap. This plant is an ornamental plant that is in great demand because of the uniqueness of its leaves which have a combination of green and white colors (Asih et al., 2022). Sente also contains phenol derivative compounds such as flavonoids, polyphenols and cyanogenetic glycosides (Masykuroh & Puspasari, 2022). Indonesian people has used sente to help treat coughs (Anjelia et al., 2021). So far, sente variegata is collected directly from the forest. Until now, no cultivation effort from the flower producer for sente variegata propagating. Direct exploitation from the forest can provoke the extinction of a species (Fay, 2018; Williams et al., 2018). Therefore, it is necessary to have a propagation effort using plant tissue culture techniques to commercialize sente Variegata.

Plant tissue culture techniques have proven to be effective in producing plants in a short time (Sjahril et al., 2019; Masykuroh & Puspasari, 2022; Bhaswatimayee et al., 2021). Recently, this technique has been used for conservation of endangered species (Oseni et al., 2018). First, the type and optimal concentration of plant growth regulators (PGR) or hormones for shoots and root inducing needs to know (Heriansyah,

2019). The application of PGR in a plant tissue culture is essential for growing and controlling organogenesis and organ morphogenesis of explants (Hesami et al., 2018; Soumare et al., 2021; Ahuja, 2021; Yachya et al., 2022).

This study aimed to determine the effect of 6-Benzyl Amino Purine (BAP) and kinetin on the root formation and growth, a stage of the shoot multiplication of sente variegata bulb explants. The type and concentration of optimal hormones for the root formation and growth are essential (Justamante et al., 2022). According to Yachya et al (2020), high concentrations of hormones could be toxic for plants, but low concentration can cause no growth response. Variations in BAP and kinetin concentrations used separately in this study were 0, 2, 5, and 10 mg L^{-1}. Knowing the optimal concentration of both hormones could accelerate the supply of sente variegata plantlets on the market. Finally, it could reduce the exploitation activities, and the sustainability of sente variegata is safer.

Materials and Methods

The study used materials, such as autoclaves, Petri dishes, tweezers, analytical balances, stirrers, pipettes, hotplate magnetic stirrers, pH meters, culture bottles, Erlenmeyer glass, beaker glass, laminar air flow cabinet, 70% (v/v) alcohol, bleach solution, equates, antibacterial, fungicides, MS (Murashige and Skoog) mineral components, agar, filter paper, sterile water, tissue paper, aluminum foil, 1 N KOH solution, and 1 N HCl solution, BAP, kinetin, bulbs of the plant *A. macrorrhiza* variegata.

1. Explant preparation

This study used *A. macrorrhiza variegata*. Bulbs as explants from local ornamental plant sellers in Blitar, East Java Province, Indonesia. The explants preparation used a brush and a 5% (v/v) detergent solution under running water for 30 minutes to remove the soil. Surface sterile used 5% (v/v) detergent solution for 10 minutes and then washed in sterile distilled water three times. The sterile was continued again using 10% (v/v) bleaching solution (contain 5,25 % sodium hypochlorite) for 10 minutes. The sterile was followed by rinsing in sterile distilled water three times then the explants were ready to use.

2. Medium Preparation

The basal medium used Murashige and Skoog (MS) composition (Dalton, 2020), supplemented with 8 g L^{-1} agar, 3% (w/v) sucrose, BAP, and kinetin. Both hormones were applied separately at concentrations 0, 2, 5, and 10 mg L^{-1}. Each treatment was repeated 6 times. The acidity of the medium was set at 6.0 using KOH or HCl. Sterilization used an autoclave at 121°C for 20 minutes.

3. Culture condition

The cultures were incubated for six weeks on an incubation rack with a fluorescent light intensity of 2000-2500 lux. The setting of the lighting period was 12 hours of light and 12 hours of darkness. The temperature in the incubation room during culture was manipulated at 24-26°C.

4. Analysis dates

The data obtained at the end of the culture were the time of emergence, number, and length of roots. The data were analyzed using the ANOVA test with a significance level of $\alpha = 0.05$. If there were a significant difference, the test was continued to Tukey.

Results and Discussion

Results

This study aimed to determine the best type and concentration of both cytokinins, such as kinetin and BAP, for root growth of sente variegata (*A. macrorrchiza variegata*) bulb explants. The indicators were used, such as by the time of emergence, number, and length of roots. The hormones (BAP and kinetin) were applied separately at 0-10 mg L-1. Observations were conducted during eight weeks of incubation.

1. Root emergence time

The results of this study showed that kinetin treatment had a significant effect on the root emergence time. An increase in kinetin concentration was followed by an increase in the root emergence time (Figure

1 A). The initial emergence time in all kinetin treatments (2-10 mgL^{-1}) was relatively the same and longer (23-25 days after planting) than the control (14.67 days after planting). The BAP treatment also showed the same results (Figure 1 B). However, the best root emergence time was achieved at 2 mg L^{-1} BAP (such as 14 days after planting), which was not significantly different from the control (such as 12.67 days after planting).

2. Number of roots

The results showed that kinetin treatment had no significant effect on the number of roots. However, the root numbers at 10 mg L^{-1} kinetin (7.33 roots/explant) were higher than the control (5.67 roots/explant) (Figure 2 A). The same results were observed on BAP treatment (Figure 2 B). Application of 2-10 mg L^{-1} BAP had no significant effect on the root numbers. However, 2 mg L^{-1} BAP (4.33 roots/explant) was higher.

3. Root length

The results showed that the root length was significantly affected by Kinetin and BAP (2-10 mg L^{-1}). Increased concentrations of kinetin and BAP had a positive impact on the root length (Figure 3 A and B). The root length at 2-10 mgL^{-1} was relatively similar, but higher than the control. The application of kinetin and BAP at the same concentration (10 mg L^{-1}) achieved the best root length (such as 1.10 and 0.47 cm/explant, respectively). The root morphology of the two hormonal treatments at various concentrations is presented in Figure 4.

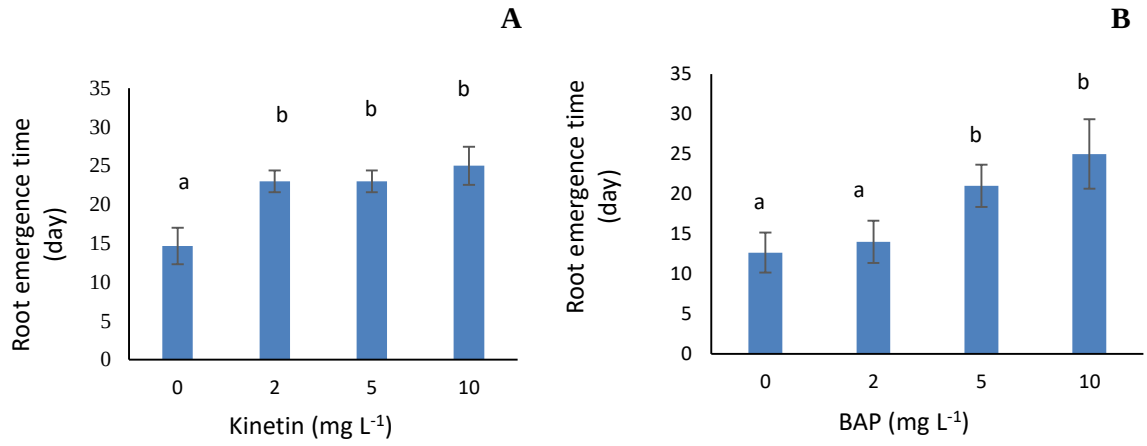

Figure 1. Emergence time of roots of sente variegata (*A. macrorrhiza* variegata) bulb explants treated with A. Kinetin and B. BAP for eight weeks of incubation. Means with common letters are not signifificantly different at $P > 0.05$ according to Tukey's multiple range test.

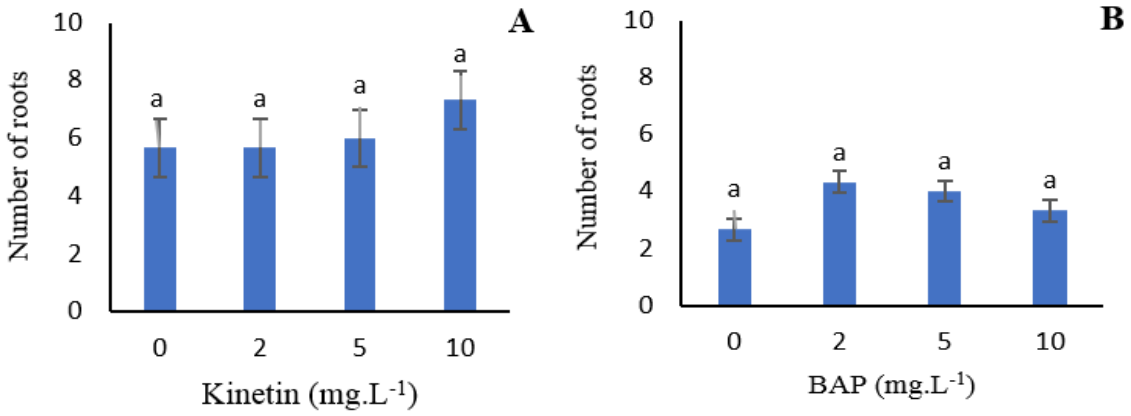

Figure 2. The average number of roots of sente variegata (*A. macrorrhiza* variegata) bulb explants in A. kinetin and B. BAP. Hormone. Means with common letters are not signifificantly different at $P > 0.05$ according to Tukey's multiple range test.

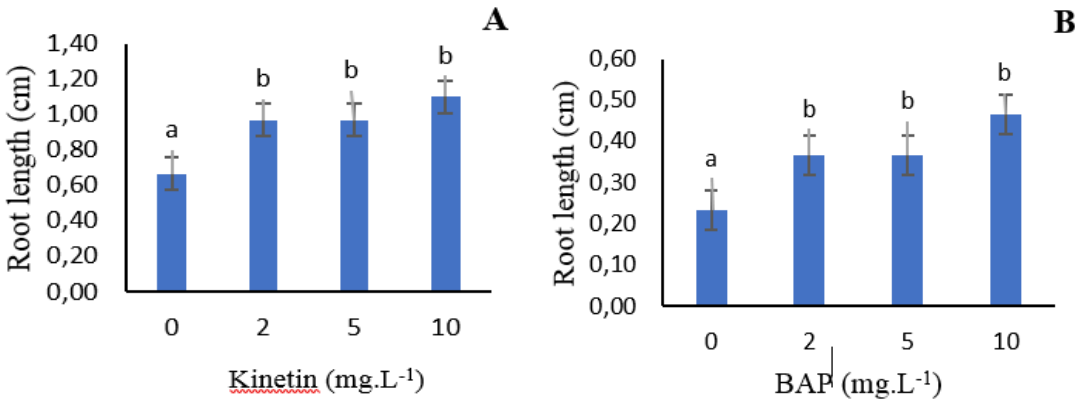

Figure 3. Root length of sente Varigata (*A. macrorrhiza* variegata) bulb explants treated with A. kinetin and B. BAP during eight weeks of incubation. Means with common letters are not significantly different at $P > 0.05$ according to Tukey's multiple range test.

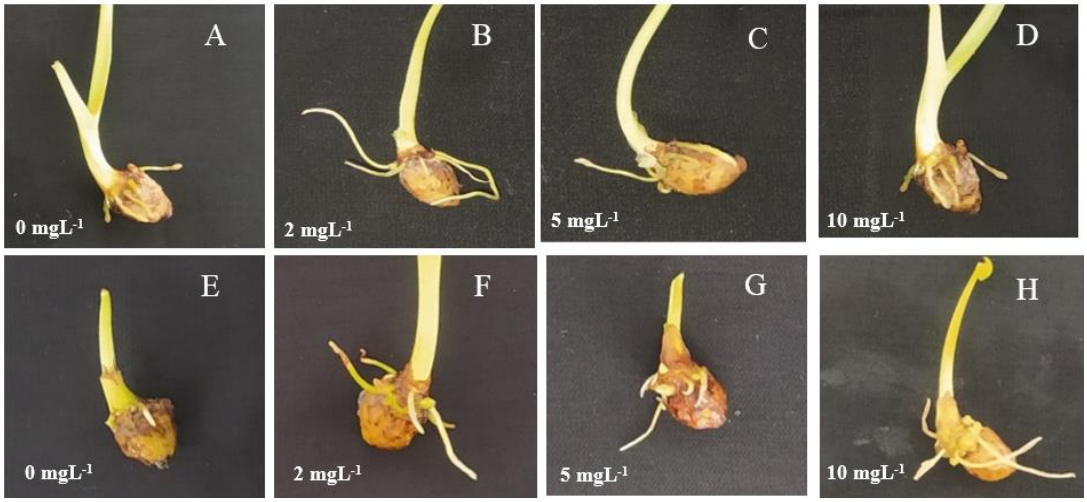

Figure 4. Root growth of sente variegata (*A. macrorrchiza* variegata) explants in (A-D) BAP and (E-H) kinetin treatments with varying concentrations of 0-10 mg L^{-1}.

Discussion

Kinetin treatment (2-10 mg L^{-1}) for eight weeks of culture inhibited the root emergence in sente variegata bulb explants (Figure 1 A). This result is similar to the report of Mawaddah et al (2021), when 0.1-10 mg L^{-1} kinetin was applied to *Globba leucantha*. Delayed root emergence also occurred in the BAP treatment (Figure 1 B). This result was similar to the report of Sulasiah et al (2015), where the use of low concentrations of BAP (0.75-1.25 mg L^{-1}) had an impact on slowing down the emergence time of *Dendrobium sp*. Application of BAP and kinetin was also reported negatively for the root formation of *Cattleya maxima* shoots, but was positively for rooting of *Phalaenopsis amabilis* (Saravia-Castillo et al., 2022). The negative influence of BAP and kinetin application on the root emergence time of sente variegata bulb explants indicated that cytokinins did not play a critical role in root initiation. Yachya et al (2020), have demonstrated that auxin (IBA) and ethylene are crucial in the root initiation in *Talinum paniculatum* cuttings.

In the number of roots, the treatment of kinetin and BAP (2-10 mg L^{-1}) did not accelerate the root number (Figure 2). However, 10 mg L^{-1} kinetin and 2 mg L^{-1} BAP could induce the roots with the highest number, such as 7.3 and 4.33 roots/explant, respectively, compared to other treatments

and control. A similar increase in the root formation using 10 mg L^{-1} kinetin on *Globba leucantha* var. bicolor Holttum (3.67 roots/explant) was reported by Mawaddah et al (2021). Application of 0.5-1 mg L^{-1} kinetin on *Dendrobium phalaenopsis* was recorded to increase number of roots (2.5 roots/eksplant) compared to the control (1.5 roots/explant) (Mahadi, 2017). Yulia et al (2020), reported the application of BAP in low concentration (2 mg L^{-1}) increased the number of *Cymbidium orchid* roots. In contrast, different results showed 2.5-10 mg L^{-1} BAP inhibited the root numbers of *Garlic sp* (Karjadi & Buchory, 2007). According to Rishu et al (2019), the application of kinetin with various concentrations does not affect the root formation because kinetin plays a role in the cell division and is dominant in the shoot multiplication rather than the root formation.

The application of kinetin and BAP has also impacted on the root length (Figure 3 A and B). The treatment of kinetin at 2-10 mg L^{-1} accelerated all root growth of sente Variegata, mainly at 10 mg L^{-1} (1.10 cm/explant). Obaid (2018), reported a relation result, a concentration of 4 mg L^{-1} kinetin significantly increased the *Citrus aurantifolia* root length. Similarly, the treatment of kinetin, 10 mg L was significant for growing roots in BAP treatment. This result corresponded with Zulkifli & Sari. (2019), where the BAP 10 mg L^{-1} significantly accelerated the root growth of *Musa paradisiaca*. L. In contrast, Nehnevajova et al (2019), reported that cytokinins was negative regulators of *Brassica napus* root growth. Finally, 10 mg L^{-1} kinetin is the concentration and type of cytokinins recommended for the formation and root growth of sente variegata bulb explants.

Conclusions

The addition of BAP and Kinetin hormones significantly affected the root growth of sente variegata (*Alocasia maccrorrhiza*). Kinetin 10 mg L-1 was the best hormone and concentration for growing sente variegata (*A. macrorrhiza* variegata) roots.

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