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Optimization of Jasmonic Acid and Benzylaminopurine on the Growth of the Raja Bulu Banana in MS Media

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ABSTRACT

Cultivation of the raja bulu banana (*Musa paradisiaca* var. *raja bulu*) is carried out on a large scale through tissue culturing. However, the slow growth of raja bulu banana seeds necessitates the use of PGRs to improve the growth of the cultured explants. This optimization was studied at the Plant Physiology and Biotechnology Laboratory, Faculty of Agriculture, UNS Surakarta, April–August 2024. A completely randomized study design was used with two factors: the concentrations of jasmonic acid (JA) and benzylaminopurine (BAP). The same concentrations were used (0, 0.5, 1, and 1.5 ppm) with three repetitions. Observational data were analyzed using analysis of variance, and if the treatments were significantly different, then processed by DMRT at a 5% significance level. The results showed that a combination of JA and BAP in MS media could increase the root lengths of Raja Bulu banana subcultures. The number of leaves could be increased in the absence of additional JA. JA at 0.5 ppm shortened the time of root emergence by 15.9%, and at 1 ppm shortened the time of shoot emergence by 8.92%. BAP at 1.5 ppm could increase the number of shoots of Raja Bulu banana subcultures by 64.72%. JA at 1 ppm and BAP at 0 ppm was the best combination for increasing the length of the roots of Raja Bulu banana subcultures, by up to 12.12%.

Key words: cytokinin, horticultural plants, plant hormone, tissue culture

INTRODUCTION

Indonesia is one of the top ten banana-producing countries in the world, ranking third behind India and China with a production of 7,280,659 tons (FAO, 2019). The largest banana-producing regions in Indonesia are the provinces of East Java, West Java, and Lampung, growing multiple varieties. The Raja Bulu banana (*Musa paradisiaca* var. raja bulu) has a large market in the Middle East, so cultivation is carried out on a larger scale using tissue culture seeds. The obstacle in conventional banana cultivation is the difficulty of obtaining quality seedlings in large quantities quickly and sustainably; to overcome this, banana seedlings are often multiplied in vitro or as tissue cultures (Sutejo et al., 2017).

Tissue culture can produce healthy, disease-free planting material in large quantities in a short time, with uniform growth (Ziraluo, 2021). The obstacle to in vitro propagation of raja bulu banana seeds is their slow growth caused by the specific nature of the explants, including very strong apical dominance and difficulties in proliferating to form shoots (Maulida et al., 2018). The endogenous hormones of raja bulu banana plantlets are not sufficient to stimulate leaf growth, so exogenous hormones and growth regulators at precise concentrations must be added to the tissue culture medium (Nofiyanto et al., 2019).

Plant hormones that can regulate physiological events in plants include jasmonic acid (JA), while the most commonly used PGR is benzylaminopurine (BAP) from the synthetic cytokinin group. BAP exhibits high activity in stimulating cell division in plant tissue culture. Research from Liang et al. (2024) showed that low concentrations of JA—of 0.5 and 5 μmol L^{-1}—may promote tuber morphogenesis by inducing cell enlargement in the tuber perimedullary zone in potato plants grown in vitro. The use of BAP is also common in tissue culture, for example, to induce shoot multiplication in bananas (Reddy et al., 2014; Pereira et al., 2018). However, the concentration of BAP needs to be well controlled, as higher concentrations can lead to lower numbers of shoots produced (Pereira et al., 2018) or the production of abnormal shoots (Reddy et al., 2014). In this study, we will investigate the optimum concentrations of JA and BAP to be applied in raja bulu banana subculture, which has not previously been reported. In addition, this study aims to describe the interaction between JA and BAP in

MS media and the effects on the growth of the raja bulu banana subculture, and determine the optimal combination of concentrations for its growth.

MATERIALS AND METHODS

The research was conducted in the Plant Physiology and Biotechnology Laboratory, Faculty of Agriculture, Universitas Sebelas Maret, from April to August 2024. The raja bulu banana multiplication explants used were derived from a secondary subculture of young banana plant stumps with an explant diameter of approximately 0.7–1 cm. Other materials used included basic MS media stock solution, JA, and BAP. The research equipment included an autoclave, LAF, pH meter, hotplate, and magnetic stirrer. A completely randomized design was implemented, using two treatment factors, the concentrations of JA and BAP. Concentrations used of each were 0, 0.5, 1, and 1.5 ppm, resulting in 16 treatment combinations. The entire experiment was replicated three times, resulting in 48 experimental units. The JA dose level was selected based on the research of Vural et al. (2018), who studied the growth of potato tubers in vitro and obtained optimal results at a dose of 1 ppm. In addition, according to Hewedy et al. (2023), higher concentrations of JA, can produce worse outcomes in cultured plants. The selection of BAP concentration was based on the research of Rodinah et al. (2018), who studied the growth of banana shoots at various BAP concentrations and found that 0.5 ppm produced the largest number of shoots.
The research procedure consisted of preparing equipment and materials, sterilization, preparation of ZPT stock solution, media preparation, subculture, maintenance and care, and observations. Data were analyzed using analysis of variance (ANOVA). In cases where significant differences were found, this was followed by the Duncan Multiple Range Test (DMRT) at the 5% level. Descriptive analysis was also carried out and presented in the form of tables and figures.

RESULTS AND DISCUSSION

Time of Shoot Emergence

Shoot growth was recorded daily after it was first seen on the planted explants. According to Ubaidah et al. (2019), emergence time of the first shoot is an indicator of treatment effects. ANOVA showed that JA significantly affected the shoot emergence time of raja bulu banana subcultures. The fastest shoot emergence time was observed with the application of 1 ppm JA, significantly different from the other treatments (Table 1), with an increase of 8.92% compared to control.

Table 1. Effect of JA on shoot emergence time of raja bulu banana subcultures.

JA (ppm)	Shoot Emergence Time (days)
0	6.50 ± 0.692 c
0.5	7.75 ± 0.191 b
1	5.92 ± 1.104 d
1.5	9.75 ± 1.605 a

Note: Numbers followed by the same letter in the same column are not significantly different according to Duncan's Multiple Range Test (DMRT) at the 5% level.

JA plays an important role in regulating plant growth. Yang et al. (2019) explain that this occurs due to interaction or crosstalk between JA and other hormones. Research by Park et al. (2019) demonstrated that pre-treatment of *Arabidopsis hypocotyl* seeds with JA derivates significantly increased the rate of de novo explant shoot regeneration. This may be due to the involvement of the CORONATINE-INSENSITIVE 1 (COI1) protein, an essential component of the JA perception machinery. COI1 plays a key role in this process, and shoot regeneration was significantly reduced in JA-pretreated COI1-1 mutant explants. Additionally, COI1 might have other roles in facilitating crosstalk between auxin and cytokinin signaling, or in regulating pluripotency acquisition by influencing cell division rates (Park et al., 2019). Therefore, JA is thought to play a role in influencing the time of shoot emergence in callus of the raja bulu banana. The DMRT 5% test data (Table 1) shows that treatment with 1.5 ppm JA (the highest concentration) delayed the time of shoot emergence by 50% compared to the control treatment. Hewedy et al. (2023) explained that high concentrations of JA inhibit cell mitosis triggers through the disruption of meristem activity, ultimately inhibiting cell division and resulting in delayed emergence of shoots. JA is known to have both positive and negative effects on plant growth, depending on the concentration and interactions with other hormones. In this study, the application of 1 ppm JA had an optimal effect on shoot emergence time, while the application of 1.5 ppm JA delayed shoot emergence.

Number of Shoots

Quantification of the number of shoots is performed by counting those that appear on the explants at the end of each observation. ANOVA

indicated that each JA and BAP treatment had a highly significant effect on the number of shoots of raja bulu banana subcultures. The number of shoots of raja bulu banana plants with various concentrations of JA and BAP are shown below (Table 2).

Table 2. Effect of JA and BAP on the number of shoots of raja bulu banana subcultures.

JA (ppm)	Average	BAP (ppm)	Average
0	3.17 ± 0.059 c	0	2.58 ± 0.471 d
0.5	2.00 ± 0.883 d	0.5	3.25 ± 0.000 b
1	4.33 ± 0.766 a	1	2.92 ± 0.236 c
1.5	3.50 ± 0.177 b	1.5	4.25 ± 0.707 a

Note: Numbers followed by the same letter in the same column were not significantly different according to Duncan's Multiple Range Test (DMRT) at the 5% level.

In this study, applying 1 ppm JA had the greatest effect on the number of shoots. This concentration of JA is thought to promote cell division and differentiation without causing growth inhibition. The application of 1.5 ppm BAP also had a significant positive effect on the number of shoots. This is thought to be because this concentration of BAP stimulates cell division and shoot proliferation.

The number of raja bulu banana subculture shoots was significantly different after the application of 1 ppm JA than the other treatments (Table 2). Treatment with 1 ppm JA produced the most shoots, with an increase of 36.59% compared to the control treatment. The application of 0.5 ppm JA decreased the number of shoots by 36.91% compared to the control treatment. Heinrich et al. (2021) state that the concentration of JA should be adjusted to balance growth and protection; while increasing the concentration of JA increases protection, it also causes plant growth inhibition. Low concentrations of JA will have a stimulatory effect that triggers shoot growth. JA applied exogenously at high concentrations can increase plant defense but disrupt plant growth and fitness.

The application of 1.5 ppm BAP resulted in significantly higher numbers of shoots compared to the other treatments (Table 2) This indicates that 1.5 ppm BAP is the most effective concentration for promoting shoot proliferation, resulting in a 64.72% increase over the control treatment. Elma et al. (2017) reported that applying BAP to a raja bulu banana culture medium induced shoot formation in a short time. The treatment with 1.5 ppm BAP in the medium has the most shoots, (4.25), while the treatment with 0 ppm BAP had the least shoots (2.58). Consistent with the results reported by Trisnawati et al. (2023), the addition of 0.5, 1.0, and 1.5 ppm BAP to the treatment medium produced more shoots than the control (0 ppm BAP). BAP is a PGR that belongs to the cytokinin group, which plays a role in various plant growth and development processes, including shoot growth. The number of shoots increases because BAP plays a role in stimulating cell division and differentiation and can accelerate shoot proliferation.

Shoot Height

Measurement of shoot height was carried out at the end of the observation. Saputri et al. (2019) measured shoot height using a ruler or tape measure by attaching it parallel to the media in the culture bottle containing the explant. The measurement was made from parallel to the shoot growth point to the end point of the shoot. ANOVA indicated that BAP significantly affected the shoot height of raja bulu banana subcultures (Table 3). JA and the interaction of JA and BAP had no significant effect on the shoot height.

Table 3. Effect of BAP on the shoot height of raja bulu banana subcultures.

BAP (ppm)	Shoot height (cm)
0	5.54 ± 0.367 b
0.5	7.92 ± 1.313 a
1	5.50 ± 0.396 b
1.5	5.28 ± 0.549 b

Note: Numbers followed by the same letter in the same column are not significantly different according to Duncan's Multiple Range Test (DMRT) at the 5% level.

The shoot height of raja bulu banana subcultures was significantly different after the application of 0.5 ppm BAP than other treatments (Table 3), indicating this concentration as the optimal treatment in terms of shoot height. Height increased 42.96% compared to the control treatment. It is suspected that at this concentration, a balance is achieved between the concentration of PGRs and endogenous hormones for stimulating cell division. According to Rasud & Anwar (2019), a BAP concentration of 0.5 ppm is the appropriate concentration to obtain the equilibrium or gradient required to stimulate explant growth. The entry of these exogenous growth regulators changes the balance of these effectors in the plant body. Faridah et al. (2017) explained that increasing BAP concentrations can decrease shoot height in some tissue cultures. It is possible that high concentrations of cytokinin or BAP—exceeding the optimum level—can cause the number of shoots to decrease and hinder shoot development. Another cause may be increases in the number of shoots on each explant. The more shoots that

grow on an explant, the lower the average shoot height will be, because the allocation of resources in the media is divided among the shoots. In this study, the application of 0.5 ppm BAP had the optimal effect on shoot height, stimulating cell division and elongation in the stem meristem without causing excessive elongation.

Time of Root Emergence

The timing of root emergence was recorded as the time when roots were first seen after the explant was planted. ANOVA indicated that JA significantly affected the root emergence time of raja bulu banana subcultures (Table 4). The time of root emergence of raja bulu banana plants with various concentrations of JA is shown below (Table 4).

Table 4. Effect of JA on root emergence time of raja bulu banana subcultures.

JA (ppm)	Root emergence time (days)
0	10.50 ± 1.635 c
0.5	8.83 ± 2.813 d
1	12.75 ± 0.044 b
1.5	19.17 ± 4.493 a

Note: Numbers followed by the same letter in the same column are not significantly different according to Duncan's Multiple Range Test (DMRT) at the 5% level.

The root emergence time of raja bulu banana subcultures treated with 0.5 ppm JA was significantly different from the treatment with 1.5 ppm JA (Table 4) and other treatments. This indicates that 0.5 ppm JA is the optimal treatment for accelerating root emergence time, with an average difference in root emergence of 1.67 days and a percentage increase of 15.9% compared to the control treatment. The treatment with the longest root emergence time was the 1.5 ppm JA treatment (19.17 days), with a difference in root emergence time of 8.67 days (a 82.57% decrease compared to the control treatment). According to Gomi (2020), JA applied to the planting medium affects the root system. This is in line with the statement of Huang et al. (2021), who reported that JA plays a role in the process of adventitious root formation in plants. It can therefore be concluded that low concentrations of JA can shorten the root formation process. The slowing of root emergence by JA at high concentrations may be explained by a mechanism of interaction with endogenous auxin hormones, by which JA reduces the expression of genes that regulate auxin transport. This process could reduce auxin activity and subsequently inhibit root growth, especially in primary roots (Ishimaru et al., 2018). This data is supported by data from Ghorbel et al. (2021), showing that a higher concentration of JA in the medium can inhibit the growth of explant organs, one of which is the root.

Number of Roots

Quantification of the number of roots was carried out at the end of the observation. The method, based on Riono (2019), involves looking at the banana explant from outside the culture bottle and counting the total number of roots that grow on each explant. According to ANOVA, BAP significantly affected the number of roots of raja bulu banana subcultures. The number of roots counted with various concentrations of JA are shown below (Table 5).

Table 5. Effect of BAP on root number of raja bulu banana subcultures.

BAP (ppm)	Number of roots
0	6.33 ± 0.015 bc
0.5	9.00 ± 1.870 a
1	6.00 ± 0.250 b
1.5	4.08 ± 1.606 d

Note: Numbers followed by the same letter in the same column are not significantly different according to Duncan's Multiple Range Test (DMRT) at the 5% level.

Treatment with 0.5 ppm BAP was significantly different from other treatments, with a 42.18% increase in root number compared to the control treatment (Table 5); it can be concluded that 0.5 ppm BAP is the optimal treatment to increase the number of roots. Treatment with 1.5 ppm BAP had the least number of roots compared to the other BAP treatments, with a decrease of 33.94% compared to the control treatment. The higher the concentration of BAP, the lower the number of roots, and vice versa.
Low concentrations of BAP can increase the number of roots formed through the stimulation of cell division in root tissue. However, high concentrations can inhibit root growth because BAP, as a cytokinin, can hinder the role of auxin in plants. Fauziah et al. (2019) state that BAP given at low concentrations can increase root numbers, presumably because the endogenous auxin content in explants is already high, the addition of BAP still facilitates root induction. According to Pamungkas & Puspitasari (2019), the addition of cytokinin can restrict the activity of auxin and physiologically inhibit the growth of apical dominance in root explants at high concentrations. In this study, the application of 0.5 ppm BAP had the optimal effect on root number. This is thought to be because this concentration of BAP stimulated cell division and root initiation in the meristem without inhibiting growth. The application of higher

concentrations of BAP (1.0 ppm and 1.5 ppm) had a negative effect on root number, likely due to inhibition of root growth at these concentrations.

Root Length

Root length measurements were carried out at the end of the observation period. Following the method of Putri et al. (2018), the longest root length for each treatment was measured after the 8th week using thread. Measurements were made from the base to the tip of the root, then the thread was measured with a ruler. ANOVA indicated that the interaction of JA and BAP significantly affected the root length of raja bulu banana subcultures. The effects of various combinations of JA and BAP concentrations on root length can be seen in Table 6.

Table 6. Effect of JA and BAP interaction on root length (cm) of raja bulu banana subcultures.

JA (ppm)	BAP (ppm)			
	0	0.5	1	1.5
0	18.40 ± 6.448 b	11.40 ± 1.498 e	6.03 ± 2.297 h	8.10 ± 0.835 f
0.5	14.43 ± 3.643 c	12.80 ± 2.488 d	3.97 ± 3.758 ij	4.57 ± 3.334 i
1	20.63 ± 8.027 a	4.53 ± 3.357 i	6.30 ± 2.108 gh	3.13 ± 4.35 j
1.5	18.57 ± 6.57 b	7.10 ± 1.54 g	6.07 ± 2.27 h	0.47 ± 4.82 j

Note: Numbers followed by the same letter in the same column are not significantly different according to Duncan's Multiple Range Test (DMRT) at the 5% level.

Table 6 shows that increasing concentrations of JA and BAP can suppress root length. The highest average root length was observed in the treatment with 1 ppm JA and 0 ppm BAP and was significantly different from the other treatments. This combination is the optimal concentration to increase root length, with an increase of 12.12% compared to the control treatment. Research conducted by Maia & Pedroso-de-Moraes (2017) shows that JA has a positive effect on root growth. Miclea et al. (2020) added that JA interacts with other PGRs to trigger the expression of related genes, and in this context, JA can also stimulate root growth.

BAP affects root growth in a complex way. According to Hirliana & Ariati (2021), roots are formed when the ratio of auxin to cytokinin concentration is low. Root growth, in addition to being influenced by the application of exogenous auxin, can also be influenced by genetic differences related to the endogenous cytokinin content of the explants used. The treatment that resulted in the shortest root length was the treatment with 1.5 ppm JA and 1.5 ppm BAP, producing a root length of 2.47 cm, 86.58% smaller than the control treatment. Yulia et al. (2020) explained that the application of BAP at high concentrations does not have a positive effect and can even inhibit root initiation and growth.

The regression graph in Figure 1 shows that BAP with increasing JA concentrations resulted in a quadratic pattern. R^2 indicates the model's fit to the data, and the treatment with the most significant influence of BAP on root length was at BAP 1.5 ppm, which is 99.5% and statistically significant. The negative value of the −7.9667x influence coefficient indicates an inverse effect. Applying JA up to a concentration of 1.5 ppm, without BAP, can increase root length, which decreases with the addition of BAP at higher concentrations. This is shown by the linear regression equation model $Y = 2.8667x^2 - 7.9667x + 8.0333$ with a significant p-value = 0.032.

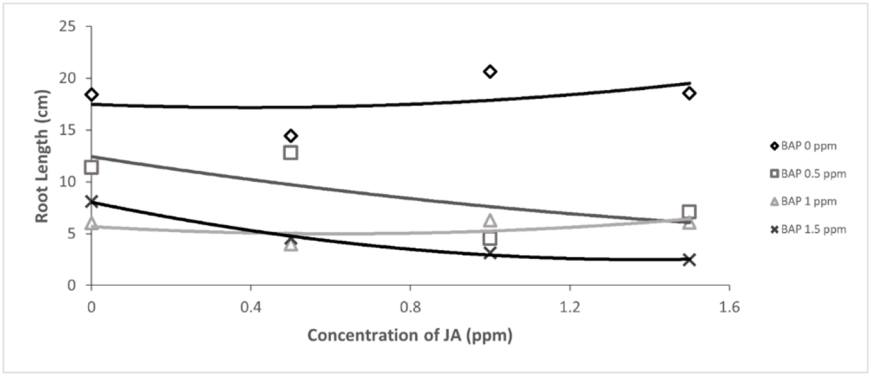

Fig. 1. Interaction regression of JA and BAP combination on root length of raja bulu banana subcultures. Note: Y (BAP 0 ppm) → = $1.9x^2 - 1.51x + 17.478$ → $R^2 = 0.1563$; Y (BAP 0.5 ppm) → = $1.1667x^2 - 5.9833x + 12.425$ → $R^2 = 0.5198$; Y (BAP 1 ppm) → = $1.8333x^2 - 2.2633x + 5.685$ → $R^2 = 0.3189$; Y (BAP 1.5 ppm) → = $2.8667x^2 - 7.9667x + 8.0333$ → $R^2 = 0.9953$.

Leaf Emergence Time

Leaf emergence time is recorded as when the first leaf appears after the explant is planted. Based on the ANOVA test, no treatments or interactions significantly affected leaf emergence time in raja bulu banana subcultures. The histogram of leaf emergence time for raja bulu banana plants with various concentrations of JA and BAP can be seen in Figure 2. It shows that the optimal leaf emergence time response was after treatment with 0.5 ppm JA and 1.5 ppm BAP, 16 days after subculture; this combination gave an increased time by 5.88% compared to the control. The longest leaf emergence time was in the treatment with 1.5 ppm JA and 0 ppm BAP, a decrease of 164.71% compared to the control treatment. The need for endogenous cytokinin hormones in plants has been met, so the media treatment does not significantly affect leaf emergence time. MS media contains several nutrients that are needed by plants Muzayyana et al. (2020), including nitrogen (N), which can accelerate plant vegetative growth. The N element contained in MS media allows optimal synthesis of amino acids and proteins, which function in plants' growth and development processes.

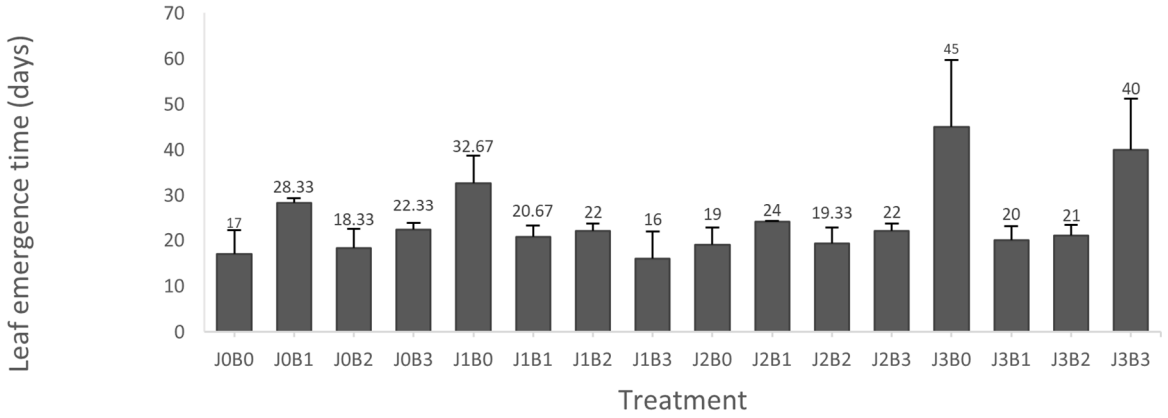

Fig. 2. Histogram of the Effect of JA and BAP on raja bulu banana leaf emergence time. Note: 1. J0B0: JA 0 ppm + BAP 0 ppm; 2. J0B1: JA 0 ppm + BAP 0.5 ppm; 3. J0B2: JA 0 ppm + BAP 1 ppm; 4. J0B3: JA 0 ppm + BAP 1.5 ppm; 5. J1B0: JA 0.5 ppm + BAP 0 ppm; 6. J1B1: JA 0.5 ppm + BAP 0.5 ppm; 7. J1B2: JA 0.5 ppm + BAP 1 ppm; 8. J1B3: JA 0.5 ppm + BAP 1.5 ppm; 9. J2B0: JA 1 ppm + BAP 0 ppm; 10. J2B1: JA 1 ppm + BAP 0.5 ppm; 11. J2B2: JA 1 ppm + BAP 1 ppm; 12. J2B3: JA 1 ppm + BAP 1.5 ppm; 13. J3B0: JA 1.5 ppm + BAP 0 ppm; 14. J3B1: JA 1.5 ppm + BAP 0.5 ppm; 15. J3B2: JA 1.5 ppm + BAP 1 ppm; 16. J3B3: JA 1.5 ppm + BAP 1.5 ppm.

Leaf emergence time was different for each treatment combination (Figure 2). Fauziah et al. (2021) explained that leaf formation is influenced by the number of shoots that appear. The more shoots that form on explants, the more leaf initiation is inhibited. The energy needed for leaf formation is used to elongate other shoot cells, which can slow down leaf emergence time. The histogram of shoot emergence time shows that various concentrations of BAP in the MS media slow down leaf emergence time, followed by increasing concentrations of JA. The lowest average leaf emergence time (i.e., the fastest) was found in various treatments with the addition of JA with 1 ppm BAP. This is consistent with the work of Kartiman et al. (2018), who showed that auxin and cytokinin have effects that encourage and inhibit the process of cell division, depending on the presence of other phytohormones.

Number of Leaves

This variable was measured by counting the number of leaves that appeared on the explants at the end of the observation period. Saputra et al. (2021) describe this measurement as manually counting the number of banana leaves for each treatment and replication. According to ANOVA, JA significantly affected the number of leaves in raja bulu banana subcultures. The number of leaves of raja bulu banana plants with various concentrations of JA is shown in Table 7.

Table 7. Effect of JA on the Number of Leaves of Raja Bulu Banana Subcultures.

JA (ppm)	Number of Leaves (sheets)
0	4.67 ± 0.633 a
0.5	3.83 ± 0.044 bc
1	3.92 ± 0.103 b
1.5	2.67 ± 0.781 d

Note: Numbers followed by the same letter in the same column are not significantly different according

to Duncan's Multiple Range Test (DMRT) at the 5% level.

The number of leaves in raja bulu banana subcultures treated with 0 ppm JA significantly differed from the other treatments (Table 7), indicating this to be the optimal treatment for increasing leaf numbers. The treatment with 0 ppm JA had the highest number of leaves, with 4.67 blades, while the treatment with 1.5 ppm JA had the lowest number of leaves, 42.83% fewer than the control treatment. The concentration of JA is essential in determining the effect on plant leaf growth. Applying JA at high concentrations can cause significant inhibition of leaf development, resulting in fewer leaves. Uyehara et al. (2023) state that several studies have established JA as a plant growth suppressor. Exogenous JA application to explants can suppress cell proliferation, resulting in smaller and fewer leaves. Noir et al. (2018) demonstrated that JA inhibits organ growth by suppressing organ differentiation and duplication in plants, further supporting its role in maintaining the balance between cell proliferation and differentiation.

CONCLUSIONS

The interaction of JA and BAP combinations in MS media can increase the root length of raja bulu banana subcultures. Meanwhile, treatments without JA in MS media can increase the number of leaves; JA 0.5 ppm can accelerate root emergence time; JA 1 ppm can accelerate shoot emergence time; and BAP 1.5 ppm in MS media can increase the number of shoots. The application of JA 1 ppm without BAP is the best combination to increase the root length of raja bulu banana subcultures.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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