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Micropropagation of Wooden Banana (*Musa paradisiaca L. cv. Kayu*) Through In Vitro

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ABSTRAK

Wooden banana (Musa paradisiaca L. cv. Kayu) is a local banana variety that had unique characteristics, including a sweet taste, fragrant aroma, thicker skin, longer shelf life, and high economic value. This banana variety had a long growth period, requiring approximately 9 to 11 months to flower and bear fruit. Additionally, a single plant usually produced only one to three suckers, making wooden banana seedlings rare and difficult to obtain. To prevent its potential extinction, seedling propagation was necessary, with one possible solution being tissue culture techniques. This study aimed to determine the optimal concentration of Benzyl Amino Purine (BAP) and the most effective culture medium for the growth of wooden banana shoots. The research employed an experimental method, testing various BAP concentrations (1 ppm, 2 ppm, 3 ppm, 4 ppm, and 5 ppm) on solid and semi-solid culture media. Data were analyzed descriptively based on the number of shoots produced under each treatment. The results showed that concentration of 3 ppm Benzyl Amino Purine (BAP) produced the highest shoot proliferation. The solid-liquid medium was the most effective culture medium, which produced an average of 4.33 shoots per explant. These findings indicate that appropriate BAP concentration and culture medium significantly enhance in vitro micropropagation of wooden banana.

Keywords: *Benzyl Amino Purine, BAP, Tissue Culture, Micropropagation, Culture Medium, Wooden Banana*

1. INTRODUCTION

Banana were horticultural crops that contributed significantly to Indonesia's agricultural sector due to suitable land, climate, and human resources. According to data from the Central Statistics Agency (BPS) in 2014, banana cultivation was carried out in 33 provinces, including West Nusa Tenggara (NTB). Based on data from the Department of Agriculture and Plantations (2023), banana production in NTB was 837,839 quintals in 2020 and increased to 1,182,336 quintals in 2021.

West Nusa Tenggara (NTB) consisted of two major islands: Lombok Island and Sumbawa Island. Lombok Island had a wide variety of banana cultivars, each with unique advantages and characteristics. According to data from BPTP NTB, the banana cultivars cultivated on Lombok Island included wooden banana, ketip, kelak, kombol, haji, mas jamak, lomak, candi, and others (Rahayu and Fitrahtunnisa, 2014). Local bananas were genetic resources that grew and reproduced naturally in specific regions. These bananas had superior traits often inherited from their parent

plants, such as resistance to climate stress, pest and disease resistance, and distinctive flavors.

Wooden banana was classified into three usage categories: as food, for religious ceremonies, and as medicine (Rai et al., 2018). Based on data from the Directorate General of Public Health (2018), 100 grams of wooden banana contained 23.60 grams of carbohydrates, 1.40 grams of protein, 2.60 grams of dietary fiber, 11 mg of vitamin C, 15 mg of calcium, 493 mg of potassium, and 74.40 grams of water. Wooden banana was one of the commodities with relatively high economic value. Its market price ranged from IDR 30,000 to IDR 40,000 per bunch.

Wooden banana had a longer growth period compared to other banana varieties, requiring approximately 9 to 11 months to flower and bear fruit. A single wooden banana plant typically produced only one to three suckers, making its seedlings scarce and increasingly rare. Farmers often preferred to cultivate other banana cultivars that were easier to propagate and had shorter growth periods, which threatened the genetic resources of wooden banana (Widiastuti et al., 2016). One solution to address this issue was through seedling propagation.

Seedling propagation was usually carried out using two methods: conventional methods and tissue culture. Seedling propagation using tissue culture techniques (in vitro) could produce healthy and uniform banana seedlings in large quantities within a shorter time frame, independent of climatic conditions, ensuring a reliable seedling supply (Riono, 2019). Mass production of seedlings or shoots through this method was known as micropropagation (Gupta et al., 2020).

The success of this technique was determined by several key factors, including plant growth regulators (PGRs) as hormones to stimulate explant growth and tissue culture media as the growth substrate for explants. The PGR used for shoot propagation was typically from the cytokinin group, with *Benzyl Amino Purine* (BAP) being one of the most effective options. BAP functioned to promote shoot growth and had several advantages compared to other cytokinins, such as greater stability, resistance to oxidation, higher effectiveness in stimulating shoots, and affordability (Agustina et al., 2020).

In vitro shoot propagation using micropropagation techniques supported the development of wooden banana cultivation, contributing to increased production. This study focused on the micropropagation of wooden banana (*Musa paradisiaca* L. cv. Kayu) as an essential initial step toward in vitro propagation of this banana variety.

2. MATERIAL AND METHODS

2.1 Research Time and Location

This study was conducted in the Immunology Laboratory and Tissue Culture Laboratory Room, Faculty of Mathematics and Natural Sciences, University of Mataram, located in Mataram, West Nusa Tenggara. The research was conducted from February 2023 to October 2023. The location for collecting wooden banana shoot explants was Mahajani Agro Tourism Garden, Senaru Village, Bayan District, North Lombok Regency.

2.2 Treatment Variables

This study was an experimental study that used various experimental designs. The treatment variables in this study included different concentrations of BAP, which were 1 ppm, 2 ppm, 3 ppm, 4 ppm, and 5 ppm, and different types of culture media, namely solid MS medium and semi-solid MS medium.

2.3 Tools and Materials

The tools used in this study included an autoclave, culture bottles, petri dishes, knife handles, measuring glasses, scissors, hand sprayers, a hot plate, volumetric flasks, a laminar air flow cabinet, a Bunsen burner, a magnetic stirrer, blades, an oven, a pH meter, tweezers, a culture rack, a double-door refrigerator, and an analytical balance. The materials used in this study included agar, distilled water, 70% and 96% alcohol, aluminum foil, the bactericide Agrept, bleach/disinfectant solution (5.25% *sodium hypochlorite*), the fungicide Benlate, sugar, rubber bands, a complete MS medium pack, transparent plastic, plastic wrap, adhesive tape, spirit, tissue, Tween, BAP growth regulator solution at 1000 ppm, and wooden banana (*Musa paradisiaca* L. var. Kayu) shoot explants.

Table 1. Treatment Variables

Code	AP Concentration	Culture Media
MP 1	1 ppm	Solid MS Media + 1 ppm BAP
MP2	2 ppm	Solid MS Media + 2 ppm BAP
MP3	3 ppm	Solid MS Media + 3 ppm BAP
MP4	4 ppm	Solid MS Media + 4 ppm BAP
MP5	5 ppm	Solid MS Media + 5 ppm BAP
MS 0 + 1	1 ppm	Solid-Liquid MS Media + 1 ppm BAP
MS 0 + 2	2 ppm	Solid-Liquid MS Media + 2 ppm BAP
MS 0 + 3	3 ppm	Solid-Liquid MS Media + 3 ppm BAP
MS 0 +4	4 ppm	Solid-Liquid MS Media + 4 ppm BAP
MS 0 + 5	5 ppm	Solid-Liquid MS Media + 5 ppm BAP

2.3 Tools and Materials

The tools used in this study included an autoclave, culture bottles, petri dishes, knife handles, measuring glasses, scissors, hand sprayers, a hot plate, volumetric flasks, a laminar air flow cabinet, a Bunsen burner, a magnetic stirrer, blades, an oven, a pH meter, tweezers, a culture rack, a double-door refrigerator, and an analytical balance. The materials used in this study included agar, distilled water, 70% and 96% alcohol, aluminum foil, the bactericide Agrept, bleach/disinfectant solution (5.25% *sodium hypochlorite*), the fungicide Benlate, sugar, rubber bands, a complete MS medium pack, transparent plastic, plastic wrap, adhesive tape, spirit, tissue, Tween, BAP growth regulator solution at 1000 ppm, and wooden banana (*Musa paradisiaca* L. var. Kayu) shoot explants.

Materials and methods should be described sufficiently to allow others to replicate and build on published results. Please note that the publication of your manuscript implies that you must make all materials, data, computer code, and protocols associated with the publication available to readers.

2.4 Preparation of BAP Solution

A 1000 ppm stock solution of BAP was prepared. To create different concentrations, 1 mL of the stock solution was diluted to 1 ppm, 2 mL was diluted to 2 ppm, 3 mL was diluted to 3 ppm, 4 mL was diluted to 4 ppm, and 5 mL was diluted to 5 ppm. Each concentration was diluted in a total volume of 1000 mL.

2.5 Preparation of Solid MS Medium with BAP

A volumetric flask was filled with 5000 mL of distilled water, which was divided into five treatments. MS medium powder, weighing 22.15 grams (4.43 grams/L), was added to the volumetric flask. Then, 150 grams of sugar (30 grams/L) was added and stirred until homogeneous. Once the mixture became homogeneous, it was divided into five portions and poured into five separate 1000 mL Erlenmeyer flasks. In Erlenmeyer flask 1, 1 mL of BAP was added; in flask 2, 2 mL of BAP; in flask 3, 3 mL of BAP; in flask 4, 4 mL of BAP; and in flask 5, 5 mL of BAP.

The five Erlenmeyer flasks were stirred until the solutions were homogeneous, and the pH of each solution was adjusted to 5.8. If the pH was below 5.8, KOH was added dropwise to raise it, and if the pH exceeded 5.8, HCl was added dropwise to lower it. Next, 8 grams of agar were added to each Erlenmeyer flask, stirred until homogeneous, and heated on a hot plate. Once the medium boiled, it was poured into culture bottles. The bottles were then covered with aluminum foil, sealed with plastic wrap, labeled, and sterilized.

2.6 Preparation of Solid MS Medium without BAP

A volumetric flask was filled with 5000 mL of distilled water, which was divided into five treatments. MS medium powder, weighing 22.15 grams (4.43 grams/L), was added to the volumetric flask. Then, 150 grams of sugar (30 grams/L) was added and stirred until the solution became homogeneous. Afterward, the medium was divided into five portions and poured into five separate 1000 mL Erlenmeyer flasks.

The solutions in the Erlenmeyer flasks were then adjusted to a pH of 5.8. If necessary, KOH or HCl was added dropwise to achieve the correct pH. Next, 8 grams of agar were added to each Erlenmeyer flask, stirred until the mixture

was homogeneous, and heated on a hot plate. Once the medium boiled, it was poured into culture bottles. The bottles were covered with aluminum foil, sealed with plastic wrap, labeled, and sterilized.

2.7 Preparation of Liquid MS Medium with BAP

A volumetric flask was filled with 5000 mL of distilled water, which was divided into five treatments. MS medium powder, weighing 22.15 grams (4.43 grams/L), was added to the volumetric flask. Then, 150 grams of sugar (30 grams/L) was added and stirred until the solution became homogeneous. The medium was then divided into five portions and poured into five separate 1000 mL Erlenmeyer flasks. In Erlenmeyer flask 1, 1 mL of BAP was added; in flask 2, 2 mL of BAP; in flask 3, 3 mL of BAP; in flask 4, 4 mL of BAP; and in flask 5, 5 mL of BAP.

The five flasks were stirred until homogeneous, and the pH of each solution was adjusted to 5.8. The medium was then boiled using a hot plate. Once boiled, the medium was poured into culture bottles. The bottles were covered with aluminum foil, sealed with plastic wrap, labeled, and sterilized.

2.8 Sterilization of Tools and Materials

Clean culture tools were wrapped in paper and secured with adhesive tape. Materials such as distilled water were poured into bottles, covered with plastic, and tied with rubber bands, while square-cut tissue pieces were placed in bottles.

The prepared tools, materials, and media were placed in an autoclave basket and neatly arranged. The basket was then placed inside the autoclave and sterilized for 15–20 minutes at 121°C and 1 atm pressure. After sterilization, the culture tools and materials were transferred to an oven to maintain their sterility. The sterilized media were allowed to cool and then stored on culture racks in the culture room.

2.9 Sterilization of Explants Outside the Laminar Air Flow

The explants used were young banana shoots along with their corms. The shoots and corms were cut and washed under running water, and the outer layers of the corms were peeled with a knife until they appeared clean and white. The leaf sheaths of the banana shoots were trimmed, leaving only 3–4 layers. The shoots were placed in a solution of tween soap, soaked, and occasionally shaken. After soaking, the shoots were rinsed with sterile distilled water. The rinsed shoots were then transferred to a laminar air flow cabinet for further sterilization.

2.10 Sterilization of Explants Inside the Laminar Air Flow

The explants were soaked in a Benlate fungicide solution for 1 hour, occasionally shaken, and then rinsed three times with sterile distilled water. Next, the explants were soaked in an Agrept bactericide solution for 1 hour, occasionally shaken, and rinsed three times with sterile distilled water. The explants were then soaked in 70% alcohol for 1 minute and rinsed once with sterile distilled water. Subsequently, the explants were soaked in a 30% Bayclin solution for 30 minutes and rinsed three times with sterile distilled water. Afterward, the explants were soaked in a 20% Bayclin solution for 20 minutes and rinsed three times with sterile distilled water. The cleaned explants were rinsed again with sterile distilled water. Any damaged parts of the explants caused by the sterilization process were removed, peeling the leaf sheaths until only 2–3 layers remained. Finally, the explants were cut vertically into two parts.

2.11 Culture of Explants on Solid Media

The sterilized explants were placed and planted into culture bottles containing solid MS media with growth regulators (ZPT) in a sterile condition. The explants, which had been cut into two parts previously, were positioned almost horizontally at a slanted angle in the media. After the explants were planted, the culture bottles were sealed with aluminum foil and secured with plastic wrap. The culture bottles were then placed on the culture rack to be observed and incubated at room temperature, approximately 25°C, with light intensity of 1000 lux.

2.12 Culture of Explants on Solid-Liquid Media

The sterilized explants were placed and planted into culture bottles containing solid MS media without growth regulators (ZPT) in a sterile condition. The explants, which had been cut previously, were positioned almost horizontally at a slanted angle in the media. Next, liquid MS media containing ZPT was added until the explants were submerged. The culture bottles were then sealed with aluminum foil and secured with plastic wrap. The culture bottles were placed

on the culture rack for observation and incubated at room temperature, approximately 25°C, with light intensity around 1000 lux.

2.13 Observation

The observation of the response of the banana plant explants (*Musa paradisiaca* L. cv. Kayu) was conducted once a week. The parameters observed included the growth of the number of shoots on different culture media with the same concentration of the growth regulator BAP in different media. The explants were observed for 5 weeks to monitor the growth of shoots that occurred on the explants.

2.14 Data Analysis

The data collected from the observations were analyzed using descriptive data analysis. The observation data consisted of qualitative and quantitative data. The qualitative data included visual observations of the shoots that grew during the 5-week observation period after planting (WAP). The quantitative data included the number of shoots that grew from the explants in each replicate bottle for each type of media over the 5-week observation period (WAP). Furthermore, the quantitative data were subjected to analysis of variance (ANOVA) using RStudio to evaluate statistically significant differences among treatment means, with the assumptions of normality and homogeneity of variances taken into account.

3. RESULT AND DISCUSSION

The results of the study on shoot growth obtained from the in vitro initiation of *Musa paradisiaca* L. var. Kayu explants, grown on solid and solid-liquid Murashige and Skoog (MS) media with the addition of the cytokinin plant growth regulator BAP at different concentrations over a 6-week period after planting (WAP), are shown in Tables 2 and 3.

Based on the results of the ANOVA presented in Table 4 and 5, it can be concluded that the application of different BAP concentrations did not result in a statistically significant effect on the observed parameters, as all p-values were greater than 0.05. Therefore, the null hypothesis (H_0) was accepted. This indicates that the variations in BAP concentration were not sufficient to produce significant differences in the response variables under the conditions of this study.

Table 2. The average number of shoot growth on the in vitro initiation of (*Musa paradisiaca* L. cv. Kayu) explants on solid MS media

Treatment of BAP Concentration	Number of Shoots per Bottle			
	1	2	3	Average
1 ppm	3	1	3	2,33
2 ppm	2	3	3	2,67
3 ppm	3	4	3	3,33
4 ppm	2	4	3	3
5 ppm	3	3	3	3

Table 3. The average shoot growth in the in initiation of explants of banana plants (*Musa paradisiaca* L. cv. Kayu) in vitro on solid-liquid MS media

Treatment of BAP Concentration	Number of Shoots per Bottle			
	1	2	3	Average
1 ppm	1	5	2	2,67
2 ppm	4	1	4	3
3 ppm	6	3	4	4,33
4 ppm	4	3	3	3,33
5 ppm	3	3	4	3,33

Table 4. Analysis of Variance (ANOVA) on Solid MS Media

Source of Variation	df	Sum Sq	Mean Sq	F value	p-value
BAP	4	1,73	0,433	0,722	0,596
Residuals	10	6,00	0,600		
Total	14	7,73			

Table 5. Analysis of Variance (ANOVA) on Solid-Liquid MS Media

Source of Variation	df	Sum Sq	Mean Sq	F value	p-value
BAP	4	4.66	1.167	0.565	0.694
Residuals	10	20.66	2.067		
Total	14	25.33			

The growth response of the banana explant shoots showed the best shoot growth at each BAP concentration cultured on solid MS media and solid-liquid MS media for 5 weeks after planting (MST), could be seen in the figure 1- Fugure 10.

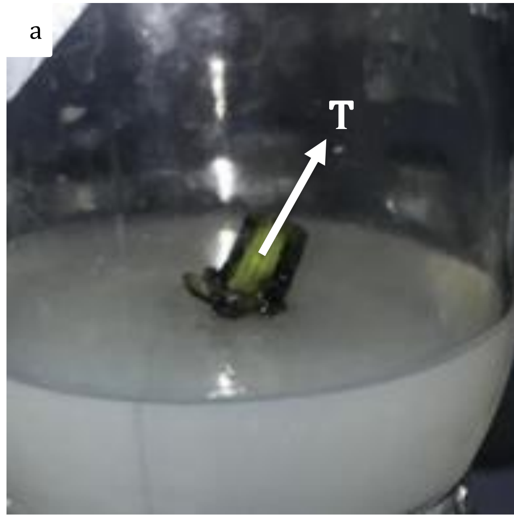

Figure 1. The growth response of banana shoots (*Musa paradisiaca* L. cv. Kayu) on solid MS media (BAP 1 ppm) for 5 weeks after planting (MST).

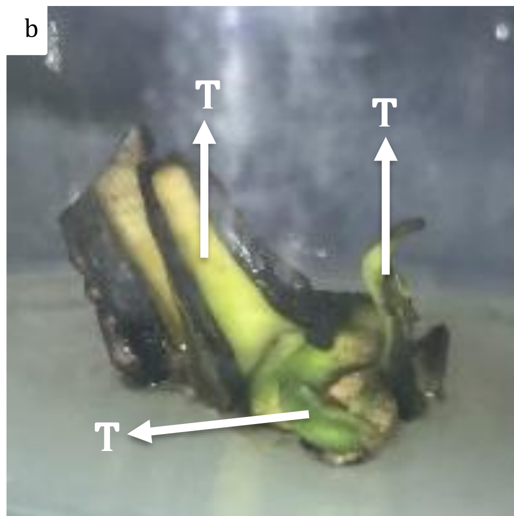

Figure 2. The growth response of banana shoots (*Musa paradisiaca* L. cv. Kayu) on solid MS media (BAP 2 ppm) for 5 weeks after planting (MST).

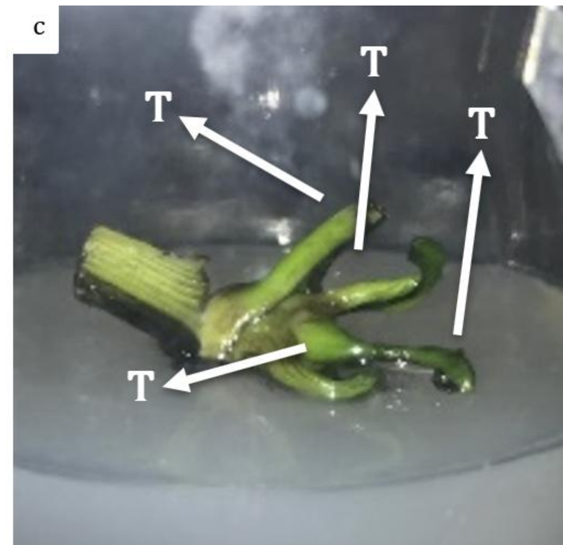

Figure 3. The growth response of banana shoots (*Musa paradisiaca* L. cv. Kayu) on solid MS media (BAP 3 ppm) for 5 weeks after planting (MST).

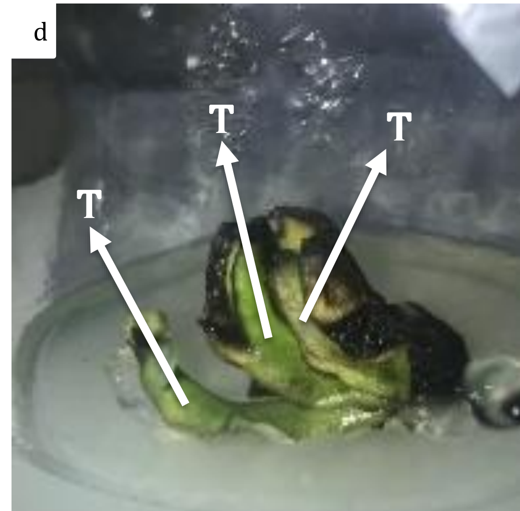

Figure 4. The growth response of banana shoots (*Musa paradisiaca* L. cv. Kayu) on solid MS media (BAP 4 ppm) for 5 weeks after planting (MST).

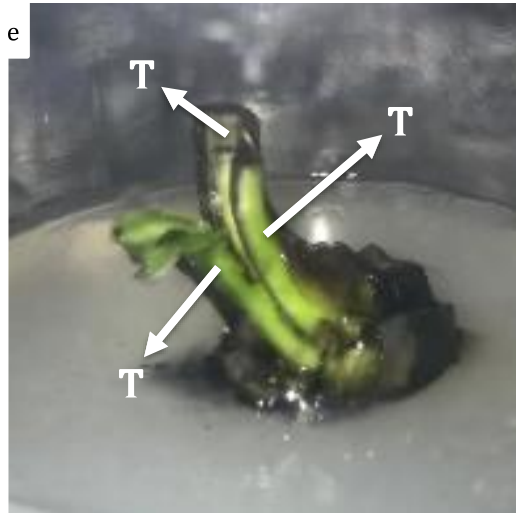

Figure 5. The growth response of banana shoots (*Musa paradisiaca* L. cv. Kayu) on solid MS media (BAP 5 ppm) for 5 weeks after planting (MST).

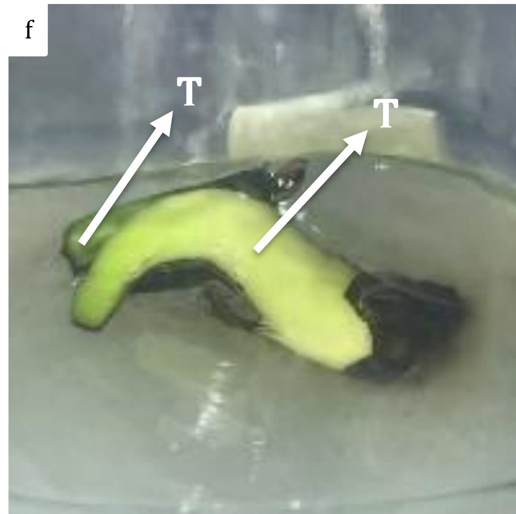

Figure 6. The growth response of banana shoots (*Musa paradisiaca* L. cv. Kayu) on solid-liquid MS media (BAP 1 ppm) for 5 weeks after planting (MST).

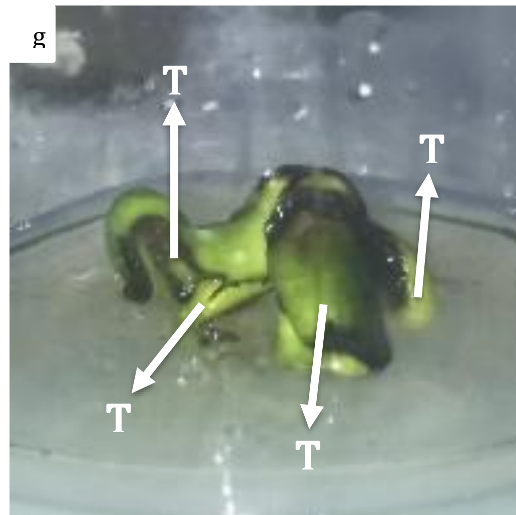

Figure 7. The growth response of banana shoots (*Musa paradisiaca* L. cv. Kayu) on solid-liquid MS media (BAP 2 ppm) for 5 weeks after planting (MST).

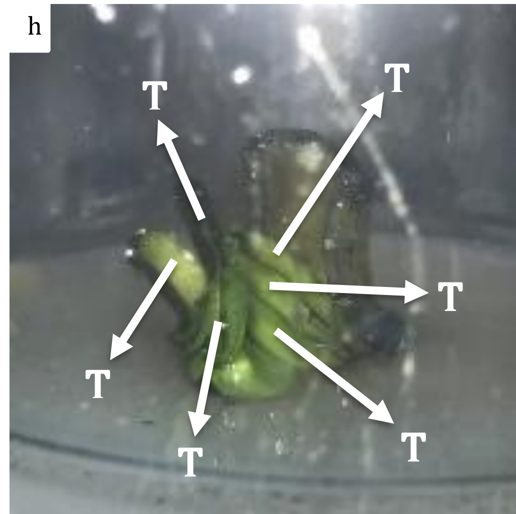

Figure 8. The growth response of banana shoots (*Musa paradisiaca* L. cv. Kayu) on solid-liquid MS media (BAP 3 ppm) for 5 weeks after planting (MST).

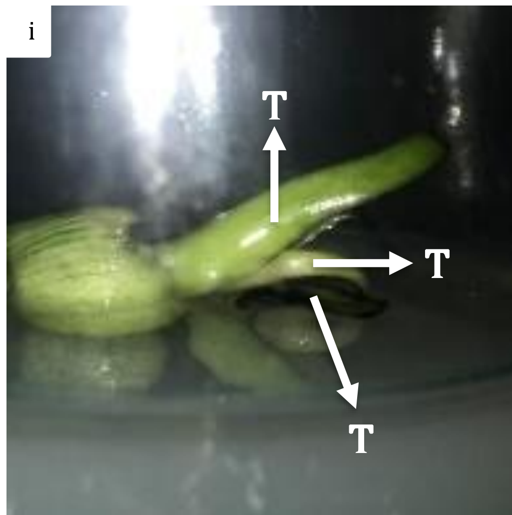

Figure 9. The growth response of banana shoots (*Musa paradisiaca* L. cv. Kayu) on solid-liquid MS media (BAP 4 ppm) for 5 weeks after planting (MST).

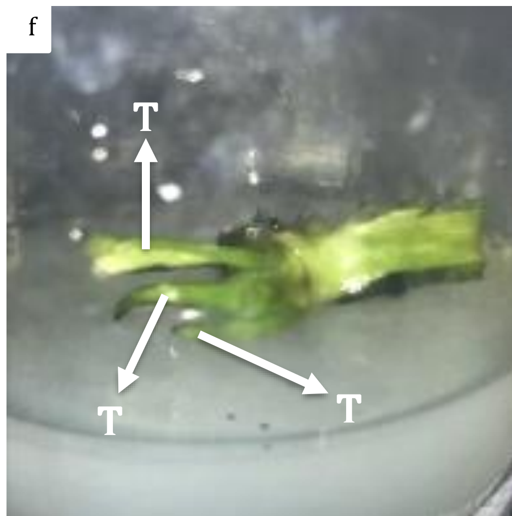

Figure 10. The growth response of banana shoots (*Musa paradisiaca* L. cv. Kayu) on solid-liquid MS media (BAP 5 ppm) for 5 weeks after planting (MST).

Plant growth regulators (PGRs) played a very important role in controlling the growth rate of each tissue in plants. One type of PGR commonly used in tissue culture was auxin and cytokinin. The role of auxin was to induce root formation, callus induction, cell and organ elongation, apical dominance, and stimulate flowering. Cytokinin had a role in increasing cell division and plant morphogenesis. Cytokinin was also very effective in stimulating the initiation of axillary and adventitious buds. Cytokinin was widely used in initiation media and bud multiplication media (Sulistiani and Yani, 2012). This study used treatments with different concentrations of cytokinin without auxin. The application of cytokinin alone was able to produce optimal buds when applied at the right concentration. However, at certain concentrations, it could lead to bud abnormalities (George et al., 2008). The formation and growth of buds were also influenced by cytokinin, with the most effective type of cytokinin for culture media being BAP (6-benzylaminopurine) (Gowen, 1995 in Pamungkas, 2015).

The growth response of bud initiation from banana wood explants (*Musa paradisiaca* L. var. Kayu) on solid media showed different numbers of buds for each treatment (Table 4.1 and Figure 4.1). The results indicated that the highest number of buds was found on solid MS media with a BAP concentration of 3 ppm, which produced an average of 3.33 buds. This might have been caused by BAP's ability to reduce apical meristem dormancy, leading to the formation of axillary or adventitious buds from the banana explant (Suminar et al., 2017). BAP also played a role in cell division, organ formation, and the formation of bud eyes. A study by Sivakumar and Visalakshi (2021) on in vitro micropropagation of banana cv. Poovan (AAB) also showed optimal results for bud growth with 3 ppm BAP, producing an average of 5.1 buds, which was considered the optimal concentration for bud initiation. The effect of BAP in the media showed better results than other types of cytokinin for bud initiation and multiplication.

According to the study by Pratiwi et al. (2023), it was stated that giving cytokinin at too high a concentration could inhibit the bud formation process. This was proven in the research, which showed a decrease in bud growth at concentrations of 4 ppm and 5 ppm, with 3 buds produced at each concentration. This result was also supported by research from Gubbuk and Pekmezci (2004) on the in vitro propagation of several new banana varieties, which stated that cytokinin at moderate or sufficient concentrations could increase bud proliferation. However, high concentrations of cytokinin could lead to a decrease in bud growth, particularly in bud elongation. The use of BAP at high concentrations could enhance bud formation during the initiation stage. However, the

formation of each bud could be inhibited, so determining the right concentration in tissue culture media needed to be done carefully to establish an optimal culture system and obtain normal plantlets (Jafari et al., 2011). High concentrations of hormones for propagation were often detrimental because they could cause various chromosomal abnormalities, leading to the production of plants that did not meet expectations (D'Amato, 1978 in Jafari et al., 2011). Although BAP could stimulate growth and development of buds in banana, it also had mutagenic effects when given at high concentrations, resulting in abnormal plants (Bairu et al., 2008).

The initiation of buds from banana wood explants (*Musa paradisiaca* L. var. Kayu) on solid-liquid media showed different numbers of buds for each treatment (Table 4.2 and Figure 4.2). The highest number of buds was observed on the solid-liquid MS media with a BAP concentration of 3 ppm, which produced an average of 4.33 buds. However, there was a decrease in the average number of buds at BAP concentrations of 4 ppm and 5 ppm, with average bud numbers of 3.33 and 3.33, respectively. The correct use of BAP could effectively stimulate bud multiplication. BAP played an active role in the organogenesis process in tissue culture (Fitriawati et al., 2020). BAP also served to stimulate and induce bud growth, but its concentration depended on the plant species used (George and Sherrington, 1984 in Fitriawati et al., 2020). Applying too high a concentration of BAP could lead to metabolic imbalance in plant tissues, which slowed down the bud proliferation process (Nuraini et al., 2022). This was because the hormone ratio became unbalanced, leading to the failure of new bud formation (Soelaiman and Ernawati, 2013).

The results of the study on bud initiation in banana wood (*Musa paradisiaca* L. var. Kayu) on both solid and solid-liquid media showed the same result, which was the optimal BAP concentration of 3 ppm. This was also demonstrated in the study by Babu (2019) on an efficient protocol for in vitro regeneration of banana cv. Nanjangudu rasabale (AAB), which stated that media supplemented with 3 ppm BAP showed rapid bud growth, requiring only 16 days to grow, compared to media with other concentrations of BAP.

The number of buds produced on solid media was lower compared to the number of buds produced on solid-liquid media (Table 4.1 and Table 4.2). Explants cultured on solid MS media showed differences in their growth responses, where cells on the opposite sides or those farther from the cells in direct contact with the media had to undergo nutrient assimilation and process regulatory signals through diffusion from neighboring cells. This resulted in the signals received by the more distant cells being different from those received by the cells in direct contact with the media, leading to less efficient nutrient and BAP absorption. This process did not occur in solid-liquid cultures, as the entire surface of the explant was submerged, and all cells were in direct contact with the media containing BAP, allowing them to capture signals directly. The use of liquid MS media on top of solid MS media allowed for more even absorption of nutrients and BAP, thus increasing the growth rate (Ascough and Fennel, 2004).

The addition of liquid media can submerge the tissue, which helps dilute and reduce the accumulation of toxic compounds that may be released by metabolites, which could inhibit growth. In contrast, toxic compounds released by the plant in solid media remain around the explant, interfering with its growth (Aitken-Christie and Jones, 1987). One of the toxic compounds is phenolic compounds. These phenolic compounds are oxidation products of sap released when cells are wounded. The injury causes the release of various enzymes that help protect the plant by catalyzing reactions to eliminate reactive oxygen species and heal the wound. This reaction produces melanin, a dark pigment responsible for the browning on the media and explants (Bhatia, 2021).

The addition of liquid media aimed to prevent browning in explants. Browning often occurs in tropical plants with high levels of phenolic compounds, such as banana plants (Sulistiani and Yani, 2012). A study by Aitken-Christie and Jones (1987) on the soaking of explants in liquid media for the propagation of pine trees also showed that liquid MS media placed on top of solid MS media provided aeration to the plant tissue and allowed contact with all parts of the explant, ensuring even nutrient absorption. This finding was also supported by research conducted by Rahman et al. (2017) on the multiplication of cassava shoots in culture,

which showed that bud, leaf, and root growth occurred more quickly in liquid media. This was because nutrient absorption from the media to the plant tissue was more efficient in liquid media than in solid media. Liquid MS media could reduce the hydration level of the explant, leading to a higher number of buds (Wawrosch et al., 2005).

The use of liquid media was more efficient and beneficial for large-scale micropropagation in many plant species because it could enhance in vitro bud proliferation, regeneration, somatic embryogenesis, rooting, and microtuberization processes. The liquid culture system ensured that plant tissues were always in contact with the media, stimulating the absorption of nutrients and plant growth regulators, which then resulted in uniform and faster bud growth (Debnath and Arigundam, 2020). The type of media could affect the osmolarity of the solution and the availability of oxygen for explant growth. Liquid media had a high water potential, which facilitated the transport of nutrients from the media to the explants (Putri et al., 2022). The liquid in the media, when placed in a container, exerted pressure in all directions. This pressure acted perpendicular to the surface it contacted, in this case, the plant explants. As a result, in liquid media, the molecules could experience cohesive and adhesive forces. This was also related to viscosity, where the viscosity of the liquid was very low, allowing it to flow easily and be transported within the plant during the transportation process. Viscosity was influenced by cohesive forces, making the liquid flow more easily (Ningrum and Toifur, 2014).

The growth response of explants on liquid media was caused by better contact between the explants and the media, which increased cytokinin absorption in its liquid form (Pati et al., 2005). A study by Zuraida et al. (2011) on the micropropagation of pineapple (Ananas comosus L.) cultured on liquid and solid media showed that explants cultured on liquid media produced the highest number of buds, with more buds formed on liquid media compared to solid media. The results showed that explants on liquid media exhibited a higher growth rate compared to those on solid media. The growth rate of explants on solid media was hindered by the diffusion rate of nutrients, which was limited by agar, leading to suboptimal absorption and slower growth. In contrast, liquid media allowed for faster growth, provided oxygen was available (Caplin and Steward, 1949). The growth and bud multiplication rate on liquid media were higher because the buds were submerged in nutrients, providing a larger surface area for nutrient and cytokinin absorption, which entered the cell surface quickly through diffusion, leading to the formation of new buds and elongation. Solid media required a longer process, which slowed down bud growth (Vyas et al., 2008).

The ANOVA results revealed that BAP he ANOVA results revealed that BAP concentration had no statistically significant effect on the observed parameters. In Table 2, the analysis produced an F value of 0.722 with a p-value of 0.596, while in Table 3, the F value was 0.565 with a p-value of 0.694. Since all p-values exceeded the 0.05 significance level, the null hypothesis (H_0) was accepted. These findings indicate that variations in BAP concentration did not result in significant differences in the measured responses under the conditions of this study.

The absence of a significant effect suggests that the range of BAP concentrations applied may not have been sufficient to influence the observed parameters, or that the response of the studied material to BAP was relatively uniform across treatments. Further research using a wider range of BAP concentrations or additional growth regulators may be necessary to elucidate potential treatment effects.

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4. CONCLUSIONS

Based on the results and discussions, it can be concluded that a BAP concentration of 3 ppm was the most effective in promoting optimal shoot growth of wooden banana (*Musa paradisiaca* L. cv. Kayu). In addition, the solid-liquid culture medium significantly enhanced shoot bud growth compared to the solid medium, which produced an average of 4.33 shoots per explant. These results confirm that the combination of an appropriate BAP concentration and suitable culture medium plays a crucial role in enhancing the efficiency of *in vitro* micropropagation of wooden banana.

Therefore, the use of a solid–liquid MS medium supplemented with 3 ppm BAP is strongly recommended as an effective protocol for the initiation and early multiplication stages of wooden banana.

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