Agrotech Journal
Url: http://usnsj.com/index.php/ATJ
Email: editor.atj@usnsj.com

Organogenesis of Cavendish Banana (*Musa acuminata* L.) Plant in Various Concentration of ZPT IAA (*Indole Acitid Acid*) and BAP (*Benzyl Amino Purine*) in Invitro Culture

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ARTICLE INFO

e-ISSN: 2548-5148
p-ISSN: 2548-5121
Vol. 6 No. 1, June 2021
URL: http://dx.doi.org/10.31327/atj.v6i1.1563

Abstract

Bananas are a horticultural commodity that originates from Southeast Asia and the West Pacific, including Indonesia. Banana cultivation is carried out with the aim of increasing the economic value of the community by cultivating the Cavendish banana species. Cavendish banana (Musa acuminata L.) is one type of banana that is widely exported, and has high economic value. The constraints in the cultivation of this Cavendis banana plant are the small, high-quality tillers produced in large quantities and in a short time. Propagation of seeds with tissue culture technology is one solution in dealing with the problem of providing these seeds. This study aims to determine the effect of ZPT IAA and BAP concentrations on the growth of cavendish banana shoots in vitro. This research was conducted at the Tissue Culture Laboratory of the Faculty of Agriculture, Cokroaminoto University, Palopo, on Jalan Lamaranginang, Batu Pasi Village, Wara Utara District, Palopo City. Starting from January to May 2020. This research was arranged in a completely randomized design (CRD), which consisted of 5 treatments and 3 replications with a total of 15 experimental units. By giving treatment concentrations of P0: Control, P1 (IAA 0.5 mg L-1 and BAP 1 mg L-1), P2 (IAA 1 mg L-1 and BAP 2 mg L-1), P3 (IAA 1.5 mg L-1 and BAP 3 mg L-1), P4 (IAA 2 mg L-1 and BAP 4 mg L-1). With research parameters callus color, callus texture, root emergence time, shoot emergence time, number of shoots, and number of leaves. The results showed that at the concentration with treatment P3: IAA 1.5 mg L-1 and BAP 3 mg L-1 produced the highest number of leaves, namely 3 strands. The fastest root emergence time was 25.33 days and the highest number of shoots was 2.33. While for the time of emergence of treatment shoots P0: Control showed the best results, namely 7 DAS (days after planting). This is because a balanced amount of auxins and cytokinins can affect the growth of banana plants in vitro

Keywords: BAP, IAA, in vitro, cavendish banana (*Musa acuminata* L.)

A. Introduction

Banana is a tropical fruit commodity that is very popular in the world. The total consumption of bananas in 2013 reached 5.63 kg / capita / year (PUSDATINTA, 2014). Currently, it is very important to increase production output and quality to meet the needs of public consumption

which continues to increase every year. It is hoped that with improved cultivation and good techniques, Indonesia can increase the export value of bananas, which in 2007-2011 was only 0.06%.

Cavendish banana (*Musa acuminata* L.) is one type of banana that is widely exported, and has high economic value. The constraints in the cultivation of this Cavendis banana plant are the small, high-quality tillers produced in large quantities and in a short time.

Tissue culture technology is a method used to be able to produce healthy seeds in large quantities and in a relatively short time. The new individual pups that are produced will be genetically the same as their parent (Dwiyani R, Yuswanti H, Darmawati IAP, Suada K & Mayadewi NNA., 2015). In vitro propagation is carried out with the composition of the planting medium and the addition of appropriate growth regulators (ZPT) as a requirement for good plant growth and development, in fulfilling macro nutrients, micro nutrients, vitamins, carbon sources, and various growth regulators. synthetic and natural from the auxin and cytokinin group. (Eriansyah *et al*., 2014). The explants used come from the weevil, on the part of the weevil there are buds containing meristematic tissue that actively divides the plant roots into plantlets.

Ilham *et al.* (2017), conducted a study on red banana callus using a combination of auction hormone with a combination of 2 ppm 2,4-D + 3 ppm BAP which was effective in inducing, with a callus appearing time of 4 MST (weeks after planting); green callus color, compact callus texture; and the highest callus weight was 0.0203 grams.

Bella *et al*. (2016), conducted research on banana micro shoots on the provision of genes and cytokinin concentrations against shoot formation time and shoot height of yellow Kepok bananas showed that the use of 2 mg L-1 BAP was able to produce high multiplication heights based on changes in shoot explant percentage (%) and shoot (cm).

Based on the above background, the use of auxin ZPT and cytokinin types IAA and BAP in appropriate amounts can increase growth. Therefore, in this study, the researcher will give a combination of IAA and BAP with different concentrations to be able to see the effect on the growth of Cavendish bananas.

B. Methodology

This research is one of the requirements in completing the undergraduate study in the form of a thesis. This research was conducted at the Tissue Culture Laboratory of the University of Cokroaminoto Palopo and took place from January to April 2020. The explants used came from the cavendish banana hump (Musa acuminata L.). The materials used in this study were cavendish banana weevil, sterile distilled water, 70% alcohol, spirit, coconut water, MS media, 7 grams of agar, sugar, sterile distilled water, types of growth regulators (ZPT) (IAA and BAP).). The tools used in this study included scarpel blade, measuring cup 1000 ml, analytic naraza, organ curl bottle, autoclave, LAP (Laminar Air Flow) sprayer, 70% alcohol, tweezers, petri dish, refrigerator, culture rack, air conditioner. , lamps, paper towels, aluminum foil, warp plastic, labels, matches.

This study used a completely randomized design (CRD) P0 = (MS + 0 mg / L-1 IAA + 0 mg / L-1 BAP) P1 = (MS + 0.5 mg / L-1 IAA + 1 mg / L- 1 BAP) P2 = (MS + 1 mg / L-1 IAA + 2 mg / L-1 BAP) P3 = (MS + 1.5 mg / L-1 IAA + 3 mg / L-1 BAP) P4 = (MS + 2 mg / L-1 IAA + 4 mg / L-1 BAP). This experiment was analyzed using a completely randomized design (CRD), with 5 treatments and 3 replications in order to obtain 15 experimental units, then to determine the effect between treatments, Analysis of Variance (ANOVA) was carried out. If there is a real difference, it is followed by the BNJ test (Honestly Real Difference) at the level of 0.5%. The observation parameters used callus color, callus texture, root emergence time (days), shoot appearance time (days), number of shoots (fruit), number of leaves (strands).

C. Result and Discussion

1. Callus Color

Callus color can be observed by looking at the changes that occur, there are several colors that appear in the observation, including white, green and brown. The difference in color that appears indicates the cell activity that occurs whether the cell remains alive or dead.

Treatments with IAA concentrations of 2 mg L -1 and 4 mg L-1 showed a different color change compared to treatments with other concentrations. Callus color of Cavendish banana (*Musa acuminata* L.) which showed different and varied callus colors (table 1) the variations that occurred in this study were caused by the presence of different cell metabolism in each part of the explants planted.

Table 1. Callus color of Cavendish banana (*Musa acuminate* L.) on MS medium with a combination of ZPT (growth regulator) IAA and BAP.

Treatment	Deuteronomy		
	1	2	3
P0	White	Green	White
P1	White	White	White
P2	White	White	White
P3	White	White	White
P4	Chocolate	White	White

a b c

Figure 1. Color change in callus (a: green, b: brown, c: white)

The callus color in several experiments was different, such as the media with a green IAA concentration of 0 mg L-1 and BAP 0 mg L-1 (Figure 4.1a). Furthermore, at concentrations of IAA 2 mg L-1 and BAP 4 mg L-1 there was a difference in the color of the callus to brown (Figure 1b), then at the IAA concentration of 1.5 mg L-1 and BAP 3 mg L-1 the color of the callus showed a white color. (Figure 1 c).

Indication of the difference in callus color can determine the level of development that is formed. According to Ali S.K., A.A. Elhassan, O.S. Ehiweris and E.H. Maki. (2007), there are several callus colors that are formed including yellow, greenish, and bright green. This color difference is due to the chloroplast content, when the callus is white there are starch grains, which will then become a membrane system when exposed to light the callus will turn green, indicating the callus is in good condition.

The change in the color of the callus from green to brown and then to death can indicate the presence of phonic compounds coming out of the explants. The browning event that occurs naturally is a process of change due to the occurrence of an injury when cutting the cavendish banana weevil, the change in callus color to brownish also indicates that no chlorophyll is formed so that the green color of the callus does not appear.

2. Callus Texture

Based on the observations from the research, it can be seen that the callus textures that are formed are crumb and The compact difference can identify that the different concentrations of IAA and BAP can affect the texture of the cavendish banana callus.

The results of the above research indicated that there were two types of callus texture that appeared, namely compact and crumb in the treatment with a combination of 2 mg L-1 IAA and BAP 4 mg L-1 in the treatment resulting in a crumb texture while in the other treatments it was compact.

Callus textures formed on cavendish banana weevil explants showed that each treatment with different IAA and BAP concentrations had a different texture, as seen in (Table 2). Formed a compact callus texture and a crumbly callus texture.

Callus textures in several experiments had differences, such as media with a concentration of 0 mg L of IAA^{-1} and BAP 0 mg L-1 had a crumb callus texture, then at a concentration of IAA 2 mg L-1 and BAP 4 mg L-1 there was a difference in one of the replications in the experiment with a crumb callus texture.

The results of the study with different callus textures could identify that the media contained nutrient composition, growth regulators and environmental conditions of the culture. A good callus to use is a callus with a compact texture because it can accumulate a lot of secondary metabolisme.

Table 2. Callus texture of Cavendish banana (Musa acuminata L.) on MS medium with a combination of ZPT IAA and BAP.

Treatment	Deuteronomy		
	1	2	3
P0	Crumb	Compact	Compact
P1	Compact	Compact	Compact
P2	Compact	Compact	Compact
P3	Compact	Compact	Compact
P4	Crumb	Compact	Compact

Based on the observations, it is known that a compact callus texture can be seen at the concentration of media with a combination of IAA 0.5 mg L-1 and BAP 1 mg L-1, with a low concentration that can form callus with a compact texture. The formation of textured callus is the effect of cytokinins and auxins that affect the water potential in cells. This causes the absorption of water from the medium into the cells to increase so that the cells become stiff so that the use of coconut water is very useful in the formation of callus textures (Dwi, 2012).

3. Time Emerged Roots

The mean time of root emergence in treatment P3 with a concentration of IAA 1.5 mg L^{-1} and BAP 3 mg L -1 showed the fastest time, namely 25.33 days after planting (HST) and the longest root emergence time was in P2 treatment with a concentration of IAA 1 mg L-1 and BAP 2 mg L^{1}. Indicators of success in tissue culture techniques are marked by the emergence of roots on explants planted on the media, the emergence of roots is indicated by the appearance of white lumps on the bananas hump close to the place where the media is placed.

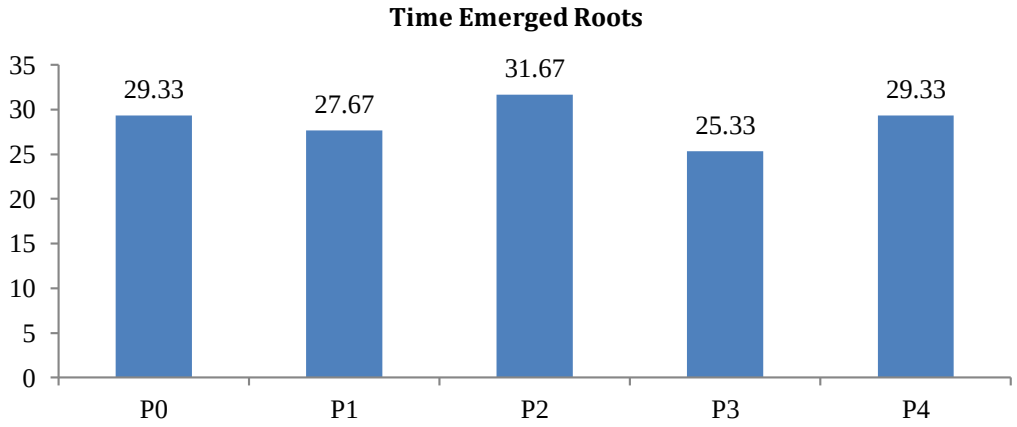

Figure 2. Effect of combination of IAA and BAP on root emergence time in Cavendish Banana (Musa acuminata) in vitro

The auxin hormone plays a role in the formation of undifferentiated lateral roots and callus. However, if the dedium is exposed to light, the explant response will decrease.

According to Yatim (2016), the number of roots is important for explant / plant growth in in vitrro tissue culture. The greater the number of roots is good for absorbing nutrients from the media. The fastest sprouting time was found in treatment P3 with a time of 25.33 HST with a combination of IAA 1.5 mg L -1 and BAP 3 mg L -1. Auxin group ZPT was able to initiate roots and accelerate root extension in tissue culture, whereas in the cytokinin group it was able to stimulate shoot growth in plantlets.

Figure 2. Explants on IAA 2 mg L-1 medium and 4 mg L-1 BAP medium

Figure 3. Explants on IAA medium 1.5 mg L-1 and BAP 3 mg L-1

4. Time Emerged Shoots

The observations from the time when shoots appeared showed that the treatment with the concentration of IAA 0 mg L-1 and BAP 0 mg L-1 showed the fastest time was 7 days after planting (HST) while in the treatment with a combination of IAA 2 mg L-1 and BAP 4 mg L-1 indicates the longest time, which is 20 days after planting (DAT).

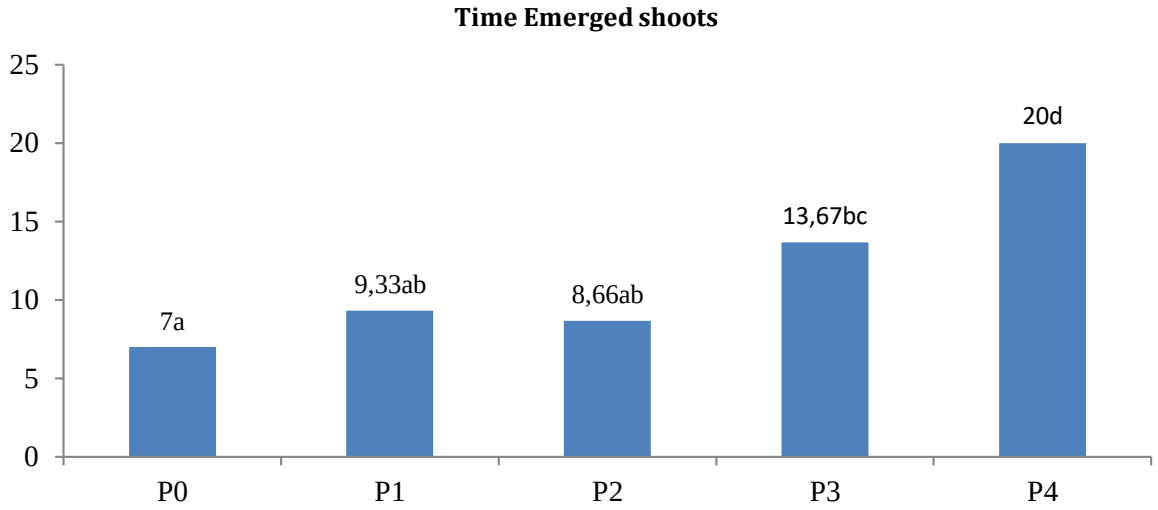

Figure 3. Effect of combination of IAA and BAP on Tuna Appearance Times Cavendish Banana (Musa acuminata) in vitro

The emergence time of cavendis banana shoots was not influenced by the combination of IAA and BAP concentrations in the P0 treatment (contral / without treatment) resulting in the fastest shoot emergence time of 7 days after planting (HST) and the longest time on P4 treatment (IAA 2 mg L-1 and BAP 4 mg L-1) that is 20 days. The administration of IAA and BAP had a significant effect on the delay in the emergence of shoots so that the BNJ (Honest Significant Difference) test was carried out at a level of 0.5%, after the BNJ result was 3.67%.

5. Number of Shoots

The mean number of shoots in treatment P3 with a concentration of IAA 1.5 mg L-1 and BAP 3 mg L-1 produced the highest number of shoots, namely 2.33 fruit while the least number of shoots was produced in treatment P0, P1, and P4 with each produce shoots of 1.33 pieces.

The addition of ZPT with a combination of IAA and BAP concentrations in each treatment showed different results, P3 treatment (1.5 mg L-1 and BAP 3 mg L-1) could produce the highest number of shoots, namely 2.33 shoots (Graph. 3). The treatment can produce the highest number of shoots, but the time needed to produce shoots is quite long, namely 13.67 days (Graph. 2). The organogenesis process that occurs is caused by the growth regulators given if the auxin is lower

than the cytokinin it will lead to shoots, if the auxin and cytokinin ratio is balanced, callus will be formed, and if the auxin ratio is higher it will affect root formation.

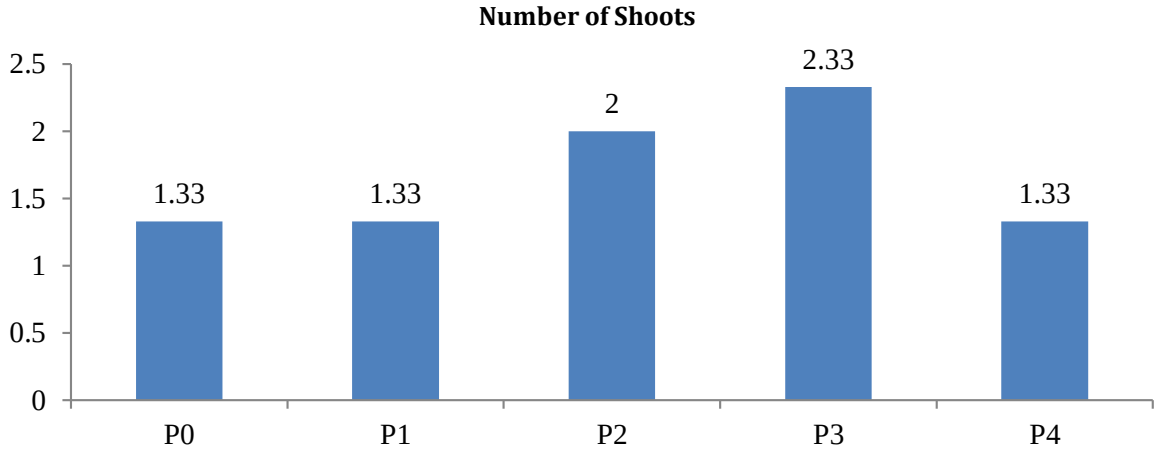

Figure 4. Effect of the combination of IAA and BAP on the Number of Cavendish Banana Shoots *(Musa acuminata)* in vitro

Treatment that produces the number of shoots 1, 33 shown in (P0, P1, and P4) this condition can be seen that the difference in the number of shoots is one of the factors indicating the success rate in the in vitro study of Cavendish banana.

Figure 5. Number of shoots on P2

Figure 6. Number of shoots at P3

6. *Number of Shoots*

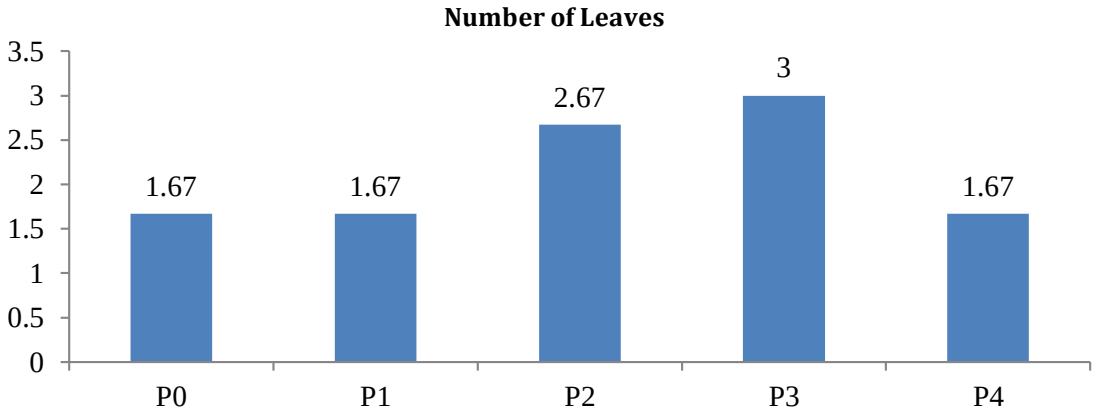

Figure 7. Effect of the combination of IAA and BAP on the number of Cavendish Banana Leaves in vitro

The results of the observation on the number of leaves showed that P3 treatment with a concentration of IAA 1.5 mg L-1 and BAP 3 mg L-1 produced the highest number of leaves, namely 3 strands. The treatments that produced relatively large numbers of leaves were 3 pieces of P3 (IAA 1.5 mg L-1 and BAP 3 mg L-1) (Pig 4), the concentrations of IAA and BAP had no significant effect on the number of leaves. The number of leaves is directly proportional to the number of shoots where the more shoots, the more leaves. As stated by Abidin (1993), Indole Acetiet Acid

(IAA), a growth hormone in the auxin group which plays a role in the process of cell enlargement and elongation. This is in accordance with the results of this study that IAA and BAP produced an average of 1.33 shoots/explant.

D. Conclusion

Based on the results of research, giving ZPT IAA and BAP with different concentrations showed an effect on the growth of cavendish banana shoots, with the highest number of shoots in the P3 treatment (IAA 1.5 mg L-1 and BAP 3 mg L-1) as many as 2.33 shoots. Other treatments showed that the interaction variety test results were not significantly different between ZPT IAA and BAP, from these results the P3 treatment (IAA 1.5 mg L-1 and BAP 3 mg L-1) gave the provision of root emergence time, namely 25.33 shoots were 2.33, and the number of leaves was 3 in the Cvendish Banana tissue culture. Whereas for the time when shoots appeared, the results obtained with P0 treatment (control / without treatment) showed significantly different results, namely 7 DAS (days after planting) so that the BNJ test at 5% level was carried out and showed 3.67% results.

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