Effect of BAP and Picloram on Shoot Induction (Musa acuminata Colla var. Mulu Bebek)

Nurul Huda Am Zen Arief¹, Enni Suwarsi Rahayu¹, Furzani Pa'ee², Noor Aini Habibah^{1*}

¹Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Kampus Sekaran, Gunungpati, Semarang, Indonesia 50229

²Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia Pagoh, KM 1, Jalan Panchor, 84600, Muar, Johor, Malaysia

*Corresponding Author: nooraini@mail.unnes.ac.id

Submitted: 2024-12-08. Revised: 2025-02-05. Accepted: 2025-04-11.

Abstract. *Musa acuminata* Colla var. Mulu Bebek is a high-quality banana from Ternate, North Maluku. Its conventional cultivation faces challenges due to Fusarium wilt, reducing productivity. In vitro culture offers a solution for rapid, disease-free seedling production. This study aims to examine the effect of BAP and picloram on shoot induction using a CRFD at various concentrations (0–3 ppm) and evaluate the percentage of live explants, emergence time, and number and length of shoots and roots. Results show that 1 ppm picloram accelerates root emergence, while 0 ppm promotes the fastest shoot emergence, the longest shoot and root lengths. 1 ppm BAP increases shoot numbers. The interaction of picloram and BAP has a significant effect on the parameters of the fastest root emergence time, namely 1 ppm picloram + 0 ppm BAP, 1 ppm and 3 ppm, the fastest shoot emergence time, namely without the addition of PGR, and the longest shoot length, 0 ppm picloram + 2 ppm BAP. The combination of 0 ppm picloram + 1 ppm BAP is the optimal condition for a percentage of 100% live explants, the fastest time for root emergence, the highest number of shoots and roots, the longest shoot and root length and the optimal condition for the fastest time for shoot emergence is 0 picloram + 0 ppm BAP. This study provides insight into the role of BAP and picloram in inducing banana shoots that has not been reported before. The micropropagation media formula obtained in this study is an effort to improve the quality of healthy banana seedlings in the future.

Keywords: Musa acuminata Colla var. Mulu Bebek; BAP (6-benzylaminopurine); in vitro; shoot induction; picloram

How to Cite: Arief, N. A. H. Z., Rahayu, E. S., Pa'ee, F., & Habibah, N. A. (2025). Effect of BAP and Picloram on Shoot Induction (*Musa acuminata* Colla var. Mulu Bebek). *Biosaintifika: Journal of Biology & Biology Education*, 17(1), 107-117.

DOI: http://dx.doi.org/10.15294/biosaintifika.v17i1.19605

INTRODUCTION

Banana (*Musa acuminata*) is a type of fruit-bearing herbaceous plant originating from Southeast Asia with a wide distribution throughout the world (OECD, 2010). According to Indonesian Central Statistics Agency (2024), banana production in Indonesia in 2023 will reach up to 9.33 million tonnes. This amount is 9.79% more than in 2021 which only reached 8.74 million tonnes. One type of banana that is cultivated, especially in Ternate, North Maluku, is the mulu bebek banana variety. Based on BPS Ternate City (2023), in 2021 banana production reached 310 tonnes but in 2022 production decreased to 129 tonnes.

The availability of quality seeds is one of the determining factors for success in agricultural development, especially bananas, now and in the

future. The main problem in cultivating banana plants using conventional methods in the field is the presence of Fusarium wilt disease pathogens which are very numerous and significant, thereby destroying the harvest every year (FAO, 2024). To overcome this problem, it is necessary to have banana plant propagation techniques that are fast, free from disease and can produce many saplings. One way is by using plant tissue culture techniques using plant growth regulator (PGR) treatment. Plant tissue culture is an activity to isolate plant parts, then culture or transfer them to sterile artificial nutrient media under controlled environmental conditions (Yali & Begna, 2021).

Tissue culture is a technique to produce quality banana seeds that are uniform, identical to the parent plant, microorganism-free, and can be mass-produced quickly, regardless of climate or season (Sadat et al., 2018). One of the factors that

plays an important role in the propagation of banana plants using tissue culture techniques is the presence of PGR because they can have a direct effect on the growth and development of explants (Rodinah et al., 2018). Exogenous PGRs, such as cytokinins and auxins, are commonly added to culture media because they are the two most important phytohormones that regulate plant tissue culture (Bielach et al., 2017; Svolacchia & Sabatini, 2023; Yang et al., 2021). As a result, research on cytokinins has increasingly intensified to better understand their role in this process. Cytokinins, such as 6-benzylaminopurine (BAP), regulate cell division, organogenesis, shoot formation, and chlorophyll production while inhibiting root growth (Unsong et al., 2022). BAP is a type of synthetic cytokinin. Cytokinins are required for cell division in merismatic tissue (Lee et al., 2019)

Apart from cytokinins, another group of PGRs is from the auxin group. Cytokinin is aimed more at shoot growth while auxin is aimed at root formation or callus formation. The use of auxin is known to cause rapid root growth and accelerate plant growth by encouraging cell division and cell enlargement. Auxin effects in the regulation of plant growth and development are largely determined by the coordination of three complex processes: auxin metabolism, auxin transport, and auxin signaling (Ma et al., 2018). One example of the auxin hormone is picloram (4-amino-3,5,6-trichloropicolinic) (Sauer et al., 2013).

Based on previous research, the novelty in this research is that apart from using banana explant varieties that have not been cultured, until now, there has been no shoot induction using picloram type auxin and a combination of picloram and BAP. This research aims to analyze the effect of picloram and BAP concentrations and determine the optimal combination of in vitro induction of Mulu bebek banana shoots to produce banana shoots free from disease. Apart from that, this research has benefits for society, namely as a basis for multiplying banana plants through shoot induction tissue culture from Mulu Bebek bananas so that healthy seedlings are obtained.

METHODS

Material and experimental design

The explant material was obtained from farmers' gardens in Marabose Village, Bacan District, South Halmahera Regency, Indonesia. The plant material that will be used is the corms of the banana, which are around 3 months old and

taken during the dry season. The important criteria to pay attention to are that the explants come from parent shoots that are healthy, productive, fertile, and free from visual stunting or viruses. The corms taken are washed until clean, then cut and peeled off the outer skin to obtain the inside of the corm up to $\pm 2x3$ cm in size, then split into two to obtain 2 explants. This research was carried out experimentally using a completely randomized factorial design (CRFD) with two factors, namely BAP and picloram concentrations, each consisting of four levels, namely 0 ppm, 1 ppm, 2 ppm, and 3 ppm. Each treatment combination was repeated three times. The treatments in this research were carried out at the Plant Tissue Culture Laboratory, Universitas Negeri Semarang (UNNES).

Preparation and sterilization of treatment media

The media used for treatment in this study was MS media with a combination of BAP and Picloram. The nutritional ingredients in making the media need to be prepared and then dissolved in distilled water, including sugar, instant Murashige, and Skoog (MS), Myo inositol, stirred until homogeneous, then added PGR picloram and BAP. The pH of the solution is then measured until it is at 5.8-6.0, and then agar is added. After that, the solution is heated for 20 minutes and poured into a culture bottle. The media is ready to use after incubating for 3-5 days.

Sterilization and explant planting

Banana explants were washed using detergent, rinsed, and left under running water for 30 minutes. The explants were then soaked in a solution of tween-20, fungicide, and bactericide each for 1 hour. The explants are then sterilized internally by Laminar Air Flow (LAF) using sodium hypochlorite 30% for 30 minutes, sodium hypochlorite 20% for 20 minutes, and sodium hypochlorite 10% for 10 minutes (Unsong et al., 2022). The explant is then placed on a petri dish, and the outer part is peeled again until it measures $\pm 1x1.5-2$ cm and then split into two parts. The explant that had been planted in it was then incubated for 3 months.

Explant incubation

The explants were incubated in a sterile incubation room at a room temperature of 24°C and illuminated with 2,000 lux fluorescent lamps. The room is kept sterile by washing and separating explants that have been contaminated by microorganisms, apart from that, the room is also sprayed with formalin every week (Corrales &

Astrin, 2023).

Data collection and statistical analysis

This research approach is experimental quantitative research by analyzes the effect of BAP and picloram concentrations. The parameters used in this research include the percentage of live explants, time for roots to emerge, time for shoots to emerge, number of shoots, number of roots, shoot length, and root length in the third month after planting. Data were analyzed quantitatively using the parametric two-way ANOVA test at a level of p \leq 0.05 if it was normally distributed and using Duncan's advanced test to analyze differences in the effect of treatments and their combinations. However, if the data is not normally distributed, then a non-parametric Kruskal-Wallis test is carried out, which is then further tested by Dunn to analyze differences in the effect of treatments and their combinations.

RESULTS AND DISCUSSION

Percentage of live explants

The survival percentage of banana stem explants with the combination of PGR picloram and BAP is 100% (Figure 1) and there is no significant difference between treatments (Table 1). The growth response of banana explants was initially indicated by enlargement and color changes of the explants, namely from white to

yellowish, then followed by swelling and elongation of the tissue, this is in accordance with Syafrilia et al. (2018). One of the factors that causes growth in explants is the growing medium used, where the medium used provides sufficient nutrients for explant growth, which in this study used BAP and picloram. This is in accordance with research conducted by Budi (2020) shows the results of the percentage of explant life (100%) caused by the culture media which is very supportive for the growth of banana explants.

Meristem tissue is young tissue, where this tissue consists of cells that are always dividing and the size of the explant is small, approximately 1 cm. Living explants show various morphologies, such as shoot formation, root formation, and some are statistical. The initial response to the explant after being planted is that there is a garden and the explant changes color. The color change in the explant from white to black does not mean that the explant has died, but is caused by the release of compounds from the injured explant tissue and still needs time to adapt to the media. Explants that do not show growth and development change color from green to brown and then die, this can be caused by the emergence of phenolic compounds that come out of the explants (Budi, 2020). In banana tissue, there are phenolic enzymes, especially the enzyme polyphenol oxidase which is a natural auxin in bananas (Onuoha et al., 2011).

Table 1. Average percentage of live explants, root emergence time, and number of shoot explants using PGR Picloram and BAP.

Growth Regulator (ppm)		I ! E14-(0/)	Transaction of the same of the	TE: eci	
Picloram	BAP	Live Explants (%)	Time of Roots Appear	Time of Shoots Appear	
0	0	100±0.00	1.67±0.57 ^{ab}	1.33±0.57 ^a	
	1	100±0.00	2.33 ± 0.57^{abc}	2.00 ± 0.00^{ab}	
	2	100±0.00	1.67 ± 0.57^{ab}	1.67±0.57 ^a	
	3	100±0.00	1.67 ± 0.57^{ab}	2.00 ± 0.00^{ab}	
	0	100±0.00	1.33 ± 0.57^{a}	1.67 ± 0.57^{a}	
	1	100±0.00	1.33 ± 0.57^{a}	2.00 ± 0.00^{ab}	
1	2	100±0.00	2.67 ± 1.15^{bc}	1.67±0.57 ^a	
	3	100±0.00	1.33 ± 0.57^{a}	2.00 ± 0.00^{ab}	
	0	100±0.00	1.33 ± 1.15^{a}	2.33 ± 0.57^{abc}	
2	1	100±0.00	2.33 ± 0.57^{abc}	2.00 ± 1.00^{ab}	
2	2	100±0.00	1.67 ± 0.57^{ab}	1.67 ± 0.57^{a}	
	3	100±0.00	2.00 ± 0.00^{abc}	2.67 ± 0.57^{bc}	
	0	100±0.00	2.67 ± 0.57^{bc}	3.00 ± 0.00^{c}	
3	1	100±0.00	3.33 ± 0.57^{c}	2.33 ± 0.57^{abc}	
	2	100±0.00	3.00 ± 1.00^{bc}	2.67 ± 0.57^{bc}	
	3	100±0.00	3.67±0.57°	2.67 ± 0.57^{bc}	

Notes: superscript letters indicate no significant difference if the notation is the same. This statistical analysis used the Kruskall-Wallis test.



Figure 1. Morphology percentage of live banana shoots against various combinations of treatment levels (a) 0 picloram + 0 BAP; (b) 0 picloram + 1 BAP; (c) 0 picloram + 2 BAP; (d) 0 picloram + 3 BAP; (e) 1 picloram + 0 BAP; (f) 1 picloram + 1 BAP; (g) 1 picloram + 2 BAP; (h) 1 picloram + 3 BAP; (i) 2 picloram + 0 BAP; (j) 2 picloram + 1 BAP; (k) 2 picloram + 2 BAP; (l) 2 picloram + 3 BAP; (m) 3 picloram + 0 BAP; (n) 3 picloram + 1 BAP; (o) 3 picloram + 2 BAP; (p) 3 picloram + 3 BAP.

Note: Scale line (I) = 1 cm

Time of Roots appear

The root emergence time parameter is an indicator to see the ability of explants to grow roots quickly in in vitro media. This parameter is calculated from the week after planting (WAP) mulu bebek banana stem explants were planted in the treatment medium until roots 0.5 cm long were formed.

The single concentration of picloram and the combination of picloram BAP had a significant effect on the time of root emergence so further tests were carried out using the Dunn test. The average root emergence time for the combination treatment can be seen in Table 1. which shows the best root emergence time, namely in the combination treatment of 1 picloram + 0 BAP, 1 picloram + 1 BAP, and 1 picloram + 3 BAP with an average value of 1.33 WAP. The results of the analysis from Dunn's further picloram test can be concluded that the best treatment is at a picloram concentration of 1 ppm which is not significantly different from 0 ppm and 2 ppm. In research conducted by Tini et al. (2022) found that one of the endogenous hormones in banana explants was present in IAA 94.2 ppm and zeatin 138.53 ppm. This indicates that endogenous auxin is sufficient for root induction. Auxin is known to cause rapid root growth and accelerate plant growth by encouraging cell division and cell enlargement (Zhang et al., 2022). Auxin has a promotive effect on the process of adventitious root formation (Wei et al., 2019). Auxin plays a vital role in controlling plant growth and development via the promotion of cell division (proliferation), growth (expansion, elongation) and differentiation. Enlargement of the cell occurs prior to cell division, however, no changes are observed in the vacuole size at this stage. On the other hand, cell expansion includes vacuole extension and is defined as a turgor-driven increase in cell size, which is controlled by the cell wall's capacity to extend. Cell expansion is related to an increased ploidy level (endoreduplication), cellular vacuolization and differentiation (Maida & Robert, 2018)

Apart from auxin, cytokinins can play a role in stimulating ethylene production under certain conditions, where ethylene can stimulate the formation of adventitious roots by synthesizing injured plant parts and making them a place for the formation of adventitious roots in parts or tissue injured by explant cutting activities (Sari et al., 2016). The process of root formation occurs in four stages, namely the formation of a meristematic locus from the dedifferentiation of

one or several cells, the multiplication of cells into a group of cells, the division of a group of cells located in the same plot simultaneously to form a root meristem and the elongation of cells at the base of the root meristem so that the roots that form begin to appear (Tyas et al., 2016).

Time of Shoots appear

The shoot emergence time parameter is an indicator to see the ability of the explant to grow shoots quickly in in vitro culture. This parameter was calculated from the WAP when mulu bebek banana stem explants were planted in the treatment medium until shoots 0.5 cm high were formed.

The single concentration of picloram and the combination of picloram BAP had a significant effect on the time of shoot emergence so further tests were carried out using the Dunn test. The average time for shoot emergence in the combination treatment can be seen in Table 1. which shows the best shoot emergence time, namely in the combination treatment of 0 picloram + 0 BAP with an average value of 1.33 WAP. From the results of the analysis from Dunn's further picloram test can be concluded that the best treatment is at a picloram concentration of 0 which is not significantly different from 1 ppm and 2 ppm. This shows that the banana stem explants already contain natural cytokinins and auxins, so there is no need to add exogenous PGR to accelerate the growth of shoots of the mulu bebek banana stem explants. This is in line with research conducted by Rodinah & Nisa (2018) that the best time for shoot emergence is on media without PGR, because in banana stem explants there are sufficient endogenous cytokinins and auxins to produce shoots. In other research too Tini et al. (2022) found that the endogenous hormone auxin was present in banana explants IAA 94.2 ppm and zeatin 138.53 ppm and cytokinin in banana explants, namely kinetin 178.82 ppm, sufficient for bud formation in the early stages.

There are banana stem explants, shoot formation begins with thickening or swelling at the bottom of the explant planted in the media. This thickening or swelling occurs because there is endogenous auxin activity that triggers the mobilization of cells for the formation of new individuals. This is in accordance with the role of auxin which can accelerate the induction of cell enlargement due to the activation of ATP-ase which pumps protons in the cell membrane, increasing cell size (activation of cell expansion) and cell enlargement due to loosening of the cell

wall (Perrot-Rechenmann, 2010; Sari et al., 2016). In research conducted by Ngomuo et al. (2013), found that cytokinins in the media not only determine the regeneration response of banana meristem cultures but also influence the regeneration method. The initial response of shoot formation explants due to the addition of cytokinin is mediated by an increase in cytosolic calcium concentration which is driven by high uptake from the media. It affects the cytoskeleton and regulates exocytosis. CK signaling in higher plants is a complex mechanism that operates through a binary signaling system involving histidine kinase (HK), phosphotransfer proteins (HP), and response regulators (RR). The HK protein family is primarily found in the cell membrane, where it functions as a CK receptor to detect active CK. In Arabidopsis, AHK2, AHK3, and AHK4 are predominantly located in the endoplasmic reticulum membrane. exhibiting distinct distributions and varying affinities for CK to fulfill the specific CK requirements in different plant tissues. During CK signaling, HK proteins first undergo autophosphorylation and subsequently

transfer phosphate groups, either directly or indirectly, to conserved aspartic acid residues, thereby modulating the activity of the ligand signaling region (Bai et al., 2023; Zubo & Schaller, 2020).

Figure 2 demonstrated the role of cytokinins in leaf primordium formation. Cells in the shoot apical meristem (SAM) are organized into layers L1, L2, and L3 as well as four distinct zones: central zone (CZ), peripheral zone (PZ), organizing center (OC), and rib zone (RZ). KNOX is expressed almost throughout the SAM. KNOX (KNOTTED-LIKE homeobox) positively regulates cytokinin synthesis and keeps their levels high. Cytokinins promote WUS expression through signal transduction and transcription factors, which maintain high cell division rates in OCs. ERECTA inhibits the effects of cytokinins and helps transport the hormone auxin. In areas with higher auxin concentrations, leaf primordium begins to form. Solid lines indicate direct relationships; dotted lines indicate potential mechanisms (Wu et al., 2021).

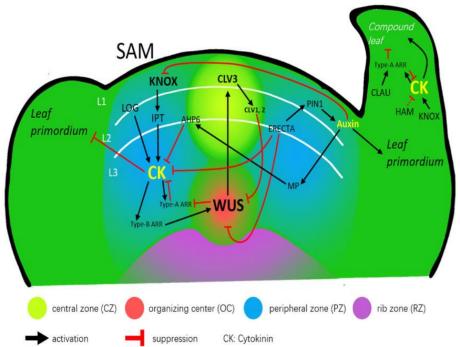


Figure 2. Schematic diagram of cytokinin (CK) regulation in leaf primordium initiation (Wu et al., 2021)

Number of shoots

Based on the mean analysis carried out, the treatment combination of 1 picloram + 1 BAP and 2 picloram + 1 BAP showed the best mean value of 2.00 (Table 2). Analysis results from the Dunn BAP follow-up test showed the best concentration, namely at a BAP concentration of

1 ppm which was significantly different from a BAP of 2 ppm. This result is different from research conducted by Manurung et al. (2021), which stated that 2.5 ppm produced the highest number of shoots. BAP is a more economical cytokinin and is often used to stimulate axillary bud multiplication. BAP affects the increase in the number of shoots (Unsong et al., 2022). Therefore,

it is necessary to add cytokinin growth regulators to stimulate high shoot multiplication (Amalia et al., 2023). Cytokinins play an important role in cell division, which, if given in appropriate concentrations, can accelerate cell division, which in this case increases the number of shoots. This is in accordance with what was stated by Shirani et al. (2009) The level of shoot proliferation is influenced by the type of cytokinin, concentration, and type of banana cultivar. If explants are planted in a medium with adequate cytokinin content, cell division will occur synchronously, whereas at high concentrations it can inhibit growth, poison and even kill the plant (Budi, 2020). This is also in accordance with opinion Nofiyanto et al. (2019) which states that the addition of cytokinin growth regulators is important in cultivating bananas using tissue culture. The results of the research carried out are in conflict with those carried out by Karamina et al. (2022), the higher the concentration of cytokinin given the greater the number of shoots formed will increase, but the formation of each shoot can be hampered so that determination Proper concentration is very

necessary considered to produce multiplication maximum banana shoots. a well-coordinated system that can optimally regulate plant growth and development (Ngomuo et al., 2013; Oktaviani & Usmadi, 2019). The direction of explant development in tissue culture is determined by the interaction and balance of PGR produced by the plant endogenously, because in the explant there is already endogenous PGR, but in in vitro growth and development exogenous PGR is still added with the aim of seeking and obtaining optimal results (Sihotang et al., 2016).

Number of roots

Based on the results of the mean analysis in Table 2, it shows that the treatment combination of 0 picloram + 1 BAP shows the highest mean value. However, based on further ANOVA tests, this treatment did not show any significant difference from other treatments. So it can be concluded that there is no effect from the combination of picloram and BAP and the single treatment of both on the root number parameter.

Table 1. Average percentage number of shoots, number of roots, shoot length and root length of mulu bebek banana stem explants using PGR Picloram and BAP

Growth Regulator (ppm)		Number of Shoots	Number of	Shoot Length	Root Length
Picloram	BAP	SHOOLS	Roots	(cm)	(cm)
	0	1.67±0.57	6.00±4.35 ^a	15.16±8.60 ^{abc}	13.66±4.04 a
0	1	1.00 ± 0.00	8.00±2.64 a	16.83 ± 6.52^{ab}	16.00±5.76 a
U	2	1.00 ± 0.00	7.00±3.46 a	20.00 ± 1.00^a	15.83±2.83 a
	3	1.67 ± 1.15	7.00±1.73 a	19.66 ± 1.52^{ab}	19.50±4.76 a
	0	1.67±1.15	6.33±2.51 a	6.33 ± 1.25^{cd}	10.83±3.32 a
1	1	2.00 ± 1.00	6.00±3.00 a	8.66 ± 3.51^{abcd}	8.50±0.50 a
1	2	1.00 ± 0.00	4.67±3.51 a	5.06 ± 3.49^{cd}	9.33±7.37 a
	3	1.00 ± 0.00	5.67±0.57 a	6.50 ± 2.59^{bcd}	8.33±5.50 a
	0	1.00 ± 0.00	2.67±2.51 a	2.66 ± 1.04^{d}	3.33±2.88 a
2	1	2.00 ± 1.00	5.33±3.21 a	6.66 ± 3.51^{bcd}	10.66±10.01 a
2	2	1.00 ± 0.00	5.50±2.78 a	7.00 ± 4.76^{abcd}	6.00±4.82 a
	3	1.00 ± 0.00	3.17±2.25 a	5.50 ± 3.50^{cd}	2.36±2.80 a
	0	1.33 ± 0.57	6.00±5.56 a	7.00 ± 3.60^{abcd}	9.50±3.77 a
3	1	1.33 ± 0.57	5.00±1.00 a	8.83 ± 1.04^{abc}	9.50±6.06 a
3	2	1.00 ± 0.00	4.33±3.21 a	4.66 ± 2.25^{cd}	8.16±6.93 a
	3	1.00 ± 0.00	4.83±2.25 a	5.83 ± 3.32^{cd}	7.16±4.50 a

Notes: superscript letters indicate no significant difference if the notation is the same (Statistical analysis of the number and length of shoots using the Kruskal-Wallis test, statistical analysis of the number and length of roots using the ANOVA test)

The highest number of roots was found at a picloram concentration of 0 ppm because the endogenous auxin was still high in the explant, so it could still form roots in good numbers (Olatunji et al., 2017). In addition, the presence of cytokinins such as BAP has an important role in increasing organ regeneration in explants. BAP increases cell division and shoot proliferation, also increases root growth in explants, as well as auxin, which influences cell development and induces axillary roots (Yulianti et al., 2024). The number of roots formed is closely related to the balance between endogenous and exogenous PGRs in the explant (Supalal, 2015).

Shoot length

The single concentration of picloram and the combination of picloram BAP had a significant effect on shoot length so further tests were carried out using the Dunn test. The average shoot length of the combination treatment can be seen in Table 2 which shows the best shoot length, namely in the combination treatment of 0 picloram + 2 BAP with an average value of 20.00 cm. The results of the analysis of the Dunn picloram follow-up test can be concluded that the best treatment is at a picloram concentration of 0 ppm and is significantly different from other treatments.

The greater number of shoots growing on the explants from each treatment tends to result in a lower average shoot height (Sari et al., 2016). Ramesh & Ramassamy (2014), states that plant height is thought to be influenced by the number of shoots that appear, so that the fewer shoots that appear, the higher the plant height, and vice versa. This is in accordance with the research carried out, the results show that the plants with the most shoots, namely at a concentration of 1 ppm picloram + 1 ppm BAP and 2 ppm picloram + 1 ppm BAP with an average number of shoots of 2.00, have a low average shoot height, namely respectively - respectively 8.66 cm and 6.66 cm compared to explants in the treatment with a concentration of 0 picloram + 2 BAP with the highest shoot average of 20.00 cm producing only 1.00 shoots. According to Ramesh & Ramassamy (2014), this is because the energy needed for shoot elongation is used to form other potential shoots, so that shoot height can be inhibited.

Root length

Based on data analysis in Table 2, the treatment combination of 0 picloram + 3 BAP showed the highest mean value, namely 19.50 cm and 2 picloram + 3 BAP which showed the lowest

mean, namely 2.36 cm.

Table 2. Average root length of banana stem explants using Pikloram PGR

Concentration of Picloram	Average root length (cm)
	$Mean \pm SD$
P0	16.25 ± 4.40^{a}
P1	9.25 ± 4.30^{b}
P2	5.59 ± 6.05^{b}
P3	$8.58 \pm 4.77^{\text{b}}$

Notes: superscript letters indicate no significant difference if the notation is the same. This statistical analysis uses the Duncan test.

Apart from that, normality and homogeneity tests were also carried out, from these results the significance value was normally distributed or > 0.05, so the Anova test was continued. Duncan's further test was carried out which showed that a picloram concentration of 0 ppm was the best concentration because it had the highest mean value, namely 16.25 cm and was significantly different from other concentrations (Table 3).

This is in accordance with research conducted by Zulkifli & Sari (2017) that the use of cytokinins can also stimulate the development of organs, one of which is the roots. In other research conducted by Olatunji et al. (2017), showed that in response to the environment, explants modulate endogenous auxin in sufficient amounts for plant development. growth and Low auxin concentrations will increase adventitious root formation, while high auxin concentrations will formation stimulate callus and suppress morphogenesis. Both endogenous and exogenous auxins play a role in increasing plant roots at optimal concentrations. The auxin hormone is able to influence physiological processes in cells which can later increase cell development and elongation so that the cells will expand, elongate to absorb nutrients (Ultimate, 2015). The existence of roots for plant growth plays an important role in absorbing nutrients and water, apart from that it also plays a role in supporting the upright growth of a plant (Pratiwi et al., 2023).

The benefit of this research for science is as a source of information regarding the induction of shoots in mulu bebek banana plants and knowledge about the influence of PGR BAP and picloram which play a role in inducing shoots. Apart from that, this research also has benefits for society, namely as a basis for multiplying banana plants through shoot induction tissue culture from

Mulu Bebek bananas so that healthy seedlings are obtained.

CONCLUSION

Picloram at 1 ppm accelerates root emergence. BAP at 1 ppm increased the number of shoots. The interaction of picloram and BAP shows the fastest root emergence time, namely 1 ppm picloram + 1 and 3 ppm BAP, and the longest shoot length, namely 0 ppm picloram + 2 ppm BAP. This study has the potential to open new avenues and perspectives regarding strategies for increasing the production of mulu bebek bananas that are free from disease. Further research needs to be developed to find out other types of PGR combinations to see the best combination for the induction of mulu bebek banana.

REFERENCES

- Amalia, A. H., Habibah, N. A., & Rahayu, E. S. (2023). Pengaruh Intensitas Cahaya, Jenis Pemadat Media, dan Konsentrasi BAP terhadap Kadar Klorofil dan Pertumbuhan Krisan (Chrysanthemum indicum L.) secara In Vitro. *Life Science*, *12*(1), 10–17. http://journal.unnes.ac.id/sju/index.php/LifeSci
- Bai, Y., Cai, M., Dou, Y., Xie, Y., Zheng, H., & Gao, J. (2023). Phytohormone Crosstalk of Cytokinin Biosynthesis and Signaling Family Genes in Moso Bamboo (Phyllostachys edulis). *International Journal of Molecular Sciences*, 24(13), 10863. https://doi.org/10.3390/ijms241310863
- Bielach, A., Hrtyan, M., & Tognetti, V. (2017). Plants under Stress: Involvement of Auxin and Cytokinin. *International Journal of Molecular Sciences*, 18(7), 1427. https://doi.org/10.3390/ijms18071427
- BPS Kota Ternate. (2023). Kota Ternate Dalam Angka 2023 (Vol. 22).
- Budi, R. S. (2020). Uji Komposisi Zat Pengatur Tumbuh Terhadap Pertumbuhan Eksplan Pisang Barangan (Musa paradisiaca L.) Pada Media MS Secara in vitro. *BEST Journal*, *3*(1), 101–111.
- Corrales, C., & Astrin, J. J. (2023). *Biodiversity Biobanking a Handbook on Protocols and Practices*. Pensoft Publishers. https://doi.org/10.3897/ab.e101876
- FAO. (2024). Banana Market Review 2023. *FAO*, 1–19.
- Indonesian Central Statistics Agency. (2024, February 26). *Fruit Crop Production*.

- https://www.bps.go.id/id/statistics-table/2/NjIjMg==/produksi-tanaman-buah-buahan.html
- Karamina, H., Indawan, E., & Agustina, F. I. K. (2022). Efektifitas Perbedaan Konsentrasi BAP terhadap Pertumbuhan Planlet Pisang Cavendish dengan Teknik Thin Cells Layer. *Kultivasi*, 21(2). https://doi.org/10.24198/kultivasi.v21i2.35373
- Lee, Z. H., Hirakawa, T., Yamaguchi, N., & Ito, T. (2019). The Roles of Plant Hormones and Their Interactions with Regulatory Genes in Determining Meristem Activity. *International Journal of Molecular Sciences*, 20(16), 4065. https://doi.org/10.3390/ijms20164065
- Ma, Q., Grones, P., & Robert, S. (2018). Auxin Signaling: A Big Question to be Addressed by Small Molecules. In *Journal of Experimental Botany* (Vol. 69, Issue 2, pp. 313–328). Oxford University Press. https://doi.org/10.1093/jxb/erx375
- Majda, M., & Robert, S. (2018). The Role of Auxin in Cell Wall Expansion. *International Journal of Molecular Sciences*, *19*(4), 1–21. https://doi.org/10.3390/ijms19040951
- Manurung, B. Y., Dewi, P. S., & Dwiati, M. (2021). Effects of BAP and Lighting Duration on Banana (Musa paradisiaca cv. Raja Bulu) Micropropagation. *Biosaintifika*, 13(3), 284–289.
 - https://doi.org/10.15294/biosaintifika.v13i3.25 173
- Ngomuo, M., Mneney, E., & Ndakidemi, P. (2013). The Effects of Auxins and Cytokinin on Growth and Development of (Musa sp.) Var. "Yangambi" Explants in Tissue Culture. *American Journal of Plant Sciences*, *04*(11), 2174–2180. https://doi.org/10.4236/ajps.2013.411269
- Nofiyanto, R. T., Kusmiyati, F., & Karno. (2019). Peningkatan Kualitas Planlet Tanaman Pisang Raja Bulu (Musa paradisiaca) dengan Penambahan BAP dan IAA pada Media Pengakaran Kultur In Vitro. *Journal of Agro Complex*, *3*(3), 132–141. https://doi.org/
- OECD. (2010). Safety Assessment of Transgenic Organisms in the Environment, Volume 8 (Vol. 4). OECD. https://doi.org/10.1787/ 9789264302235-en

10.14710/joac.3.3.132-141

Oktaviani, M. A., & Usmadi. (2019). The Effect of Bio-Slurry and Phosphorus on Growth Andyield of Lowland Cauliflower (Brassica oleracea L.). *Jurnal Bioindustri*, 01(02), 125– 137.

- Olatunji, D., Geelen, D., & Verstraeten, I. (2017). Control of Endogenous Auxin Levels in Plant Root Development. *International Journal of Molecular Sciences*, 18(12), 2587. https:// doi.org/10.3390/ijms18122587
- Onuoha, I. C., Eze, C. J., & Unamba, C. I. N. (2011). In Vitro Prevention of Browning in Plantain Culture. *OnLine Journal of Biological Sciences*, 11(1), 13–17.
- Pamungkas, S. S. (2015). Pengaruh Konsentrasi NAA dan BAP terhadap Pertumbuhan Tunas Eksplan Tanaman Pisang Cavendish (Musa paradisiaca L.) Melalui Kultur In Vitro. *Gontor Agrotech Science Journal*, 2(1), 31–45.
- Perrot-Rechenmann, C. (2010). Cellular Responses to Auxin: Division versus Expansion. *Cold Spring Harbor Perspectives in Biology*, 2(5), 1–17.
 - https://doi.org/10.1101/cshperspect.a001446
- Pratiwi, B. I., Nugrahani, P., & Augustien K., N. (2023). Pengaruh Nutrisi AB Mix dan Benzyl Amino Purine (BAP) terhadap Pertumbuhan Pisang (Musa acuminata) Var. Cavendish In Vitro. *Agro Bali : Agricultural Journal*, *6*(1), 231–240.
 - https://doi.org/10.37637/ab.v6i1.1163
- Ramesh, & Ramassamy. (2014). Effect of Gelling Agents in In Vitro Multiplication of Banana Var. Poovan. *International Journal of Advanced Biological Research*, 4(3), 308–311.
- Rodinah, Hardarani, N., & Ariani, H. D. (2018). Modifikasi Media dan Periode Subkultur pada Kultur Jaringan Pisang Talas (Musa paradisiaca Var. Sapientum L.). *Jurnal Hexagro*, 2(1), 30–35
- Rodinah, & Nisa, C. (2018). Growth Regulator Formulation with Subculture Time Interval on Initiation and Multiplication of Talas Banana (Musa paradisiaca var sapientum L) as Invitro). *Ziraa'ah*, *43*(2), 141–148.
- Sadat, M. S., Siregar, A. M., & Setiado, H. (2018).

 Pengaruh IAA dan BAP Terhadap Induksi
 Tunas Mikro dari Eksplan Bonggol Pisang
 Kepok (Musa paradisiaca L).

 JurnalAgroekoteknologi FP USU, 6(1), 107–
 112.
- Sari, B. D. R., Suminar, E., Nuraini, A., & Ismail, A. (2016). The Experiment Of Effectiveness With Concentration Of Cytokinin On Micro Shoot Multiplication Banana (Musa Paradisiaca L.) On In Vitro Culture. *Jurnal Kultivasi*, 15(2), 74–80.
- Sauer, M., Robert, S., & Kleine-Vehn, J. (2013). Auxin: Simply Complicated. *Journal of Experimental Botany*, 64(9), 2565–2577.

- https://doi.org/10.1093/jxb/ert139
- Shirani, S., Mahdavi, F., & Maziah, M. (2009). Morphological Abnormality Among Regenerated Shoots of Banana and Plantain (Musa spp.) after In Vitro Multiplication with TDZ and BAP from Excised Shoot-Tips. *African Journal of Biotechnology*, 8(21), 5755–5761. http://www.academicjournals.org/AJB
- Sihotang, S., Kardhinata, E. H., & Riyanto. (2016). Stimulasi Tunas Pisang Barangan (Musa acuminata L.) secara In Vitro dengan Berbagai Konsentrasi IBA (Indole-3-butyric acid) DAN BA (Benzyladenin). *BioLink*, *3*(1), 18–30. http://ojs.uma.ac.id/index.php/biolink
- Supalal. (2015). Modifikasi Zat Pengatur Tumbuh dalam Budidaya Jaringan untuk Perbanyakan Bibit Tebu (Saccharum officinarum). *Bioma*, *4*(1), 1–20.
- Svolacchia, N., & Sabatini, S. (2023). Cytokinins. *Nat Rev Mol Cell Biol*, *33*(1), 10–13. https://doi.org/10.1016/j.cub.2022.11.022
- Syafrilia, R. P., Roostika, I., Adiredjo, A. L., & Saptadi, D. (2018). Regenerasi Embrio Pisang Liar Melalui Kultur In Vitro dengan Aplikasi Sukrosa, Benzyl Adenine dan Polyvinylpirrolidone. *Jurnal Produksi Tanaman*, 6(6), 935–942.
- Tini, E. W., Sakhidin, Saparso, & Haryanto, T. A. D. (2022). Kandungan Hormon Endogenous pada Tanaman Hortikultura. *Jurnal Galung Tropika*, *11*(2), 132–142. https://doi.org/10.31850/jgt.v11i2.955
- Tyas, K. N., Susanto, S., Dewi, I. S., & Khumaida, N. (2016). Organogenesis Tunas secara Langsung pada Pamelo (Citrus maxima (Burm.) Merr.). *Buletin Kebun Raya*, 19(1).
- Unsong, N., Tilaar, W., & Sumayku, B. R. (2022). Sterilisasi dan Penggunaan Zat Pengatur Tumbuh BAP (Benzile Amino Purin) terhadap Pertumbuhan Eksplan Tunas Pisang Abaka (Musa textilis Nee) Melalui Teknik In Vitro. *Agri-SosialEkonomi Unstrat*, 18(3), 717–724.
- Wei, K., Ruan, L., Wang, L., & Cheng, H. (2019).
 Auxin-Induced Adventitious Root Formation in Nodal Cuttings of Camellia sinensis.

 International Journal of Molecular Sciences, 20(19), 1–10. https://doi.org/10.3390/ijms20194817
- Wu, W., Du, K., Kang, X., & Wei, H. (2021). The Diverse Roles of Cytokinins in Regulating Leaf Development. In *Horticulture Research* (Vol. 8, Issue 1, pp. 1–13). Springer Nature. https://doi.org/10.1038/s41438-021-00558-3
- Yali, W., & Begna, T. (2021). Plant Tissue Culture and Its Application in Modern Crop

- Improvement. *International Research Journal of Plant and Crop Sciences*, 6(4), 201–210. www.advancedscholarsjournals.org
- Yang, W., Cortijo, S., Korsbo, N., Roszak, P., Schiessl, K., Gurzadyan, A., Wightman, R., Jönsson, H., & Meyerowitz, E. (2021). Molecular Mechanism of Cytokinin-Activated Cell Division in Arabidopsis. *Science*, *371*(6536), 1350–1355. https://doi.org/10.1126/science.abe2305
- Yulianti, S., Noli, Z. A., & Idris, M. (2024). Shoot Induction of Black Stem Potato (Solanum tuberosum L. var. Cingkariang) with Several Concentration of 6-Benzylaminopurine (BAP). *Jurnal Biologi Tropis*, 24(1), 52–58. https://doi.org/10.29303/jbt.v24i1.6237
- Zhang, Q., Gong, M., Xu, X., Li, H., & Deng, W. (2022). Roles of Auxin in the Growth, Development, and Stress Tolerance of Horticultural Plants. In *Cells* (Vol. 11, Issue 17, pp. 1–15). MDPI. https://doi.org/10.3390/cells11172761
- Zubo, Y. O., & Schaller, G. E. (2020). Role of the Cytokinin-Activated Type-B Response Regulators in Hormone Crosstalk. *Plants*, *9*(2), 166. https://doi.org/10.3390/plants9020166
- Zulkifli, & Sari, P. L. (2017). Pengaruh Konsentrasi Bayclin pada Pencucian II dan BAP pada Media MS terhadap Pertumbuhan Eksplan Tanaman Pisang Klutuk (Musa paradisiaca. L) secara In Vitro. *Dinamika Pertanian*, *13*(1), 163–169.