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The Effect of Benzyl Amino Purine (BAP) on Indirect Organogenesis of the Titan Arum (*Amorphophallus titanum* (Becc.))

Putri Salsa Afifah, Yusniwati*, Aprizal Zainal, Ryan Budi Setiawan
Department of Agrotechnology, Faculty of Agriculture, Universitas Andalas, Padang, Indonesia

*Corresponding author: yusniwati@agr.unand.ac.id

ABSTRACT

The titan arum (*Amorphophallus titanum* (Becc.)), an endangered and endemic flora from the island of Sumatra, faces a significant risk of extinction. Conservation efforts for the titan arum were undertaken, including in vitro conservation through tissue culture. The study aimed to determine the optimal concentration of the growth regulator Benzyl Amino Purine (BAP) for inducing shoot formation in titan arum and to observe cellular changes during the shoot stage through histological test. This research was conducted from November 2023 to March 2024 at the Tissue Culture Laboratory, Andalas University. The experiment was arranged in a Completely Randomized Design (CRD) with five treatment levels: 2, 3, 4, 5, and 6 ppm. Data analysis was performed using an F-test at a 5% significance level. If the F-test showed significant differences, further testing was carried out using Duncan's Multiple Range Test (DMRT) at a 5% significance level. Meanwhile, data on explant rooting percentage and root count were presented as means and standard deviations. Results indicated that various concentrations of BAP could induce shoots formation of *Amorphophallus titanum* Becc., with the percentage of explants forming shoots reaching 80–95%. The concentration of 5.0 ppm BAP was the most effective, yielding the highest average number of shoots at 7.80. Histological test revealed cell enlargement at the shoot tips. This research is pivotal for the conservation of titan arum and promotes further studies in in vitro culture techniques.

Keywords: Conservation; Endemics; Explants; Propagation; Shoot

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INTRODUCTION

Indonesia is the world's largest archipelagic country with a very high level of floral diversity. As of 2017, Indonesia's floral diversity has been identified as comprising 31,750 plant species, with 25,000 of these categorized as flowering plants (Retnowati et al., 2019). The threat of extinction to Indonesia's floral diversity is becoming increasingly severe. This is due to land conversion for agricultural purposes, deforestation for settlement areas, industrial and mining activities, as well as infrastructure development such as bridges and roads (Widyatmoko, 2019). Setiawan (2022) states that Indonesia ranks sixth globally in terms of the highest rate of biodiversity decline (both flora and fauna). A total of 490 plant species in Indonesia are facing extinction threats, including 135 species classified as Critically Endangered, 120 species classified as Endangered, and 235 species classified as Vulnerable (BRIN, 2023).

One of the rare plant species in Indonesia is *Amorphophallus titanum* (Becc.). *A. titanum* is an endemic Indonesian species originating from Sumatra and is commonly known as the "giant corpse flower" from the genus *Amorphophallus*. *A. titanum* was first discovered by Dr. Odoardo Beccari in 1878 in the Anai Valley, West Sumatra (Hetterscheid & Ittebach, 1996). The corpse flower is listed as a protected plant in Indonesia under Government Regulation No. 7 of 1999 and the Regulation of the Minister of Environment and Forestry of the Republic of Indonesia No. P.92/MENLHK/SETJEN/KUM.1/8/2018 (KLHK, 2015; KLHK, 2018).

According to IUCN data from 2018, the population of A. titanum in Sumatra is less than 1,000 individuals and is classified as Endangered due to a decline in its wild population (Yuzammi & Hidayat, 2018). This plant is distinguished by its unique flower morphology compared to other plants and emits an unpleasant odor, in addition to having distinctive biological cycles and rarity status (Arianto *et al*., 2019). Furthermore, *A. titanum* can be utilized as an ornamental plant due to its unique form, as a subject of scientific research, and as a food source from its tubers (Widyawati *et al*., 2019).

The rarity of A. *titanum* in Indonesia is due to the plant's long flowering period in the wild, which requires cross-pollination to produce seeds and involves a mismatch in the timing of pollen and stigma maturation (Lobin *et al*., 2007). Cross-pollination is challenging because the plant is already rarely found. Other factors contributing to the plant's rarity include land conversion, illegal logging, new land clearing, the removal of young corpse flowers growing on private lands, and the perception of the corpse flower as a nuisance due to its unpleasant odor (Hidayat & Yuzammi, 2008). Effective conservation efforts for *A. titanum* need to be implemented, including both in situ and ex situ conservation strategies.

Warseno (2015) notes that in situ conservation is

currently very challenging due to habitat destruction caused by exploitation activities, making ex situ conservation a viable alternative. Ex situ conservation of plants is typically carried out through botanical gardens and plant collections. Various methods such as seed banks, in vitro techniques, and cryopreservation have been developed for plant conservation (Ruta *et al*., 2020). Issues related to the availability of plant seedlings can be addressed through in vitro propagation via tissue culture. This technique uses plant cells, tissues, or organs under aseptic conditions to produce new individual plants (Putri et *al*., 2021). In addition to producing new plant seedlings with identical characteristics to the parent plant, this method also yields disease-free seedlings in a shorter time (Rahmawati *et al*., 2021).

Bhatia and Bera (2015) state that in vitro propagation through tissue culture can be achieved using two methods: somatic embryogenesis and organogenesis. Somatic embryogenesis can be carried out either directly or indirectly without involving gamete fusion. The indirect method of somatic embryogenesis involves initial callus formation, where callus cells subsequently differentiate into meristematic tissues (dos Santos & Paz, 2016). Meanwhile, organogenesis can also be performed either directly or indirectly. Direct organogenesis uses explants with or without shoot primordia, bypassing the callus stage. In contrast, indirect organogenesis involves initial callus formation on the explants (Dwiyani, 2015, as cited in Rahman *et al*., 2023).

The success of tissue culture is influenced by plant growth regulators, which include cytokinins and auxins. Raspor *et al*. (2021) report that cytokinins play a crucial role in stimulating cell division and cytokinesis, while auxins are involved in promoting cell growth and elongation. One commonly used cytokinin for inducing shoot growth is BAP (6-Benzyl Amino Purine). Wirianto (2014) reported that a combination of 2.0 ppm BAP and 0.1 ppm IBA for 28 weeks resulted in 162 shoots on the upper explants and 14.2 shoots on the lower explants during the shoot regeneration of *A. decus-silvae* from seeds. Similarly, Nurfadhilah (2019) found that a combination of 1.0 ppm BAP and 1.0 ppm NAA in leaf stalk cultures of *A. titanum* resulted in a low percentage of explants forming shoots, at 18.75%.

Isnaini and Novitasari (2020) reported that MS medium supplemented with a combination of 2.0 ppm BAP and 0.5 ppm NAA is optimal for stimulating shoot growth in *A. paeoniifolius* from leaf stalks, achieving a shoot formation percentage of 50%. In line with this, Wati (2021) indicated that 2.0 ppm BAP is the best concentration for inducing shoots in *A. titanum*, with an average of 3.6 shoots and the highest shoot formation percentage of 55%. Furthermore, Firdaushi (2023) found that 2.0 ppm BAP yields the fastest shoot development from *A. muelleri* callus cultures, with an average of 7.8 days after transfer and the highest number of shoots, 17.2 shoots. The most recent study by Rahmah (2024) demonstrated that 2.5 ppm BAP can produce an average of 6.50 shoots per explant over 140 days after transfer. This study aims to identify the optimal BAP concentration for shoot formation and to observe cell characteristics at the shoot stage of *A. titanum* in vitro through histological analysis.

MATERIAL AND METHOD

This study was conducted at the Plant Tissue Culture Laboratory of the Faculty of Agriculture, the Anatomical Pathology Laboratory of the Faculty of Medicine, and the Central Laboratory of Andalas University from November 2023 to March 2024.

The equipment used in this experiment included 100 ml culture bottles (baby bottles), an analytical balance, graduated cylinders, Petri dishes, Erlenmeyer flasks, stirring rods, a hot plate, a magnetic stirrer, a Laminar Air Flow Cabinet (LAFC), a Carl Zeiss Stereo Microscope STEMI 305 with Camera, a microtome, a refrigerator, an autoclave, a measuring flask, a scalpel, a measuring cylinder, a spray flask, an oven, beakers, a Bunsen burner, dropper pipettes, a culture rack, scissors, glass plastic, buckets, a hand sprayer, an 18-watt fluorescent light, matches, a camera, ImageJ software, and stationery.

The materials used in this experiment included callus obtained from *A. titanum* petiole cultures maintained at the Plant Tissue Culture Laboratory, Faculty of Agriculture, Andalas University. The media used were Murashige and Skoog (MS) medium, 30 g/L sucrose, and 8 g/L bacto agar. Other materials included 70% alcohol, 96% alcohol, Bacylin (NaClO 5.25%), liquid detergent, plastic wrap, aluminum foil, label paper, spirit, rubber bands, glass plastic, tissues, HVS paper, sterile distilled water, pH meter paper, 1 N HCl or 1 N KOH, fixation solution (10% formalin), and *Hematoxylin and Eosin* (HE) staining solution. The plant growth regulator used was BAP (6-*Benzyl Amino Purine*).

The experimental design employed was a Completely Randomized Design (CRD) with 5 treatments and 5 replications, resulting in 25 experimental units. Each experimental unit consisted of 4 culture bottles. Each culture bottle contained 1 explant, totaling 100 culture bottles, all of which were observed. The BAP treatments applied were: 2.0 ppm (A1); 3.0 ppm (A2); 4.0 ppm (A3); 5.0 ppm (A4); and 6.0 ppm (A5). The data obtained were statistically analyzed using an F-test at a significance level of 5%. If the calculated F-value was greater than the F-table value, it was followed by *Duncan's Multiple Range Test* (DMRT) at the 5% level. Data analysis was performed using Statistical Tool for Agricultural Research (STAR) software. The percentage of rooting explants and the number of roots were presented as means with standard deviations.

RESULT AND DISCUSSION

Shoot Emergence Time, Percentage of Explants With Shoots, and Number of Shoots of *A. titanum*

The analysis of variance for the shoot emergence time of A. titanum indicated that the application of BAP concentrations had no significant effect. The average time for shoot emergence in A. titanum callus ranged from 15.59 to 18.45 days after transfer (DAT). The application of BAP, a cytokinin hormone, plays a crucial role in stimulating cell division, which underlies shoot formation. Shoot emergence begins with swelling of the callus; this differentiation process results in small, white to greenish protrusions that develop into shoots during weeks 2 to 3 after transfer. Subsequently, the shoots will continue to develop and increase in size over time (Table 1). Swelling in A. muelleri callus results in white to greenish protrusions that further develop into shoots (Firdaushi, 2023).

Table 1. Shoot emergence time, percentage of explants with shoots, and number of shoots of A. titanum at various BAP concentrations at 12 Weeks After Transfer (WAT).

BAP Concentration	Shoot Emergence Time (DAT)	Percentage of Explants with Shoots (%)	Number of Shoots
2.0 ppm	18.45	95	4.05 c
3.0 ppm	17.21	90	5.33 b
4.0 ppm	16.50	95	5.75 b
5.0 ppm	17.18	95	7.80 a
6.0 ppm	15.59	80	5.60 b
KK (%) =	8.73%	25.74%	8.66 %

Note: Numbers in columns followed by different lowercase letters indicate significantly different effects according to Duncan's Multiple Range Test (DMRT) at the 5% level. Shoot emergence time data were transformed using √x %, percentage data for shoot emergence time were transformed using Arc sin √x %, and shoot emergence time data were transformed using √x %.

BAP is a cytokinin hormone that plays a crucial role in cell division. Sagai *et al.* (2016) state that BAP is essential for cell division as it enhances protein synthesis and accelerates the transition from the G1 phase (Gap 1) to the synthesis phase, and from the G2 phase (Gap 2) to mitosis. All plants undergo growth and development beginning with cell division, followed by cell differentiation and morphogenesis. In line with this, Hnatuszko *et al.* (2021) state that cytokinins affect competent cells during shoot organogenesis by leading to cell phosphorylation and regulating gene expression that governs organogenesis in shoot formation.

The application of BAP cytokinin to the culture medium of *A. titanum* callus induces shoot formation. Rasud *et al*. (2015) report that applying BAP to wounded explant tissues triggers cell division and differentiation, resulting in shoot formation. Similarly, Raspor *et al.* (2021) report that BAP can stimulate cell division, organogenesis, induce shoot formation, and promote the proliferation of axillary shoots. BAP and the endogenous hormones within the explants are thought to influence the timing of shoot emergence. The appropriate interaction between endogenous hormones in the explant, such as the kesturi orange nodal explants, and the exogenous BAP applied affects the speed of shoot emergence (Yanti & Isda, 2021). Additionally, Kartiman *et al.* (2018) state that the balance between endogenous and exogenous cytokinins and auxins is crucial for plant growth and development in vitro. Variations in shoot emergence among explants affect shoot growth and development (Wati, 2021).

The percentage of explants forming shoots refers to the proportion of explants that produce shoots out of the total explants planted. The average percentage of *A. titanum* explants forming shoots ranges from 80% to 95%. This study shows an improvement compared to previous research, with Wati (2021) and Rahmah (2024) reporting shoot formation percentages ranging from 5% to 55% in indirect organogenesis of *A. titanum*. This indicates that BAP application effectively promotes shoot formation and growth. BAP has been reported to enhance protein synthesis, thereby stimulating cell division which drives shoot formation (Harjadi, 2009, as cited in Lutfiani *et al.,* 2022).

Based on the research by Nurfadhilah (2019) and Wati (2021), BAP concentrations of 2.0 and 4.0 ppm did not significantly improve shoot growth in *A. titanum* petiole cultures. In contrast, Firdaushi (2023) reported that BAP concentrations of 2.0 and 4.0 ppm significantly enhanced shoot growth in *A. muelleri* petiole cultures, but concentrations of 6.0 ppm resulted in a decline. This suggests that an optimal condition may have been reached, and higher concentrations of BAP could inhibit shoot growth, leading to a decrease in the percentage of explants forming shoots. The results indicate that the percentage of explants forming shoots decreased at 6.0 ppm BAP. It is assumed that high concentrations of BAP are ineffective for shoot formation due to an imbalance between endogenous and exogenous hormones in the explants. The success of explants in forming shoots is influenced by the balance of endogenous hormones affected by the addition of exogenous plant growth regulators (Munggarani *et al*., 2018).

Figure 1. illustrates the growth of A. titanum shoots at 4, 8, and 12 months after subculture (MAS), showing variations in both the number and size of the shoots. Explants with a higher number of shoots generally exhibit smaller and shorter shoots, while those with fewer shoots tend to have larger and taller ones. This observation suggests that shoot formation is influenced not only by the applied hormones but also by other factors. Ngomuo *et al*. (2013) reported that poor shoot formation in explants is attributed to endogenous factors within the explant. The selection of explants, culture media, phytohormones, genotype, carbohydrates, and gelling agents, as well as environmental factors such as humidity, light, and temperature, are critical in the processes of organogenesis and embryogenesis (Ramage *et al.,* 2003 as cited in Bidabadi & Jain, 2020).

Analysis of variance for the number of shoots per explant indicated significant differences in response to varying concentrations of BAP. Observations of shoot numbers are summarized in Table 4. A concentration of 5.0 ppm BAP resulted in the highest average number of shoots, 7.80, compared to 4.05 shoots with 2.0 ppm BAP and 5.33 to 5.75 shoots with 3.0, 4.0, and 6.0 ppm BAP. Shoot formation occurs through cell division. del Pozo *et al.* (2006) as cited in Qi and Zhang (2020) reported that the plant cell cycle comprises four phases: G1 (post-mitotic interphase), S (DNA synthesis phase), G2 (pre-mitotic interphase), and M (mitosis/cytokinesis). The progression through these phases is regulated by cyclin-dependent kinases (CDKs) in conjunction with cyclins. Cyclin classes A, B, and D play specific roles: Cyclin D regulates the G1 to S transition, Cyclin A controls the S to M phase transition, and Cyclin B manages the G2 to M transition. During the G1 to S transition, the CDKA/CYCD complex phosphorylates retinoblastoma

(RBR) protein, activating the S-phase transcription factor "E2F," which promotes G1-S transition by driving gene expression involved in DNA replication, cell cycle progression, and chromatin remodeling. Additionally, during the G2-M transition, CDKA and CDKB bind with CYCA, CYCB, or CYCD, facilitating cell division.

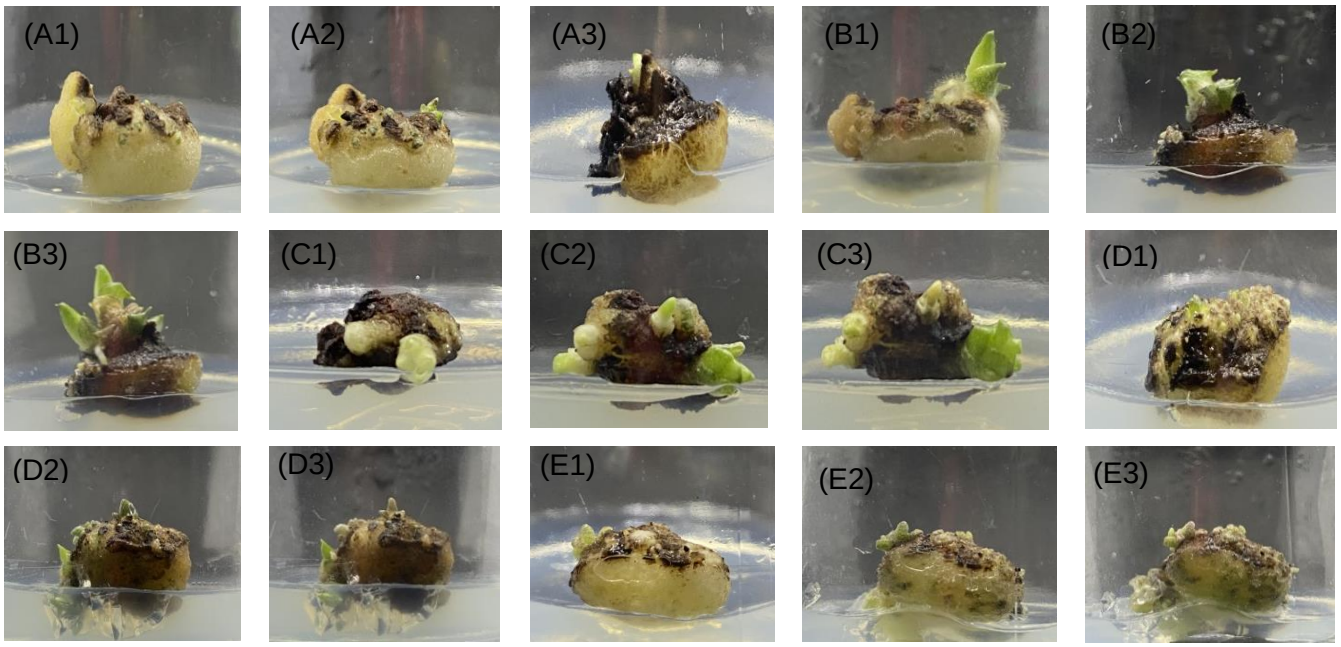

Figure 1. Appearance of *A. titanum* Shoots at 4, 8, and 12 Weeks After Transfer (WAT) : A. 2.0 ppm; B.3.0 ppm BAP; C. 4.0 ppm BAP; D. 5.0 ppm BAP dan E. 6.0 ppm BAP

Increasing BAP concentrations, a type of cytokinin, enhances the number of shoots across all treatments. Kieber & Schaller (2014) stated that cytokinins are essential in in vitro plant propagation, not only for cell division leading to undifferentiated callus formation but also for promoting the development of new shoot apical meristems. Correspondingly, Ferdous et al. (2015) as cited in Munggarani et al. (2018) reported that high concentrations of cytokinins can increase banana shoot numbers. The number of shoots observed in this study is higher compared to similar studies. Wati (2021) reported that 2.0 ppm BAP produced an average of 3.6 shoots/explant after 12 week after plant (WAP), while Nurfadhillah (2019) found that 1.0 ppm BAP combined with 1.0 ppm NAA resulted in 12 shoots/explant after 32 WAP. Additionally, Firdaushi (2023) observed that 2.0 ppm BAP produced an average of 13.2 shoots/explant after 6 WAP, and Rahmah (2024) found that 2.5 ppm BAP resulted in 6.50 shoots/explant after 140 days after subculture (DAS). Variations in shoot numbers are influenced by different responses of each explant to the exogenous hormones. The size of the shoots in this study varied, with smaller sizes associated with higher shoot numbers and larger sizes with fewer shoots (Figure 1). The response of each explant to the added growth regulators varies (Prasetyo *et al*., 2020).

The ratio of cytokinin to auxin also affects shoot formation. Kurepa & Smalle (2022) indicated that higher concentrations of cytokinin relative to auxin favor shoot formation, whereas higher auxin concentrations lead to root formation. This study also noted a decrease in the number of shoots at a 6 ppm BAP concentration. Firdaushi (2023) similarly reported a reduction in A. muelleri shoots at 6 ppm BAP. Elevated BAP concentrations may affect explant physiology, leading to decreased shoot numbers. Shoot formation is significantly influenced by the balance of endogenous and exogenous hormones within the explant. Rasud & Anwar (2019) previously reported that the balance and levels of endogenous and exogenous hormones drive shoot formation and growth, contributing to increased numbers of Siam orange shoots.

Shoot Emergence Time, Percentage of Explants Forming Shoots, and Number of Roots *A. titanum*

The analysis of variance for the height of A. titanum shoots indicates a significant effect of BAP concentration. A concentration of 2.0 ppm BAP was found to be the most effective in promoting shoot height growth in A. titanum. Specifically, 2.0 ppm BAP resulted in the tallest shoots, with an average height of 0.56 cm, while 5.0 ppm BAP produced the shortest shoots, with an average height of 0.32 cm. Shoot height growth is attributed to the process of cell elongation. Widiastoety (2014) reported that increases in plant height result from cell division, elongation, and enlargement occurring in the apical meristems and stem internodes, leading to overall stem elongation.

The application of high concentrations of the cytokinin BAP is thought to increase the number of shoots while potentially inhibiting shoot height growth. Munggarani *et al.* (2018) reported that higher BAP concentrations in potato plants are inversely related to the number of shoots, where fewer shoots result in increased shoot height and vice versa. An increased number of shoots leads to competition among them for nutrients in the medium. Azizi *et al.* (2017) noted that the reduction in shoot height is due to competition among shoots for nutrients, which is exacerbated by an increased number of shoots in sugarcane (Saccharum officinarum L.). Similarly, Novianti *et al.* (2022) observed that a higher number of shoots leads to decreased plant height in red banana plants

Table 2. Shoot emergence time, percentage of explants forming shoots, and number of roots of *A. titanum* at various BAP concentrations at 12 Weeks After Transfer (WAT).

BAP Concentration	Shoot Eight	Percentage of Explants Forming Shoots (%)	Number of Roots
2.0 ppm	0.56 a	10 ± 3.49	3 ± 1.73
3.0 ppm	0.46 b	10 ± 3.49	2 ± 1.15
4.0 ppm	0.41 b	5 ± 3.16	1 ± 0.00
5.0 ppm	0.32 c	0 ± 0.00	0 ± 0.00
6.0 ppm	0.34 c	0 ± 0.00	0 ± 0.00
KK (%) =	11.66%		

Note: Numbers in columns followed by different lowercase letters indicate significantly different effects according to Duncan's Multiple Range Test (DMRT) at the 5% level. The percentage of rooting explants and the number of roots were presented as means with standard deviations.

The reduction in shoot height may be attributed to sufficient endogenous cytokinin levels, making the addition of exogenous hormones unnecessary. Saepudin *et al.* (2020) reported that the interaction and balance between exogenous and endogenous growth regulators influence the rate and direction of orchid development in vitro. Both exogenous and endogenous hormones in explants affect shoot height growth. Ainipasha et al. (2024) stated that shoot height growth is influenced by several factors, including the focus of explant growth and both exogenous and endogenous hormones.

The percentage of explants that form roots refers to the proportion of explants that develop roots out of the total number planted. The observed data on the percentage of rooted explants is presented in Table 2. High concentrations of BAP can inhibit root formation. Su *et al.* (2011) reported that media without cytokinin are more effective for root development, as cytokinin can inhibit the biosynthesis of endogenous auxins necessary for root formation. High concentrations of BAP suppress endogenous auxins within the explants, thereby impeding root development. Mirah *et al.* (2021) stated that increasing concentrations of cytokinin increasingly inhibit root growth.

Auxin plays a critical role in cell elongation, cell division, vascular tissue differentiation, and root initiation (Astutik *et al.,* 2021). The effectiveness of auxin and cytokinin is dependent on their concentrations. Kurepa & Smalle (2022) indicated that a higher ratio of cytokinin to auxin promotes shoot formation, whereas a lower ratio favors root formation. Consistent with these findings, Nurfadhilah (2019) reported that a combination of 1.0 ppm BAP and 1.0 ppm NAA resulted in the highest percentage of rooted explants, which was 25%.

Based on Table 2, it is evident that BAP application was not effective in inducing root formation across all treatments. A concentration of 2.0 ppm BAP resulted in an average of 3 roots, while concentrations of 3.0 and 4.0 ppm BAP produced only 1 root each, and concentrations of 5.0 and 6.0 ppm BAP did not yield any roots. These results are lower compared to those reported by Wati (2021), who found that 2.0 ppm BAP produced an average of 2.30 roots in A. titanum. This lower performance may be attributed to the relatively high endogenous auxin levels in the explants, which suggests that low concentrations of exogenous BAP may not be effective in inducing root formation in *A. Titanum*.

Auxins and cytokinins interact to stimulate root formation. Widiastoety (2014) reported that the levels of endogenous auxins and cytokinins in plant tissues are related to root formation, leading to subsequent cell elongation and enlargement. In line with this, Schaller *et al*. (2014) noted that cytokinins play a crucial role in regulating meristems, promoting the formation of Shoot Apical Meristems (SAM) through cell division stimulation, while auxins drive the formation of Root Apical Meristems (RAM) through enhanced cell differentiation. Auxins are essential for root development, activating genes involved in root formation such as WUSCHEL RELATED HOMEOBOX (WOX5) (Pierre-Jerome *et al.,* 2018).

High concentrations of BAP alone are suspected to suppress endogenous auxins, thereby inhibiting root formation (Figure 1). Mirah *et al.* (2021) reported that increased cytokinin concentrations can hinder root growth. The optimal effect of auxins and cytokinins on certain plants requires precise concentration levels. Hnatuszko *et al*. (2021) stated that a higher ratio of cytokinin to auxin promotes shoot growth, whereas a lower ratio favors root growth. Consistent with this, Wati (2021) reported that a combination of 0 ppm BAP and 1 ppm NAA resulted in the highest average number of roots in A. titanum, with 8.65 roots

Histological Analysis of Shoots

Histological observations of A. titanum shoots at 4, 8, and 12 months after subculture (MAS) reveal that cell division and cell elongation are integral to shoot formation. The application of cytokinin, specifically BAP, stimulates shoot development. As illustrated in Figure B, changes in cell shape through elongation in the shoot region indicate active cell division. Sagai *et al*. (2016) reported that cell division is facilitated by increased protein synthesis and the acceleration of the transition from the G1 phase (Gap 1) to the S phase and from the G2 phase (Gap 2) to mitosis following the application of cytokinin in the form of BAP. Genes involved in the cell cycle, such as EF2a (Ikeda *et al*., 2006), ESR1 (Iwase *et al*., 2017), and ESR2 (Ikeuchi *et al*., 2019) as cited in Hnatuszko *et al*. (2021), are crucial for this process. Additionally, auxins play a significant role in shoot formation. Auxins, as supplementary signals in the CYCD/CDK pathway, promote cell division in shoots and influence the progression of the cell cycle into the S phase via the cyclin-dependent kinase A/D-type cyclin (CYCD) pathway (Schaller *et al*., 2015).

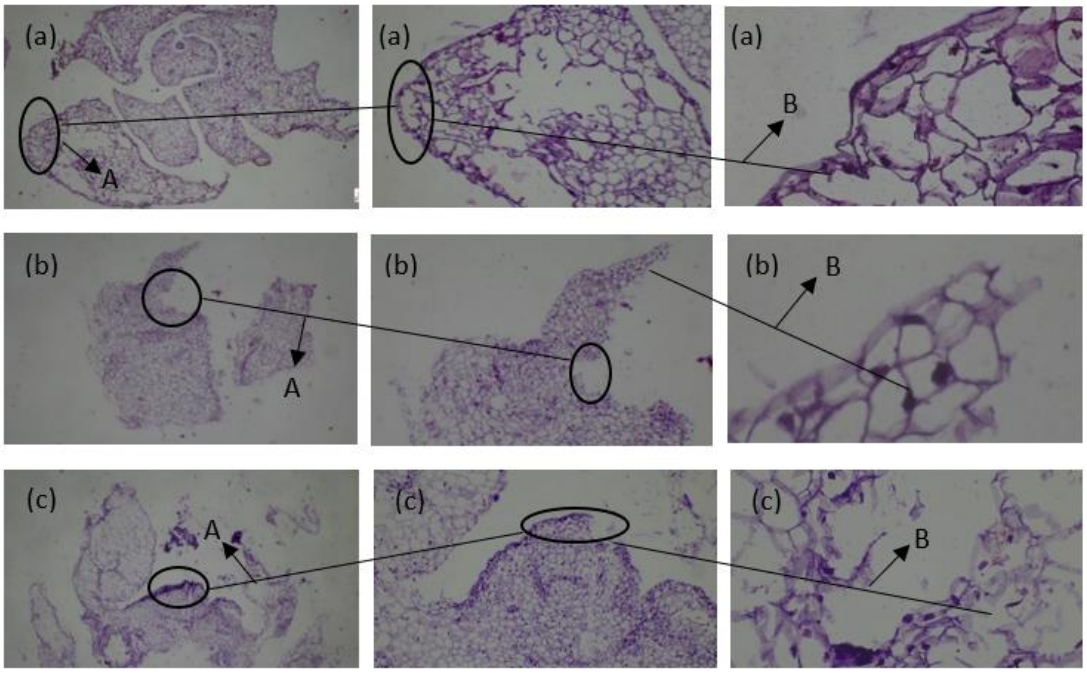

Figure 2. Histological observations of A. titanum shoots at a concentration of 4 ppm BAP, with objective lens magnifications of 4x, 10x, and 40x. A) Shoot tip, B) Cell elongation, and C) (a) 4 Months After Subculture (MAS), (b) 8 MAS, and (c) 12 MAS.

The application of cytokinins activates genes that promote shoot formation, such as WUSCHEL (WUS). Yadav *et al*. (2011) reported that WUSCHEL plays a role in shoot induction through the interaction with other shoot meristem genes, such as the Shoot Meristem-Less (STM) gene, which is expressed in the promeristem. In agreement, Klawe *et al.* (2020) stated that WUS is involved in the formation of apical meristems and the differentiation of new tissues. Additionally, several genes, including ARR1, ARR2, ARR10, and ARR12, interact with HD ZIP III to activate WUS expression, thereby promoting shoot regeneration (Zhang *et al*., 2017, as cited in Smeringai *et al.,* 2023).

As shown in Figure 2.A, there is an interaction between cytokinins and auxins in shoot formation, evident from the elongation of cells at the shoot tip. Cytokinins facilitate cell division. Hayati (2020) reported that cytokinins promote cell division by increasing the rate of protein synthesis. Auxins are crucial for cell elongation. Auxins signal specific proteins to interact with H+ ATPase in the plasma membrane, leading to phosphorylation and cell elongation in Arabidopsis plants (Lin *et al.,* 2021).

CONCLUSION

Based on the conducted research, the following conclusions can be drawn: The application of various concentrations of BAP can induce shoot formation in *Amorphophallus titanum* (Becc.) with shoot formation percentages ranging from 80% to 95%. Among the tested concentrations, 5.0 ppm BAP proved to be the most effective, yielding the highest average number of shoots at 7.80. Histological analysis revealed that explants underwent cell division and elongation, as evidenced by the increase in cell size at the shoot tips.

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