Jurnal Riset Biologi dan Aplikasinya
https://journal.unesa.ac.id/index.php/risetbiologi

Somatic Embryo Enhancing of *Phalaenopsis amabilis* (L.) Blume Orchid with 6-Benzyl Amino Purine (BAP)

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Article History

Received : 6 February 2025
Revised : 25 February 2025
Accepted : 17 March 2025
Published : 31 March 2025

Keywords
Tissue culture; PGRs; BAP; propagation

ABSTRACT

Phalaenopsis amabilis (L.) Blume is an orchid with high economic value because of its beautiful white flowers, yellow labellum, and long stems. Its existence in nature is threatened with extinction, so efforts are needed to cultivate and preserve *P. amabilis*, one of which is through somatic embryos, which generally require Plant Growth Regulators (PGRs) in the form of 6-Benzyl Amino Purine (BAP). 6-Benzyl Amino Purine (BAP) is used because it is one of the cytokinin hormones proven to induce somatic embryos. This study aims to examine and determine the use of the right BAP concentration to propagate *P. amabilis* through somatic embryo induction. The explants used were *P. amabilis* protocorms; because protocorms produced a greater number of somatic embryos than leaf explants, the development of the explants was observed every week for 2 months. This study used a single factor Completely Randomized Design (CRD) in the form of BAP concentrations of 0, 1, 2, 3, and 4 ppm with 20 replications. The results showed that the most optimal treatment for the propagation of *P. amabilis* somatic embryos was on media with an average number of somatic embryos at a concentration of BAP 3 ppm (65.00) embryo/explain.

How to cite: Karend AHA., Setiari N., Nurchayati Y & Rashid FA. (2025). Somatic Embryo Enhancing of *Phalaenopsis amabilis* (L.) Blume Orchid with 6-Benzyl Amino Purine (BAP). *Jurnal Riset Biologi dan Aplikasinya*, 7(1):88-98. DOI: 10.26740/jrba. V7n1.p.88-98.

INTRODUCTION

One of the largest and most diverse families of flowering plants is the Orchidaceae species. Indonesia has around 5000 species of the 20,000 orchid species that live naturally throughout the world (Mose et al., 2020). One of the orchids in Indonesia is *Phalaenopsis amabilis* (L) Blume. The *P. amabilis* orchid has a high economic value because, in Deli Serdang Regency, Indonesia, this plant is valued at IDR 234,696/stem, with a need of up to 100 stems per month (Zega et al., 2021). In addition, the *P. amabilis* orchid has appeal because of its beautiful flower color and shape (Salama et al., 2024).

The *P. amabilis* orchid has CITES appendix II status, which means that this species is not currently threatened with extinction but is at risk of becoming so if trade in the species is not controlled (CITES, 2021). Currently, the availability of *P. amabilis* in the wild is low due to the extensive deforestation in its natural habitat, forest fires, and land conversion into housing and highways. The slow growth of *P. amabilis* orchids has also contributed to a decline in the orchid population in the wild (Siron et al., 2019). Therefore, efforts are needed to cultivate and conserve *P. amabilis*, one of which is through tissue culture.

Tissue culture is one of the methods used for cultivation and conservation. The advantage of propagation by tissue culture is that the offspring produced are more numerous than in conventional methods. Moreover, the seedlings produced are uniform and have the same character as the parent (Oseni et al., 2018). One such tissue culture technique involves somatic embryos. Propagation using somatic embryos can produce uniform offspring (Destinugrainy & Semiarti, 2016). According to Ibrahim & Hartati (2017), somatic embryos have two poles (bipolar), enabling the formation of root and shoot buds. This technique offers a solution for conserving and mass-producing

endangered orchid plants, such as *P. amabilis*, which naturally grow slowly. With the formation of somatic embryos, orchid populations can increase more quickly and efficiently. Forming somatic embryos through tissue culture techniques requires a suitable culture media to increase the propagation process in orchid plants. Culture media generally contain complete nutrients. These complete nutrients include micro and macro elements, sucrose, myoinositol amino acids, and vitamins (Bait et al., 2024). One of these tissue culture media is Murashige and Skoog (MS) (Nur'riyani, 2021). In addition, the determinant of somatic embryo success is adding plant growth regulators (PGRs) at specific concentrations (Lestari, 2011). The addition of BAP as an exogenous cytokinin causes the levels of cytokinin hormones in plants to be higher than auxin, thereby stimulating cell division. Generally, cytokinin hormones stimulate cell division (Muchsin et al., 2022).

One of the cytokinins often added to in vitro culture media is 6-benzyl Amino Purine (BAP) (Siron et al., 2019), according to Fithriyandini et al. (2015), who stated that the addition of BAP concentrations of two and 2.5 ppm to the nodes of the flower stalks of the moth orchid resulted in a higher number of Protocorm Like Bodies (PLB) by 66.7%. In another study conducted by Ario and Setiawan (2020), administering BAP at a concentration of 1.5 ppm increased the number of shoots in *Dendrobium spectabile* explants. This study aims to examine and determine the use of the right BAP concentration to propagate P. amabilis through somatic embryo induction. This research is necessary because there hasn't been much information on using BAP to induce somatic embryos in *P. amabilis*.

MATERIALS AND METHODS

This research was conducted in the plant tissue culture room, Plant Structure and Function Biology Laboratory, Department of Biology, Faculty of Science and Mathematics, Diponegoro University, Semarang. The research began in August 2023 and ended in June 2024.

The materials used in the study are protocorm derived from *P. amabilis* orchid seed plantlets in Murashige and Skoog (MS) media, 70% alcohol, 95% alcohol, distilled water, ready-to-use MS basic culture media, 6-Benzyl Amino Purine (BAP) stock solution, HCL, NaOH, sucrose, gelatin for compacting planting media, water, and laundry soap. Meanwhile, the tools used in this study were Petri dishes, culture bottles, aluminium foil, plastic wrap, tweezers, scalpels, spatulas, beakers, droppers, magnetic stirrers, autoclaves, Erlenmeyers, labels, analytical balances, laminar airflow (LAF), pH meter paper, sprayers, tissues, refrigerators, measuring cups, culture racks, scissors, stationery, sterile opaque paper, sponges, brushes.

The design used in this study was a completely randomized design (CRD) in a factorial manner consisting of one factor, the BAP factor. The BAP factor consists of five treatment levels: BAP 0 ppm (control), BAP 1 ppm, BAP 2 ppm, BAP 3 ppm, and BAP 4 ppm. Each treatment consisted of 20 replications.

The implementation of the research includes the preparation of plant materials, sterilization of orchid seed capsules, preparation of research sites, sterilization of tools, making Murashige and Skoog (MS) media, giving BAP treatment, installing labels, planting explants, maintenance and observation parameters by measuring the percentage of live and bleached explants, morphology of explants that successfully live and grow to form somatic and bleached embryos, the number and percentage of somatic embryos (20 replication) formed in *P.amabilis* orchid explants with BAP treatment at eight weeks after planting (WAP), morphology of the somatic embryo formation stage, development of somatic embryos per 2 weeks, and the average number of somatic embryos in each phase of the *P. amabilis* orchid.

The quantitative data obtained were analyzed using ANOVA. If a significant difference exists, further testing is carried out using the DMRT Test at the test level $\alpha = 0.05$. Qualitative data was analyzed descriptively with the help of an *OptiLab* advanced 2.2 camera and a stereo microscope at 2x magnification.

RESULTS AND DISCUSSION

The results of the study on the induction of somatic embryos of *P. amabilis* orchids with BAP include the percentage of live and bleached explants, the morphology of explants that successfully survived and grew somatic and bleached embryos which undergo chlorophyll degradation, the number and percentage of somatic embryos formed in *P. amabilis* orchids for eight weeks, the morphology of the somatic embryo formation stage, the development of somatic embryos per two weeks, and the average number of

somatic embryos in each phase of *P. amabilis* orchids.

Percentage of Live Explants and Bleaching

The analysis showed that BAP treatment affected the percentage of live explants and bleaching in *P. amabilis* orchids that remained alive. The percentage of live explants with BAP 1, 2, and 3 ppm was more alive than the percentage of live explants planted in BAP zero and four ppm media (Figure 1). Treatment with the right BAP concentration prevented the bleaching process.

The percentage of living explants planted on MS media with BAP 1 ppm (95%), BAP 2 ppm (95%), and BAP 3 ppm (80%) treatments was more significant than *P. amabilis* explants planted on BAP 0 ppm (70%) and BAP 4 ppm (60%) media (Figure 1). This difference is because BAP helps the formation of chloroplasts in explants (eight WAP) so that cells in the *P. amabilis* orchid remain alive and growing. The mechanism of cytokinin is to receive the cytokinin hormone from the receptor. In the receptor, signal transduction will occur (signal transmission), which causes phosphorylation (addition of phosphate groups) to the transcription factor. This transcription factor will activate the gene that encodes the phosphatase enzyme so that the phosphatase enzyme is synthesized. The phosphatase enzyme in cell division breaks one of the phosphate bonds in cyclin-dependent kinase (CDK), so CDK becomes active, which drives the transition process from G2 to the mitosis phase or cell division. When cell division continues, the cells will swell and differentiate into somatic embryos. When cell division continues, the cells will swell and differentiate into somatic embryos. Cytokinin can maintain leaf chlorophyll through plants' high isopentenyl transferase (IPT) gene expression. Cytokinin signal transduction genes delay the ageing process of explants by increasing chlorophyll content and reducing sucrose accumulation (Wu et al., 2021). This is supported by Cortleven et al. (2016), who stated that the presence of the cytokinin hormone could increase the rate of chloroplast formation and can stimulate ultrastructural changes that are characteristic of the transition from etioplast to chloroplast so that explants can survive by maintaining chlorophyll and preventing changes in explant colour. This result is in accordance with the research of Yulia et al. (2020) that BAP can stimulate increased chlorophyll production, which affects the photosynthesis process. Fithriyandini et al. (2015) reported that the administration of BAP with the right concentration increased the percentage of survival of *P. amabilis* flower stalk node explants. In addition, Hartati et al. (2016), at the right concentration, the use of cytokinin was proven to affect inhibiting color changes in Sansevieria macrophylla explant callus.

In the percentage of bleaching explants with BAP 1 ppm (5%), BAP 2 ppm (5%), and BAP 3 ppm (20%), fewer explants experienced bleaching compared to BAP concentrations of 0 ppm (30%) and BAP 4 ppm (40%). This is because low BAP concentrations (0 ppm) and high (4 ppm) have not been able to maintain the survival of *P. amabilis* explants optimally, so the number of explants experiencing bleaching is higher. Explants that experience bleaching show signs of damage, such as a change in colour to pale white due to degraded chlorophyll content.

In the control medium (0 ppm), as many as 30% of explants experienced bleaching. This is due to the absence of cytokinin hormone. Wu et al. (2021) stated that one of the functions of the cytokinin hormone is to maintain chlorophyll. This is supported by research by Mose et al. (2017), that several *P. amabilis* explants in control media (TDZ 0 ppm) could not live or died after 4 MST, and only 70% of protocorms succeeded in forming somatic embryos. In addition, at a concentration that is too high (BAP 4 ppm), as many as 40% of explants experience bleaching. This result is because BAP given at a concentration too high is toxic to plants, so explant growth is inhibited. In accordance with research by Sulaiman et al. (2020), giving BAP at high concentrations can inhibit *Ananas comosus* L. Merr explants.

Formation of somatic embryos

The results of the ANOVA test showed that BAP treatment affected the average number of somatic embryos in each explant. The results of the DMRT test showed that explants planted in media supplemented with BAP concentrations of 1, 2, and 3 ppm significantly effect on the multiplication of somatic embryos (Table 1).

The average number of somatic embryos of *P. amabilis* on MS media supplemented with one ppm BAP (52.23), two ppm BAP (62.67), and three ppm BAP (65.00) was higher compared to *P. amabilis* explants grown on control media (29.67) and four ppm BAP (17.67). Media with BAP concentrations of 1, 2, and 3 ppm produced a higher number of embryos because the administration of

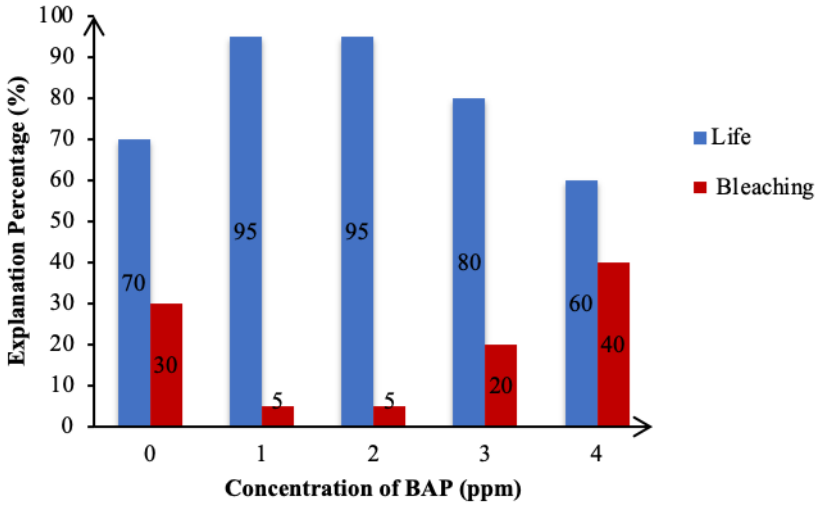


Figure 1. Percentage of live and bleached explants in 20 *Phalaenopsis amabilis* orchid explants planted with BAP treatment (0, 1, 2, 3, and 4 ppm) after eight WAP

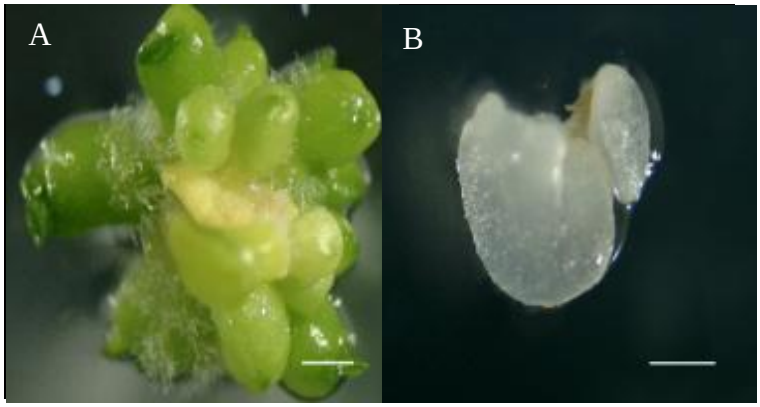


Figure 2. *Phalaenopsis amabilis* orchid explants that successfully survive (A) and experience bleaching (B). Explants observed at 0 ppm BAP concentration. Bar = 1 mm

Table 1. Percentage, Number, and Average of Phalaenopsis amabilis Orchid Explants that Form Somatic Embryos with 6-Benzyl Amino Purine (BAP) treatment at 8 MST

Concentration of BAP (ppm)	Percentage of Explants Forming Somatic Embryos	Total Somatic Embryo	Mean Somatic Embryo/Explant ± Standard Deviation
0	100 %	89	29.67 ± 9.02[b]
1	100 %	157	52.23 ± 17.21[a]
2	100 %	188	62.67 ± 2.52[a]
3	100 %	195	65.00 ± 13.75[a]
4	100 %	53	17.67 ± 1.53[b]

BAP at the right concentration was able to increase the number of higher cell divisions, resulting in a more optimal number of somatic embryos (Kępczyńska & Kępczyński, 2023). This is in accordance with research (Mose et al., 2017) where the protocorm and stem explants of *Phalaenopsis amabilis* (L.) Blume produced the most somatic embryos supplemented with TDZ (1, 2, and 3 ppm).

Another study by Britto et al. (2022) found that BAP 3 ppm is optimal for callus growth in *Cimbidium pandurate* orchid explants. This callus growth is due to the role of BAP as a signal in cells that functions to regulate cell division. One of them is a signal in activating phosphatase, which breaks one of the phosphate bonds in CDK so that CDK becomes active. Furthermore, CDK regulates the transition process from G2 to the mitosis phase or cell division (Thomas & Jiménez, 2005). When cell division occurs continuously, the cells will swell and differentiate into somatic embryos (Riyadi et al., 2016).

The number of somatic embryos produced was lower in media with a BAP concentration of 0 (control). This is thought to be because, at BAP 0 ppm, there was no addition of BAP, and endogenous cytokinins in *P. amabilis* explants were not optimal in increasing somatic embryo induction, so the signal received by the receptors in the cells would be lower, and cause a suboptimal cell division process. This result is in accordance with research by Fithriyandini et al. (2015), which showed that the addition of BAP with a concentration of 2.5 ppm can increase the thickness of callus in *Phalaenopsis amabilis* orchids, which is an early indication of somatic embryo induction. This result was supported by research by Mose et al. (2017), which found that at a TDZ concentration of 0 ppm, the somatic embryos produced were 70% in the *P. amabilis* protocorm.

Furthermore, the number of somatic embryos decreases at concentrations that are too high (4 ppm BAP). This is suspected to be due to the cytokinin hormone with a high concentration, causing the receptors in the cells not to work optimally because there is hormone competition, which disrupts the role of cytokinins to encourage the cell division process optimally, resulting in a decrease in in in the number of cell divisions and producing fewer somatic embryos. This is in accordance with the opinion of Lee et al. (2021) that when the concentration of ZPT is too high, the growth of somatic embryos is disrupted, as seen in studies on other plants, namely the Dendrobium spectabile orchid. This is supported by research by Ningrum et al. (2017), who found that the use of ZPT that is too high can inhibit plant growth and tend to have no effect.

The use of BAP can play a role in accelerating the orchid propagation process (Lee et al., 2021). Determining the right concentration of ZPT is very important to increase the success of orchid propagation because it can increase plant growth optimally (Hartati et al., 2024). This is supported by research by Mose et al. (2017), that at a TDZ concentration of 0 ppm, the somatic embryos produced were 70% compared to TDZ concentrations of 1, 2, and 3 ppm of 100% in *P. amabilis* protocorms.

Morphology of Somatic Embryos of *P. amabilis*

The morphology of the somatic embryo of the *P. amabilis* orchid has several phases, including the embryogenic callus phase, globular embryo with buds, elongated shoots, and leaf-forming embryos. The initial stage is the formation of embryogenic callus. The next is the formation of the globular phase, which is marked by green circles. The next phase is the embryo with buds, which is marked by the appearance of buds at the tip of the embryo. The elongated shoot phase is formed, which is marked by an increase in the length of the shoots, and the last is when the embryo begins to form leaves (Figure 3).

The explant forms callus tissue that has the potential to develop into a somatic embryo called proembryo callus or phase I (Figure 3A). Proembryo callus is undifferentiated plant tissue (not yet developed into a specific structure) that has the potential to develop into an embryo (Suo et al., 2021). This somatic proembryo is formed from totipotent cells. At this stage, the explant tissue undergoes dedifferentiation, forming a callus morphologically appearing as an unorganized mass (Fehér, 2019). In phase II, the explant begins to appear as a small green spherical structure, indicating early embryo formation (Figure 3B). This is called the globular phase, the initial stage of embryo formation. It is still simple and has not yet been differentiated into a bud (Wahyudiningsih & Sumardi, 2016). Over time, the embryo develops into an embryo phase with a bud, which is marked by the appearance of a small bud structure at the tip of the embryo (Figure 3C). This stage indicates the beginning of embryo differentiation towards forming vegetative organs. This is in accordance with the research of Mose et al. (2017) that the

globular phase differentiates to form an embryo with buds.

The embryo then enters the shoot elongation phase, where the shoot's size increases in length due to cell division and elongation (Figure 3D) (Figure 3D). The embryo begins to show a more precise direction of growth toward forming a complete plant structure. Finally, the leaf formation phase occurs, where the embryo begins to form a leaf structure that will later become part of a more complex plant (Figure 3E). This research is supported by Utami et al. (2007), who state that embryos with buds will form shoots and then form leaf primordia.

The proper concentration of BAP can optimize the formation of somatic embryos. Fithriyandini et al. (2015) state that the addition of BAP can increase the process of cell division in plants. Based on the research results, the use of BAP affected the formation of somatic embryos of *P. amabilis* orchids. It could produce several stages of somatic embryo development in *P. amabilis* explants during 8 weeks after planting (WAP) (Table 2).

The explants used in this study were *P. amabilis* orchid protocorms. Orchid protocorms were chosen because of their high regenerative capacity (Cardoso et al., 2020). Research by Yeung & Stasolla (2024) supports this. Orchid protocorms have meristem cells with high regenerative potential, which allows them to form embryo structures.

The development of somatic embryos of *P. amabilis in vitro* at the age of 0 WAP or the beginning of planting, with BAP concentrations of 0, 1, 2, 3, and 4 ppm, did not show any significant changes. This is an initial condition where the growth of somatic embryos is seen without swelling as a sign of somatic embryo development. This is in accordance with the research of Wisman (2018), that the process of somatic embryo induction begins with the swelling of leaf explants in *Dendrobium lineale* orchids.

The second week of growth (2 WAP) began with the swelling of explants planted in BAP media 1, 2, 3, and 4 ppm, while in the control media (0 ppm), the explants began to form proembryo callus. This is in accordance with the statement of Elhiti & Stasolla (2016) that the formation of somatic embryos begins with several cells that undergo dedifferentiation, returning to meristematic (similar to embryonic cells) and forming proembryogenic. Treatment in control media (0 ppm) (Table 3) is characterized by cells that gather, which will later form a globular phase.

In the BAP treatments of 1, 2, 3, and 4 ppm, at 4 WAP, the embryo began to swell and form a proembryo callus, which was characterized by a smooth and compact texture on the surface of the explant. Furthermore, in the control medium (0 ppm), the globular phase began to form, which was characterized by the formation of circles on the

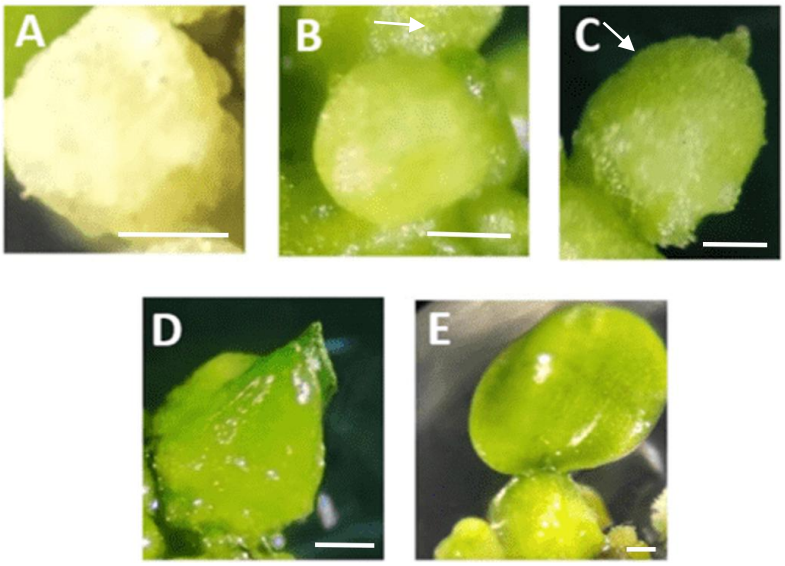
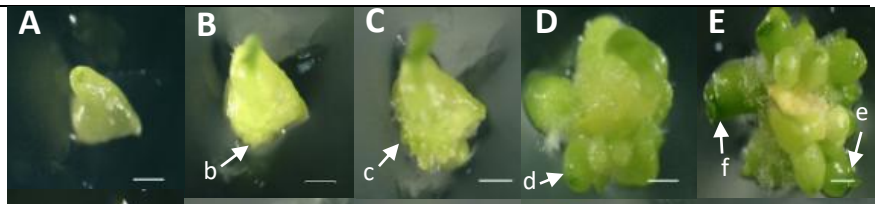
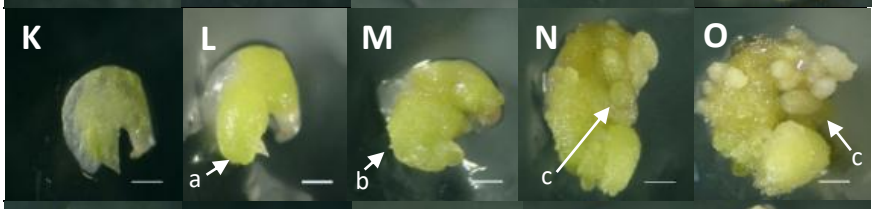
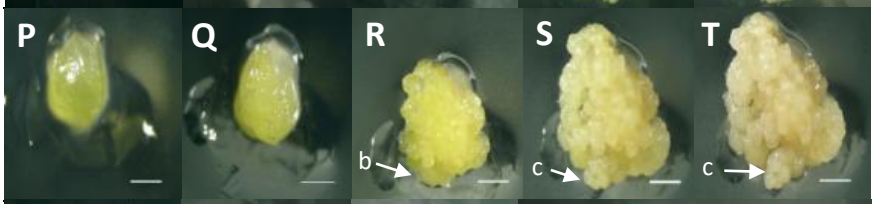
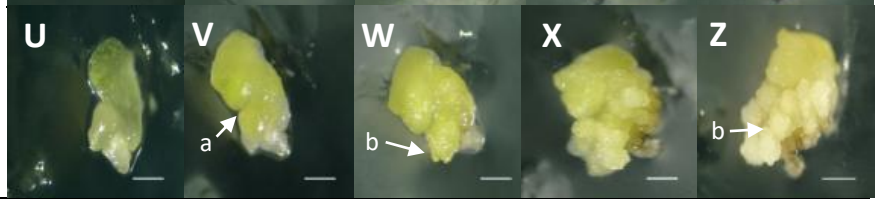


Figure 3. Stages of somatic embryo formation. A. Phase I (proembryo callus), B. Phase II (globular phase), C. Phase III (embryo with bud), D. Phase IV (elongated bud), and E. Phase V (leaf-forming embryo). Protocorm development on control media (BAP 0 ppm). Bar = 1 mm

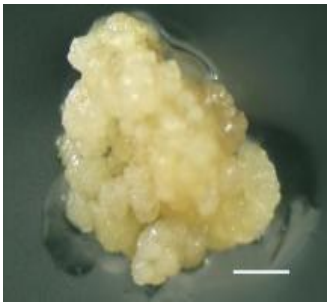
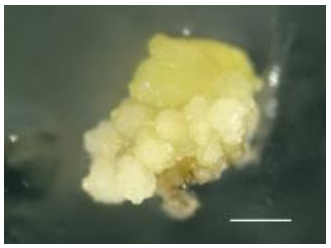
Table 2. Development of *P. amabilis* orchid somatic embryos from protocorms with BAP treatments of 0.1, 2, 3, and 4 ppm

BAP Concentration (ppm)	Somatic Embryo Development of *P. amabilis* in the 2nd week (WAP)				
	0	2	4	6	8
0					
1					
2					
3					
4					

Note:

1. In week 0 (A, F, K, P, U): No changes have occurred in the explant
2. In week 2 (B, G, L, Q, V): The explant swelled at concentrations of 0, 1, 2, 3, and 4 ppm, and the proembryo callus phase began to form in the control medium (0 ppm).
3. In week 4 (C, H, M, R, W): The explant began to grow proembryo callus in the BAP treatment with concentrations of 1, 2, 3, and 4 ppm and somatic embryos in the globular phase in the BAP treatment with concentrations of 0 ppm.
4. In week 6 (D, I, N, S, X): The explant formed a globular phase in the BAP treatment with concentrations of 1, 2, 3, and 4 ppm. At a BAP concentration of 0 ppm, the globular phase changed into an embryo with buds and elongation.
5. In the eight weeks (E, J, O, T, Z): the Proembryo callus and globular phase divide more in the BAP concentration treatment of 1, 2, 3, and 4 ppm. In addition, at a BAP concentration of 0 ppm, the embryo begins to form leaves.
6. a. cell swelling; b. proembryo callus; c. globular phase; d. embryo with buds; e. elongated shoots; f. embryo forms leaves (Bar = 1 mm)

Table 3. Average number of somatic embryos in each phase of *P. amabilis* orchid at 8 MST with BAP treatments of 0, 1, 2, 3, and 4 ppm

BAP concentration (ppm)	Picture	Mean Number of Embryos in Phase ± Standard Deviation				
		I	II	III	IV	V
0		18.00 ±1.00^{c}	6.00 ±3.6^{ef}	2.33 ±4.04^{g}	2.33 ±3.22^{m}	1.00 ±1.00^{k}
1		38.00 ±7.81^{b}	13.33 ±8.51^{e}	0.33 ±0.58^{g}	0,33 ±0.58^{m}	0.33 ±0.58^{k}
2		28.33 ±5.51^{bc}	33.33 ±3.22^{d}	0.33 ±0.58^{g}	0.33 ±0.58^{m}	0.33 ±0.58^{k}
3		53.33 ±10.21^{a}	10.67 ±8.51^{ef}	0.33 ±0.58^{g}	0.33 ±0.58^{m}	0.33 ±0.58^{k}
4		17.00 ± 2.65^{c}	0.00 ±0.00^{f}	0.33 ±0.58^{g}	0.33 ±0.58^{m}	0.00 ±0.00^{k}

Note: Phase I (somatic proembryo); phase II (globular); phase III (embryo with buds); phase IV (elongated shoots); and phase V (leaf-forming embryo). (Bar = 1 mm). The same letters in one column indicate results that are not significantly different. at p-value≤0.05 based on DMRT test. Observed in 20 eksplant at eight WAP

surface of the explant. This is in accordance with the statement of Méndez-Hernández et al. (2019) that the stages of somatic embryo formation begin with cell division into a collection of cells forming the globular phase.

At 6 WAP in control media (0 ppm), somatic embryo induction was faster and more apparent. This change was indicated by the formation of a shoot apical meristem (SAM) structure, SAM indicate with the appearance of protrusions on protocorm. Shoot apical meristem will develop to form stems and leaves, which indicated that the embryo was moving toward the embryo phase with buds. At a BAP concentration of 2 ppm, the globular phase began to form, indicated by roun and green explants. At BAP concentrations of 1, 3, and 4 ppm, it was seen that the cells in the *P. amabilis* explants were increasingly actively dividing.

At the age of 8 WAP in control media (0 ppm), somatic embryos began to enter the embryo phase with leaves. This is indicated by the leaves forming and developing more clearly in the SAM section. Somatic embryo cells continue to grow and develop in the BAP treatment (1, 2, 3, and 4 ppm). This is in accordance with the research of Utami et al. (2007), which states that globular phase somatic embryos begin to form embryos with buds and elongated leaf primordia. Furthermore, leaves form on *P. amabilis* orchids after several weeks of planting.

Phalaenopsis amabilis somatic embryos grown on control media (BAP 0 ppm) showed faster growth and development of somatic embryos compared to BAP treatments (1, 2, 3, and 4 ppm) (Table 3). This is because the endogenous hormones in the plant are sufficient. The addition of cytokinin hormones in the form of BAP to the explants causes the cytokinin hormones in the explants to increase, thus inhibiting the formation of somatic embryos in *P. amabilis* explants. This is due to the competition for binding cytokinin signals to receptors, so the explants need to adjust the conditions with the media supplemented with BAP. This is supported by research by Ningrum et al. (2017), which states

Until the research ended within two months of research, the average number of somatic embryos in phase I with a BAP concentration of 3 ppm was significantly different from BAP concentrations (0, 1, 2, and 4 ppm) of 53.33 (a). Furthermore, phase II, with a BAP concentration of 2 ppm, was significantly different from BAP concentrations (0, 1, 3, and 4 ppm), with an average number of somatic embryos of 33.33 (d). The BAP hormone concentration of 2 and 3 ppm given to the explants stimulated the formation of more optimal phase II and I somatic embryos. This is due to the presence of the cytokinin hormone, which encourages the transition of cells from the callus phase to somatic embryos. This can happen because BAP can induce the isopentenyl transferase (IPT) enzyme, which will then stimulate the synthesis of endogenous cytokinin's so that endogenous cytokinin's will encourage cell transition (Uniyal et al., 2022).

In phases III, IV, and V, the results were not significantly different from each other. Administration of BAP causes endogenous hormones in the form of cytokinin's to increase, thus inhibiting the formation of somatic embryos in phases III, IV, and V. This is because the work of cytokinin hormones as signals is inhibited and causes competition for binding of cytokinin signals to receptors. This is supported by the findings of Nic-Can & Loyola-Vargas (2016), which state high cytokinin concentrations, can inhibit the development of somatic embryos.

The results of this study emphasize the importance of selecting the right BAP concentration for the somatic embryo formation process. The use of BAP at low concentrations (0 ppm) was able to induce *P. amabilis* somatic embryos, but the number of somatic embryos formed was lower than BAP 1, 2, 3 ppm. At the proper concentrations, such as BAP (1, 2, and 3 ppm), it was able to encourage embryo formation efficiently and produce a more significant number. In comparison, BAP concentrations that were too high (4 ppm) triggered a decrease in the average somatic embryo formation.

CONCLUSION

In conclusion, MS media treated with three ppm BAP (65) somatic embryos are optimal media for inducing somatic embryos from all *P. amabilis* treatments. Easy procedures for somatic embryo production using media containing BAP will support and provide high benefits for orchid conservation and the orchid industry.

ACKNOWLEDGMENTS

The author would like to express his gratitude to the parties who have made significant contributions to this research, namely the University of Diponegoro, Plant Laboratory, research funds from the Faculty of Science and Mathematics with contract number 20/UN7.F8/HK/II/2024.

REFERENCES

Ario, A., & Setiawan, S. (2020). The Effect of Benzyl Amino Purine (BAP) Concentration on the Growth Amount of the Explant of *Dendrobium spectabile* Orchid by In-Vitro. *International Journal of Multi Discipline Science (IJ-MDS)*, *3*(2), 33. https://doi.org/10.26737/ij-mds.v3i2.2397

Bait, M., B, V. S., & Malik, F. (2024). Kultur Jaringan PT Pupuk Kaltim. In F. Malik & F. Yanti (Eds.), *Kultur Jaringan PT Pupuk Kaltim*. PT Pupuk Kaltim. https://www.pupukkaltim.com/public/assets/files/ebook/TJSL/Kultur_Jaringan.pdf

Britto, J. De, Kamsinah, & Prayoga, L. (2022). Addition of IAA and BAP to the Growth of Callus on Leaf Explants of *Coelogyne pandurata* Lindl. Orchid. *Jurnal Ilmiah Biologi Unsoed*, *3*(2), 112. https://doi.org/10.20884/1.bioe.2021.3.2.4255. [Indonesian]

Cardoso, J. C., Zanello, C. A., & Chen, J. T. (2020). An Overview of Orchid Protocorm-Like Bodies: Mass Propagation, Biotechnology, Molecular Aspects, and Breeding. *International Journal of Molecular Sciences*, *21*(3). https://doi.org/10.3390/ijms21030985

CITES. (2021). *Appendices I , II and III* (Issue February).

Cortleven, A., Marg, I., Yamburenko, M. V., Schlicke, H., Hill, K., Grimm, B., Schaller, G. E., & Schmülling, T. (2016). Cytokinin Regulates the Etioplast-Chloroplast Transition through the Two-Component Signaling System and Activation of Chloroplast-Related Genes. *Plant Physiology*, *172*(1), 464–478. https://doi.org/10.1104/pp.16.00640

Destinugrainy, P., & Semiarti, E. (2016). The Effect of *Thidiazuron* and *Naphthalene* Acetic Acid on Somatic Embryogenesis Induction from Leaf Explants of Phalaenopsis 'Sogo Vivien' Orchid. *Dinamika*, *07*(1), 31–40. [Indonesian]

Elhiti, M., & Stasolla, C. (2016). Somatic Embryogenesis: The Molecular Network Regulating Embryo Formation BT - Somatic Embryogenesis in Ornamentals and Its Applications (A. Mujib (ed.); pp. 217–229). *Springer India*. https://doi.org/10.1007/978-81-322-2683-3_14

Fehér, A. (2019). Callus, Dedifferentiation, Totipotency, Somatic Embryogenesis: What These Terms Mean in the Era of Molecular Plant Biology? *Frontiers in Plant Science*, 10(April), 1–11. https://doi.org/10.3389/fpls.2019.00536

Fithriyandini, A., Dawam, M., & Wardiyati, T. (2015). The Effect of Basic Media and 6-Benzylaminopurine (BAP) on the Growth and Development of Flower Stem Node Explants of Moon Orchid (*Phalaenopsis amabilis*) in In Vitro Propagation. *Jurnal Produksi Tanaman*, *3*(1), 43–49.

Hartati, S., Budiyono, A., & Cahyono, O. (2016). The Effect of NAA and BAP on the Growth of Subculture Orchids from Hybridization *Dendrobium biggibum* X *Dendrobium liniale*. *Caraka Tani: Journal of Sustainable Agriculture*, *31*(1), 33. https://doi.org/10.20961/carakatani.v31i1.11938. [Indonesian]

Hartati, S., Yunus, A., & Iistikharoh, I. (2024). Effect of Auxin and Cytokinin on Orchid Subculture Resulting from Coelogyne Crosses. *Journal of Biodiversity and Biotechnology*, *3*(2), 73. https://doi.org/10.20961/jbb.v3i2.80626

Ibrahim, M. S. D., & Hartati, R. S. (2017). Enhancement of Embryogenic Callus Induction and Somatic Embryo Conversion in Robusta Coffee Clone BP 308 *Jurnal Tanaman Industri dan Penyegar*, *4*(3), 121. https://doi.org/10.21082/jtidp.v4n3.2017.p121-132. [Indonesian]

Kępczyńska, E., & Kępczyński, J. (2023). Hormonal Regulation of Somatic Embryogenesis in Medicago spp. *Plant Cell, Tissue and Organ Culture*, *155*(3), 613–625. https://doi.org/10.1007/s11240-023-02593-5

Lee, H. B., Im, N. H., An, S. K., & Kim, K. S. (2021). Changes of Growth and Inflorescence Initiation by Exogenous Gibberellic Acid3 and 6-Benzylaminopurine Application in Phalaenopsis Orchids. *Agronomy*, *11*(2). https://doi.org/10.3390/agronomy11020196

Lestari, E. G. (2011). The Role of Plant Growth Regulators in Plant Propagation through Tissue Culture. *Jurnal AgroBiogen*, *7*(1), 63. https://doi.org/10.21082/jbio.v7n1.2011.p63-68. [Indonesian]

Méndez-Hernández, H. A., Ledezma-Rodríguez, M., Avilez-Montalvo, R. N., Juárez-Gómez, Y. L., Skeete, A., Avilez-Montalvo, J., De-La-Peña, C., & Loyola-Vargas, V. M. (2019). Signaling Overview of Plant Somatic Embryogenesis. *Frontiers in Plant Science*, *10*(February), 1–15. https://doi.org/10.3389/fpls.2019.00077

Mose, W., Daryono, B. S., Indrianto, A., Purwantoro, A., & Semiarti, E. (2020). *Direct Somatic Embryogenesis and Regeneration of an Indonesian orchid Phalaenopsis amabilis (L.) Blume under a Variety of Plant Growth Regulators, Light Regime, and Organic Substances*. *13*(4), 509–518.

Mose, W., Indrianto, A., Purwantoro, A., & Semiarti, E. (2017). The Influence of Thidiazuron on Direct Somatic Embryo Formation from Various Types of Explant in Phalaenopsis amabilis (L.) Blume Orchid. *HAYATI Journal of Biosciences*, *24*(4), 201–205. https://doi.org/10.1016/j.hjb.2017.11.005

Muchsin, M. E., Supriatna, A., Adawiyah, A., & Darniwa, A. V. (2022). The Effect of Various Concentration BAP

(6-Benzyl Amino Purine) on Orchid Growth (Macodes petola (Blume) Lindl.) In-Vitro. *Berkala Sainstek*, *10*(1), 25. https://doi.org/10.19184/bst.v10i1.27091

Nic-Can, G. I., & Loyola-Vargas, V. M. (2016). *The Role of the Auxins During Somatic Embryogenesis BT - Somatic Embryogenesis: Fundamental Aspects and Applications* (V. M. Loyola-Vargas & N. Ochoa-Alejo (eds.); pp. 171–182). Springer International Publishing. https://doi.org/10.1007/978-3-319-33705-0_10

Ningrum, E. F. C., Rosyidi, I. N., Puspasari, R. R., & Semiarti, E. (2017). Early Development of Protocorm in Phalaenopsis amabilis Orchid In Vitro after the Addition of Plant Growth Regulator α-*Naphtaleneacetic* Acid and *Thidiazuron*. *Biosfera*, *34*(1), 9. https://doi.org/10.20884/1.mib.2017.34.1.393. [Indonesian]

Nur'riyani, N. (2021). Suitable Tissue Culture Media for Propagation of *Cavendish Banana* Plants (Musa acuminata L.). *Bioscientiae*, *18*(1), 37. https://doi.org/10.20527/b.v18i1.4068. [Indonesian]

Oseni, O. M., Pande, V., & Nailwal, T. K. (2018). A Review on Plant Tissue Culture, A Technique for Propagation and Conservation of Endangered Plant Species. *Excellent Publisher*, *7*(July), 07. https://doi.org/10.20546/ijcmas.2018.707.438

Riyadi, I., Efendi, D., Purwoko, B. S., & Santoso, D. (2016). Indirect Somatic Embryogenesis in Sago Palm (*Metroxylon sagu* Rottb.) Using Suspension Culture System, Brief Immersion, and Solid Media.. *Jurnal AgroBiogen*, *12*(1), 37–44. [Indonesian]

Salama, S. F. El, Ngadiani, & Andriani, V. (2024). Respon Growth of Moon Orchid (*Phalaenopsis amabilis*) in Response to Planting Media and Red Onion Skin POC Treatment (*Allium cepa*). *Stigma*, *25*(1), 89–97. [Indonesian]

Siron, U., Noertjahyani, Taryana, Y., & Romiyani. (2019). The Effect of Naphthalene Acetic Acid and Benzyl Amino Purine Growth Regulator Concentrations on the Growth of Protocorms in Dendrobium spectabile Orchid under In Vitro Culture. *Ilmiah Pertanian*, *7*(1), 16–23. [Indonesian]

Sulaiman, S., Yusuf, N. A., & Awal, A. (2020). Effect of plant growth regulators on in vitro culture of pineapple (Ananas comosus L. Merr) MD2 variety. *Food Research*, *4*, 110–114. https://doi.org/10.26656/fr.2017.4(S5).017

Suo, J., Zhou, C., Zeng, Z., Li, X., Bian, H., Wang, J., Zhu, M., & Han, N. (2021). Identification of regulatory factors promoting embryogenic callus formation in barley through transcriptome analysis. *BMC Plant Biology*, *21*(1), 145. https://doi.org/10.1186/s12870-021-02922-w

Thomas, C., & Jiménez, V. M. (2005). Mode of action of plant hormones and plant growth regulators during induction of somatic embryogenesis: Molecular aspects. *Plant Cell Monographs*, *2*(November), 157–175. https://doi.org/10.1007/7089_040

Uniyal, S., Bhandari, M., Singh, P., Singh, R. K., & Tiwari, S. P. (2022). Cytokinin biosynthesis in cyanobacteria: Insights for crop improvement. *Frontiers in Genetics*, *13*(September), 1–12. https://doi.org/10.3389/fgene.2022.933226

Utami, E. S. W., Soemardi, I., Taryono, T., & Semiarti, E. (2007). Somatic Embryogenesis of Moon Orchid *Phalaenopsis amabilis* (L.) Bl: Structure and Development Pattern. *Berkala Penelitian Hayati*, *13*(1), 33–38. https://doi.org/10.23869/bphjbr.13.1.20075. [Indonesian]

Wahyudiningsih, T. S., & Sumardi, I. (2016). Structure and Development of Somatic Embryos from Leaf Explants of Dyera lowii Hook.f. Through In-Vitro Technique. *Jurnal Hutan Tropika*, *X*(2), 39–47. [Indonesian]

Wisman, P. (2018). *Induksi Embrio Somatik Pada Eksplan Daun Dari Kultur In Vitro Anggrek Dendrobium lineale Rolfe*. Universitas Gadjah Mada.

Wu, W., Du, K., Kang, X., & Wei, H. (2021). The diverse roles of cytokinins in regulating leaf development. *Horticulture Research*, *8*(1). https://doi.org/10.1038/s41438-021-00558-3

Yeung, E. C.-T., & Stasolla, C. (2024). *Protocorm Regeneration and Protocorm-Like Bodies BT - Orchid Propagation: The Biology and Biotechnology of the Protocorm* (E. C.-T. Yeung & Y.-I. Lee (eds.); pp. 65–81). Springer US. https://doi.org/10.1007/978-1-0716-4031-9_4

Yulia, E., Baiti, N., Handayani, R. S., & Nilahayati, N. (2020). Response to the Application of Several Concentrations of BAP and IAA on the Growth of Sub-Culture Cymbidium Orchid (*Cymbidium finlaysonianum* Lindl.) In-Vitro *Jurnal Agrium*, *17*(2). https://doi.org/10.29103/agrium.v17i2.5870

Zega, N. M. S., Barus, R. H. B., & Sinaga, A. H. (2021). Analysis of the Efficiency of Moon Orchid (*Phalaenopsis Amabilis*) Business in Limau Manis Village, Tanjung District. *Jurnal Agribizda*, *5*(2), 118–125. [Indonesian]