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Effect of Light Color and Auxin on Callus Induction and Development in *Amorphophallus muelleri* Blume.

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ABSTRACT

Porang (*Amorphophallus muelleri* Blume) is a significant player in the international market, but its slow seedling growth rate is a major concern. This study, which holds significant implications for porang cultivation, aimed to accelerate the seedling growth phase through the induction of adventitious shoots from callus proliferation. We explored the use of plant growth regulators (PGRs), specifically auxins (NAA and 2,4-D) and cytokinins (BAP), in combination with variations in light color (white and white-blue). The observed parameters included the time of first callus and adventitious shoot emergence, callus growth, number of adventitious shoots, and callus coloration. Data were subjected to two-way analysis of variance (ANOVA) to evaluate the effects of plant growth regulator (PGR) type and light quality as independent variables. Statistical significance was assessed at a 95% confidence level using SPSS software. The combination of 1.0 ppm NAA and 0.5 ppm BAP in MS medium under white-blue light effectively accelerated callus induction from bulbil explants, with visible callus formation by day 8 and the fastest proliferation over 7 weeks. Under white light, the same PGR combination also promoted the earliest adventitious shoot formation by day 16 and yielded the highest number of shoots, totaling seven by the end of the culture period.

Keywords: *adventitious shoots; blue light; callus induction; white light*

INTRODUCTION

Global demand for porang (*Amorphophallus muelleri* Blume) has continued to rise steadily. Indonesian export volumes increased from 5.7 thousand tons in early 2019 to 14.8 thousand tons by mid-2021 (Ministry of Agriculture, 2021). This trend accelerated in 2024, marked by a significant export breakthrough. In May 2024, Indonesia began shipping porang chips to China, with an initial 25-ton export from Semarang (Bea dan Cukai Republik Indonesia, 2024). These developments reflect Indonesia's strengthening position in the global porang market, driven by both high international demand and supportive government policy.This upward trend underscores the pressing need for optimization efforts in seedling production to sustainably meet the growing global demand. One of the major hurdles in porang propagation is the prolonged time required to produce transplant-ready seedlings using conventional methods, such as tubers, bulbils, seeds, or leaf cuttings. Porang tubers necessitate a dormancy period of approximately four months before producing new shoots, while seeds undergo dormancy for four to five months (Harefa *et al*., 2023).

Plant tissue culture emerges as a promising alternative to overcome these

constraints by accelerating callus induction, proliferation, and uniform adventitious shoot formation under disease-free conditions (Sutarsih *et al*., 2022). This innovative technique, which relies on plant growth regulators (PGRs) to stimulate cell division and tissue differentiation in a controlled manner, offers a new direction in porang propagation research.

PGRs are compounds that play a crucial role in promoting plant growth and development. The application of PGRs at appropriate concentrations can effectively support optimal plant cell division and differentiation. Among the commonly used synthetic auxins are Naphthalene Acetic Acid (NAA) and 2,4-dichlorophenoxyacetic Acid (2,4-D). NAA is effective in promoting cell division, cell elongation, and the induction of root formation (Song *et al*., 2023). NAA is effective in promoting cell division, cell elongation, and the induction of root formation (Song *et al*., 2023). At lower concentrations, typically around 0.5–1 mg/L, NAA is commonly used to induce adventitious roots in various plants, including *Lilium longiflorum* (Deswiniyanti *et al*., 2020). In contrast, higher concentrations of NAA are more suitable for callus induction (Lestari *et al.,* 2018).

Meanwhile, 2,4-D is known as a strong synthetic auxin capable of inducing callus formation with high biomass and distinct coloration (Rasud & Bustaman, 2020). In addition to auxins, cytokinins also play a critical role in tissue culture, particularly in callus cell division, morphogenesis, and lateral shoot formation (Anisa *et al*., 2016). Benzyl Aminopurine (BAP), one of the widely used synthetic cytokinins, has been proven effective in promoting in vitro shoot proliferation in *Clitoria ternatea* (Jannah & Prihantoro, 2023). Restanto *et al.* (2024) used BAP to stimulate meristematic tissue division, thereby enhancing the regeneration of embryogenic calli in porang.

Previous studies have reported that the optimal callus induction medium for porang bulbil explants is Murashige and Skoog (MS) medium supplemented with 1.0 ppm NAA and 2.0 ppm 2,4-D, resulting in the highest callus induction percentage (Khoirunnisa & Mercuriani, 2022). Furthermore, Hardjo *et al*. (2023) found that the optimal shoot induction medium for porang bulbils was MS medium supplemented with 5.0 $mg \cdot L^{-1}$ BAP and 0.2 $mg \cdot L^{-1}$ NAA, which produced 15 adventitious shoots with an average shoot height of 7.2 cm.

In addition to internal factors such as PGRs, light also significantly influences plant growth. Appropriate light spectra can enhance photosynthetic efficiency, support callus growth, and accelerate adventitious shoot formation. Light-emitting diodes (LEDs) are preferred as a light source due to their specific light spectra, low energy consumption, high efficiency, and long lifespan (Hasibuan *et al.,* 2020). According to Huan *et al.* (2004), LED lighting composed of 25% red and 75% blue light was most suitable for enhancing the formation of protocorm-like bodies (PLBs) from callus and the regeneration of PLBs in Cymbidium orchids. Additionally, Li *et al.* (2024) reported that red-blue light (1:1), as well as blue and white light, were optimal for promoting cotton callus proliferation.

This study not only examines the effects of PGRs such as NAA, 2,4-D, and BAP but also integrates variations in light color (white and white-blue) on callus induction from bulbil explants, callus proliferation and adventitious shoot formation in *A. muelleri.* This comprehensive approach, which has the potential to enhance the efficiency of uniform porang seedling production, also offers practical and scientific benefits for the agribusiness sector and plant propagation initiatives, thereby contributing to the advancement of the field.

MATERIALS AND METHOD

From March to September 2024, the study was conducted at the Plant Biotechnology Laboratory, Faculty of Biotechnology, University of Surabaya. Sterile bulbil explants of porang (*Amorphophallus muelleri*), each measuring approximately 1.5 × 1.5 cm, were used as explants. These explants were sourced from stock cultures maintained in the Plant Biotechnology Laboratory of the University of Surabaya. The explants were cultured on Murashige and Skoog (MS) medium supplemented with either Naphthalene Acetic Acid (NAA) or 2,4-dichlorophenoxyacetic acid (2,4-D) at concentrations of 1.0 ppm and 2.0 ppm, respectively, in combination with 0.5

ppm Benzylaminopurine (BAP). The cultures were incubated under two different lighting conditions: white light and a combination of white-blue light, both provided by LED lamps. All cultures were maintained at a controlled temperature of 25°C. Equipment used in this study included a laminar air flow (LAF) cabinet for sterilization, forceps, scalpel, and 300 mL Petri dishes for culturing.

A completely randomized factorial design (CRD) was employed, consisting of two factors: (1) type and concentration of plant growth regulators (PGRs)—NAA 1.0 ppm + BAP 0.5 ppm, NAA 2.0 ppm + BAP 0.5 ppm, 2,4-D 1.0 ppm + BAP 0.5 ppm, and 2,4-D 2.0 ppm + BAP 0.5 ppm; and (2) light quality—white light and combined white-blue light conditions during the culture incubation period, as presented in Table 1. Each treatment was replicated five times.

Data collection was carried out weekly over a 7-week period after planting. Changes in the porang bulbil explants that indicated the onset of callus formation were observed and recorded each week. The implementation of light quality treatments white and combined white-blue LED was adapted from a previous study by Hardjo *et al.* (2022), which demonstrated that variations in light spectrum significantly influence callus induction and growth in porang. Measurements of explants exhibiting callus development were taken using a ruler along three dimensions—length, width, and surface inclination—and the average value was calculated. The appearance of green spots on the callus tissue indicated the initiation of shoot primordia.

Callus color was identified visually based on the dominant color occupying at least 80% of the explant surface. Color identification followed a reference system from the Coolors website, and classification was made according to color categories listed in Table 2, which were assigned scores ranging from 1 to 5. The color assessment was conducted subjectively and qualitatively by observing the dominant color (≥80%) on the explant and subsequently quantifying it according to the color scale in Table 2.

Table 1. Treatment combinations of plant growth regulators and light spectra

Code	Treatment Combination	
	Light	PGR
PN1	White	NAA 1.0 ppm + BAP 0.5 ppm
PN2	White	NAA 2.0 ppm + BAP 0.5 ppm
PD1	White	2,4-D 1.0 ppm + BAP 0.5 ppm
PD2	White	2,4-D 2.0 ppm + BAP 0.5 ppm
PBN1	White-Blue	NAA 1.0 ppm + BAP 0.5 ppm
PBN2	White-Blue	NAA 2.0 ppm + BAP 0.5 ppm
PBD1	White-Blue	2,4-D 1.0 ppm + BAP 0.5 ppm
PBD2	White-Blue	2,4-D 2.0 ppm + BAP 0.5 ppm

Table 2. Callus Color Reference Scale

Numerical Scale	Color	Color Name
1		*Arylide yellow*
2		*pear*
3		*Citron*
4		*Olive*
5		*Apple green*

(Source: Coolors, 2024)

The data collected were analyzed using a two-way analysis of variance (ANOVA), with the first factor being the type of plant growth regulator (PGR) and the second factor being light quality. Statistical significance was evaluated at a 95% confidence level for each measured variable, ensuring the reliability and accuracy of the results. Further Analysis was conducted using Duncan's Multiple Range Test (DMRT) at the same 95% confidence level.

RESULTS AND DISCUSSION

The application of auxins (NAA and 2,4-D) combined with different light spectra (white and white-blue) significantly affected the development of *Amorphophallus muelleri* Blume bulbil explants (Table 3).

MS medium supplemented with either NAA or 2,4-D did not show a statistically significant difference in callus proliferation under both white and white-blue light exposure. However, the most effective treatment combination for callus induction and growth was found to be NAA at 1.0 and 2.0 ppm combined with BAP at 0.5 ppm (PBN1 and PBN2) under white-blue light, producing average callus sizes of 1.1 ± 0.27 cm and 1.6 ± 0.05 cm, respectively.

Auxins, including synthetic types like Naphthalene Acetic Acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D), play a fundamental role in plant cell enlargement. They promote cell elongation by stimulating the activity of plasma membrane H^+-ATPases, which pump protons into the cell wall. This acidifies the apoplast, activating enzymes such as expansins that loosen the cell wall structure. As a result, the cell wall becomes more extensible, allowing water uptake and turgor-driven expansion of the cell (Taiz *et al.,* 2015). This promising result, along with the acceleration of callus formation from bulbil explants, indicates that light quality plays a crucial role in the initiation and proliferation of porang callus, and opens up new possibilities for plant growth research.

White-blue light exposure significantly enhanced both the rate of callus formation and callus biomass production. The increase in size and accelerated development of callus are likely associated with auxin distribution within the cells, influenced by the phototropin activity. This activity mediates cell division in response to light intensity (Ruíz-Rivas *et al.,* 2022), shedding light on the intricate mechanisms of plant growth. Additionally, blue light enhances cellular energy, promotes a more compact cell structure, and supports optimal absorption of plant growth regulators (Cavallaro *et al*., 2022).

Table 3. Size of *Amorphophallus muelleri* Blume. Callus

Treatment	Callus Formation Start (Day-)	Average Size of Callused Explants (cm)		Increase in the Size of Callused Explants Over 7 Weeks (cm)
		Initiate (0 week)	Final (7 weeks)	
PN1	11.4±0.54^{c}	0.5±0.15	0.8±0.34	0.4±0.18^{a}
PN2	10.3±0.55^{b}	0.4±0.15	0.7±0.30	0.6±0.18^{a}
PD1	13.6±0.55^{d}	0.4±0.14	0.8±0.27	0.5±0.13^{a}
PD2	12.6±0.55^{cd}	0,5±0.13	0.8±0.11	0.4±0.02^{a}
PBN1	7.6±0.54^{a}	0.8±0.31	1.9±0.57	1.1±0.27^{b}
PBN2	6.8±0.83^{a}	0.5±0.12	1.3±0.19	1.6±0.05^{b}
PBD1	9.8±0.84^{b}	0.5±0.01	1.1±0.18	1.0±0.06^{b}
PBD2	8.8±0.83^{b}	0.5±0.05	1.0±0.35	0.7±0.03^{b}

Description: MST = Weeks After Planting; Numbers within the same column followed by different letters indicate significant differences based on the DMRT test (α = 5%).

Table 4 shows that explants exposed to white-blue light exhibited lower color score values compared to white light, indicating a shift towards more yellowish coloration according to the reference scale in Table 2. This finding is consistent with the study by Lian *et al.* (2019), which reported that culturing *Gynura procumbens* callus under blue light resulted in greenish-brown callus formation. The brownish coloration is attributed to the accumulation of reactive oxygen species (ROS), which stimulate the production of phenolic compounds as a defense response to environmental stress (Faralli *et al*., 2022). These phenolic compounds are subsequently oxidized by the enzyme polyphenol oxidase, leading to browning in wounded areas of the explant tissue (Helena *et al.,* 2022).

Moreover, the light intensity under white-blue illumination which averaged approximately 4580 lux was higher than that of white light alone, which averaged around 3480 lux during culture incubation. This increase in light intensity, combined with the spectral quality of blue wavelengths, has been shown to enhance phenolic compound synthesis in explants (Lian *et al.,* 2019; Usman *et al.,* 2020). This browning is driven by enzymatic oxidation catalyzed by polyphenol oxidase (PPO) and peroxidase (POD), converting phenolics into o-quinones that polymerize into brown pigments (Sharma *et al*., 2020; Amente *et al*., 2018). This research has practical implications for the cultivation of *A. muelleri* in particular, as it provides insights into the role of light in the synthesis of phenolic compounds.

Table 4. Callus Color Score of *Amorphophallus muelleri* Blume. During the 7-Weeks Culture Period.

Treatment	Color Score Value	
	Initiate(0 week)	Final (7 weeks)
PN1	4,7±0,52	4,0±0,26
PN2	5,0±1,1	5,3±1,97
PD1	4,3±1,03	4,8±1,17
PD2	5,0±0,89	5,0±0
PBN1	4,5±0,55	3,0±1,10
PBN2	4,3±0,52	4,0±2
PBD1	4,5±0,55	4,2±1,17
PBD2	5,0±0	3,5±1,64

Table 5. Number of Adventitious Shoots from *A. muelleri* Callus under Combinations of Auxin Treatments and Light Color

Treatment	First Appearance of Shoots (Day-)	Number of shoots	
		Initiate (0 week)	Final (7 weeks)
PN1	15,6±1,14^{a}	0	6,7±2,88^{d}
PN2	18,0±0,71^{b}	0	5,3±1,97^{bc}
PD1	-	0	0^{a}
PD2	-	0	0^{a}
PBN1	17,8±0,83^{b}	0	5,5±1,94^{cd}
PBN2	19±0,70^{b}	0	5,2±2,59^{bc}
PBD1	-	0	0^{a}
PBD2	-	0	0^{a}

Numbers within the same column followed by different letters indicate significant differences based on the DMRT test (α = 5%).

The optimal treatment for the earliest adventitious shoot formation—observed on day 16 after planting—was obtained using MS medium supplemented with 1.0 ppm NAA and 0.5 ppm BAP under white light exposure (Table 5, Fig. 4A). This combination also yielded the highest number of adventitious shoots, with an average of seven shoots over the seven-week culture period. This result can be attributed to white light encompassing the full visible light spectrum, providing maximal energy for photosynthesis and shoot proliferation (Heo *et al*., 2017). In contrast, white-blue light contains a higher proportion of blue wavelengths, which may result in suboptimal shoot differentiation and reduced activation of photosynthetic pathways (Zhang *et al*., 2018).

In both light treatments (white and white-blue), the redlight component remained constant and played a crucial role in cell elongation and shoot initiation by activating phytochrome signaling pathways (Huang et al., 2018). However, the dominance of blue light in the white-blue treatment may disrupt the spectral balance necessary for shoot induction, raising concerns about the potential negative effects of light imbalance on shoot induction.

Moreover, the higher blue light intensity in the white-blue combination appeared to favor callus proliferation over shoot regeneration. This could be linked to the upregulation of stress-responsive genes under blue light exposure, which may suppress shoot development (Huang *et al*., 2018). In this treatment, adventitious shoots were not found specifically in MS media supplemented with 2.0 ppm 2,4-D and 0.5 ppm BAP under either white or white-blue light. This is likely due to the high concentration of 2,4-D, a strong synthetic auxin known to promote callus formation rather than organogenesis. At excessive concentrations, 2,4-D can inhibit shoot regeneration by maintaining cells in an undifferentiated state and suppressing cytokinin-mediated pathways required for shoot initiation. Additionally, the combination of high 2,4-D and blue light exposure may further enhance stress signaling, which can divert cell fate toward callogenesis rather than shoot organogenesis (Zulaikha *et al.,* 2022).

The absence of shoot formation in treatment PD1 reinforces that 2,4-D at 1.0 ppm—even when combined with BAP—does not support shoot organogenesis in porang. Research shows that exposure to 2,4-D-rich media can suppress endogenous IAA biosynthesis through a negative feedback mechanism (Lee *et al.,* 2024). That's why the cell fate transitions necessary for shoot initiation despite callus proliferation.

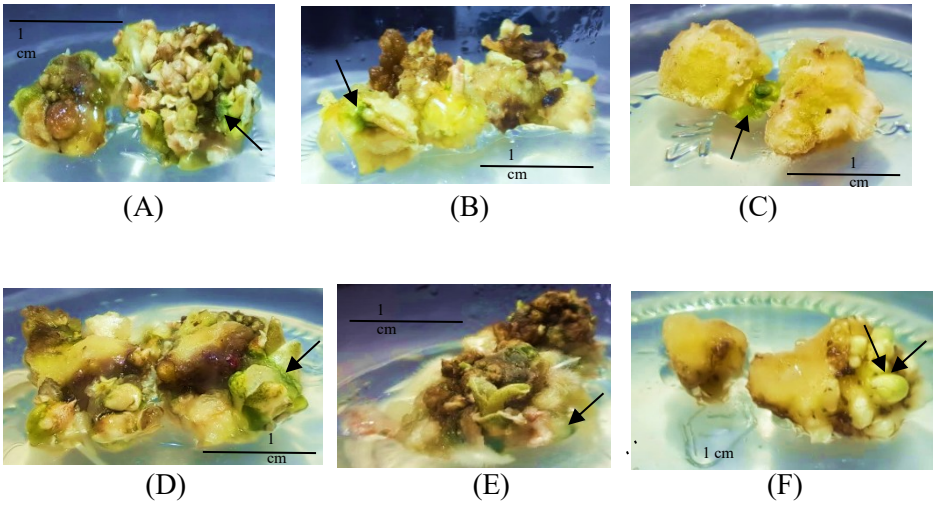

Figure 1. Response of *A. muelleri* Callus to Plant Growth Regulator Treatments and Light Color
Description: Shoots on MS medium with 1.0 ppm NAA and 0.5 ppm BAP under white light (A), Shoots on MS medium with 2.0 ppm NAA and 0.5 ppm BAP under white light (B), Green spot on MS medium with 1.0 ppm 2,4-D and 0.5 ppm BAP under white light (C), Shoots on MS medium with 1.0 ppm NAA and 0.5 ppm BAP under white-blue light (D), Shoots on MS medium with 2.0 ppm NAA and 0.5 ppm BAP under white-blue light (E), Green spot on MS medium with 1.0 ppm 2,4-D and 0.5 ppm BAP under white-blue light (F)

Explant coloration also serves as an important indicator in the regeneration process. Figures 1A, 1B, 1D, and 1E show callus formation with adventitious shoots under NAA treatments (1.0 and 2.0 ppm) under both white and white-blue light. In contrast, the 2,4-D 1.0 ppm treatment produced green spots under both white and white-blue light, as seen in Figures 1C and 1F. The combination of NAA (1.0 and 2.0 ppm) with BAP 0.5 ppm under white or white-blue light exposure can also produce callus capable of forming roots.

The study demonstrates that the combination of NAA with either white or white-blue light can induce root formation in plant tissue culture. The addition of NAA has been shown to significantly enhance rooting efficiency, as evidenced in *Lamprocapnos spectabilis* cultures, where NAA supplementation improved rooting compared to the control without NAA (Wojtania *et al.,* 2020). The addition of 1.0 ppm 2,4-D led to the formation of green spot protrusions, which contained chlorophyll and played a crucial role in the initiation of adventitious shoots (Ekawati *et al.,* 2022). However, over a more extended incubation period, these green spots did not develop into shoots but instead proliferated into callus, bud formation may require longer time or lower concentrations

The formation of green spots is due to chlorophyll production, although the area of these spots may decrease over time (Shwe, 2020). In MS media containing 2,4-D (1.0 and 2.0 ppm) combined with 0.5 ppm BAP, under both white-blue and white light exposure, only callus proliferation occurred, and the callus failed to regenerate into adventitious shoots. This is a cautionary note, as 2,4-D, a potent synthetic auxin, is more dominant than NAA in stimulating callus formation, thereby inhibiting organogenesis (Harnelly *et al.,* 2017). This finding is in agreement with Wijaya *et al.* (2022), who stated that increased concentrations of 2,4-D suppress shoot initiation and growth in *Alyxia reinwardtii* Blume.

CONCLUSION

The results of This study demonstrates that the application of MS medium supplemented with 1.0 ppm NAA and 0.5 ppm BAP significantly enhances the in vitro development of porang (*Amorphophallus muelleri*) by accelerating both callus proliferation and adventitious shoot formation. Callus induction from bulbil explants was most effectively promoted under white-blue LED light, with visible formation observed as early as day 8. In contrast, the fastest adventitious shoot initiation occurred under white light exposure with the same PGR combination, yielding the highest number of shoots seven per explant by the end of the 7-week culture period. These findings highlight the combined influence of plant growth regulators and light quality in optimizing early-stage porang propagation.

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