AGROMIX
Jurnal Ilmiah Fakultas Pertanian, Universitas Yudharta Pasuruan
pISSN (Print): 2085-241X; eISSN (Online): 2599-3003
Website: https://jurnal.yudharta.ac.id/v2/index.php/agromix

# The effectiveness of growth regulators and light color spectrum on callus growth of *Amorphophallus muelleri Blume.* var. Madiun 1

*Efektivitas zat pengatur tumbuh dan spektrum warna cahaya terhadap pertumbuhan kalus Amorphophallus muelleri Blume. var. Madiun 1*

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***Article history***
*Received : August 25, 2022*
*Accepted : August 25, 2023*
*Published : September 30, 2023*

***Keyword***
*6-benzyl amino purine, calli, naphthalene acetic acid, light color spectrum*

***ABSTRACT***

***Introduction:*** *Plant Growth Regulators (PGRs) play a role in regulating organogenesis and morphogenesis in shoots, roots, and callus formation. Color spectrum of light is one of the quality light factors that affects plant physiological processes. This study aimed to determine the effect of cytokinin and auxin on Murashige-Skoog (MS) medium and light on callus induction and proliferation of porang (Amorphophallus muelleri Blume.) var. Madiun1.* ***Methods:*** *This study used completely randomized design, with the first factor was PGRs (combination auxin and cytokinin) and the second was color spectrum of light (white light, blue light, and its combination) during incubation. Variables observed were emergence time, color, texture, structure and calli growth, also shoots emerging from calli.* ***Results:*** *The results showed an interaction between PGRs with a combination of light color spectrum on callus growth. The fastest callus growth occurred in combination 5.0 mg.l$^{-1}$ 6-benzyl amino purine (BAP) with 0.2 mg.l$^{-1}$ naphthalene acetic acid (NAA) which was incubated in a combination of white and blue light for 16 hour irradiation. The combination 5.0 mg.l$^{-1}$ BAP with 0.2 mg.l$^{-1}$ NAA was able to induce callus emergence time, and the shoots appearing were faster, whereas combination of white and blue light was able to accelerate callus emergence from bulbil and adventitious shoots emergence.* ***Conclusion:*** *The combination of white and blue light color spectrum for 16 hours irradiation can accelerate callus emergence from bulbil and adventitious shoots emerging from calli, and interaction with combination of 5.0 mg.l$^{-1}$ BAP and 0.2 mg.l$^{-1}$ NAA can accelerate porang's callus growth.*

**Riwayat artikel**
Dikirim : 25 Agustus, 2022
Disetujui : 25 Agustus, 2023
Diterbiktkan : 30 September, 2023

**Kata Kunci**
asam asetat naftalin, 6-benzil amino purin, kalus, spektrum warna cahaya

**ABSTRAK**

**Pendahuluan:** Zat pengatur tumbuh (ZPT) berperan mengatur organogenesis dan morfogenesis pembentukan tunas, akar, dan kalus. Spektrum warna cahaya merupakan salah satu faktor kualitas cahaya yang berpengaruh terhadap proses fisiologi tumbuhan. Penelitian ini bertujuan untuk mengetahui pengaruh ZPT sitokinin dan auksin pada medium *Murashige-Skoog* (MS) medium serta cahaya terhadap induksi dan proliferasi kalus porang (*Amorphophallus muelleri* Blume.) var. Madiun1. **Metode:** Penelitian menggunakan Rancangan Acak Lengkap, dengan faktor pertama ZPT (kombinasi auksin dan sitokinin) dan faktor kedua spektrum warna cahaya (cahaya putih, biru, serta kombinasi putih dan biru) saat inkubasi. Variabel yang diamati waktu muncul kalus, warna, tekstur, struktur dan pertumbuhan kalus, serta waktu muncul tunas dari kalus. **Hasil:** Hasil penelitian menunjukkan ada interaksi antara ZPT dengan kombinasi spektrum warna cahaya terhadap pertumbuhan kalus. Pertumbuhan kalus tercepat pada kombinasi 6-benzil amino purin (BAP) 5,0 mg.l$^{-1}$ dengan asam asetat naftalein (NAA) 0,2 mg.l$^{-1}$, diinkubasi pada kombinasi spektrum warna cahaya putih dan biru selama 16 jam penyinaran. Kombinasi BAP 5,0 mg.l$^{-1}$ dengan NAA 0,2 mg.l$^{-1}$ mampu menginduksi waktu muncul kalus dan tunas adventif lebih cepat, sedangkan kombinasi cahaya putih dan biru mampu mempercepat waktu muncul kalus dari eksplan dan kemunculan tunas adventif. **Kesimpulan:** Kombinasi spektrum cahaya putih dan biru selama 16 jam penyinaran mempercepat waktu muncul kalus dari bulbil dan waktu muncul tunas adventif dari kalus., dan interaksi dengan kombinasi BAP 5,0 mg.l$^{-1}$ dan NAA 0,2 mg.l$^{-1}$ berefek mempercepat pertumbuhan kalus porang.

**Sitasi:** Wijaya, A. N., Poernomo, C., Savitri, W. D., Irawati, F., & Hardjo, P. H. (2023). The effectiveness of growth regulators and light color spectrum on callus growth of Amorphophallus muelleri Blume. var. Madiun 1. *Agromix, 14*(2), 182-188. https://doi.org/10.35891/agx.v14i2.3309

## INTRODUCTION

Porang tubers (*Amorphophallus muelleri* Blume.) which have high economic value are well known to have many benefits resulting in the increasing demand by farmers to cultivate them. Generally, porang tubers are exported in the form of chips or porang flour. Porang tubers are processed into flour, shirataki, konyaku, and various processed foods in the food industry. In the pharmaceutical industry, porang tubers are efficacious in lowering cholesterol and blood glucose, preventing obesity, and overcoming constipation.

However, the cultivation of porang is constrained due to the expensive price of frog seedlings (bulbil) and the difficulty to obtain due to the high demand for the seedlings. The application of tissue culture techniques for mass production of seedlings is an alternative to increase the availability of porang seedlings with uniform seedling quality, identical, and not depending on the season. Tissue culture techniques have resulted in various micropropagation protocols, for example in plants *Vanda pumila* Hook.f. (Maharjan et al., 2019) and *Prunus africana* (Hook.f.) Kalkman (Komakech et al., 2020). External and internal factors of culture affect the growth and development of each stage of culture. The internal factor to be studied in this study is ZPT, and the external factor is the color spectrum of light.

The method of micropropagation using callus is aimed at mass production of seedlings. Callus is a set of cells that are actively and continuously dividing, and it can be induced by auxins and/or cytokinins through organogenesis or embryogenesis forming planlets (Mayerni et al., 2020). Hariyanto et al. (2022) reported auxin, such as 1 ppm 2,4-dichlorophenoxyaceticacid (2.4-D) and 1.5 ppm Naphthaleneacetic Acid (NAA) were able to induce embryogenic callus from the explants of porang leaf buds (*Amorphophallus muelleri* Blume.).

One of the abiotic external factors affecting the growth and development of culture is the color spectrum of light. Monochromatic light-emitting diodes (LEDs) emit light at certain wavelengths. LEDs can be set to produce only the spectrum that plants need for a morphogenic response. In micropropagation, the response to LED light depends on the light intensity, photoperiod, and wavelength (Bello-Bello et al., 2017). The light factor determines the formation of callus. Some plant species require light at certain growth periods, but some do not require light at the beginning of callus induction. Baharan et al. (2015) reported that darkness can increase the callus induction of date palm *(Phoenix dactylifera* L.) from leaves. Furthermore, according to Xu et al. (2020), *Cunninghamia lanceolata* planlets exposed to red (610-700 nm), blue (425-490 nm), purple for 8 and 16 hours produce more roots than white light. White light affects the root in vitro, the number and length of new shoots, chlorophyll pigments, and carotenoids. Ansori et al., (2019) reported that the addition of blue and yellow light for 4 hours increased the content of antioxidant compounds, and the addition of blue light increased plant height and fresh weight of *Chrysanthemum* plants in the greenhouse.

This study aims to determine the effectiveness of cytokinin and auxin on a combination of white and blue light color spectrum to induce callus and callus growth of *Amorphophallus muelleri* Blume.

## METHODS

### Tools and materials

The Plant used in this research is frog/bulbil porang tuber (*Amorphophallus muelleri* Blume.) variety Madiun1. The light source used white (400-720 nm) and blue (440-480 nm) LED lights. The equipment used to conduct research was the Laminar Air Flow Cabinet (LAF), autoclave, beaker glass, spatula, erlenmeyer, micropipette, petridish, hotplate magnetic stirrer, analytical balance, gas stove, gas cylinder, and refrigerator.

### Place of execution

This research was conducted at Plant Biotechnology Laboratory, Faculty of Biotechnology, University of Surabaya.

### Surface explant sterilization and callus induction

Bulbil was cleaned in running water for 30 minutes, washed with liquid soap, and peeled off. After that, it was soaked in a solution of fungicide for 1 hour, then the bulbil was rinsed and soaked in a bactericidal solution for 1 hour and rinsed many times with distilled water (Aquadest). Further sterilization of the bulbil surface was inside the LAF. The size of the bulbil was reduced to several pieces, then soaked into a 0.1% $HgCl_2$ solution for 5 minutes, then rinsed with sterile Aquadest. The size of the bulbil was reduced, then soaked in a solution of NaOCl:Aquadest (1:1), with 2 drops of Tween-20 solution for 15 minutes. After that, it was repeatedly soaked in NaOCl:Aquadest (1:2) for 15 minutes and rinsed many times with sterile Aquadest. Pieces of bulbil were reduced to a size of 1.5 cm and drained in filter paper. The bulbil cut explants were ready to be planted in the callus induction treatment medium; each bottle contains 3 explants. The culture was placed in an incubation room with a duration of irradiation of 16 hours of light and 8 hours of darkness, according to the treatment of light colors, at a room temperature of 24±1°C for 90 days.

### Experimental design

The research used a complete randomized design (CRD) and a factorial treatment design consisting of Plant Growth Regulators (PGRs) factor (a combination of auxin, i.e. NAA with cytokinin, i.e. BAP) and light color spectrum factors (white, blue, and combinations both of them) with five replications. The PGRs treatment was as follows:

1. 2 mg.l$^{-1}$ BAP + 0.2 mg.l$^{-1}$ NAA
2. 3 mg.l$^{-1}$ BAP + 0.2 mg.l$^{-1}$ NAA
3. 4 mg.l$^{-1}$ BAP + 0.2 mg.l$^{-1}$ NAA
4. 5 mg.l$^{-1}$ BAP + 0.2 mg.l$^{-1}$ NAA

The light color spectrum treatment was as follows (photoperiod 16 h light and 8 h dark):

1. White light LED
2. Blue light LED
3. Combination of white and blue light LED

**Data analysis**

Observed variables included the time of callus appearing, callus color, callus texture, and callus growth. The quantitative data, such as the time of callus to appear, time of adventitious shoots to appear from callus, and callus growth are tested for normality and homogeneity of the data, followed by testing the difference between treatments and DMRT tests at α=5%. Qualitative data, such as color, texture, and structure of the callus are displayed with photos. Callus growth data were calculated based on the average size gain of the callus diameter area measured from 3 directions calculated from the beginning of induction to 60 days of callus life.

## RESULTS AND DISCUSSIONS

Callus induction from explant bulbil porang is influenced by PGRs concentrations, where 0.2 mg.l$^{-1}$ NAA combined with 5.0 mg.l$^{-1}$ BAP can induce the fastest callus compared to other PGRs treatments, while the color and texture of callus are not different during the 30 days incubation period (Table 1). Similarly, the difference in light color also has no effect on the color and texture of callus during the 30 days incubation period. The treatment of PGRs and light color has no effect on the color, texture and structure of the callus as shown in Figure 1. The combined treatment of 0.2 mg.l$^{-1}$ NAA (group of auxins) and 2.0-5.0 mg.l$^{-1}$ BAP (group of cytokinins) encourages induction and growth of callus in bulbil porang, as also reported by Ikeuchi et al. (2013) that the addition of auxins and balanced cytokinins will promote callus formation. Callus is white at the beginning of formation for up to 30 days of incubation in all PGRs treatments. The same was obtained by Paul et al. (2013), on the callus *Amorphophallus campanulatus* Blume. cultured on 0.5-1 mg.l$^{-1}$ BAP + 0.5-2.0 mg.l$^{-1}$ NAA and 3 mg.l$^{-1}$ Kinetin + 0.5-2.5 mg.l$^{-1}$ NAA. The following is a table of the effect of PGRs combination treatment on time, color, texture, and callus structure:

Tabel 1. The time of the appearance of callus, the color and texture of the callus *Amorphophallus muelleri* on the combined treatment of BAP and NAA concentrations

| PGRs (mg.l$^{-1}$) | Average time of Callus Appears (day-to-day) | Callus Color | | Callus Texture | | Callus Structure |
|---|---|---|---|---|---|---|
| | | 30 days | 60 days | 30 days | 60 days | 60 days |
| 2BAP+0.2NAA | 26.11±3.98$^{c}$ | white | white | compact | compact | nodular callus |
| 3BAP+0.2NAA | 21.11±3.82$^{b}$ | white | white yellowish | compact | compact | enlarged nodular callus |
| 4BAP+0.2NAA | 20.22±4.74$^{b}$ | white | yellow greenish | compact | compact | elongated nodular callus |
| 5BAP+0.2NAA | 16.22±3.03$^{a}$ | white | yellow greenish | compact | compact | elongated nodular callus |

Note: Values followed by the different letter in the same column were significantly different at $p < 0.05$ (α=5%) by DMRT test

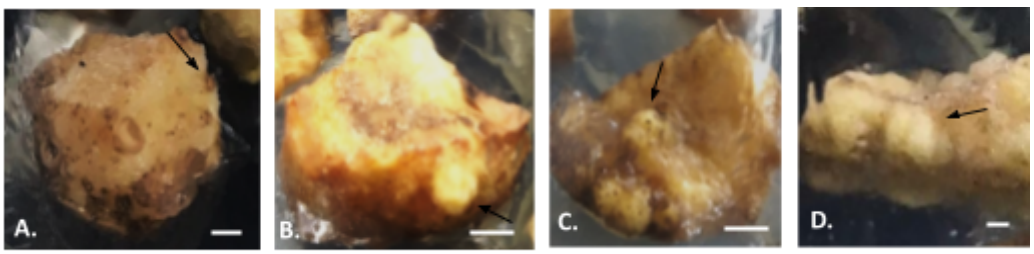


Figure 1. Callus initiation from bulbil of *Amorphophallus muelleri* at combination light white and blue color during 30 days after planted

Notes: (Scale bar = 1 mm)
A.) 2 mg.l$^{-1}$ BAP + 0.2 mg.l$^{-1}$ NAA
B.) 3 mg.l$^{-1}$ BAP + 0.2 mg.l$^{-1}$ NAA;
C.) 4 mg.l$^{-1}$ BAP + 0.2 mg.l$^{-1}$ NAA
D.) 5 mg.l$^{-1}$ BAP + 0.2 mg.l$^{-1}$ NAA

Figure 2 shows the callus formed from the bulbil explant porang has a nodular and compact structure. The callus cultured in Murashige and Skoog (MS) media with a low concentration of 2-3 mg.l$^{-1}$ BAP + 0.2 mg.l$^{-1}$ NAA, is still white nodular structure, in contrast, the callus cultured in MS media with 4-5 mg.l$^{-1}$ BAP + 0.2 mg.l$^{-1}$ NAA mostly regenerates to form prospective shoots with green color. The increase in cytokinin concentrations in the form of BAP accelerates the regeneration of nodular callus to form buds within an incubation period of 60 days.

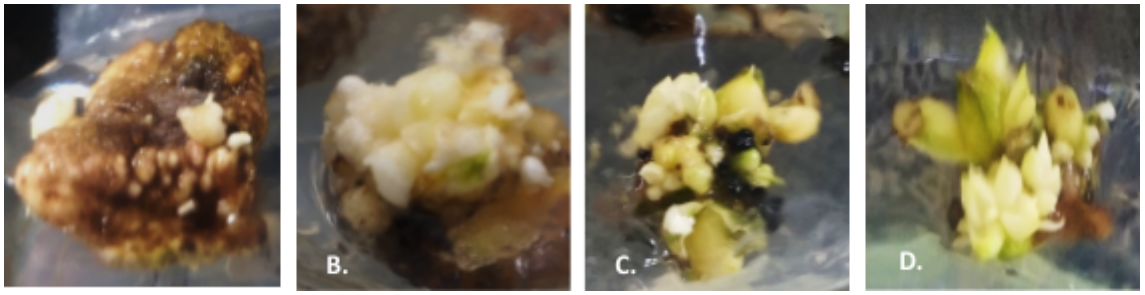


Figure 2. Callus initiation from bulbil of *Amorphophallus muelleri* explants at 60 days after planted

Notes: (Scale bar = 1 mm)
A.) 2 mg.l$^{-1}$ BAP + 0.2 mg.l$^{-1}$ NAA
B.) 3 mg.l$^{-1}$ BAP + 0.2 mg.l$^{-1}$ NAA
C.) 4 mg.l$^{-1}$ BAP + 0.2 mg.l$^{-1}$ NAA
D.) 5 mg.l-1 BAP + 0.2 mg.l-1 NAA

Nodular callus has changed color to greenish at BAP concentrations of 2-3 mg.l$^{-1}$ + 0.2 mg.l$^{-1}$ NAA during increasing incubation period up to 90 days, while higher BAP concentrations, 4-5 mg.l$^{-1}$ + NAA 0.2 mg.l$^{-1}$ shoots, elongate higher (Figure 3).

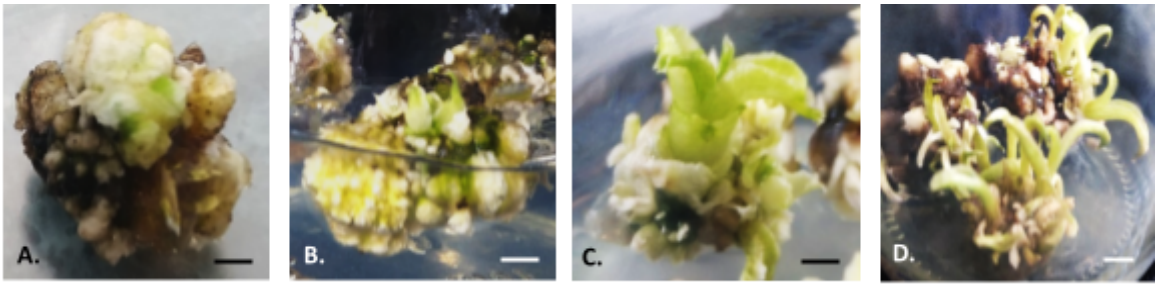


Figure 3. Callus initiation from bulbil of *Amorphophallus muelleri* explants at 90 days after planted

Notes: (Scale bar = 1 mm)
A.) 2 mg.l$^{-1}$ BAP + 0.2 mg.l$^{-1}$ NAA
B.) 3 mg.l$^{-1}$ BAP + 0.2 mg.l$^{-1}$ NAA
C.) 4 mg.l$^{-1}$ BAP + 0.2 mg.l$^{-1}$ NAA
D.) 5 mg.l-1 BAP + 0.2 mg.l-1 NAA

According to Table 2, the average time of the fastest callus grew in the white light color treatment and PGRs combination of 5 mg.l$^{-1}$ BAP + 0.2 mg.l$^{-1}$ NAA were 16.22±3.03 and 18.17±4.30. PGRs affect the time of callus growth because it affects the cell proliferation process as well, so that the emergence of callus is faster along with an increase in the concentration of BAP which is classified as a cytokinin.

In this study, it was also observed that the influence of LED light color on the time of callus appearance (Table 2). LEDs have the potential to be a substitute for light sources for in vitro cultures based on wavelength specificity (Dutta Gupta & Jatothu, 2013). The influence of the color of light on the appearance of callus was due to the light provided can be an elicitor which affects the morphology, physiology, and morphogenesis of plants. Based on the research of Suhartanto et al. (2022), it is known that light has a positive influence on callus induction in srikandi putih maize (*Zea mays* L.) culture. Light intensity is one of the important factors for callus formation and bud regeneration in howartia plant propagation in vitro (Chen et al., 2019). Based on Table 2, the fastest appearance of callus on white light color and the combination between white and blue. This can be due to higher light energy compared to blue light energy. This high light energy can spur the growth of callus or other physiologies of the plant. In addition, the presence of high-energy light is able to cause morphological changes in plants as well (Usman et al., 2020). The following is a table of the influence of PGRs and color of light on the time of appearance of the callus *Amorphophallus muelleri* Blume.:

Table 2. Effect of PGRs factor and light color to appearance time of callus *Amorphophallus muelleri* Blume.

| Average time of callus appearance (days after explant planted) | | | | |
|---|---|---|---|---|
| | Light color | | | |
| PGRs (mg.l$^{-1}$) | White | Blue | White & Blue | Average |
| 2BAP+0.2NAA | 24±2.00 | 31±1.00 | 23.33±2.08 | 26.11±3.98$^{c}$ |
| 3BAP+0.2NAA | 18.33±3.06 | 25±3.00 | 20±2.00 | 21.11±3.82$^{b}$ |
| 4BAP+0.2NAA | 16.33±1.53 | 26±2.65 | 18.33±1.53 | 20.22±4.74$^{b}$ |
| 5BAP+0.2NAA | 14±2.00 | 19.67±2.08 | 15±1.00 | 16.22±3.03$^{a}$ |
| Average | 18.17±4.30$^{a}$ | 25.42±4.64$^{b}$ | 19.17±3.46$^{a}$ | |

Note: Values followed by the different letter in the same column or line were significantly different at $p < 0.05$ (α=5%) by DMRT test

Light is one of the abiotic elicitors that can help change the physiological processes of plants, such as helping the process of plant development and biosynthesis of secondary metabolites, which are commonly found in the callus. A high accumulation of secondary metabolites will lead to an increase in callus weight (Fazal et al., 2016). The increase in callus weight can be in harmony with the increase of callus diameter. Based on the research of Usman et al. (2020), the highest wet and dry weight of *S. xanthocarpum* callus was obtained successively in the treatment of white light and blue light because the higher the energy level can produce a higher rate of photosynthesis, so that biomass production and the callus diameter area can increase. In addition, light can cause stress which has an impact on the accumulation of phytochemicals and antioxidants that have the potential to regulate metabolites in plant development and physiology. Based on the research of Khurshid et al. (2020), the use of white light can produce the callus *Eclipta alba* L. with the most wet weight compared to the use of blue light. It can also be seen in this study that the increase in the area of the callus diameter of *Amorphophallus muelleri* Blume. is higher in treatments with exposure to white light than in treatments with exposure to blue light (Table 3). The increased area of callus diameter is in line with the increase of the wet weight of callus. This is because exposure to white light has a higher level of energy compared to blue light, so it can cause an increase in biomass accompanied by an increase in the callus diameter area. However, the increased area of callus diameter is the highest in the combination treatment of white and blue light, which can cause higher energy levels, resulting in physiological changes to the accumulation of secondary metabolites in the callus and indirectly affecting the increase in the callus diameter area.

In addition, based on Table 3, the increasing concentration of BAP leads to an increased area of callus diameter as well. This can be due to the combination of NAA and BAP, where NAA which is classified as auxin plays a role in spurring the formation of roots and callus, while BAP which is classified as cytokinin can play a role in cell division known as cytokinesis (Mayerni et al., 2020). Thus, BAP growth can help in continuous cell division which has an impact on the growth of the callus diameter area. The following is a table of the Interaction of ZPT with light color on the increase area of callus diameter *Amorphophallus muelleri* Blume.:

Table 3. Interaction of PGRs with light color to increasing area of callus diameter *Amorphophallus muelleri* Blume. after 60 days incubation at MS media

| | Light Color | | |
|---|---|---|---|
| PGRs (mg.l$^{-1}$) | White | Blue | White & Blue |
| 2BAP+0.2NAA | 0.69±0.05$^{bc}$ | 0.49±0.03$^{a}$ | 0.70±0.03$^{bc}$ |
| 3BAP+0.2NAA | 0.74±0.06$^{cd}$ | 0.59±0.30$^{ab}$ | 0.76±0.03$^{cd}$ |
| 4BAP+0.2NAA | 0.79±0.07c$^{de}$ | 0.69±0.04$^{bc}$ | 0.84±0.08$^{def}$ |
| 5BAP+0.2NAA | 0.94±0.04$^{f}$ | 0.89±0.03$^{ef}$ | 1.23±0.15$^{g}$ |

Note: Values followed by the different letter were significantly different at $p < 0.05$ (α=5%) by DMRT test

There is no interaction of PGRs with light color to the time emergence of adventitious budding from the callus, but there remains an influence of each factor singularly as shown in Table 4. An increase in the concentration of cytokinin BAP up to 5 mg.l$^{-1}$ can accelerate the emergence of shoots from the nodular callus. Similarly, Cioć et al. (2019) found that increased concentrations of BA (5 µM) resulted in increased multiplication of in vitro buds of *Gerbera jamesonii* Bolus illuminated by LED lamps with intensity 80 µmol m$^{-2}$.s$^{-1}$. The following is a table of the influence of PGRs factors and light color on the time emergence of *Amorphophallus muelleri* Blume.'s shoots:

Table 4. Effect of PGRs and light color to time emergence of *Amorphophallus muelleri* Blume.'s shoots

| Average time emergence of shoots (days after planted) | | | | |
|---|---|---|---|---|
| | Light color | | | |
| PGRs (mg.l$^{-1}$) | White | Blue | White & Blue | Average |
| 2BAP+0.2NAA | 92±1.00 | 100±2.00 | 85.33±4.73 | 92.44±6.88$^{d}$ |
| 3BAP+0.2NAA | 74.67±3.06 | 85±4.58 | 65.67±2.01 | 75.11±8.88$^{c}$ |
| 4BAP+0.2NAA | 64.33±4.04 | 73±2.00 | 60±2.00 | 65.78±6.24$^{b}$ |
| 5BAP+02NAA | 58.33±2.08 | 66.67±3.51 | 54.67±2.52 | 59.89±5.84$^{a}$ |
| Average | 72.33±13.55$^{b}$ | 81.17±13.56$^{c}$ | 66.42±12.38$^{a}$ | |

Note: Values followed by the different letter in the same column or line were significantly different at $p < 0.05$ ($\alpha$=5%) by DMRT test

## CONCLUSION

PGRs combination of 0.2 mg.l$^{-1}$ auxin NAA and 5.0 mg.l$^{-1}$ cytokinin BAP induces earlier emergence of callus from bulbil explants and the time of emergence of adventitious buds from the callus *Amorphophallus muelleri* Blume. The combination of white and blue light induces earlier emergence of callus from the bulbil explant and the time of adventitious bud emerges from the callus *Amorphophallus muelleri* Blume. The combination of 0.2 mg.l$^{-1}$ auxin NAA and 5.0 mg.l$^{-1}$ cytokinin BAP accelerates the growth of callus *Amorphophallus muelleri* Blume which was incubated on a combination of white and blue light colors for 16 hours of irradiation.

## ACKNOWLEDGEMENT

The research team would like to thank the KemendikbudRistek-LPDP (the Ministry of Education, Culture, Research and Technology – Education Fund Management Agency) for funding this research through the Scientific Research Grant for the Independent Lecturer scheme with contract number 159/E4.1/AK.04.RA/2021.

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Alamat Redaksi: Jl. Yudharta No 07, Sengonagung
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Terakreditasi Nomor: 36/E/KPT/2019

Vol 14 No 1 (2023): Maret

Frekuensi Terbit: 2 kali per tahun (Maret & September)
DOI: https://doi.org/10.35891/agx
P-ISSN: 2085-261X
E-ISSN: 2685-3000
Penerbit: Fakultas Pertanian, Universitas Yudharta Pasuruan
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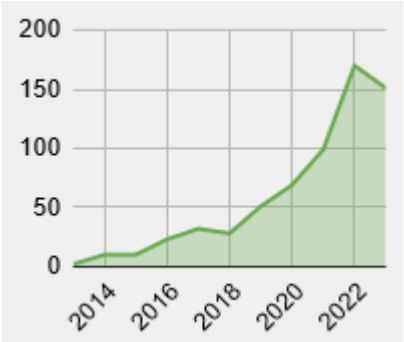


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Name of Journal: Agromix
*Publisher: Agriculture Faculty, Yudharta University, Indonesia*
Address: Yudharta Street No. 7, Sengonagung, Purwosari, Pasuruan

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