El-Hayah

**JURNAL BIOLOGI**
Journal Homepage: http://ejournal.uin-malang.ac.id/index.php/bio/index
e-ISSN: 2460-7207, p-ISSN: 2086-0064

*Original research article*

# *The Effect Of 2,4-D (2,4-Dichlorophenoxyacetic Acid) and Kinetin (6-Furfuryl Amino Purine) Concentrations on The Induction of Embryogenic Callus In Porang (Amorphophallus muelleri Blume) In Vitro*

**Nadya Rahadianti Agung[1], Darul Huddi[2*], Amalia Nasyira Putri[2], Ruri Siti Resmisari[1]**

[1]*Department of Biology, Faculty of Science and Technology, UIN Maulana Malik Ibrahim Malang, Jl Gajayana No. 50 Malang, 65144*
[2]*Master of Biology, Faculty of Science and Technology, UIN Maulana Malik Ibrahim Malang, Jl Gajayana No. 50 Malang, 65144*

[*]*Corresponding author*
*Email: darulhuddi10@gmail.com*
DOI: 10.18860/elha.v9i2.26105

**Article Info**

*Article history:*
Received 10 Augustus 2022
Received in revised form 15 October 2022
Accepted 10 January 2023

*Key Word:*
*2,4-D*
*Amorphophallus muelleri*
*Embryogenic callus*
*In vitro*
*Kinetin*

**Abstract**

*Porang (Amorphophallus muelleri Blume) belongs to the family Araceae and holds significant potential for cultivation in Indonesia. It contains more than 50% glucomannan in its corms. Porang is commonly commercialized as flour and chips and has found extensive use in the food, cosmetics, chemical, pharmaceutical, and coating industries. Conventional propagation of Porang often takes a long time, approximately 2-3 years, making it unable to meet the demand for Porang seedlings quickly. In vitro propagation using plant growth regulators 2,4-D and kinetin is being explored to induce embryogenic callus, offering a solution for large-scale cultivation of high-quality Porang within a shorter timeframe. This research adopts an experimental approach employing a completely randomized design (CRD) with two factors. The first factor is the auxin 2,4-D (2,4-Dichlorophenoxyacetic acid), and the second factor is the cytokinin kinetin. The optimal concentration for inducing embryogenic callus in Porang is found to be 2,4-D at 1 mg/L and kinetin at 0 mg/L, resulting in intermediate callus with a yellowish-brown color and a wet weight of 2.43 grams.*

## 1. INTRODUCTION

Porang is a type of tuberous plant with potential and opportunities for cultivation in Indonesia **[1]**. Porang contains a high level of glucomannan in its tubers. Porang tubers contain more than 50% glucomannan, depending on the location, soil, climate, and

age of the tubers. Porang is commonly commercialized as flour and chips derived from processed Porang tubers. Porang has been widely used in the food industry, cosmetics, chemicals, petroleum, pharmaceuticals, and coating industries **[2]**.

The propagation of Porang plants naturally can occur through vegetative organs, namely leaf bulbs (bulbils) and stem tubers, while generative propagation employs seeds. Propagation through bulbils, when directly planted in a seedling medium, undergoes a dormancy period of approximately 5-6 months, thus failing to meet the demand for Porang seedlings in a short time **[3]**. The supply of bulbils is further constrained because on average, Porang plants produce 1 bulbil in the first growth phase, 4 - 7 in the second phase, and 10 - 20 in the third phase. The use of stem tubers is also not recommended because harvested tubers, when used for reseeding, may reduce Porang tuber production **[4].** Tuber seedlings typically require about one year before they can be harvested **[5].** Additionally, Porang tubers experience shrinkage and a decrease in glucomannan content (degradation) after Porang flowers bloom, so Porang tubers are usually harvested before flowering **[6].**

One solution to obtain a large quantity of high-quality Porang seedlings in a short time is through in vitro culture. One of the in vitro culture techniques that can be utilized is callus culture. Callus culture is an initial stage of in vitro culture technique that produces and multiplies callus cells massively, where each callus cell has the ability to form new individuals through embryogenesis and organogenesis stages **[7].** Callus as a collection of amorphous cells (undifferentiated cells) derived from continuously dividing cells in culture bottles. **[8].** The advantage of callus culture is its ability to generate new plants with superior quality that are free from diseases, and it can produce higher levels of secondary metabolites compared to the original plants **[9].** Factors such as the type of media used, the type of explants utilized, and growth regulators are some factors that can influence callus growth during the callus propagation process **[10]**.

Previous research on the induction of embryogenic callus has been conducted. Induced callus from embryo explants of maize seeds (*Zea mays* L.) with a concentration of 1.5 mg/L 2,4-D and 0.3 mg/L kinetin, resulting in embryogenic callus with a friable texture and whitish-yellow color, with the highest percentage of callus formation achieved within 15 days **[11]**. Induced callus from barley plant (*Hordeum vulgare* L.) caryopsis explants, showing that the primary callus gradually transformed into a homogeneous embryogenic mass, with a solid and brittle texture ranging from white to yellowish on media containing 2 mg/L 2,4-D and 2.5 mg/L kinetin **[12]**.

## 2. MATERIALS AND METHODS

### Tools

The tools used in this research includes Petri dishes, tissue paper, label paper, pH meter, autoclave, plastic measuring cups, micropipette, glass beakers, analytical balance, hot plate and magnetic stir bar, culture bottles, rubber bands, forceps, blade no. 22, scalpel, laminar air flow (LAF), lighter, Bunsen burner, aluminum foil, incubation rack, and oven.

### Materials

The materials used in this research include subculture explants of *Amorphophallus muelleri Blume* callus, MS (Murashige & Skoog) media 4.43 g/L, and growth regulators 2,4-D (0 mg/L, 1 mg/L, 2 mg/L, 3 mg/L, and 4 mg/L), kinetin (0 mg/L, 0.1 mg/L, 0.2 mg/L, and 0.3 mg/L), sucrose (30 g/L), agar-agar (10 g/L), betadine, 70% alcohol, 96% alcohol, sterile distilled water, distilled water, and methylene blue.

**Porang Callus Induction**

Porang callus (*Amorphophallus muelleri* Blume) resulting from three subcultures on NAA 2 mg/l media were removed from the bottle. They were then cut on a Petri dish to a size of 1 cm and soaked in sterile distilled water previously treated with betadine, before being planted in each treatment media. Explant initiation was conducted inside a Laminar Air Flow (LAF). After initiation, the culture bottles were tightly closed and placed in the incubation chamber.

**Data Collection Technique**

Data were obtained from final observations, which were conducted 45 days after explantation. The observed parameters include: callus color, callus texture, callus weight, and callus anatomy. The observation parameters are as follows:

a. Wet weight observation of the callus was conducted on the final day, by weighing the callus using an analytical balance.
b. Callus color observation was based on the color that appeared on each callus.
c. Callus texture was visually observed to classify the formed callus as friable callus, intermediate callus, or compact callus. Callus anatomy was observed using an Olympus binocular microscope

## 3. Results and Discussion

**Callus Weight**

The results of the analysis of variance (ANOVA) for the addition of growth regulators 2,4-D and kinetin in the induction of embryogenic callus of Porang (*Amorphophallus muelleri* Blume) showed a significant effect on the variable of callus wet weight in the induction of Porang embryogenic callus. Therefore, further testing was conducted using Duncan Multiple Range Test (DMRT) with a significance level of 5%. The results of the DMRT calculation are presented in Table 1.

**Table 1.** The Effect of 2,4-D and Kinetin Combination on the Wet Weight of Porang Callus (*Amorphophallus muelleri* Blume)

| Treatment (mg/l) | Wet Weight of Callus (g) |
|---|---|
| 0 2,4-D + 0 Kinetin | 1,13a |
| 0 2,4-D + 0,1 Kinetin | 1,49ba |
| 0 2,4-D + 0,2 Kinetin | 1,40ab |
| 0 2,4-D + 0,3 Kinetin | 2,30cd |
| 1 2,4-D + 0 Kinetin | **2,43d** |
| 1 2,4-D + 0,1 Kinetin | 1,80bc |
| 1 2,4-D + 0,2 Kinetin | 1,14a |
| 1 2,4-D + 0,3 Kinetin | 1,61ab |
| 2 2,4-D + 0 Kinetin | 1,38ab |
| 2 2,4-D + 0,1 Kinetin | 1,69ab |
| 2 2,4-D + 0,2 Kinetin | 1,55ab |
| 2 2,4-D + 0,3 Kinetin | 1,44ab |
| 3 2,4-D + 0 Kinetin | 1,25ab |
| 3 2,4-D + 0,1 Kinetin | 1,31ab |
| 3 2,4-D + 0,2 Kinetin | 1,44ab |
| 3 2,4-D + 0,3 Kinetin | 1,13a |
| 4 2,4-D + 0 Kinetin | 1,65ab |
| 4 2,4-D + 0,1 Kinetin | 1,13a |
| 4 2,4-D + 0,2 Kinetin | 1,21ab |
| 4 2,4-D + 0,3 Kinetin | 1,61ab |

Note: Mean values followed by the same letter notation indicate that the combination of 2,4-D and Kinetin does not significantly differ based on the Duncan Multiple Range Test (DMRT) at a significance level of 5%.

Based on Table 1, it is known that the best combination of 2,4-D and Kinetin for callus induction is a concentration of 1 mg/l 2,4-D + 0 mg/l Kinetin, with an average wet weight of callus of 2.43 g. From the observations based on Figure 1, the addition of growth regulators into the media is crucial in determining the direction of growth of an explant. At a concentration of 0 mg/l 2,4-D + 0 mg/l Kinetin, no embryogenic callus formation was observed for all observation variables. The absence of embryogenic callus formation in samples without the addition of 2,4-D and kinetin is attributed to the presence of two crucial types of growth regulators in vitro culture, namely auxin and cytokinin, which influence the growth and morphogenesis of explants **[13].** Auxin and cytokinin added to the media will interact with endogenous auxin and cytokinin hormones, thus

determining the direction of growth and development of the explants. Different types of plants, explant ages, and different growth regulators also give different responses to explants. Growth regulators will diffuse into the tissue through the cut portion of the explant, then stimulate cell division to form callus **[14].**

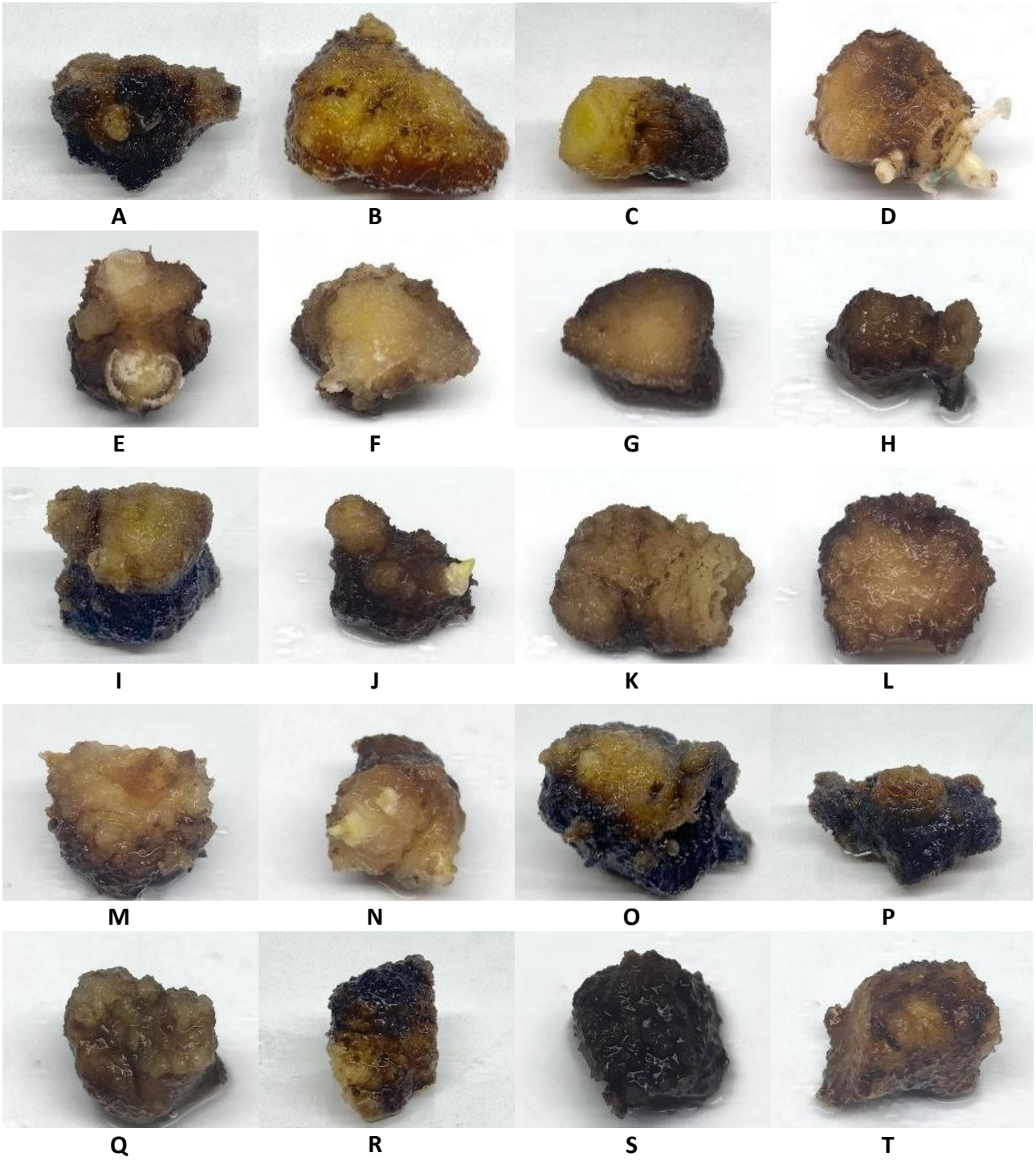


**Figure 1.** The Effect of 2,4-D and Kinetin Combination on the Morphology of Porang Callus (Amorphophallus muelleri Blume) (A) 0 mg/l 2,4-D + 0 mg/l Kinetin (B) 0 mg/l 2,4-D + 0,1 mg/l Kinetin (C) 0 mg/l 2,4-D + 0,2

mg/l Kinetin (D) 0 mg/l 2,4-D + 0,3 mg/l Kinetin (E) 1 mg/l 2,4-D + 0 mg/l Kinetin (F) 1 mg/l 2,4-D + 0,1 mg/l Kinetin (G) 1 mg/l 2,4-D + 0,2 mg/l Kinetin (H) 1 mg/l 2,4-D + 0,3 mg/l Kinetin (I) 2 mg/l 2,4-D + 0 mg/l Kinetin (J) 2 mg/l 2,4-D + 0,1 mg/l Kinetin (K) 2 mg/l 2,4-D + 0,2 mg/l Kinetin (L) 2 mg/l 2,4-D + 0,3 mg/l Kinetin (M) 3 mg/l 2,4-D + 0 mg/l Kinetin (N) 3 mg/l 2,4-D + 0,1 mg/l Kinetin (O) 3 mg/l 2,4-D + 0,2 mg/l Kinetin (P) 3 mg/l 2,4-D + 0,3 mg/l Kinetin (Q) 4 mg/l 2,4-D + 0 mg/l Kinetin (R) 4 mg/l 2,4-D + 0,1 mg/l Kinetin (S) 4 mg/l 2,4-D + 0,2 mg/l Kinetin (T) 4 mg/l 2,4-D + 0,3 mg/l Kinetin

The combination of 2,4-D and Kinetin also affects the wet weight of the callus. At a concentration of 1 mg/l 2,4-D + 0.1 mg/l Kinetin, it was able to produce a callus weighing 1.80 g. The wet weight of the callus indicates growth related to an increase in the number and volume of cells. The combination of 2,4-D and Kinetin can enhance cell division, thus increasing the wet weight of the callus. The provision of these growth regulators likely occurs in a balanced concentration with endogenous auxin and cytokinin, thereby interacting in cell enlargement and division. The presence of auxin will decrease the pH in the cell wall, causing the cell wall to soften and cell enlargement to occur. Furthermore, with the presence of cytokinin, cell division in the meristematic tissue is stimulated, which is related to the process of RNA transcription and translation in protein synthesis during the interphase stage. With the interaction of hormones in this balanced concentration, it can increase the speed of enlargement, division, and proliferation of cells, thereby increasing the weight of the callus **[15].**

Based on the research results, the addition of 2,4-D and Kinetin at both the lowest and highest concentrations resulted in poor callus formation. At these concentrations, many calluses exhibited browning and entered a phase of death due to decreased or halted growth. This is suspected to be a sign of decreased callus growth due to either a deficiency or excess of exogenous hormones, leading to an imbalance between exogenous and endogenous hormones. A concentration that is too low does not stimulate growth significantly, while a concentration that is too high can lead to growth inhibition because high concentrations of exogenous hormones can be toxic to explants/plants [16]. Browning in explants is related to the oxidation of phenolic compounds. Polyphenol oxidase (PPO) catalyzes the reaction between phenolic compounds and oxygen to produce quinone. Quinone, which is highly reactive, reacts to polymerize proteins and other cellular components, resulting in the formation of dark amorphous melanin pigments [17].

## Morphology of Callus

Qualitative parameters in this research are divided into two categories: morphology and anatomy. The qualitative parameter of callus morphology includes callus color and texture. Callus color is a visual representation of callus growth that indicates whether the callus cells are still actively dividing or not, while callus texture is an indicator to determine the quality of the callus [18]. The observation results regarding the effect of adding combinations of 2,4-D and Kinetin on callus morphology are presented in Table 2.

**Table 2.** The Effect of 2,4-D and Kinetin Combination on the Morphology of Porang Callus (*Amorphophallus muelleri* Blume)

| Treatment (mg/l) | Colour | Texture |
|---|---|---|
| 0 2,4-D + 0 Kinetin | Black Hex: #060101 | Compact |
| 0 2,4-D + 0,1 Kinetin | Seal Brown Hex: #2A1506 | Compact |
| 0 2,4-D + 0,2 Kinetin | Seal Brown Hex: #2E1705 | Compact |
| 0 2,4-D + 0,3 Kinetin | Brown Pod Hex: #431F09 | Compact |
| 1 2,4-D + 0 Kinetin | Antique Brass Hex: #6D4319 | Intermediate |
| 1 2,4-D + 0,1 Kinetin | Tuscok Hex: #C49449 | Intermediate |

| | | |
|---|---|---|
| 1 2,4-D + 0,2 Kinetin | Anzac Hex: #C48F44 | Compact |
| 1 2,4-D + 0,3 Kinetin | Seal Brown Hex: #2F1506 | Compact |
| 2 2,4-D + 0 Kinetin | Seal Brown Hex: #261607 | Intermediate |
| 2 2,4-D + 0,1 Kinetin | Seal Brown Hex: #281306 | Compact |
| 2 2,4-D + 0,2 Kinetin | Antique Brass Hex: #674820 | Intermediate |
| 2 2,4-D + 0,3 Kinetin | Dark Brown Hex: #633E18 | Compact |
| 3 2,4-D + 0 Kinetin | Brown Pod Hex: #48280D | Compact |
| 3 2,4-D + 0,1 Kinetin | Brandy Punch Hex: #BF8340 | Compact |
| 3 2,4-D + 0,2 Kinetin | Seal Brown Hex: #241207 | Compact |
| 3 2,4-D + 0,3 Kinetin | Black Hex: #080101 | Compact |
| 4 2,4-D + 0 Kinetin | Seal Brown Hex: #2D1A07 | Compact |
| 4 2,4-D + 0,1 Kinetin | Black Hex: #080000 | Compact |
| 4 2,4-D + 0,2 Kinetin | Black Hex: #0D0508 | Compact |
| 4 2,4-D + 0,3 Kinetin | Seal Brown Hex: #281306 | Compact |

The changes in callus color are influenced by pigmentation, nutrient availability in the culture media, and environmental factors such as light [19]. Treatment combinations of 2,4-D 1 mg/l + Kinetin 0.1 mg/l and 2,4-D 3 mg/l + Kinetin 0.1 mg/l are the best combinations that can produce whitish to brownish callus, indicating that the callus has embryogenic capacity. This is supported by the literature of Marthani et al [20], which states that whitish callus with intermediate texture has embryogenic potential. Leupin et al [21] added that whitish callus consists of embryonic tissue that does not contain chloroplasts yet but contains plastids filled with high-starch granules, which serve as polysaccharide or food reserves in plants. Whitish callus indicates that the callus is young, gradually changing to brownish as it ages.

Treatment combinations of 2,4-D 0 mg/l + Kinetin 0 mg/l; 2,4-D 3 mg/l + Kinetin 0.3 mg/l; 2,4-D 4 mg/l + Kinetin 0.1 mg/l; and 2,4-D 4 mg/l + Kinetin 0.2 mg/l resulted in dark brownish callus. The dark brownish color of the callus indicates physiological deterioration because the cells have reached maximum growth and are entering the cell death phase, or it may be due to the presence of high levels of phenolic compounds leading to oxidation. This aligns with the literature of Setiawati *et al* **[22]** which states that the brownish coloration of callus is caused by several factors such as excessive metabolism of phenolic compounds or plant adaptation processes due to wound stress response.

Treatment combinations of 2,4-D 1 mg/l + Kinetin 0.1 mg/l and 2,4-D 3 mg/l + Kinetin 0.1 mg/l resulted in friable callus texture. Friable texture in this research indicates characteristics such as being brittle and easily detachable due to its low cell density and high water content. According to Thao *et al* **[23]** friable callus contains a lot of water because its cell walls have not undergone lignification, making it easy to separate one cell from another. Yelnititis **[24]** adds that visually, friable callus is easily separable and fragile, and it easily sticks when picked up with forceps. Callus with this friable texture is very useful for cell suspension cultures because undifferentiated cell clusters can grow rapidly in liquid culture media **[25].**

Most of the treatment combinations of 2,4-D and Kinetin resulted in compact callus texture. Compact callus has characteristics of being hard, solid, and difficult to separate. According to Mahadi *et al* **[26]**, the compact texture is due to the lignification process (thickening of cell walls), which makes the callus hard and rigid because of the involvement of endogenous and exogenous hormones in nutrient transport processes. High levels of auxin and cytokinin hormones also affect the formation of compact callus

because the water potential inside the cells causes increased water absorption from the culture media into the cells, making them more rigid **[27].** Litz *et al* **[28]** also added that the compact callus texture is related to sucrose content because sucrose, as one of the components in cell wall formation, consists of cellulose chains that are not easily separable **[29]**. On the other hand, some treatment combinations resulted in callus with an intermediate texture. Intermediate callus has characteristics where the cell clusters consist of both friable and compact callus textures.

**Callus Anatomy**

To ensure the type of callus resulting from the treatment combinations of growth regulators 2,4-D and Kinetin is embryogenic callus, anatomical observations were conducted. Three types of callus were observed, namely friable, compact, and intermediate callus. Anatomical observation was carried out using an Olympus binocular microscope with a magnification of 400x. The anatomical observation results of the callus are shown in Figure 2.

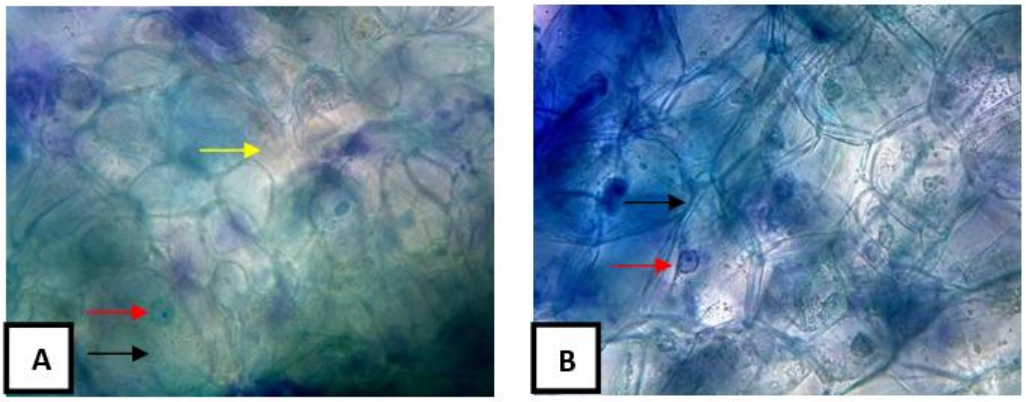


**Figure 2.** Porang (*Amorphophallus muelleri* Blume) callus anatomy at 400x magnification. (A) Friable (3 mg/L 2,4-D and 0.1 mg/L Kinetin), (B) Compact (4 mg/L 2,4-D and 0.1 mg/L Kinetin): black arrow : cell wall, red arrow : nucleus, and yellow arrow : intercellular space

The anatomical observations in Figure 2 indicate that the cells typically exhibit a rounded and elongated shape, comprising cellular components such as the nucleus, cell wall, and intercellular spaces. The anatomical differences among friable, intermediate, and compact calluses are discernible based on the size and number of nuclei as well as the intercellular spaces. In this study, the friable callus displays noticeable intercellular spaces, resulting in loosely packed cell units with numerous large nuclei and distinct cell walls. This observation aligns with Pauline **[30]**, where friable callus exhibits clustered cells with meristematic properties, characterized by evident intercellular spaces, clearly visible nuclei, dense cytoplasm, and high cell division activity.

Thomy **[31]** suggests that the friable texture of callus facilitates the detachment and separation of its cells into single units, thereby enhancing oxygenation between cells. Consequently, friable callus texture can be utilized for suspension culture in liquid media. Conversely, compact callus displays cells with smaller nuclei compared to those in friable callus, with fewer nuclei observed overall. The cell walls appear distinct, and the cell arrangement is dense, resulting in the absence of intercellular spaces. Ariati **[27]** further asserts that compact callus is characterized by its dense and firm texture, typically featuring small and tightly packed cells with dense cytoplasm. The nuclei are often small and may not be easily discernible, and the cells contain starch granules. In observations of

intermediate callus anatomy, both large and small nuclei are visible, with intercellular spaces present and distinct cell walls. According to Rasud & Bustaman **[7]** intermediate callus comprises a mixture of compact and friable cell masses.

## 4. CONCLUSION

The combination of growth regulators 2,4-D at 1 mg/L and Kinetin at 0 mg/L significantly influenced the wet weight parameter of the callus, resulting in a wet weight of 2.43 grams. The callus exhibited a yellowish-brown color with an intermediate texture.

## 5. REFERENCES


1. D. Suheriyanto, R. Romaidi, dan R.S. Resmisari, "Pengembangan Bibit Unggul Porang (Amorphophallus oncophilus) Melalui Teknik Kultur In Vitro Untuk Mendukung Ketahanan Pangan Nasional," El-Hayah: Jurnal Biologi, vol. 3, no. 1, pp. 1-6, 2012.
2. R. Impraprasert, C. Borompichaichartkul, dan G. Srzednicki, "A new drying approach to enhance quality of konjac glucomannan extracted from Amorphophallus muelleri," Drying Technology, vol. 32, no. 7, pp. 851-860, 2014.
3. Sumarwoto, S. 2004. Pengaruh Pemberian Kapur Dan Ukuran Bulbil Terhadap Pertumbuhan Iles-Iles (Amorphophallus muelleri Blume) Pada Tanah Beraliran Tinggi. Ilmu Pertanian (Agricultural Science). 11(2).
4. S. Sumarwoto, "Uji Zat Pengatur Tumbuh dari Berbagai Jenis dan Konsentrasi pada Stek Daun Iles-Iles (Amorphophallus muelleri Blume)," Agroland: Jurnal Ilmu-ilmu Pertanian, vol. 15, no. 1, pp. 7-11, 2008.
5. Y. Supriati, "Keanekaragaman Iles-Iles (Amorphophallus sp.) dan Potensinya untuk Industri Pangan Fungsional, Kosmetik, dan Bioetanol," Jurnal Litbang Pertanian, vol. 35, no. 2, pp. 1-4, 2016.
6. B. Budiman dan E. Arisoesilaningsih, "Predictive Model of Amorphophallus muelleri Growth In Some Agroforestry In East Java by Multiple Regression Analysis," Biodiversitas Journal of Biological Diversity, vol. 13, no. 1, pp. 1-5, 2012.
7. Y. Rasud dan B. Bustaman, "Induksi Kalus secara In Vitro dari Daun Cengkeh (Syzygium aromaticum L.) dalam Media dengan Berbagai Konsentrasi Auksin," Jurnal Ilmu Pertanian Indonesia, vol. 25, no. 1, pp. 67-72, 2020.
8. Sudarmadji. (2003). Penggunaan Benzil Amino Purin pada Kalus Kapas secara In Vitro. Buletin Teknik Pertanian. 8(1).
9. Yustina. (2003). Kultur Jaringan: Cara Memperbanyak Tanaman Secara Efisien. Agro Medika Pustaka. Jakarta.
10. Trisnawarti, N dan N.I. Sumardi. (2000). Kultur Ovule Jeruk Nipis (Citrus aurantifolia) Terhadap Keberhasilan Embriogenesis Somatik. Balai Penelitian Buah. Solok.
11. Malini, N., Ananadakumar, C. R., & Ramakrishnan, S. H. (2015). Regeneration of Indian maize genotypes (Zea mays L.) from immature embryo culture through callus induction. Journal of Applied and Natural Science, 7(1), 131-137
12. Bouamama, B., Ben Salem, A., Ben Youssef, F., Chaieb, S., Jaafoura, M. H., Mliki, A., & Ghorbel, A. (2011). Somatic embryogenesis and organogenesis from mature caryopses of North African barley accession “Kerkena”(Hordeum vulgare L.). In Vitro Cellular & Developmental Biology-Plant, 47(2), 321-327.
13. Karjadi, A. K., & Buchory, A. (2007). Pengaruh NAA dan BAP terhadap Pertumbuhan Jaringan Meristem Bawang Putih pada Media B5. Jurnal Hortikultura. 17(3) : 217-223.
14. Arianto, D., Basri, Z., & Bustami, M. U. (2013). Induksi Kalus Dua Klon Kakao (Theobroma cacao L.) Unggul Sulawesi pada Berbagai Konsentrasi 2,4 Dichlorophenoxy Acetic Acid Secara In Vitro. Agrotekbis 1 (3) : 211-220.
15. Catala, C., Rose, J. K., & Bennett, A. B. (2000). Auxin-Regulated Genes Encoding Cell Wall-Modifying Proteins Are Expressed

During Early Tomato Fruit Growth. Plant physiology. 122(2): 527-534.
16. Dwijasaputro. (2004). Fisiologis Tumbuhan. Yogyakarta: Gadjah Mada Press.
17. Leng, P., Su, S., Wei, F., Yu, F., & Duan, Y. (2009). Correlation Between Browning, Total Phenolic Content, Polyphenol Oxidase and Several Antioxidation Enzymes During Pistachio Tissue Culture. Acta horticulturae. 829: 127- 132
18. Indah, P.N dan D. Ermavitalini. (2013). Induksi Kalus Daun nyamplung (Calophyllum inophylum Linn.) pada Beberapa Kombinasi Konsentrasi 6 Benzylaminopurine (BAP) dan 2,4-Dichlorophenoxyacetic acid (2,4-D). Jurnal Sains dan Seni Pomits, 2(1).
19. Evans, D. E., Coleman, J. O. D., Kearns, A. (2003). Plant Cell Culture. New York: Bios Scientific.
20. Marthani, Q. K., Anggraito, Y. U., & Rahayu, E. S. (2016). Kalogenesis Eksplan Setengah Biji Koro Benguk (Mucuna pruriens L.) secara In Vitro menggunakan BAP dan NAA. Life Science. 5(1): 72-78.
21. Leupin, R. E., Leupin, M., Ehret, C., Erismann, K. H., & Witholt, B. (2000). Compact Callus Induction and Plant Regeneration of A Non-Flowering Vetiver from Java. Plant Cell, Tissue and Organ Culture. 62(2): 115-123.
22. Setiawati, T., Ayalla, A., & Witri, A. (2019). Induksi Kalus Krisan (Chrysanthemum morifolium Ramat.) dengan Penambahan Berbagai Kombinasi Zat Pengatur Tumbuh (ZPT). EduMatSains: Jurnal Pendidikan, Matematika dan Sains. 3(2): 119-132
23. Thao, N. T. P., Ozaki, Y., & Okubo, H. (2003). Callus Induction and Plantlet Regeneration in Ornamental Alocasia Micholitziana. Plant Cell, Tissue and Organ Culture. 73(3): 285-289.
24. Yelnititis. (2012). Pembentukan Kalus Remah dari Eksplan Daun Ramin (Gonystylus bancanus (Miq) Kurz). Jurnal Pemuliaan Tanaman Hutan. 6: 181-194.
25. Ramulifho, E., Goche, T., Van As, J., Tsilo, T. J., Chivasa, S., & Ngara, R.(2019). Establishment and Characterization of Callus and Cell Suspension Cultures of Selected Sorghum bicolor (L.) Moench Varieties: A Resource for Gene Discovery in Plant Stress Biology. Agronomy. 9(5): 218.
26. Mahadi, I., Syafi'i, W., & Sari, Y. (2016). Induksi Kalus Jeruk Kasturi (Citrus microcarpa) menggunakan Hormon 2, 4-D dan BAP dengan Metode In Vitro. Jurnal Ilmu Pertanian Indonesia. 21(2): 84-89.
27. Ariati, S.N. (2012). Induksi Kalus Tanaman Kakao (Theobroma cacao L.) pada Media MS dengan Penambahan 2,4-D, BAP dan Air Kelapa. Jurnal Natural Science. 1(1): 78-84.
28. Litz, R. E., Moon, P. A., & Chavez, V. M. (2000). Somatic Embryogenesis from Leaf Callus Derived from Mature Trees of The Cycad Ceratozamia Hildae (Gymnospermae). Plant cell, tissue and organ culture. 40(1): 25-31.
29. Campbel, N.A. 2003. Biologi Edisi Kelima Jilid II. Jakarta: Erlangga.
30. Pauline, D. K. (2016). Perkembangan Kalus Embriogenik Sagu (Metroxylon sagu Rottb.) pada Tiga Sistem Kultur In Vitro. E-Journal Menara Perkebunan. 76(1).
31. Thomy, Z. (2012). Effect of plant growth regulator 2, 4-D and BAP on callus growth of plants producing gaharu (Aquilaria malaccensis Lamk.). In Proceeding Seminar Hasil Nasional Biologi. Medan (Vol. 11)