LenteraBio
Berkala Ilmiah Biologi

LenteraBio, 2026; Volume 15, Issue 2: 122-130

p-ISSN: 2252-3979 e-ISSN: 2685-7871
https://journal.unesa.ac.id/index.php/lenterabio/index

In-vitro Callus Growth of *Amorphophallus muelleri* with Corn Organic Compound Supplementation in Murashige and Skoog (MS) Media

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Article History:
Received:
29-September-2025
Revised:
12-May-2026
Available online:
31-May-2026
Published regularly:
31-May-2026

Abstract
Porang (*Amorphophallus muelleri* Blume) has prospects as an export commodity as alternative to rice. However, both generative and vegetative propagation remains resulted in limited productivity, thus necessitating alternative propagation through plant tissue culture. This study examined the effect of corn organic compound on the growth of *A. muelleri* growth in vitro. The research employed a completely randomized design with 0%, 9%, 18%, and 27% concentration of corn filtrate. The texture and colour callus data were analyzed descriptively. Furthermore, the fresh weight of callus was examined using nonparametric Kruskal-Wallis test. The results revealed the callus showed active growth. The callus texture was compact in 9% treatment and friable in 0%, 18%, and 27% treatments. The colour of callus was creamy white in all of the corn filtrate treatments. Callus from the 18% corn filtrate treatment showed the best result, with fresh weight of 5.044 ± 4.627 g but the treatment was not significant statistically compared to other groups. The most optimal callus growth was found in 18% corn filtrate treatment.

Keywords: fresh weight; callus texture; callus colour; plant tissue culture; micropropagation; crops

How to Cite: Fatimah N & Dewi SK, 2026. In-vitro Callus Growth of *Amorphophallus muelleri* with Corn Organic Compound Supplementation in Murashige and Skoog (MS) Media. LenteraBio; 16(2): 122-130.

DOI: https://doi.org/10.26740/lenterabio.v15n2.p122-130

INTRODUCTION

Porang or *Amorphophallus muelleri* is a tuberous plant with high carbohydrate and glucomannan content (Afifah *et al.*, 2024) closely related to konjac. The content of glucomannan in porang tubers is quite high, making it a valuable raw material for use in industry, food, and health sectors (Tanjung *et al.*, 2022). Glucomannan functions as binding and thickening agent in foods such as meatballs (Rahmi *et al.*, 2021). Glucomannan in *porang*-processed rice from porang can reduce Low-Density Lipoprotein (LDL) levels in patients with diabetes mellitus (Faizal *et al.*, 2022). Glucomannan in porang tubers can be utilized as basic material for the production of edible films (Falah *et al.*, 2021).

The various benefits of *A. muelleri* led to an increase in demand for its production. *Porang* offers promising crop opportunities due to its high economic value, provide significant benefits to *porang* farmers (Utami, 2021). *Porang* has been exported to various countries, including China, Japan, Thailand, Vietnam, Pakistan, Malaysia, Cambodia, Bangladesh, Korea, New Zealand, Italy, the United Kingdom, Sri Lanka, and Australia (Dermoredjo *et al*, 2021; Aldillah *et al*, 2023). According to the Center for Agricultural Data and Information Systems (2022), *porang* exports increased from 841 tons in 2021 to 1,119 tons, with export value rising from 1.794 million USD to 3.446 million USD. However, only 20% of export demand is met, thus porang remained has significant potential to be developed as an export commodity (Utami, 2021). However, one of the challenges for porang exports is the long harvesting period for its tubers (Aldillah *et al.*, 2023).

In general, porang propagation is carried out using two methods; generative and vegetative. Vegetative propagation of porang can occur through leaf cuttings, stem tubers, and leaf tubers (bulbils), while generative propagation uses seeds. Propagation of porang using leaf tubers (bulbils) does not grow immediately because they undergo a long dormancy period, lasting approximately four to five months (Riptanti *et al.*, 2022). Seed propagation also faces similar issue. Flowering only happens after the tuber is four years old, and the maturation of seeds takes around 12 months (Santosa *et al.*, 2016).

Due to long period of growth, alternative cultivation techniques such as plant tissue culture can be applied to accelerate porang production.

Plants produced from tissue culture results in identical characteristics as the parent plant, a large number of seedlings, better health and quality plants, as well as reducing land requirements for cultivation (Harapan *et al.*, 2019). Tissue culture of porang has been studied before. Addition of 2 ppm of 2,4-D and leaf stalk explants produced the largest callus weight at 92.97 g at 12.25 days induction time. Growth regulator 2 mg/L 2,4-D showed the biggest effect on callus induction percentage at 83.33%, while leaf stalk explants succeeded to induce callus at 91.67% (Haring *et al.*, 2023). The best combination for porang planlet growth was 0.5 ppm IBA and 1.5 ppm TDZ (Haq *et al.,* 2022). The supplementation of BAP and Kinetin increased the number of shoots but reduced the height of porang planlets. BAP at 3 mg/L yielded the best treatment with 8.13 shoots for three months after subculture (Ibrahim *et al.*, 2024). Girsang *et al.* (2023) reported 1.5 mg/L of IAA and 10% coconut water in Murashige and Skoog (MS) medium resulted in the shortest callus induction time at 15 ± 2.83 days after inoculation.

Murashige and Skoog media contains complex nutrients from macro and micronutrients, most of which are essential for many plant species (Silalahi, 2015).To support plant growth, media in general must contain inorganic substances, inorganic salts, organic substances, compacting agents, pH, growth regulators, and other additives (Suaib and Sadimantara, 2014; Sunghun, 2021). The additives in the growing media can be substituted with organic compound. The supplementation using organic compounds is friendly to the environment, safe to use, easy to get, and cheaper (Kaffi, 2018). In addition, organic compounds has also contain carbohydrates, vitamins, growth regulators, amino acids, and minerals that can help plants to grow (Ambarwati *et al.*, 2021). Corn is one of the organic compounds can be used as growth regulator in the tissue culture media (Damiska *et al.*, 2015).

Corn can be applied as organic compound in growing media because corn contains carbohydrates, amino acids, vitamins, minerals, auxins, and cytokinins (Damiska *et al.,* 2015), as well as 62.20 ppm of IAA; 128.25 ppm of kinetin; 45.76 ppm of zeatin; and 269.75 ppm of gibberellin (Tini *et al.*, 2022). Corn extract also found to contain a cytokinin, zeatin, which functions in cell division, chloroplast maturation, inhibits cell aging, and induces shoot growth. Furthermore, it also has nitrogen, potassium, sulfur, and iron, which are crucial minerals for plant development and growth (Febryanti *et al.*, 2017). The single application of corn extract to the growing medium has previously studied to significantly affected germination time of dragonfruit (Widasari et al., 2021). The combination of NAA and corn extract had an effect on the number of leaves, roots, and shoots in *Dendrobium* sp. (Herawati *et al.*, 2021).

The levels of endogenous growth regulators significantly influence physiological processes in explants, controlling the plant growth cycle (Xue *et al.*, 2022). A balanced ratio of cytokinin and auxin resulted in the formation of callus (Bano *et al.*, 2022). Nevertheless, every plant contains different growth regulators at various level, expressed by the genotype and affected by physiological condition of the explant (Nguyen *et al.*, 2020). Thus, it is necessary to identify the optimal composition of exogenous growth regulators (auxins and cytokinins) to produce abundant callus (Normasari *et al.,* 2023). Based on this elaboration, this study analyzes the effect of corn filtrate supplement on the callus growth of *porang* (*A. muelleri* Blume) on MS media in vitro.

MATERIALS AND METHODS

This experimental study was held at PT. Pawitra Jaya Sakti Biotek from October 2024 to June 2025. The study employed completely randomized design. The environmental conditions, media, and porang callus were adjusted to ensure homogeneity. Four treatments were used: 0% (control), 9%, 18%, and 27% corn filtrate, with 2 ppm BAP and 1 ppm IBA added to each treatment. Callus used were subcultured callus at age one month obtained from Plant Biotechnology Laboratory, Biotechnology Faculty, Universitas Surabaya.

Preparation of corn filtrate was performed by blending 600 g of corn, in 600 mL of distilled water. The blended corn was filtered using filter paper. The preparation of 9%, 18%, and 27% corn

filtrate was made by diluting corn filtrate in distilled water (v/v). Negative control used was distilled water. Murahige and Skoog (MS) media was prepared diluted 41.71 grams of instant MS media in distilled water. The media was added 2 mL of 2 ppm BAP and 1 mL of 1 ppm IBA (Oktavia *et al*., 2024). The corn filtrate was put into the MS Media according to the established concentrations (0%, 9%, 18%, and 27%). The MS media was homogenized using a magnetic stirrer. The pH of each media used was adjusted to 5.7. The MS media was heated on a hot plate and stirred using a magnetic stirrer until boiling. Then, 50 mL of MS media was poured into culture jars, capped with plastic and rubber. The MS media was sterilized using an autoclave at 121^{o}C for 20 minutes.

Callus were inoculated after sterilization in Biological Safety Cabinet (BSC). The porang callus was cut using scalpel and weighed at 0.5 g. A pair of callus were placed into a culture bottles using tweezers. The bottle rim was sterilized over a flame, then the culture bottles were rewrapped with plastic wrap and labeled.

Culture bottle were placed ± 25 cm below 20-watt fluorescent lamp at tempreature 25^{o}C. These conditions were maintained under control. The growth of porang callus was observed for 12 weeks or 3 months. The parameters of callus growth were texture, color, and fresh weight of callus. The texture was visually observed at the end of the period, which was week 12. Callus color was visually observed every two weeks during the observation period. The callus fresh weight was measured by weighing the fresh weight using a digital scale at week 12.

Data on texture and color of porang callus were analyzed using descriptive methods (Harahap *et al*., 2024). Data on fresh weight of porang callus were analyzed for normality test by the Kolmogorov-Smirnov test and for homogeneity test with the Levene Test. The nonparametric Kruskal-Wallis test using SPSS version 23.0 was used to analyze the effect of corn filtrate on the fresh weight of porang callus.

RESULTS

The results obtained indicate active callus growth, marked by callus proliferation. Observations of callus texture and color can be presented in the following Table 1. Results showed that callus at concentrations of 0%, 18%, and 27% filtrate had a friable texture. Meanwhile, 9% filtrate produced callus with a compact texture. The cell of friable texture has been easy to separate and brittle. The compact callus texture is characterized by cells arranged tightly, dense, and difficult to separate. The friable callus can form embryogenic callus (Mardiana *et al.*, 2023), while the compact callus represents the callus is non-embryogenic (Rivai *et al.*, 2014). On the other hand, all treatments mostly produced yellowish-white callus, indicated fairly good callus growth. Figure 1 shows the visual observation of callus from each treatment.

Table 1. The effect of adding corn filtrate on the porang (*A. muelleri* Blume) callus texture and color

Corn Filtrate (%)	Callus Texture	Callus Color
0	Friable	White-yellowish
9	Compact	White-yellowish
18	Friable	White-yellowish
27	Friable	White-yellowish

The addition of 18% corn filtrate to MS media led to the highest callus fresh weight of 5.044 ± 4.627 g. The supplementation of 0% corn filtrate to MS media affected callus fresh weight of 1.586 ± 2.195 g. 27% corn filtrate in MS media showed callus fresh weight of 0.776 ± 0.201 g. The 9% corn filtrate in MS media indicated in the lowest callus fresh weight of 0.574 ± 0.05 g. However statistical test showed no significant difference between different treatments. Comparation of callus fresh weight is presented in Table 2.

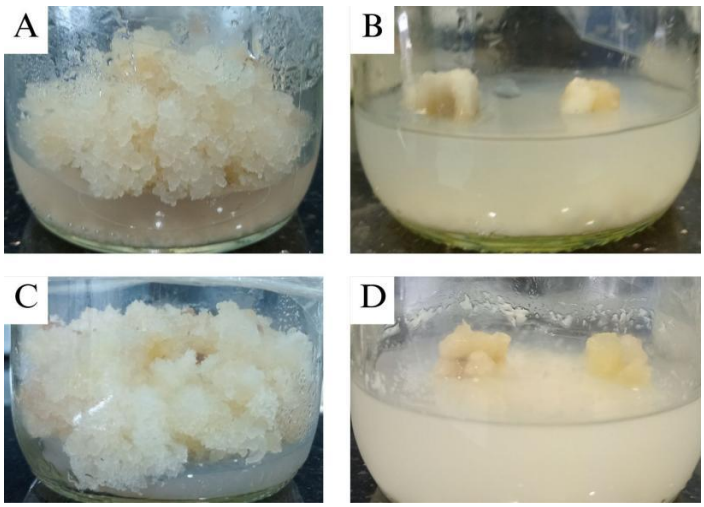

Figure 1. Visual observation on callus. A) 0% filtrate; B) 9% filtrate; C) 18% filtrate; and D) 27% filtrate.

Table 2. The effect of corn filtrate on the fresh weight of porang callus (*A. muelleri* Blume)

Corn Filtrate Concentration (%)	Callus Fresh Weight (g)
0	1.586 ± 2.195
9	0.574 ± 0.050
18	5.044 ± 4.627
27	0.776 ± 0.201

DISCUSSION

Callus observation showed increased growth after supplementation of corn filtrate, marked by callus proliferation. Callus growth was observed from texture, fresh weight, and color. Callus fresh weight was measured using an analytical balance in the last observation period. The results showed that the supplementation of 18% corn filtrate to MS simultaneously with 1 ppm IBA and 2 ppm BAP, led to the highest callus fresh weight of 5.044 ± 4.627 g.

The high standard deviation indicates a wide spread in the data relative to the average callus fresh weight. This was because callus used had previously been grown in the laboratory, so the initial callus conditions could not be fully controlled. The observed differences in callus growth response was influenced by the genetic factors of the explants. Nguyen *et al.* (2020) stated rose's ability to form callus highly relies on the originating genotype, even under the same media conditions and treatments. This difference in genotype is due to the competence of the callus. Some plant cells have the competence to regenerate and show totipotency, so the cells respond to growth regulators in the growing media to produce callus cells (Rosyidah *et al.*, 2014).

Callus fresh weight indicates its growth. Fresh weight shows the accumulation of callus biomass formed during the culture period. Physiologically, the fresh weight is caused by intake of water and carbohydrates (Nasution and Nasution, 2019). Cell division and cell elongation in callus cells are caused by high water content (Haring *et al.*, 2023).

Supplementaion of 18% corn filtrate to MS media resulted in highest callus fresh weight, indicated that it was the most optimal treatment for callus growth. The interplay of exogenous and endogenous growth regulators is one of the factors that influence callus growth (Astutik *et al.*, 2022). Optimum callus growth is affected by a balanced ratio of cytokinin and auxin (Bano *et al.*, 2022).

The addition of corn filtrate containing growth regulators induces cell division and elongation in the callus. According to Tini *et al.* (2022), 5-10 g dry sample of corn extract contained 62.20 ppm IAA, 128.25 ppm kinetin, 45.76 ppm zeatin, and 269.75 ppm gibberellin. Cytokinin induces cell division and callus formation in plants (Wulannanda *et al.*, 2023). The cytokinin will increase protein synthesis in cells, thereby stimulating plant cell enlargement and division, driven by mRNA formation to maintain its stability. This process causes an increased rate of translation of genetic messages into proteins

(Fadilah, 2014). Cytokinins directly influence the cell cycle by regulating protein productions in spindle fibers (Hazrati *et al*., 2022).

Auxin activates proton pumps in the cell wall and secretes H^+ ions out of the cell. Expansin enzyme activates and partially breaks the hydrogen bonds in the cellulose molecular chains in cell walls, making the cell wall flexible and stretchable (Rosyidah *et al*., 2014). At the same time, the release of H^+ ions makes the cell wall acidic. The acidification induce K^+ ions to be drawn out of the cell and decrease the cell's water potential, consequently. The water in the media penetrates the cell walls through osmosis, triggering the cell to enlarge and elongate (Fadilah, 2014).

Callus texture represents quality of callus growth visually. Two types of callus textures were observed from this study, compact and friable callus. Compact callus contains tightly packed cells with dense and hard structure, while friable callus has soft and easily separated cells arranged by cells with numerous intercellular spaces (Ulva *et al*., 2019).

Friable callus were formed in 0%, 18%, and 27% corn filtrate. This callus texture tends to be embryogenic callus generally (Mardiana *et al*., 2023). The friable callus forms due to auxin stimulation of cell elongation. The auxin can increase the plasticity of the cell wall, making it loose. This situation allows water to penetrate the cell wall and leads the cell to elongation in consequense. This callus indicates the cells have not yet undergone lignification of the cell walls (Ulva *et al*., 2019). The cells in friable callus are relatively easy to separate because the cell walls are not yet lignified and hold a lot of water (Setiawan *et al.*, 2020).

The compact and friable callus indicates good callus growth. Compact callus has the potential to develop into organogenesis (Rivai *et al.*, 2014). Compact callus is formed due to lignification resulting from the interaction of cytokinin and auxin (Farlisa *et al*., 2022). This lignification causes cell density and restricts oxygen flow, leading to hypoxic conditions. This condition induces de novo shoot growth through primordial growth mediated by Aintegumenta (ANT) under environmental stress. Furthermore, Monopteros (MP) induces auxin maxima (high auxin levels) (Han *et al*., 2024).

High auxin levels in certain tissues regulate the fate of vascular cells (Nanda and Melnyk, 2018). Meanwhile, cytokinins stimulate cell division and differentiation in callus. Cytokinins activate signal transduction pathways involving protein kinase (AHK4) and response regulators. This pathway leads to the activation of the WUSCHEL (WUS) gene, which is involved in the production and preservation of the shoot apical meristem (Cheng *et al.*, 2013). The SHOOTMERISTEMLESS (STM) gene is activated to maintain and nurture the shoot apical meristem in an undifferentiated form from WUS. The STM is required to encourage the cell division (Hnatuszko-Konka *et al*., 2021). The opposing pathways of ANT-auxin and STM-CK manage the apical meristem and shoot primordial growth correspondingly (Han *et al*., 2024).

A friable callus has the possibilities to form somatic embryogenic callus (Mardiana *et al*., 2023). A protein encoded by YUCCA4 (YUC4) gene catalyzed the inhibition of IAA biosynthesis. LEAFY COTYLEDON 2 (LEC2) interacts with FUS3 and binds to the YUC4 promoter, allowing LEC2 to rapidly activate YUCCA2 (YUC2) and YUC4. Embryogenic competence induced by LEC 2 is strongly linked to auxin (Li et al., 2019). High auxin levels enhance somatic embryo development in callus (Eshagi *et al*., 2021). Auxin is crucial in the early stages for triggering essential genes in cells for the somatic embryo development pathway. Auxin also inhibits the IPT gene and cytokinin signaling, so endogenous cytokinin levels tend to be low early in the somatic embryo development process. Cytokinin will begin to activate after cells transform into somatic embryos due to auxin activity. Cytokinins regulate the formation of important structures in the embryo (the shoot and root apical meristem), direct embryonic cell fate toward shoot or root growth, and activate the WUS-RELATED HOMEOBOX 5 (WOX5) and WUS genes for embryonic tissue formation (Avilez-Montalvo *et al.*, 2022).

Callus color indicates physical characteristics of callus cells to establish the quality of callus growth. The callus color seen in all treatments was yellowish-white, which indicated good callus growth as supported by Mahadi *et al*. (2016). Embryogenic callus indicates good quality because cell division still actively occurred in the callus cells, resulting in easy proliferation of the callus (Farlisa *et al.*, 2022).

Color can also identify the age of the callus cells. Rasud and Bustaman (2020) pointed that yellowish white callus indicated maturity of cells. Callus color is also influenced by the interaction between endogenous and exogenous growth regulators. Wahyuni *et al.* (2020) revealed that boosting auxin in media affected callus color; the higher the auxin concentration, the more yellow the resulting callus. This increase reduced chlorophyll content due to interrupted carbohydrate metabolism.

In all treatments, *porang* callus was unable to form shoots until the end of the observation. The shoot induction time can be quite long depending on the physiological activity of the callus in response to the treatment. Therefore, a longer observation period is required to confirm the process of shoot formation and growth of porang callus. The application of growth regulators and different light colors affected time needed for shoots to emerge from callus. The mixture of 0.2 mg/L NAA and 5 mg/L BAP in white and blue light resulted in 54.67 days induction time porang shoot induction time, 2 ppm BAP and 0.2 ppm NAA in blue light, induced shoot at 100 days after inoculation (Wijaya *et al*., 2023). The ability of callus to regenerate into plant organs varies from plant to plant. This may be due to the callus' competence (Ekawati *et al*., 2022). Some cells possess the competence to regenerate and exhibit totipotency, enabling them to respond to growth regulators in the media (Rosyidah *et al*., 2014).

Callus structure can also affected its ability to regenerate into shoots and roots. Globular or nodular callus with translucent white color commonly has greater shoot regeneration capabilities than compact callus with blackish-brown color (Purnamaningsih, 2006). Hardjo *et al.* (2023) stated that porang callus characterized by compact callus with a nodular structure and a greenish-white color can induce and grow porang shoots. Callus regeneration can be affected by nutrients in the growing medium. Undifferentiated callus resulted from lack of organic nitrogen in the treatment medium (Khumaida and Handayani, 2010). Furthermore, the balance of cytokinin and auxin in the medium and endogenous hormones in the callus is a factor influencing organ formation in the callus (Mardiana *et al*., 2023).

CONCLUSION

Based on the research findings above, the supplementation of corn filtrate to MS media affected the growth of porang callus, but statistical test showed no significant difference. The textures of callus were compact in 9% corn filtrate and friable in 0%, 18%, and 27% corn filtrate. The colors of the callus in all treatments were yellowish-white. The highest average fresh weight of porang callus at 5.044 ± 4.627 g, was found in the 18% corn filtrate. The supplementation of 18% corn filtrate to MS medium was the favorable treatment for callus growth, with a fresh weight of 5.044 ± 4.627 g.

ACKNOWLEDGEMENTS

We would like to thank article reviewers for their help in improving this study. We also thank Mrs. Ria and Efilia Candra Indra Sari Wahyu Nugraheni for the technical support in this research.

CONFLICT OF INTEREST

There is no conflict of interest.

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