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Research Article
Concentration of 2,4-D and BAP Combination on Callus Induction of Porang Plant (*Amorphophallus muelleri* Blume)

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Abstract

Background and Objective: One of the problems with the scarcity of porang seed availability is the high price of natural seeds commonly used by farmers. Tissue culture is one of the appropriate efforts to ensure the availability of porang plant seeds. The study aims to determine the concentration of growth regulator (2,4-D and BAP) and the best combination of interactions between the two on MS base media on the growth of porang callus. **Materials and Methods:** The study was carried out at the Plant Reproduction Bio-Science and Biotechnology Laboratory, Faculty of Agriculture, Hasanuddin University, Makassar, South Sulawesi, from January, 2022 to April, 2022. The study was structured in a 2-factor factorial design with the first factor being 2 ,4-D concentration consisting of a0 (0 mg/L), a1 (0.5 mg/L), a2 (1 mg/L) and a3 (1.5 mg/L) and the second factor is the BAP concentration consisting of b0 (0 mg/L), b1 (0.5 mg/L), b2 (1 mg/L) and b3 (1.5 mg/L). **Results:** The 2,4-D concentration had a natural effect and gave the best results on the characteristics of callus emergence time (22.65), callus percentage (100%) and callus weight, while the BAP concentration and the interaction between the two (had a natural effect and gave the best results on callus weight characteristics (1.14 g), compared to no treatment (control). The color of the callus produced at the end of the observation was green, grayish yellow-brown, brownish yellow, brownish white and greenish-white in the various treatments, while the overall texture of the callus produced was compact. **Conclusion:** The concentration of 0.5 mg/L 2,4-D and 1 mg/L BAP are the best combinations.

Key words: Growth regulator, porang seed, tissue culture, callus induction, *Amorphophallus muelleri* Blume

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Porang plant (*Amorphophallus muelleri* Blume) is a plant used as food that produces carbohydrates, proteins, fats, minerals, vitamins and dietary fiber and also exported as industrial raw materials[1,2]. In recent years, the porang plant has become popular because it contains the carbohydrate glucomannan, which is considered to be a source of healthy food that meets food needs with relatively few ingredients. It has economic value and a high level of demand. However, the insufficient availability of porang seeds means that porang plants are still not widely cultivated, so farmers generally only use and take plants that grow wild in the forest[3]. Therefore, efforts are needed to overcome the scarcity of seeds and ensure the availability of porang plant seeds.

Generally, farmers use natural seeds from tubers, leaves, or frogs, the price of which reaches 150 to 400 thousand rupiah per kilogram[3-5]. Meanwhile, the need for seeds for 1 ha is around 200 kg, so farmers have to spend around 30 to 80 million rupiah. Therefore, tissue culture is considered as an appropriate effort to overcome the scarcity of porang plant seeds. The advantage of propagation through tissue culture is that it can produce large numbers of plants in a relatively short time and is easy to distribute, especially in plantlet form[6,7].

Tissue culture requires a planting medium as a place to grow explants under aseptic conditions, the choice of which depends on the species of plant, tissue, or organ that will be used in tissue culture[8,9]. One of the most widely used media is MS primary media because it has a more complete composition compared to other primary media. The MS media contains high concentrations of mineral salts and N compounds in the form of ammonium and nitrate, which can support the growth of plant cells *in vitro*[10,11].

The use of growth regulators (ZPT) also influences the success of a tissue culture technique[12]. The right combination of base media and PGR can optimize explant growth[13]. The PGRs can stimulate or inhibit plant physiological processes, so their use in tissue culture has an important role[14,15]. The multiplication of propagules as desired can be stimulated using PGRs in the form of auxin, cytokinin, gibberellin, or a combination of other PGRs that will be used[16,17].

The balance and interaction between cytokinin and auxin can determine growth and morphology *in vitro*[18]. Embryogenic callus induction in this study used a combination of ZPT consisting of 2,4-Dichlorophenoxy Acetic Acid (2,4-D) as auxin and benzyl amino purine (BAP) as cytokinin. The 2,4-D is known to be effective in inducing callus because it can induce cell division, while BAP is known to have a role in the cell division cycle[19,20]. The use of a combination of both has also been reported by Aziz[21] and Akram and Aftab[22] using various concentrations of the hormones 2,4-D and BAP to induce callus on porang tubers and teak stems on MS media *in vitro*. Therefore, the combination of the two as ZPT in this study is expected to optimize the formation and growth of porang plant callus. This study aimed to determine the effectiveness of using multivariate analysis and determine the best combination of interactions between 2,4-D and BAP concentrations in MS base media on porang callus growth.

MATERIALS AND METHODS

Study area: The study was carried out at the Plant Reproduction Bio-Science and Biotechnology Laboratory, Faculty of Agriculture, Hasanuddin University, Makassar, South Sulawesi. The study takes place from June, 2023 to September, 2023.

Experimental design: The research was arranged in a 2-factor factorial design with the first factor being the concentration of 2,4-D consisting of a0 (0 mg/L), a1 (0.5 mg/L), a2 (1 mg/L) and a3 (1.5 mg/L) and the second factor is the BAP concentration consisting of b0 (0 mg/L), b1 (0.5 mg/L), b2 (1 mg/L) and b3 (1.5 mg/L). Each treatment was repeated three times, so there were 48 experimental units and each experimental unit contained three explants, so a total of 144 explants were used. Data analysis was carried out using One-way Analysis of Variance (ANOVA) according to a 2-factor factorial design with the least significant difference (LSD) test at a confidence level of 0.05.

Explant source: The explant material used is the tuber of the porang plant (*Amorphophallus muelleri* Blume) taken from Satoimo Sulawesi at Ruko Pondok Modern, Ir. Sutami Street, Biringkanaya, Makassar, South Sulawesi.

Study implementation

Tool sterilization: The tools and media were sterilized first using an autoclave at 121°C and a pressure of 15 lbs. Sterilization lasts 30 min for tools and 15 min for media. Planting tools such as tweezers and scalpels are sterilized again in laminar air flow using the burning method using 96% alcohol.

Material sterilization: Before the leaf tissue is isolated, the leaves are washed using soap and cleaned with running water.

After that, soak using fungicide and bactericide for 10 min each, then rinse using distilled water. Isolation was carried out in laminar air flow cabinet (LAFC) by Esco's Singapore, the leaves were soaked in an ascorbic acid solution and then sterilized with 70% alcohol, bayclin and betadine. Before inoculation, the explants are soaked in a sterile ascorbic acid solution again to reduce browning and sap, then placed in sterile filter paper to absorb the sap that comes out of the explants. The leaf tissue was then cut to ±0.5 cm and inoculated. All cultures were incubated in an air-conditioned room with a temperature of ±26°C.

Media creation: The media used was solid media with the composition Murashige and Skoog (MS) supplemented with 30 g/L sucrose and 8 g/L agar. The acidity of the media is adjusted to reach pH 5.8 by adding NaOH or HCl solution. Treatment media was made by adding treatment concentrations of 2,4-D and BAP to MS media. The medium was then dissolved in a beaker using a magnetic stirel and placed on a hot plate by IKA Indonesia until homogeneous. After that, pour it into the prepared culture bottle. Sterilize the media using an autoclave at 121°C for 15 min, then store it in the incubation room at 25°C before use.

Explant planting: Planting is carried out in laminar air flow, which has been sterilized with UV light. Porang plant explants that have been sterilized are then planted into the treatment medium. Each bottle that is planted consists of 1 explant. The culture bottle that has been planted is placed in an incubation room at a temperature of ±25°C in dark conditions.

Data collecting and analysis: The parameters observed were the time the callus appeared, the percentage of callus formed, the color of the callus, the texture of the callus and the weight of the callus.

RESULTS

Callus appearance time: The results of variance analysis in Table 1 show that the 2,4-D concentration treatment (a) has a significant effect, while the BAP concentration treatment (b) and the interaction between the two do not have a significant effect on the characteristics of the time callus appears. The 2,4-D concentration with the best callus appearance time was the concentration of 1.5 mg/L (a3) with an average value of 22.65, not significantly different from the control (a0) and significantly different from the concentration of 0.5 mg/L (a1) and 1 mg/L (a2).

Callus percentage: The results of variance analysis in Table 2 show that the 2,4-D concentration treatment (a) had a significant effect, while the BAP concentration treatment (b) and the interaction between the two had no significant effect on the character of the callus percentage. The 2,4-D concentration with the best callus percentage was the concentration of 0.5 mg/L (a1) with an average value of 100.00%, not significantly different from the concentrations of 0.5 mg/L (a1) and 1 mg/L (a2) and is significantly different from the control (a0).

Callus color: The callus color is an indicator in describing the visual appearance of callus cells to determine the level of

Table 1: Callus appearance time at various concentrations of 2,4-D and BAP

	2,4-D (a)				
BAP (b) (mg/L)	0 (mg/L) (a0)	0.5 (mg/L) (a1)	1 (mg/L) (a2)	1.5 (mg/L) (a3)	CV (a) $HSD_{0.05}$
0 (b0)	26.00	34.56	34.67	18.50	9.00
0.5 (b1)	35.44	34.00	44.00	26.00	
1 (b2)	26.33	28.00	29.56	20.78	
1.5 (b3)	27.89	39.00	37.00	25.33	
Average	28.92^{pq}	33.89^{q}	36.31^{q}	22.65^{p}	

^{p,q}Mean they are not significantly different in the $HSD_{0.05}$ test

Table 2: Callus percentage time at various concentrations of 2,4-D and BAP

	2,4-D (a)				
BAP (b) (mg/L)	0 (mg/L) (a0)	0.5 (mg/L) (a1)	1 (mg/L) (a2)	1.5 (mg/L) (a3)	CV (a) $HSD_{0.05}$
0 (b0)	66.67	100.00	100.00	83.33	15.28
0.5 (b1)	88.89	100.00	88.89	100.00	
1 (b2)	77.78	100.00	88.89	100.00	
1.5 (b3)	77.78	100.00	100.00	100.00	
Average	77.78^{q}	100.00^{p}	94.44^{pq}	95.83^{pq}	

^{p,q}Mean they are not significantly different in the $HSD_{0.05}$ test

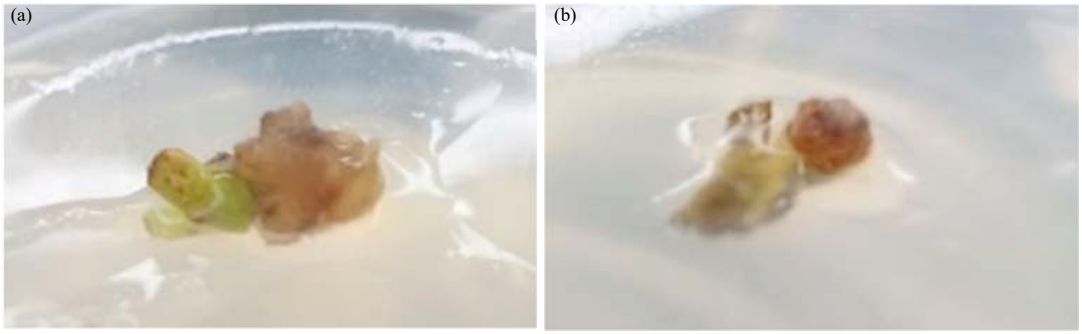

Fig. 1(a-b): Porang callus texture at various concentration of 2,4-D and BAP has, (a) Compact (active cell) and (b) Compact (active cell)

Table 3: Callus color at various concentrations of 2,4-D and BAP

Treatment	Color
a0b0	Gray brown group N199 (Dark greyish yellowish brown B)
a0b1	Grayed green group 197 (Light olive gray A)
a0b2	Gray brown group N199 (Strong yellowish brown D)
a0b3	Brown group 200 (Moderate brown C)
a1b0	Brown group N200 (Dark greyish yellowish brown A)
a1b1	Gray brown group 199 (Light olive brown B)
a1b2	Greyed orange group 164 (Pale yellow D)
a1b3	Greyed orange group 164 (Moderate orange yellow B)
a2b0	Greyed orange group 174 (Greyish reddish orange B)
a2b1	Greyed orange group 164 (Moderate orange yellow C)
a2b2	Greyed orange group 165 (Brownish orange B)
a2b3	Grayed orange group 166 (Greyish brown A)
a3b0	Greyed orange group N170 (Moderate yellowish pink C)
a3b1	Brown group 200 (Greyish reddish brown B)
a3b2	Greyed orange group 165 (Brownish orange B)
a3b3	Greyed green group 193 (Pale yellow green A)

RHS color chart

Table 4: Callus texture at various concentrations of 2,4-D and BAP

Treatment	Texture	Treatment	Texture
a0b0	Compact	a2b0	Compact
a0b1	Compact	a2b1	Compact
a0b2	Compact	a2b2	Compact
a0b3	Compact	a2b3	Compact
a1b0	Compact	a3b0	Compact
a1b1	Compact	a3b1	Compact
a1b2	Compact	a3b2	Compact
a1b3	Compact	a3b3	Compact

active cell division. The color of the callus produced at the end of the observation (Table 3) was green, grayish yellow brown, brownish yellow, brownish white and greenish white in various treatments.

Callus texture: The callus texture is a marker used to determine the quality of a callus to determine whether cells are still actively dividing or have experienced stagnation in cell division. The callus produced in all treatments (Table 4) has a compact texture. The compact texture of the callus indicates that the callus cells are still active (Fig. 1).

Callus weight: The results of variance analysis in Table 5 show that the treatment of 2,4-D concentration (a), BAP concentration (b) and the interaction between the two had a very significant effect on the character of callus weight. The interaction between the concentration of 2,4-D and BAP with the best callus weight was the concentration of

Table 5: Callus weight at various concentrations of 2,4-D and BAP

	2,4-D (a)				
BAP (b) (mg/L)	0 (mg/L) (a0)	0.5 (mg/L) (a1)	1 (mg/L) (a2)	1.5 (mg/L) (a3)	CV (a) $HSD_{0.05}$
0 (b0)	0.18^{e}	0.54^{b}	0.39^{bcde}	0.51^{bcd}	0.28
0.5 (b1)	0.34^{bcde}	0.53^{bc}	0.47^{bcd}	0.34^{bcde}	
1 (b2)	0.94^{a}	1.14^{a}	0.59^{b}	0.42^{bcde}	
1.5 (b3)	0.37^{bcde}	0.25^{cde}	0.24^{de}	0.23^{de}	

^{abcde}Mean they are not significantly different in the $HSD_{0.05}$ test

2,4-D 0.5 and BAP 1 mg/L (a1b2) with an average value of 1.14 g and significantly different from the 3 concentrations other BAPs (a1b0, a1b1 and a1b3).

DISCUSSION

The use of 2,4-D in this study had a significant effect on almost all of the observed characteristics. This is because 2,4-D is a type of auxin that plays a role in cell division and callus formation. In addition, the presence of cells that respond to auxin, which causes dedifferentiation, can stimulate cell division. This was in accordance with the opinion of Shinta *et al.*[23], Baklouti *et al.*[24], Reis *et al.*[25] and Faramayida *et al.*[26] that the addition of 2,4-D in the media can stimulate cell division in explants because 2,4-D is a hormone that plays a vital role in physiological processes such as growth, cell division and differentiation as well as protein synthesis. The difference in the concentration of 2,4-D given also has a significant influence on the character of the time for callus to appear, where the character of the fastest time for callus to appear is at a concentration of 1.5 mg/L (a3), while the character for the highest percentage of callus is at a concentration of 0.5 mg/L (a1), compared to without the addition of 2,4-D (control). This was in accordance with the opinion of Mayerni *et al.*[27] and Kamarul Zaman *et al.*[28] that the higher the concentration of 2,4-D given, up to 1.5 mg/L in callus culture, the more the callus growth will increase.

This is different from the addition of BAP, which does not have a natural effect on the characteristics of the time the callus appears and the percentage of callus. The lack of impact of BAP administration is thought to be caused by the endogenous hormone content in the callus cells, which is sufficient to trigger callus formation. Wibisono *et al.*[29] and Marimuthu and Muthuchelian[30] stated that if the concentration of BAP used is not appropriate, the callus will appear slowly, which ultimately acts as an obstacle to its growth. However, the difference in BAP concentration given had a significant effect on the characteristics of callus weight. The BAP, as a cytokinin, functions in cell division and protein synthesis, causing cells to proliferate, resulting in cell volume increasing and an increase in the weight of the callus produced. Guo and Jeong[13], Bano *et al.*[20] and Blinstrubienė *et al.*[31] added that callus induction is influenced by auxin, while cytokinin plays a more significant role in callus proliferation.

The interaction between the concentration of 2,4-D and BAP also had a significant influence on the characteristics of callus weight. This is because there is an appropriate concentration balance between exogenous and endogenous PGR contained in the explant. In accordance with the opinion of Sosnowski *et al.*[18] and Pacheco *et al.*[32], the right concentration balance between auxin and cytokinin is known to stimulate callus formation through interactions in cell enlargement and division. The success of the explant in responding to the media composition to initiate callus also depends on the condition of the explant. This was in accordance with the opinion of Mehbub *et al.*[9], Intesaful Haque *et al.*[33] and Mohammed *et al.*[34], stating that the season when the explants are taken, the overall quality of the plant, the aseptic conditions of the media and explants, the size of the explants and the physiological age of the plant influence the success of tissue culture. The success of inducing callus is more significant if the explant used is meristematic (actively dividing).

The callus formed from an explant in this study showed differences in color, which developed from 2 weeks after inoculation until 60 days after inoculation. After inoculation, almost all of the calluses in the treatments were still fresh, light yellowish, then changed according to the development of the callus in each treatment. The color of the callus at the end of the observation was dominated by green, grayish yellow-brown, brownish yellow and greenish-white in various therapies. This difference is thought to be due to differences in response to the ZPT given[12,35]. According to Indriani *et al.*[36] and Das and Banduopadhyay[37], different callus colors are influenced by cytokinins in the development of plastids. In these, namely chloroplasts, the color of the callus indicates the presence of chlorophyll in the tissue; the greener the color of

the callus, the more chlorophyll it contains. Apart from that, each treatment showed that the callus experienced browning, which was caused by the oxidation of phenolic compounds produced by the plant tissue. The synthesis of phenolic compounds is stimulated by stress or stress on plant cells in the form of injury to the tissue or stress from the media, which can be an obstacle or even toxic to explant growth[38]. So, efforts are needed to prevent browning, such as by administering ascorbic acid 50-200 mg/L before planting the explants in the media or by periodically subculturing the explants with different time treatments[39,40].

The entire treatment shows a compact callus texture. The compact callus texture indicates that the callus is attached to the explant. The texture of the callus produced is influenced by the balance between the hormones auxin and cytokinin in the cells[41]. The callus texture, which tends to be compact, is assumed to be due to the presence of auxin in the media, which is caused by the hardening of the cell walls due to the auxin transport process in carrying nutrients and water through the phloem vessels, thus affecting the osmotic potential in the cells[42,43]. Callus induced by the addition of auxin has a more compact texture than callus produced without auxin induction and causes the absorption of water from the media into the cells to increase so that the cells become stiffer[36,44].

CONCLUSION

The administration of 2,4-D, BAP and the interaction between the two had a significant effect on the characteristics of callus weight (1.14 g). The administration of the single factor, 2,4-D had a real influence on the characteristics of callus emergence time and callus percentage, while BAP had no real effect on these characteristics.

SIGNIFICANCE STATEMENT

In recent years, porang has become a healthy food ingredient with high economic value with a fairly high level of demand. However, the availability of porang plant seeds is inadequate and the price is still relatively high, so a solution is needed to overcome the scarcity of porang plant seed supplies, one of which is through tissue culture. Tissue culture requires appropriate media and use of ZPT (growth regulator). This study aims to determine the best combination of 2,4-D and BAP concentrations on MS base media for the growth of porang callus. So, the best PGR combination will be obtained for the growth of porang callus, in order to realize adequate porang seed availability.

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