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In Vitro Conservation of Titan Arum (Amorphophallus Titanum (BECC.))

Winda Purnama Sari[1)], Putri salsa Afifah[2)], Yusniwati[1)*]

[1)]Lecture of Department of Agrotechnology, Faculty of Agriculture, Universitas Andalas Padang, Indonesia

[2)]Student of Department of Agrotechnology, Faculty of Agriculture, Universitas Andalas Padang, Indonesia

[*)] Corresponding author: yusniwati@agr.unand.ac.id

Abstract: Titan arum (Amorphophallus titanum (Becc.)) was an endangered and endemic flora from the island of Sumatra, facing a significant risk of extinction. Conservation efforts for titan arum had been undertaken, including in vitro conservation through tissue culture. This study aimed to determine the appropriate concentration of paclobutrazol and MS media dose to achieve slow shoot growth in titan arum to support medium-term storage. The research was conducted from August to October 2024 at the Tissue Culture Laboratory, Andalas University. The experiment was arranged in a Factorial Randomized Block Design (RBD) consisting of two factors: the concentration of the growth inhibitor Paclobutrazol (PBZ) (0.0 ppm; 3.0 ppm; 5.0 ppm) and MS media doses (Full MS, ½ MS, ¼ MS). Data analysis was performed using an F-test at a 5% significance level. If the F-test showed significant differences, further testing was carried out using Duncan's Multiple Range Test (DMRT) at a 5% significance level. The results showed that various concentrations of PBZ could suppress shoot growth in Amorphophallus titanum Becc., with the percentage of explants forming shoots reaching 100%. The concentration of 5.0 ppm PBZ and full-dose MS was the most effective in inhibiting shoot growth of titan arum, with an average shoot height of 1.5 cm and the highest number of shoots observed at concentrations of 0 ppm and 3.0 ppm across all MS media doses. This research was crucial for the conservation of titan arum and encourages further studies on in vitro culture techniques.

Keywords: endemic, conservation, MS, paclobutrazol, shoot

I. INTRODUCTION

The corpse flower (*Amorphophallus titanum* (Becc.)) was one of Indonesia's rare and endemic plants, originating from Sumatra Island, and was known as the "giant corpse flower" from the Amorphophallus genus. A. titanum was first discovered by Dr. Odoardo Beccari in 1877 in the Anai Valley, West Sumatra [1]. *A. titanum* was unique because it had a flower shape that was different from other plants and emitted a foul odor, as well as unique biological cycles and rarity status [2]. In addition, this plant could be utilized as an ornamental plant due to its unique shape, as research material for scientists, and its tubers could be used as food [3]. According to the IUCN data from 2018, the number of *A. titanum* in Sumatra was fewer than 1,000 individuals and was classified as endangered due to a decline in its population in the wild [4].

The rarity of *A. titanum* in Indonesia was caused by several factors, including land conversion, illegal logging, land clearing, cutting down young corpse flowers growing on local lands, and a social stigma that regarded the corpse flower as a

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nuisance plant because of its unpleasant [5]. *A. titanum* in the wild required a long time to flower because cross-pollination was needed to produce seeds and there was a difference in the maturation time of pollen and stigma [6]. This cross-pollination was difficult to achieve because the plant had become rare.

Efforts to conserve A. titanum needed to be conducted properly, such as through in situ and ex situ conservation. In situ conservation within its natural habitat was very difficult at that time due to the widespread damage to its habitat from exploitation activities, making ex situ conservation outside its habitat an alternative that could be used [7]. The problem of propagating *A. titanum* could be solved through in vitro propagation via tissue culture. This technique utilized cells, tissues, or plant organs under aseptic conditions to produce new individuals or plants [8]. In addition to producing new plant seedlings with identical traits to the parent, this technique also produced disease-free seedlings, and the time required was shorter [9]. The shoots of *A. titanum* produced through tissue culture needed to undergo repeated subculturing, which could decrease yields and quality, as well as increase somaclonal variation of this plant. The use of growth inhibitors such as Paclobutrazol and MS Media was expected to slow down the growth and development of A. titanum shoots through medium-term storage, allowing the shoots obtained in vitro to be stored for a certain period.

II. MATERIALS AND METHODS

The materials used in this study were A. titanum shoots from the Tissue Culture Laboratory collection, Faculty of Agriculture, Andalas University. The media used were Murashige and Skoog (MS instant) with 8 g/L bacto agar and sucrose according to the treatment. Other materials used included 70% alcohol, 96% alcohol, Bacylin (NaClO 5.25%), liquid detergent, plastic wrap, aluminum foil, label paper, spirit, rubber bands, glass plastic, tissue, HVS paper, sterilized aquades, pH paper, 0.1 N HCl and 0.1 N KOH. The plant growth regulator (PGR) used was BAP (6-Benzyl Amino Purine), and the growth inhibitor used was PBZ (Paclobutrazol).

The tools used in this study were culture bottles (baby bottles), an analytical scale, a measuring cylinder, petri dishes, Erlenmeyer flasks, stirring rods, pH paper, a hot plate, a magnetic stirrer, a Laminar Air Flow Cabinet (LAFC), a refrigerator, an autoclave, a volumetric flask, a scalpel, a measuring glass, a spray flask, an oven, a beaker, a Bunsen burner, a dropper pipette, a culture rack, scissors, glass plastic, a bucket, a hand sprayer, an 18-watt neon lamp, matches, a camera, the ImageJ application, and stationery.

The explants used were A. titanum shoots from the Tissue Culture Laboratory collection, Andalas University. The explants were then subcultured onto treatment media with a size of approximately 0.8 - 1 cm. They were placed on the incubation rack and given light using an 18-watt neon lamp. The explants were incubated for 16 weeks, with observations made once a week. The explants in the incubation room were maintained daily by spraying with 70% alcohol and removing contaminated bottles from the culture rack.

The research was conducted using an experimental method with a Factorial Randomized Block Design (RBD), consisting of two factors: the concentration of the growth inhibitor Paclobutrazol (PBZ) (0.0 ppm; 3.0 ppm; 5.0 ppm) and the concentration of sucrose (Full MS, ½ MS, ¼ MS). Each treatment level consisted of 4 replications, resulting in 36 experimental units. Each experimental unit consisted of 1 culture bottle. Each culture bottle contained 1 explant, resulting in 36 culture bottles. The treatment media used was MS media (Murashige and Skoog). The data obtained from the study were analyzed using the F-test at a significance level of 5%, and significantly different data were further analyzed using Duncan's New Multiple Range Test (DNMRT). The variables observed were shoot height, the number of shoots per explant, the percentage of explants producing shoots, the number of roots, and the percentage of explants with roots.

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III. RESULTS AND DISCUSSION

A. The Percentage of Explants Forming Shoots

The percentage of explants forming shoots refers to the number of explants that produce shoots out of all the explants planted. The percentage of explants forming shoots can be seen in Table 1.

Table 1. The Percentage of Explants Forming Shoots

Concentration Paclobutrazol	MS Dose		
	Full Dose	½ Dose	¼ Dose
	------ % ------		
0 ppm	100	100	100
3 ppm	100	100	100
5 ppm	100	100	100

The data were not analyzed because all the explants (shoots) survived until the end of the observation.

The data in Table 1 showed that all the explants (shoots) survived until the end of the observation in each experimental unit. It can be said that the application of paclobutrazol concentration and several doses of MS media did not have a significant effect on the percentage of explants forming corpse flower shoots. The growth of shoots was influenced by three factors: explant, media, and environment [10]. Concentration was the main factor in propagation activities to achieve an optimal percentage of explants producing shoots [11]. In addition to the source and size of the plantlet, the percentage of plant growth was also influenced by exogenous and endogenous hormones [12].

The factors affecting the percentage of explants forming shoots were influenced by the growth media, plant growth regulators, and the explant source used in the in vitro culture. According to [13] the selection of explants in in vitro culture could be seen from the explant's age, as it affected plant growth regeneration. The regeneration of callus that can form shoots and indicate morphogenesis can be observed from the color change of the callus, from brownish or yellow to yellowish-white and then green [14]. Propagation with paclobutrazol tended to result in shorter shoots because paclobutrazol inhibited gibberellin biosynthesis. As a result, cell elongation was inhibited, and the shoots became shorte.

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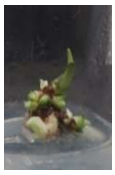
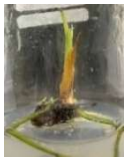
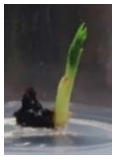
Table 2. Shoot Growth *A. titanum*

Treatment	Shoot growrh *A. titanum*		
	4 MST	8 MST	12 MST
0 ppm PBZ + Full MS			
3.0 ppm PBZ + Full MS			
5.0 ppm PBZ + Full MS			
0 ppm PBZ + 1/2 MS			
3.0 ppm PBZ + 1/2 MS			

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Treatment	Shoot growrh *A. titanum*		
	4 MST	8 MST	12 MST
5.0 ppm PBZ + 1/2 MS			
0 ppm PBZ + 1/4 MS			
3.0 ppm PBZ 1/4 MS			
5.0 ppm PBZ + 1/4 MS			

The characteristics of living corpse flower plants were the presence of green shoots, while dead plants showed explants that were brown in color. A yellowish-brown color appeared due to plant cells that were about to die because of the cut marks and the difficulty of the plant adapting to the new media provided. This statement was in accordance with the study by [15], which stated that the fine brown color indicated the synthesis of phenolic compounds, where the cells experienced stress from the wound in the cut tissue, in addition to the stress from the medium.

B. Shoot Height

The analysis of variance for the shoot height of A. titanum showed that the application of Paclobutrazol concentration and MS Media had a significantly different effect. The observed shoot height can be seen in Table 3.

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Table 3. Shoot Height *A. titanum*

Concentration Paclobutrazol	MS dose		
	Full Dose	½ Dose	¼ Dose
	------- cm -----		
0 ppm	2,38 a A	1,90 a AB	1,88 b AB
3 ppm	1,96 a A	2,88 a A	2,10 a A
5 ppm	1,50 a B	2,48 a AB	2,63 a A
KK = 17,37%			

Note: Numbers in the columns followed by different lowercase and uppercase letters indicate a significant difference based on the DMRT test at a 5% significance level. The data for shoot emergence time were transformed using √x %.

The data in Table 3 showed a significant interaction between the application of paclobutrazol concentration and several doses of MS media. The application of 0 ppm paclobutrazol resulted in the highest shoot height with full MS media dose, while the ½ dose of MS media showed the highest shoot height with 3 ppm paclobutrazol. The ¼ dose of MS media resulted in the highest shoot height at both 3 ppm and 5 ppm paclobutrazol.

The interaction between 0 ppm paclobutrazol concentration and full MS media dose had the same effect as the ½ dose of MS media but was significantly different from the ¼ MS media dose. The interaction between 3 ppm and 5 ppm paclobutrazol concentrations had the same effect across all MS media doses. The interaction between the full MS media dose and 0 ppm paclobutrazol had the same effect as 3 ppm paclobutrazol but was significantly different from 5 ppm paclobutrazol. The interaction between the ½ MS media dose and all paclobutrazol concentrations showed the same effect. Meanwhile, the interaction between the ¼ MS media dose and 0 ppm paclobutrazol was significantly different from 3 ppm and 5 ppm paclobutrazol.

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Shoots planted on ½ MS media showed the highest shoot height with 3 ppm paclobutrazol. This indicated that the application of paclobutrazol could reduce the required MS media dose for the growth of corpse flower explant shoots. Paclobutrazol is a plant growth regulator that inhibits stem elongation by blocking the action of gibberellins, which are responsible for stem elongation. According to Dicks (1979) in [14] paclobutrazol is a synthetic organic compound with physiological effects such as inhibiting cell elongation at the sub-apical meristem, shortening plant internodes, thickening stems, and extending shelf life. In addition, paclobutrazol also reduces vegetative growth, which can stimulate flower and fruit development [16].

C. Number of Shoots per Explant

The analysis of variance for the number of shoots per explant showed that the application of Paclobutrazol concentration and MS Media had a significantly different effect. The observed number of shoots can be seen in Table 4.

Table 4. Number of Shoots per Explant *A. titanium*

Concentration PBZ	MS Dose			Main Factor: Paclobutrazol Concentration
	Full Dose	½ Dose	¼ Dose	
0 ppm	6,00	4,25	3,50	4,58 a
3 ppm	5,00	6,75	4,75	5,50 a
5 ppm	1,25	1,50	2,50	1,75 b
KK = 33,66%				

Table 4 shows that there was no significant interaction between the paclobutrazol concentration and the MS media doses given on the number of shoots per corpse flower explant. Individually, paclobutrazol concentration had a significant effect on the number of shoots per explant of the corpse flower. The paclobutrazol concentration of 0 ppm had a significant effect compared to 5 ppm and was similar to the effect of 3 ppm at all doses of MS media.

Based on the results, it can be seen that as the paclobutrazol concentration increased, the number of shoots per explant decreased. This could occur because paclobutrazol inhibits the rate of cell division and elongation. This is in line with [17], who stated that the use of paclobutrazol can inhibit gibberellin activity, which is a growth regulator necessary for normal growth. According to [18] in [19], paclobutrazol, as a plant growth regulator, plays a role in reducing tissue metabolism, inhibiting vegetative growth, and inhibiting gibberellin synthesis.

D. Percentage of Rooted Explants

The percentage of rooted explants refers to the number of explants that formed roots out of all the explants that were planted. The observed percentage of rooted explants can be seen in Table 5.

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Table 5. Percentage of Rooted Explants *A. titanium*

Concentration Paclobutrazol	MS Dose		
	Full Dose	½ Dose	¼ Dose
	------ % ------		
0 ppm	50	75	25
3 ppm	50	75	75
5 ppm	75	25	100
KK = 80,29%			

Table 5 showed that there was no interaction between the paclobutrazol concentration and the MS media doses on the percentage of rooted corpse flower explants. Individually, both paclobutrazol concentration and MS media doses did not have a significant effect on the percentage of rooted corpse flower explants. Explants that had formed shoots were mostly able to produce roots, possibly because the growing shoots could produce endogenous auxins. According to [20], cytokinins can stimulate ethylene production under certain conditions, which can then stimulate the formation of adventitious roots by synthesizing the injured parts of the plant and using them as a site for adventitious root formation in the tissue damaged by explant cutting [21].

According to [22], media without the addition of cytokinins is better for root formation compared to media containing cytokinins because cytokinins can inhibit the biosynthesis of endogenous auxins in root formation. The endogenous auxin content in the explants might have been high enough to grow roots [23]. Furthermore, according to [24] the effect of cytokinin application can be suppressed or inhibited in the xylem cells, thus protecting the root formation cells from the influence of cytokinin in those cells.

E. Percentage of Rooted Explants

The number of rooted explants refers to the ability of the explants to form roots after being treated with Paclobutrazol and MS media at different concentrations. The observed number of roots can be seen in Table 6.

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Table 6. Percentage of Rooted Explants *A. titanum*

Concentration Paclobutrazol	MS Dose		
	Full Dose	½ Dose	¼ Dose
0 ppm	2,25	1,75	0,25
3 ppm	1,25	1,25	2,50
5 ppm	1,25	0,75	2,50
KK = 87,71%			

The data in Table 6 showed that there was no interaction between the concentration of paclobutrazol and the doses of MS media on the number of roots. Individually, the concentration of paclobutrazol and the doses of MS media had no significant effect on the number of roots. Both paclobutrazol concentration and MS media doses showed the same effect. It could be said that the application of paclobutrazol had no effect on the doses of MS media. This might have been due to the adaptation of the root system of the corpse flower shoots to survive and enhance nutrient absorption and efficiency [25].

The composition of the culture media consisted of essential nutrients (macro and micronutrients), hormones or growth regulators, amino acids, vitamins, and carbon sources. The process of tissue formation such as shoots or roots is linked to the interaction between exogenous growth regulators added to the media and endogenous growth regulators produced by the plant tissues [25]. In the case of the corpse flower plant treated with the retardant paclobutrazol at various concentrations, it was found that paclobutrazol significantly affected the number of shoots, plant height, number of leaves, and the number of roots [13].

IV. Conclusion

The application of paclobutrazol at various concentrations and MS media doses did not significantly affect the percentage of explants forming shoots, but it influenced shoot height and the number of shoots per explant. The concentration of 5.0 ppm PBZ and full-dose MS was the most effective in inhibiting shoot growth of titan arum, with an average shoot height of 1.5 cm and the highest number of shoots observed at concentrations of 0 ppm and 3.0 ppm across all MS media doses. Higher concentrations of paclobutrazol tended to inhibit cell elongation, resulting in shorter shoots and fewer shoots. However, paclobutrazol did not significantly affect root formation and the number of roots, which was likely influenced by the explants' adaptation to the culture medium provided.

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