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Shoot Induction of *Pandanus Tectorius* through Shoot Culture in WPM Medium Supplemented With BAP And NAA

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Abstract

This research aims to determine the best shoot induction medium for *Pandanus tectorius*, a coastal plant valued for its ecological and economic benefits. Coastal degradation and land conversion have reduced its population, necessitating propagation efforts. In vitro culture was explored to address the scarcity of *Pandanus tectorius*. Woody Plant Medium (WPM) was prepared by combining 100 mL stock solution, 30 g sugar, and distilled water to 1 L, supplemented with 1 g activated charcoal, 0.1 g ascorbic acid, and plant growth regulators at varying concentrations. The pH was adjusted to 5.6–5.8, and agar was added before sterilization at 121 °C for 20 minutes. Explants consisting of 1 cm-long shoots were sterilized and cultured in bottles, with observations conducted over 8 weeks. Results showed that the best shoot induction was achieved using WPM supplemented with $5x10^{-6}$ M BAP and $5x10^{-6}$ M NAA, producing 1 to 3 shoots per explant. Data analysis using a factorial completely randomized design confirmed significant differences among treatments. This study demonstrates the effectiveness of in vitro propagation in restoring *Pandanus tectorius* populations and supports sustainable coastal management.

Keyword: In vitro culture, *Pandanus tectorius*, WPM medium, Tsunami.

INTRODUCTION

Screw pine (*Pandanus tectorius*) is a coastal plant with several functions (ecological function, aesthetic function, material for weaving, and medicinal use). Ecologically, it helps mitigate tsunamis by forming coastal forests or a coastal greenbelt. This mitigation function requires a large number of *Pandanus tectorius* plants. Naturally, *Pandanus tectorius* can propagate through seeds and stem cuttings. However, seed propagation takes a considerable amount of time. The first flowering from seed propagation can take 10-25 years, while propagation through stem cuttings allows flowering in the sixth year (Thomson, et al., 2006; Gallaher, 2014).

The technique of propagating *Pandanus tectorius* was previously studied by Hani and Dendang in 2008, and plant propagation through seeds and stem cuttings by Rahayu et al. (2015). The research showed that seedlings from seeds take about 6 months to grow into seedlings, while stem cuttings take a much shorter time, only 2-3 months to grow. Seedlings from seeds are abundantly available in nature, but using stem cuttings in large quantities can pose a problem as it reduces the number of *Pandanus tectorius* plants in nature. Therefore, research is needed to find a propagation method to produce a large and uniform number of seedlings. This can be achieved through in vitro culture. In vitro culture for *Pandanus tectorius* has not been widely conducted, and publications on this

topic are still limited. Jose et al. (2016) conducted in vitro culture on *Pandanus fascicularis* and *Pandanus furcatus*, while Wu et al. (2023) researched the propagation of *Pandanus tectorius*. In this study, in vitro propagation of *Pandanus tectorius* was conducted using the methods of Jose et al. (2016) and Wu et al. (2023) with WPM medium supplemented with plant growth regulators BAP and NAA at different concentrations. This research aims to obtain a shoot induction medium with the appropriate plant growth regulators for the in vitro culture of *Pandanus tectorius*.

METHOD

Making WPM medium involves taking the previously made stock solution with a volume of 100 mL. Then, 30g of sugar is added. The medium solution is then added with distilled water up to 1L. The solution is then added with 1g of activated charcoal and 0.1g of ascorbic acid. Then, growth regulators are added according to the concentrations that have been determined as follows (Table 1).

Table 1. Composition of growth regulators provided in WPM medium.

BAP (M) / NAA (M)	$5x10^{-6}$	10^{-5}	$5x10^{-5}$	10^{-4}
$5x10^{-6}$	1	2	3	4

The pH is adjusted to range from 5.6 to 5.8 by adding 0.1 N NaOH or 0.1 N HCl solution. Then, 10g of agar is added. It is heated on the stove until boiling. After that, it is poured into a culture bottle. The next process is sterilizing the medium in an autoclave for 20 minutes at 121 ° C and a pressure of 15-17 psi.

Planting

The explants to be used are the shoots of screw pine branches. The prepared explants were transferred into sterile Petri dishes, and the shoots were cut to approximately 1 cm in size. The explants were sterilised with 70% alcohol for 3 minutes and 6% chlorine for 45 minutes, then soaked in liquid medium for 15 minutes twice. Then, planted in treatment bottles, repeated 5 times. The bottles that had been planted with explants were arranged in culture racks. Observations were made every day to see the results and were completed in week 8. The parameters recorded were the number of shots.

Data Analysis

The design model in data analysis is RAL in Factorial, treatment of 2 types of growth regulators, namely BAP (4 concentration levels) and NAA (1 concentration level), with five replications. The combination of BAP and NAA treatments used was 4. Each treatment was repeated 5 times so that there were 20 experimental units in total. Analysis of variance was carried out using the F test. Suppose Fcount> Ftable, the test results were declared significantly different at a 95% confidence level. Test results that were significantly different were further tested for the Least Significant Difference (LSD). Data processing was carried out using the SPSS 27 program.

RESULT

The cultured screw pine shoot explants showed significant proliferation in the treatment medium used. On the 16th day after culture initiation, the majority of explants had shown apparent shoot growth, indicating a positive response to the medium containing a combination of BAP (Benzyl Amino Purin) hormones with a concentration of $5x10^{-6}$ M and NAA (Naphthalene Acetic Acid) with a concentration of $5x10^{-6}$ M. Figure 1 visually illustrates the development of the explants, indicating that this hormone combination is effective in stimulating shoot growth in screw pine tissue culture.

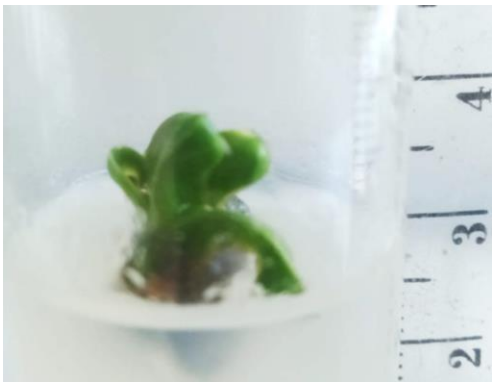

Figure 1. Shoot explants growing in medium 3.

Four combinations of plant growth regulators (BAP and NAA) planted with explants showed a positive response and produced shoot growth with the largest number of 3 shoots as seen in Table 2. The largest number of shoots was obtained in the medium added with $5x10^{-6}$M BAP and $5x10^{-6}$M NAA. Figure 3 shows that shoot growth took place in all combinations of plant growth regulators given.

Table 2. Average number of shoots growing in the treatment medium.

BAP (M) / **NAA (M)**	**$5x10^{-6}$**	**10^{-5}**	**$5x10^{-5}$**	**10^{-4}**
$5x10^{-6}$	2,4	1,4	1,8	1,4

The average number of shoots produced in the treatments showed a variation between 1 to 3 shoots per explant. In Figure 2, it is clear that the highest number of shoots, namely 3 shoots, was initiated in the treatment using a combination of BAP growth hormones with a concentration of $5x10^{-6}$ M and NAA with a concentration of $5x10^{-6}$ M.

This shows that the combination of BAP and NAA at these concentrations provides an optimal synergistic effect in stimulating shoot growth in the explants used in this study.

Figure 2. Three shoots growing from the branch tip in medium 1.

DISCUSSION

Pandanus tectorius, or commonly known as the screw pine, is a plant that plays a vital role in coastal ecosystems, particularly in mitigating erosion and serving as raw material for handicrafts and traditional medicine. This plant has distinctive morphological characteristics with ribbon-shaped leaves, prop roots, and the ability to withstand harsh coastal environments. In addition, the screw pine is also known to be adaptive to dynamic coastal conditions, though the first flowering process from seed propagation takes a long time, approximately 10-25 years, whereas through stem cutting propagation, it only flowers in the sixth year. This makes it an interesting subject for tissue culture research for vegetative propagation. (Thomson, *et al.*, 2006; Gallaher, 2014).

The culture method used in this research involved WPM medium enriched with the plant growth regulators BAP and NAA. The selection of BAP and NAA was based on their roles in stimulating cell division (cytokinesis) and cell elongation, which are essential in shoot induction. BAP, as a cytokinin, is known to effectively stimulate shoot formation by promoting cell division, while NAA, as an auxin, supports cell elongation and works synergistically with BAP in the shoot induction process, as previously demonstrated in studies by Rosita et al. (2015), Dahniar and Elvavina (2022), and Sari et al. (2017).

The number of shoots growing from the explants varied between 1 to 3 shoots at 16 days of culture. These results are consistent with the previous study by Jose et al. (2016), who cultured shoots of *Pandanus fascicularis* and produced 2-3 shoots at 5 weeks of culture. On the other hand, Wu et al. (2023), who cultured shoots of *Pandanus tectorius*, reported an average of 7.9 shoots at 35 days of culture. In other species, such as *Musa acuminata*, shoot induction produced 3.5-20.25 shoots (Jafari et al., 2011), while in *Saccharum officinarum* shoots, induction produced 6 shoots (Biradar et al., 2009). The success of this shoot induction cannot be separated from the use of exogenous plant growth regulators. In this study, the growth regulators used to induce shoot growth were different from those used by Jose et al. (2016), who used a combination of BAP and IAA, while Wu et al. (2023) used BAP and NAA. Meanwhile, the studies by Jafari et al. (2011) and Biradar et al. (2009) only used growth regulators from the cytokinin group, specifically BAP.

The study by Zainal et al. (1999) showed that the use of the growth regulator kinetin was not yet successful in producing shoots. BAP is one of the most widely used growth regulators for inducing shoots (Lestari, 2011). In in vitro culture, the use of BAP is more responsive compared to kinetin and 2-iP (Flick et al., 1983). The addition of exogenous growth regulators in the form of cytokinins and auxins can stimulate the growth of planted explants, whether applied individually or in combination by manipulating the concentrations of each growth regulator.

In line with the research findings, statistical tests were also conducted to determine data distribution and significant differences between the treatments applied to the in vitro culture of *Pandanus tectorius* shoots. The Shapiro-Wilk normality test was applied to examine whether the data obtained from various treatments followed a normal distribution. The Shapiro-Wilk test results showed that the data from three (3) treatments with a combination of BAP and NAA were not normally distributed, as indicated by a p-value of less than 0.05 (Sig. < 0.05); similarly, Treatment 3 (BAP $5x10^{-5}$ and NAA $5x10^{-6}$M) showed the same results. This condition indicates that the data did not follow a normal distribution, hence the use of non-parametric statistical tests such as Kruskal-Wallis is more appropriate for further analysis.

The non-parametric Kruskal-Wallis test was then applied to examine whether there were significant differences between several treatment groups. The results of the Kruskal-Wallis test indicated that there were significant differences in the number of shoots produced by *Pandanus tectorius* explants under varying concentrations of BAP and NAA, although no significant differences were observed between the treatment groups. Treatment one (1), with a BAP concentration of $5x10^{-6}$M and NAA $5x10^{-6}$M, produced the highest number of shoots, with an average of 2.4 (2-3 shoots per explant). These results are consistent with previous studies showing that BAP is an effective cytokinin for inducing shoot formation in various plant species. The success of this shoot induction further underscores the role of growth regulator combinations in tissue culture for in vitro propagation of *Pandanus tectorius*. The findings of this study are beneficial for the development of vegetative propagation techniques for *Pandanus tectorius*, which has high ecological and economic value, particularly in coastal areas.

CONCLUSION

Based on the results of this study, it can be concluded that WPM medium enriched with BAP ($5x10^{-6}$ M) and NAA ($5x10^{-6}$ M) is the most effective combination in inducing shoot formation. The use of these concentrations resulted in the optimal number of shoots, indicating that this combination of hormones provides an environment that supports maximum shoot growth under tissue culture conditions.

Further research is needed to evaluate the ability of successfully induced shoots to root well and to determine whether the shoots can grow well when transferred to the natural environment. This research will provide important information about the feasibility of this tissue culture method in plant conservation and cultivation efforts, especially in the context of ex situ plant development before reintroduction to its natural habitat.

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