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Tissue Culture of Purple Sweet Potato Using Murashige and Skoog (MS) Medium Based on Benzyl Amino Purine (BAP)

Kultur Jaringan Ubi Jalar Ungu Menggunakan Media Murashige dan Skoog (MS) Berbasis Benzyl Amino Purine BAP

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Abstrak

Ubi jalar ungu (*Ipomoea batatas* L.) merupakan salah satu sumber pangan lokal yang kaya akan antosianin dan berpotensi dikembangkan melalui teknik kultur jaringan. Penelitian ini bertujuan untuk mengetahui pengaruh pemberian Benzyl Amino Purine (BAP) terhadap pertumbuhan eksplan ubi jalar ungu secara *in vitro* menggunakan media *Murashige* dan *Skoog* (MS). Penelitian dilakukan menggunakan Rancangan Acak Lengkap (RAL) dengan empat perlakuan konsentrasi BAP (0; 2,5; 3,5; 5,0 mg/L) dengan masing-masing lima ulangan (20 unit percobaan). Eksplan berupa batang ubi jalar ungu (*Ipomoea batatas* L.). Eksplan kemudian dikulturkan pada media MS padat yang telah mengandung BAP selama 8 minggu. Parameter yang diamati meliputi tinggi batang, tinggi kalus dan persentase eksplan yang membentuk kalus. Hasil penelitian menunjukkan bahwa perlakuan BAP 3,5 mg/L (N2) memberikan hasil terbaik dengan rata-rata tinggi tanaman sebesar 2,7 mm, tinggi kalus 1,6 mm dan persentase pembentukan kalus mencapai 100%. Uji statistik menunjukkan bahwa pemberian BAP memberikan pengaruh nyata terhadap semua parameter yang diamati. Dengan demikian, konsentrasi BAP 3,5 mg/L direkomendasikan sebagai perlakuan optimal untuk perbanyakan tanaman ubi jalar ungu secara *in vitro*.

Kata kunci: *ubi jalar ungu, kultur jaringan, BAP, media MS, kalus*

Abstract

Purple sweet potato (Ipomoea batatas L.*) is one of the local food sources rich in anthocyanins and has great potential to be developed through tissue culture techniques. This study aimed to determine the effect of Benzyl Amino Purine (BAP) on the growth of purple sweet potato explants cultured in vitro using Murashige and Skoog (MS) medium. The experiment was conducted using a Completely Randomized Design (CRD) with four BAP concentration treatments (0, 2.5, 3.5, and 5.0 mg/L), each replicated five times, resulting in 20 experimental units. The explants used were stem segments of purple sweet potato (Ipomoea batatas L.). The explants were then cultured on solid Murashige and Skoog (MS) medium containing BAP for 8 weeks. Observed parameters included plant height, callus height, and the percentage of explants forming callus. The results showed that the 3.5 mg/L BAP treatment (N2) produced the best growth, with an average plant height of 2.7 mm, callus height of 1.6 mm, and 100% callus formation. Statistical analysis indicated that BAP application had a significant effect on all observed parameters. Therefore, a concentration of 3.5 mg/L BAP is recommended as the optimal treatment for in vitro propagation of purple sweet potato.*

***Keywords**: purple sweet potato, tissue culture, BAP, MS medium, callus*

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INTRODUCTION

Sweet potatoes (*Ipomoea batatas* L.) are a type of tuber that grows on vines [1]. Sweet potatoes are high in carbohydrates, containing 91.42–93.45% (based on dry weight), as well as vitamins A, B1, B2, B3, and C [2]. The high carotene content causes the flesh of the tuber to be yellow to red in color, which indicates a high vitamin A content [3]. Studies suggest that the plant originates from the Yucatan region (Mexico) to the Orinoco River (Venezuela) [4]. In Indonesia, sweet potatoes have proliferated since the 1960s [5].

Tissue culture is a vegetative propagation technique performed aseptically using artificial media [4]. Tissue culture media contain macro- and micro-nutrients, vitamins, carbon sources, and plant growth regulators (PGRs) [8]. MS (Murashige and Skoog) media is the most used basic medium because it has a complete and balanced nutrient composition [8].

The addition of Benzyl Amino Purine (BAP) to MS medium plays a role in stimulating bud and callus formation [7], [9]. Previous studies have reported that the application of cytokinin increases cell division and photosynthetic activity, as well as accelerates explant regeneration [6], [7]. However, information on the optimal concentration of BAP for regenerating purple sweet potatoes is still limited. Therefore, this study aims to determine the effect and optimal concentration of BAP on the growth of purple sweet potato explants. The observed parameters include stem height, the number of calli formed, and the height of the calli.

METHODS

Research Period and Location

The research team conducted this study from March to May 2025 at the STKIP Arrahmaniyah Laboratory, located at Jl. Masjid Al-Ittihad No. 22 Bojong Pondok Terong, Kec. Cipayung, Kota Depok, West Java 16437.

Equipment and Materials

The equipment used in this study included an autoclave, laminar air flow (LAF), lamp/Bunsen burner, tweezers, scalpel, surgical knife, micropipette, test tubes/culture bottles, and Petri dishes.

The materials used included purple sweet potato stem explants (*Ipomoea batatas* L.), Murashige and Skoog (MS) medium, agar powder, distilled water, alcohol, vitamins, the plant growth regulator (PGR) BAP, and spiritus.

Research Design

The research design employed was a completely randomized design (CRD) with four treatment levels and five replicates, resulting in 20 experimental units. The treatments given were as follows:

Table 1. Design of Treatments and Replications

Treatments	Description	Replications
N0	MS (without BAP)	CW1-CW5
N1	MS + BAP 2,5 mg/L	CW1-CW5
N2	MS + BAP 3,5 mg/L	CW1-CW5
N3	MS + BAP 5,0 mg/L	CW1-CW5

Notes :
MS = Murashige and Skoog Medium
ZPT = Plant Growth Regulators (BAP = Benzyl Amino Purine)

Sterilization and Inoculation Techniques

Sterilization of equipment and culture bottles is performed by washing them with detergent, drying them, wrapping them in paper, and then sterilizing them in an autoclave for 30 minutes. Researchers sterilize the prepared media in an autoclave at 121°C for 30 minutes.

We sterilize the explants in two stages. In the first stage, we perform the procedure outside the Laminar Air Flow (LAF). We rinse the cut purple sweet potato stems (approximately 1 × 1 cm) with distilled water for 1 minute, soak them in a 10% chlorine solution for 1 minute, wash them in 70% alcohol for 1 minute, and then rinse them twice with sterile distilled water.

In the second stage, the researchers work inside the Laminar Air Flow (LAF) to plant the explants aseptically. Before beginning the planting process, they place all tools and materials in the LAF and irradiate them with an ultraviolet lamp for 30 minutes to ensure aseptic conditions. The researchers then planted the sterilized explants in culture bottles containing MS and MS + BAP media according to the treatment.

A good aseptic sterilization process is a crucial factor in preventing contamination and ensuring the success of tissue culture [10].

Medium Preparation

The researchers prepared the medium using MS solution, 30 g/L sucrose, 7 g/L agar powder, and distilled water. The pH was adjusted to 5.8 according to the treatment, then the medium was poured into culture bottles (25 ml per bottle) and sterilized using an autoclave.

MS medium contains complete macro- and micro-nutrients, as well as vitamin B1, which plays a crucial role in plant cell metabolism [6], [8].

Observation Parameters

1. Stem height (cm)
2. Callus height (cm)
3. Percentage of explants forming callus

Data Analysis Techniques

The researchers tested the data for normality using the Shapiro–Wilk test. If the data did not follow a normal distribution, the analysis was continued with the Kruskal-Wallis test and a follow-up test using the Mann-Whitney U test. The test results were presented in tables and graphs to facilitate interpretation of the differences between treatments [11].

RESULTS AND DISCUSSION

This study aims to investigate the effect of varying BAP concentrations on the in vitro growth of purple sweet potato explants cultured on MS medium. The parameters observed include stem height, callus height, and the number of explants that form callus. The results of the observations are presented in tables and analyzed statistically to determine the significance of the differences between treatments.

Stem Height

Stem height measurements were taken to determine the growth response of the explants at various concentrations of BAP. Table 2 shows the average stem height for each treatment.

Table 2. Average Stem Height of Purple Sweet Potato (mm)

Treatment	Average Height (cm)	Description
N0 (MS without BAP)	0,2	a
N1 (2,5 mg/L BAP)	1,04	ab
N2 (3,5 mg/L BAP)	2,7	b
N3 (5,0 mg/L BAP)	3,3	b

Notes :
a : significantly low
b : significantly high
ab : in an intermediate position, showing similarity to both groups (not significantly different from either one)

Table 2 shows that BAP treatment has a significant effect on stem height. The average stem height obtained at a concentration of 5.0 mg/L BAP (N3) was 3.3 mm, which was not significantly different from N2. Meanwhile, the control treatment (N0) yielded a height of only 0.2 mm.

This finding aligns with the study by Behera et al. [9], which reported that moderate concentrations of cytokinin can increase photosynthetic enzyme activity and accelerate cell division in *Ipomoea batatas* explants. Cytokinin plays a role in stimulating bud formation and elongating stems through increased metabolic activity and translocation of photosynthetic products [7], [9].

Callus Height

Callus height was observed to assess the effectiveness of BAP in stimulating callus formation in purple sweet potato explants. The average callus height for each treatment is presented in Table 3.2 below.

Table 3. Average Callus Height of Purple Sweet Potato (mm)

Treatment	Callus Height (cm)	Description
N0 (MS without BAP)	0	b
N1 (2,5 mg/L BAP)	0,6	ab
N2 (3,5 mg/L BAP)	1,6	b
N3 (5,0 mg/L BAP)	1,3	ab

Notes :
a : significantly low
b : significantly high
ab : in an intermediate position, showing similarity to both groups (not significantly different from either one)

Table 3 shows that the 3.5 mg/L BAP (N2) treatment produced the highest callus height, namely 1.6 mm. The control treatment did not form any callus at all.

These results reinforce the role of BAP cytokinin in promoting cell division and callus formation [7]. Research by Vollmer et al. [6] states that the addition of vitamin B1 to the culture medium can accelerate explant regeneration by increasing oxidative enzyme activity and energy metabolism. Additionally, the presence of BAP in nitrogen-rich MS media enhances the efficiency of nutrient utilization, thereby supporting the growth of callus tissue [8].

Percentage of Explant Forming Callus

The percentage of explants that formed callus indicates the success of callus initiation in each treatment. The data are presented in Table 3.3 below.

Table 4. Percentage of Explans Forming Callus (%)

Treatment	Callus Percentage (%)	Description
N0 (MS without BAP)	0%	a
N1 (2,5 mg/L BAP)	80%	ab
N2 (3,5 mg/L BAP)	100%	b
N3 (5,0 mg/L BAP)	60%	a

Notes :
a : significantly low

b : significantly high
ab : in an intermediate position, showing similarity to both groups (not significantly different from either one.

Table 4 shows that all explants (100%) in the N2 treatment formed callus, while N0 did not show callus formation. The highest effectiveness was achieved at a concentration of 3.5 mg/L, while a concentration of 5.0 mg/L showed a decrease due to the toxic effects of BAP.

These findings are consistent with the report by Pratiwi *et al*. [11], which indicates that a combination of BAP with moderate concentrations yields the best results for callus and shoot formation in purple sweet potatoes. In addition, Sulikah [12] also reported that administering BAP at the right concentration can increase the number of shoots in Arnet yellow sweet potatoes in vitro.

CONCLUSION

The addition of BAP to MS medium had a significant effect on the growth of purple sweet potato explants, with a concentration of 3.5 mg/L producing the best increase in all parameters, including 100% callus formation. These findings align with studies by Behera *et al*. [9], Pratiwi *et al*. [11], and Sulikah [12], which demonstrate that moderate concentrations of cytokinin, particularly BAP, can enhance explant regeneration and plant propagation in vitro. This study still faces challenges in the form of contamination in some explants, which is suspected to be due to suboptimal aseptic conditions, but this does not affect the conclusions regarding the main parameters of the study.

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CONFLICT OF INTEREST

I hereby declare that there is no conflict of interest in the writing of this scientific paper.

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