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Research Article

Micropropagation of *Sweet Potato (ipomoea batatas, Keledek Anggun)*

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Abstract

The study involved two types of explants, nodal and young shoots, obtained from *Ipomoea batatas* and cultured using two different media: MS (Murashige and Skoog) and WPM (McCown's woody plant). Different concentrations of the plant growth regulator, BAP (6-benzyl aminopurine), ranging from 0 to 5 mg/L, were tested. The results revealed that culturing node explants in the MS medium resulted in better outcomes compared to the WPM medium. Among the various concentrations of BAP tested, the lowest concentration, 0.5 mg, showed the highest mean shoot length of 3.9 cm, indicating the positive role of BAP in shoot initiation. In contrast, the mean shoot length observed in the WPM medium was relatively low, ranging from 0.9 cm to 2.2 cm, across all concentrations of BAP. Another experiment focused on shoot proliferation and rooting. Low concentrations of BAP (ranging from 0.0 to 1 mg/L) were found to enhance plantlet length, ranging from 6.5 cm to 8.1 cm. However, higher concentrations of BAP (3 or 5 mg/L) led to 50% callus initiation, which inhibited the growth of plantlet length and the development of roots in Ipomoea batatas. Interestingly, the presence of NAA (α-Napthaleneacetic acid) at concentrations of 1 or 2 mg/L effectively increased the number of roots, with a recorded range of 7.2 to 7.3 roots. These findings highlight the importance of media selection, particularly the preference for MS medium, and the role of BAP and NAA in regulating shoot initiation, shoot proliferation, and rooting in the tissue culture of Ipomoea batatas.

Keywords: *Micropropagation, Sweet Potato, Keledek Anggun, Plant Growth Regulator.*

Introduction

Ipomoea batatas (L.) Lam, commonly known as sweet potato, is a large, starchy, and sweet-tasting root vegetable belonging to the Convolvulaceae family (Woolfe, 1992). It boasts a rich history, with evidence of human consumption dating back to the Palaeolithic era in Central and South America. Presently, it is cultivated in numerous countries with tropical, subtropical, and temperate climates, covering approximately 8.1 million hectares of plantation area and yielding an annual production of 106 to 110 million tonnes (Dolinski & Olek, 2013).

This herbaceous perennial vine features medium-sized flowers and heart-shaped or palmately lobed leaves. The tuberous root, which constitutes the edible part of the plant, possesses a smooth skin and a long, tapered shape. Sweet potatoes are highly valued for their sweetness, nutritional richness, and relatively short growing period of 90 to 120 days (Mohanraj & Sivasankar, 2014). Furthermore, they have been extensively utilized as a source of energy and phytochemicals in human and animal nutrition. Traditional medicine also harnesses the medicinal properties of Ipomoea batatas, with the raw consumption of the tuber used

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to address anaemia, hypertension, and diabetes, and the leaves employed in managing type 2 diabetes and oral inflammatory and infectious conditions (Abel & Busia, 2005; Groppo, *et al*., 2011).

Despite being an underutilized plant, *Ipomoea batatas* holds significant potential and benefits that warrant further exploration, particularly considering its potential therapeutic characteristics. In recent years, in vitro culture methods have been employed, recognizing their importance in the propagation process. Plant tissue culture serves as an effective tool for preserving genetic diversity, particularly for plant species at risk of extinction. Micropropagation techniques have been developed for a wide range of medicinal plants; thus, this study aims to establish an efficient micropropagation protocol for Ipomoea batatas, specifically focusing on the Anggun variety of sweet potato.

Materials and Methods

Healthy Ipomea batatas plants were cultivated in a glasshouse for several weeks before extracting explants for in vitro establishment (Figure 1a). The explants were obtained by cutting 2-3 cm pieces of young shoots (Figure 1b) or stem segments bearing nodes (Figure 1c). Subsequently, the explants were placed in a conical flask and washed with detergent (Decon), followed by thorough rinsing under running water for 1-2 hours. Next, the plantlets underwent treatment with a fungicide solution (5% Bavestin) with a few drops of Tween 20. They were then placed on a shaker for 1 hour before being rinsed with distilled water. In the laminar airflow chamber, the explants were surface sterilized using 70% ethanol for 1 minute, followed by immersion in a 10% Clorox® solution containing several drops of Tween-20 for 20 minutes on a rotary shaker. Afterward, the sterilized explants were rinsed with sterile water and allowed to dry for 4 hours before being cultured on either Murashige and Skoog's (MS) (1962) basal medium or Woody Plant Medium (WPM) (1981).

In experiments to examine the effectiveness of MS or WPM medium in shoot initiation, the culture medium was prepared with 3% sucrose and supplemented with 0, 0.5, 1, 3, or 5 mg/L of the cytokinin 6-benzylaminopurine (BAP). The pH of the medium was adjusted to 5.8 before autoclaving (15 minutes at 121°C). The cultures were then placed in a culture room maintained at 25±2°C, under white fluorescent light with an intensity of 3000 lux, following a photoperiod of 12 hours light and 12 hours darkness. Regular checks were conducted on the cultures to identify any signs of contamination. If contamination was observed, the affected cultures were immediately discarded after recording the findings. Additionally, monthly sub-culturing was performed using fresh media. After 60 days of culture, the data were expressed as follows: percent of shoot initiation, number of shoots per explant, and mean shoot length. Furthermore, some callus production was observed at the bottom of the cultures, and these observations were duly recorded.

The initial shoots obtained from the previous experiment (Figure 1d,e) were utilized for the shoot proliferation experiment, which aimed to optimize shoot multiplication. These initial shoots were cultured on basal MS medium supplemented with various concentrations of BAP (0, 0.5, 1.0, 3.0, and 5.0 g/L), NAA (0, 1.0, or 2.0 mg/L), or combinations of both growth regulators as specified in Table 2. Each treatment was replicated ten times in separate flasks, and observations were recorded after 60 days of culture. The recorded results encompassed the number of shoots per explant, the length of plantlets, and the number of roots and callus initiated. The complete plantlets produced in vitro were carefully taken out from the culture medium, and their roots were washed to eliminate the agar. Subsequently, the plantlets were transferred into polybags containing a mixture of organic soil, topsoil, and sand in the ratio of 1:1:0.5. These polybags were then placed in a net house under controlled conditions, where they experienced 75% shading and temperatures ranging from 28˚C to 32˚C. To maintain appropriate humidity levels, the plants were watered twice daily. Observations were diligently recorded,

focusing on the percentage of survival for both rooted and acclimatized plants. The degree of response was calculated, and means and standard errors of the means were determined.

Results and Discussion

Shoot Induction

This experiment focused on the usage of two different culture media, MS and WPM, containing varying concentrations of BAP (0, 0.5, 1, 3, 5 mg/L) to induce shoot initiation from nodal segments and young shoots of sweet potato (Ipomoea batatas, Keledek Anggun). Both nodal segments and young shoots exhibited different responses to the treatments. For nodal explants cultured in MS and WPM without BAP, no callus initiation was observed. However, the percentage of shoot induction differed between the two media. MS medium recorded a higher range of 80-90% shoot induction (Figure 1f,g), while WPM medium had a lower range of 50-60%. Interestingly, without the presence of BAP, the nodal segments cultured in MS medium recorded one of the highest mean shoot lengths at 3.1 cm. On the other hand, the presence of the lowest BAP concentration, 0.5 mg, resulted in the highest mean shoot length of 3.9 cm, indicating the positive effect of BAP as a plant growth regulator in shoot initiation. This finding is consistent with a study by Hussain, *et al*., (2012), which showed that the addition of BAP at a concentration of 0.5 mg/L promoted maximum shoot initiation and proliferation in black pepper callus.

However, it was observed that at higher BAP concentrations (3 and 5 mg/L), the number of explants with shoot initiation increased compared to the absence of BAP or lower concentrations. Nevertheless, these higher concentrations of BAP resulted in shorter mean shoot lengths ranging from 0.7 cm to 1.2 cm due to callus initiation, which hindered shoot growth. This observation is supported by reports stating that elevated cytokinin levels in the medium can lead to vitrification, reducing shoot quality and vitality (Espinosa, *et al*., 2006).

In comparison, for nodal explants, it was much more preferable to culture them in MS medium rather than WPM medium. The mean shoot length range in WPM medium was relatively low, recorded at 0.9 cm to 2.2 cm. The most suitable BAP concentration for shoot induction of Ipomoea batatas was found to be in the range of 0-0.5 mg/L. This result contradicts the findings of Komakech, *et al*., (2020), where they reported a substantially higher average length of axillary shoots (0.93cm) from nodal segments cultivated in WPM. In their study, WPM was identified as the medium that encouraged the best axillary shoot growth. Furthermore, the young shoot explants were also tested with both MS and WPM media using the same concentrations of BAP as before. In MS medium, a 10% shoot induction rate was recorded with the absence of BAP and at 0.5 mg/L BAP, while no percentage was recorded for other concentrations. The mean shoot length for the 10% shoot induction ranged from 2.2 cm to 2.3 cm, and only one explant produced shoots. However, in WPM medium, the results were poor, with no shoots being produced, and therefore, no mean shoot length could be obtained. Thus, it can be determined that young shoots did not respond well in both MS and WPM media, showing poor performance in terms of shoot induction.

Table 1: Effect of 2 types of medium containing different concentrations of BAP on shoot initiation from nodal segments and young shoots (after 60 days of culture)

Explants	Culture medium	BAP conc. mg/L	Shoot initiation (%)	No. of shoot/ Explants	Mean shoot length (cm)	Callus initiated (%)
Node	MS	0	80	1	3.1	0
		0.5	90	1	3.9	0
		1	80	1	1.1±0.1	20
		3	80	2	1.2±0.5	30
		5	80	2	0.7	50
	WPM	0	50	1	2.1±0.09	0
		0.5	50	1	2.2±0.2	0
		1	60	2	1.8±0.3	0
		3	50	1	0.9±0.1	30
		5	50	0	0	50
Young shoots	MS	0	10	1	2.3	0
		0.5	10	1	2.2	0
		1	0	0	0	0
		3	0	0	0	0
		5	0	0	0	0
	WPM	0	0	0	0	0
		0.5	0	0	0	0
		1	0	0	0	0
		3	0	0	0	0
		5	0	0	0	0

Shoot Proliferation and Rooting

Another experiment was conducted to optimize shoot multiplication, aiming to improve incubation conditions and nutritional media to further encourage proliferation. Plant growth regulators were also employed to promote organ growth (Chée, *et al*., 1992). Cultures were established on basal MS medium supplemented with different concentrations of BAP (0, 1.0, 3.0, and 5.0 g/L), NAA (0 or 1.0 mg/L), or combinations of both growth regulators. Table 2 shows the effect of plant growth regulators (BAP and NAA) on the percentage of callus initiated, mean number of shoots per explant, length of plantlets (cm), and mean number of roots produced after 60 days of culture on MS medium (Figure 1h,I,j).

In the absence of plant growth regulators BAP and NAA, the length of plantlets recorded the highest value at 8.1 cm, and the number of roots produced was also the highest at 8.2. No callus initiation was observed, and the mean number of explants obtained was 1. The presence and proper concentration of growth regulators are crucial factors that govern the growth and development of plants in tissue cultures, along with the composition of macro- and microelements in the culture medium. Auxins and cytokinins play vital roles in various aspects of plant development. Their interplay controls essential morphological processes, including meristem morphology and growth. During the in vitro plant multiplication stage, low doses of auxins are used in combination with high concentrations of cytokinin to promote shoot proliferation (Su, *et al*., 2011).

Using only BAP at different concentrations (0, 0.5, 1, 3, and 5 mg/L) resulted in various responses. At the lowest BAP concentration (0.5 mg/L), the length of plantlets obtained was 7.8 cm, while the number of roots obtained was 5.3, and no callus initiation was observed. It is evident that using only BAP at a low concentration boosts the length of plantlets compared to root multiplication. However, as the BAP concentration increased,

particularly at 3 and 5 mg/L, the results showed poor performance in terms of the length of plantlets (2.4-3.1 cm) and the number of roots (2.2-2.9) due to the development of callus, reaching 50% for both concentrations. Next, the combination of BAP (1, 3, 5 mg/L) and NAA (1 mg/L) resulted in the formation of a clump of undifferentiated cells known as a callus (Figure 1k), which developed when auxin and cytokinin levels were balanced, ranging from 10-30%. This affected the length of plantlets and the number of roots. On the other hand, when only NAA (1 mg/L and 2 mg/L) was used as the growth hormone, the callus did not grow. Instead, this growth hormone promoted root multiplication, resulting in a range of 7.2-7.3 roots, as high auxin concentrations generally boost root formation (Hussain, *et al*., 2012). This finding is supported by research from Komakech, *et al*., (2020), where the medium supplemented with 1.5 mg/L NAA hormone recorded the highest mean number of roots in *Prunus africana.*

Regenerated plantlets were removed from agar jar (Figure 1l) and transfer into bottle containing vermiculite for 2 weeks (Figure 1m). The seedling then were transferred onto polybag with media containing a mixture of organic soil, topsoil, and sand in the ratio of 1:1:0.5. They were maintained at about 70% relative humidity in the greenhouse with 75% shading and temperatures ranging from 28˚C to 32˚C. A survival rate 95% was achieved after 2 months (Figure no 1).

Table 2: Effect of plant growth regulators (BAP and NAA) on microshoot propagation (% of shoot explants forming microshoots), number of microshoots per explants, and mean number of roots produced after 60 days of culture on MS medium.

BAP (mg/L)	NAA (mg/L)	Callus Initiated (%)	Mean number of shoots/explant	length of plantlets (cm)	No. of roots
0	0	0	1	8.1±1.4	8.2±1.2
0.5	0	0	1	7.8±1.0	5.3±0.8
1	0	0	1	6.5±1.5	5.1±0.7
3	0	50	2	3.1±0.6	2.9±0.5
5	0	50	1	2.4±0.5	2.2±0.5
1	1	10	1	3.4±0.9	5.7±2.1
3	1	20	2	2.1±0.6	6.1±1.1
5	1	30	1	2.2±0.7	5.2±0.7
0	1	0	1	2.0±0.5	7.2±1.3
0	2	0	1	1.3±0.5	7.3±1.0

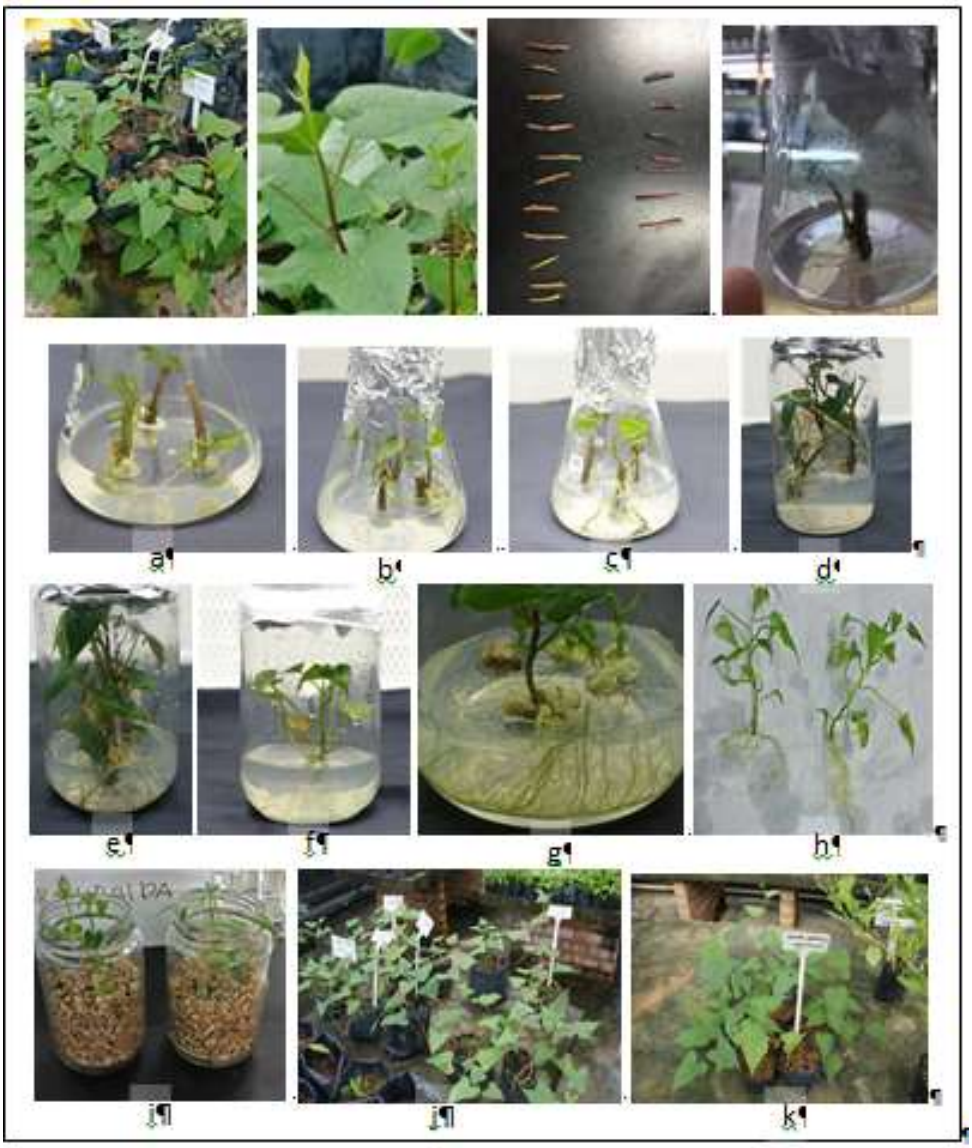

Figure 1: Micropropagation of *ipomea batatas*. (a) Planting material (exlants) is cultivated in a glasshouse, (b) Shoots are obtained from the cultivated plants , (c) Nodes are also used as explants for the micropropagation process, (d,e,f,g) shoot initiation, (h,I,j) shoot elongation and proliferation occur during this micropropagation process (k) callud initiation, (l,m) Regenerated plantlets are removed form the agar jar, (n,o) The complete plantlets are transfer into polybeg

Conclusion

In conclusion, the optimal micropropagation method for Ipomoea batatas involves using MS medium with a BAP concentration of 0 to 0.5 mg/L for shoot initiation and low concentrations of BAP (0.0 to 1 mg/L) for promoting plantlet length. Additionally, low concentrations of BAP (ranging from 0.0 to 1 mg/L) were found to be beneficial in promoting the length of plantlets, with the range recorded at 6.5 cm to 8.1 cm. However, higher concentrations of BAP should be avoided to prevent callus formation and hindered growth. The presence of NAA (1 or 2 mg/L) is essential for maximizing root production.

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