



Original Research Paper

COMPARATIVE ANALYSIS OF TWO BAP CONCENTRATIONS WITH MULTIPLE INCISION TECHNIQUES FOR ENHANCED SHOOT MULTIPLICATION IN TISSUE-CULTURED LAKATAN [MUSA ACUMINATA COLLA (AA)]

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ABSTRACT

A successful large-scale micropropagation of *Musa acuminata* Colla (AA) 'Lakatan' would greatly contribute if efficient and optimal protocol will be established on improving cytokinin-induced shoot proliferation. This experiment was to determine the effects of the concentration of 6-benzylaminopurine (BAP) and the combined impact of multiple incisions on in vitro corm proliferation. The explants were placed in the Murashige and Skoog (MS) medium under treatments T1 (20 mg·L⁻¹ BAP) and T2 (30 mg·L⁻¹ BAP) with uniformly performed multiple incisions. Thirty explants per treatment were assessed throughout their four different developmental stages (establishment of explant, protrusion, leaf-like, and plantlet) in every 15 days for the total 60 days of inoculation. The experiment was arranged following a Completely Randomized Design (CRD). Data were analysed statistically by Analysis of Variance (ANOVA) and differences were partitioned by using LSD ($p \leq 0.05$). Shoot proliferation in Treatment 1 was significantly increased over that in Treatment 2 with this effect, which is most significant when observed from protrusion to shoot bud stages. Higher 6-benzylaminopurine (BAP) concentrations (30 mg·L⁻¹ BAP) led to diminished efficiency, and this could be attributed to possible inhibition caused by excess cytokinin. Thus, an additive effect of moderate concentrations of BAP and wounding can promote both meristematic and inducing shoot responses. The reported results led to the formulation of an enhanced procedure for accelerated micropropagation of Lakatan banana.

Keywords: *Musa acuminata*, 6-Benzylaminopurine, Cytokinin optimization, Micropropagation, Shoot induction, Tissue culture

1 INTRODUCTION

Banana (*Musa* spp.) is one of the world's most important fruit crops because of its nutritional value, economic significance, and contribution to food security. In the Philippines, banana is among the leading agricultural commodities and plays a vital role in domestic consumption, employment generation, and export earnings. Among the dessert banana cultivars, Lakatan (*Musa acuminata* Colla [AA]) is highly preferred because of its excellent eating quality, attractive fruit characteristics, and high market demand. Despite its commercial importance, the sustainable production of Lakatan banana remains threatened by numerous biotic stresses, including Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense*, Banana bunchy top virus (BBTV), nematodes, and other pests and diseases that considerably reduce productivity and profitability.

Conventional propagation of banana through suckers is inadequate to meet the increasing demand for high-

quality planting materials because of its low multiplication rate and the high risk of transmitting systemic pathogens from infected mother plants. Consequently, plant tissue culture has become the preferred propagation technique for the rapid production of genetically uniform, disease-free, and vigorous planting materials throughout the year. Micropropagation has significantly contributed to the commercial banana industry by enabling the mass production of elite cultivars while maintaining genetic fidelity and phytosanitary quality.

Among the various factors influencing in vitro regeneration, plant growth regulators play a critical role in determining the efficiency of shoot multiplication. Cytokinins, particularly 6-benzylaminopurine (BAP), are the most widely used growth regulators for inducing axillary shoot proliferation in banana. BAP promotes cell division, stimulates dormant axillary buds, and enhances shoot multiplication; however, its effectiveness depends

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largely on the concentration used and the genotype being cultured. Numerous studies have shown that moderate BAP concentrations generally produce higher multiplication rates and healthier shoots, whereas excessive concentrations may lead to compact shoot clumps, reduced elongation, hyperhydricity, and abnormal plant development. Therefore, optimizing the concentration of BAP is essential for developing an efficient micropropagation protocol for a specific banana cultivar.

Recent advances in banana micropropagation have also emphasized the importance of physical manipulation techniques that enhance shoot proliferation. One such approach is the multiple incision technique, which disrupts apical dominance by creating wounds in the apical meristem, thereby exposing dormant meristematic tissues to exogenous cytokinins. This technique increases the number of active shoot initials and enhances the regeneration potential of the explants. The effectiveness of multiple incisions depends on their interaction with plant growth regulators, particularly BAP, suggesting that both factors should be optimized simultaneously to maximize shoot multiplication efficiency.

Although numerous studies have investigated the effects of BAP on banana micropropagation, information regarding the combined effects of high BAP concentrations and the multiple incision technique on the shoot multiplication of Lakatan banana remains limited. Most published protocols focus primarily on optimizing growth regulator concentrations without considering the potential synergistic effects of meristem wounding techniques. Establishing an optimized protocol integrating both hormonal and physical manipulation strategies could substantially improve multiplication efficiency, reduce production costs, and increase the availability of high-quality disease-free planting materials for commercial nurseries, research institutions, and banana growers. Therefore, this study evaluated the effects of two BAP concentrations combined with the multiple incision technique on the shoot multiplication and plantlet regeneration of tissue-cultured Lakatan banana (*Musa acuminata* Colla [AA]).

1.1 Objectives of the Study

This study will optimize *in vitro* shoot multiplication in *Musa acuminata* ‘Lakatan’ by the use of a cytokinin. The general objective of this research study is to find the best concentration of 6-benzylaminopurine (BAP) in

combination with multiple incision techniques to increase the efficiency of shoot multiplication.

Specifically, The specific objectives of this research study are: (i) to measure and estimate the effectiveness of 20 mg·L⁻¹ and 30 mg·L⁻¹ of BAP concentration on the kinetics of shoot proliferation throughout a series of developmental stages of explants; (ii) to assess the effects of a mechanical stimulation with multiple incisions to promote meristematic cell division and to induce shoots in explants of Lakatan bananas; (iii) to compare the differences in rates of shoot multiplication and in the growth of the explants treated with the different concentrations of cytokinin and multiple incisions; and (iv) to determine the combination of treatments most efficient to promote high levels of *in vitro* multiplication.

2 MATERIALS AND METHODS

2.1 Laboratory and period of the experiment

The study was carried out at the Mangrove and Bamboo Research Innovation Center (MABRIC) Tissue Culture Laboratory, Cagayan State University-Gonzaga Campus, Gonzaga Cagayan Philippines from the January, 2025 to August 2025.

2.2 Genetic materials

Mapilak-Lakatan (*Musa acuminata* Colla (AA)) was used in the experiment.

2.3 Concentrations of BAP with multiple meristem incisions

Different concentrations of BAP (20 mg·L⁻¹ and 30 mg·L⁻¹ BAP) with multiple meristem incisions of Mapilak-Lakatan were studied.

2.4 Culture media preparation for *in vitro*

The Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) served as the basal medium for the experiment. It contained essential inorganic salts and vitamins, as presented in Table 1. To provide a carbohydrate source, the MS medium was supplemented with sucrose at a concentration of 30 g/L. Additionally, 6-benzylaminopurine (BAP) was incorporated at concentrations of 20 mg·L⁻¹ and 30 mg·L⁻¹ to serve as the treatment variables, along with 100 ppm of BAP added to the base formulation. The pH of the medium was adjusted to 5.7 using 0.1 M NaOH before further processing to ensure optimal conditions for plant tissue growth and development.

Table 1 Composition and amount of stock solutions used in the preparation of media for ‘Lakatan’ shoot tip/meristem culture.

Stock Solution	Ingredients	Amount (g)	Amount of stock solution used per liter of medium (mg·L ⁻¹) at different stages		
			initiation	proliferation	rooting
A. Macro-MSI 25X	Ammonium nitrate	41.25	40	40	40

	Potassium nitrate	47.5			
	Calcium chloride	11			
	Magnesium sulfate hydrated	9.25			
	Potassium phosphate HCL	4.25			
B. Micro-MSII 100X	Potassium iodide	0.83	10	10	10
	Boric acid	0.062			
	Manganese sulfate	2.23			
	Zinc sulfate	0.86			
	Sodium molybdate	0.025			
	Cupric sulfate	0.0025			
	Cobalt chloride	0.0025			
C. Vitamins-MS III 100X	Myo inositol	1	10	10	10
	Nicotinic Acid	0.05			
	Pyridoxine HCL	0.05			
	Thiamine HCL	0.01			
	Glycine	0.2			
D. Fe EDTA 200X (500 mg·L⁻¹)	Fe SO ₄ . 7H ₂ O	2.78	5	5	5
	Na ₂ EDTA	3.73			
E. BAP	100 ppm	0.1	20	20	0
	100 ppm	0.1	30	30	0

***All media contains 30 g/L sucrose and solidified with 8 g agar ('gulaman' bar). **A-B-C-** Dissolve in distilled water and bring to a final volume of 1L, **D-** Dissolve in distilled water (nearly boiling). Slowly add FeSO₄*7H₂O while stirring. Cool and bring to final volume of 500 mg·L⁻¹, **E-** Weigh BAP. Dissolve in NaOH. Bring to a final volume of 1L.

In the final step of the process, agar was incorporated into the medium as a solidifying agent at a concentration of 8 g/L. The agar was dissolved by heating and thoroughly mixed with the medium to ensure uniform consistency. Once the different media formulations were prepared, they were dispensed into glass bottles, covered with autoclavable plastic secured with rubber bands, and topped with a layer of paper for additional protection.

The prepared media were sterilized using a pressure cooker functioning as an autoclave at 15 psi for 20 minutes. This sterilization process effectively eliminates microbial contaminants through the combined action of high pressure and temperature. After autoclaving, the media were allowed to cool and solidify at room temperature for 24 hours to ensure complete agar solidification and to reach a suitable temperature for inoculation or subsequent experimental procedures.

2.5 Growth Conditions

All cultures were incubated in a controlled-environment growth chamber maintained at 25 ± 2 °C under a 16-hour photoperiod, illuminated by cool white fluorescent light with an intensity of approximately 40 μmol·m⁻²·s⁻¹ (Murashige, T., & Skoog, F. (1962). Subculturing was performed at 30-day intervals to ensure optimal growth and development of the cultures.

2.6 Multiple incision techniques

The multiple incision technique was performed on the Lakatan banana (*Musa acuminata* Colla [AA]) shoot-tip meristem during the initial inoculation and repeated at every subculture throughout the shoot multiplication stage. Using a sterile scalpel under aseptic conditions, several shallow longitudinal and transverse incisions were carefully made on the apical meristem without completely severing the explant. The incisions were intended to disrupt apical dominance and expose dormant meristematic tissues to the culture medium containing 6-benzylaminopurine (BAP), thereby promoting the initiation and proliferation of multiple shoot primordia.

During each subculture, newly formed shoot clumps were aseptically removed from the culture vessel, and the same multiple incision procedure was repeated before transferring the explants onto fresh Murashige and Skoog (MS) medium supplemented with the designated BAP concentration. Repeating the multiple incision technique at every subculture maintained continuous activation of dormant axillary meristems, enhanced cytokinin exposure to wounded tissues, and promoted sustained shoot proliferation throughout the six-month culture period.

2.7 Experimental Design

The experiment was arranged following a Completely Randomized Design (CRD) with two treatment combinations: T1 (20 mg·L⁻¹ BAP + multiple meristem incisions) and T2 (30 mg·L⁻¹ BAP + multiple meristem incisions). Each treatment consisted of 30 replicate explants cultured individually to ensure uniformity and minimize the risk of contamination. Multiple meristem

incisions were performed by carefully making four to six longitudinal cuts on the shoot apex using a sterile scalpel to disrupt apical dominance and expose the inner meristematic tissues to the cytokinin-containing medium, thereby promoting the activation of dormant axillary meristems and enhancing shoot proliferation (Israeli et al., 1995; Strosse et al., 2004; George et al., 2008).

2.8 Statistical Analysis

The study employed a Completely Randomized Design (CRD) to evaluate the effect of two 6-benzylaminopurine (BAP) concentrations ($20 \text{ mg}\cdot\text{L}^{-1}$ and $30 \text{ mg}\cdot\text{L}^{-1}$) combined with multiple meristem incisions on shoot multiplication efficiency of Lakatan banana (*Musa acuminata* Colla [AA]). Data collected monthly over a six-month culture period were subjected to Analysis of Variance (ANOVA) using the Statistical Tool for Agricultural Research (STAR) developed by the International Rice Research Institute (IRRI). When

significant treatment effects were detected, mean comparisons were performed using the Least Significant Difference (LSD) test at the 5% level of significance ($p \leq 0.05$) (Gomez & Gomez, 1984; IRRI, 2013).

3 RESULTS AND DISCUSSION

This resulted in increase in leaf-like projections (Figure 1). The shoots produced increased from 110 to 146 (with an average of 264 for each explant) in the fifth month and formed green shoots but without roots (Figure 1) before transforming into roots, and with the increased production to 240-296 (average 3844 for each explant) at the sixth month (Figure 1). Promoting shoot proliferation of 'Lakatan' banana using BAP and Multiple Incisions of basal medium gave consistent results with the reported study by Magdalita and San Pascual (2020) at 100ppm BAP ($20 \text{ mg}\cdot\text{L}^{-1}$) + Multiple Incisions. Green plantlets obtained were presented in Figure 1 (Representative samples).

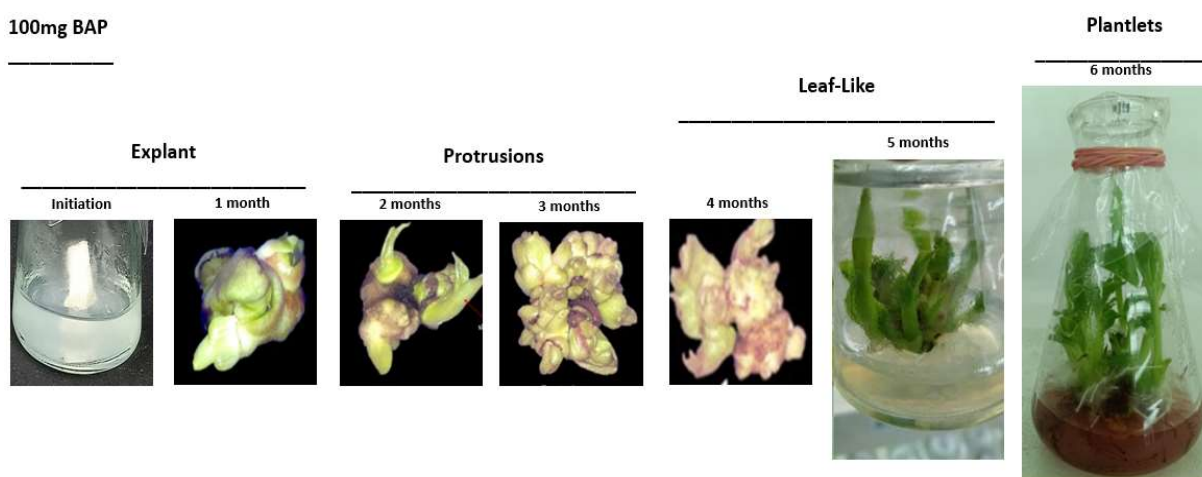


Figure 1 Different stages of tissue cultured 'Lakatan' explants grown on MS medium supplemented with 100 ppm BAP, 100 ppm BAP ($20 \text{ mg}\cdot\text{L}^{-1}$ BAP) + Multiple Incisions

Table 2 Effect of Two BAP Concentrations and Multiple Incision Techniques for Enhanced Shoot Multiplication in Tissue-Cultured Lakatan (*Musa acuminata* Colla (AA))

Treatment	Initiation (0 month)	Initiation (1 month)	Number of Protrusion (2 months)	Number of Protrusion (3 months)	Number of leaf-like (4 months)	Number of leaf-like (5 months)	Number of Plantlets (6 months)
T1 - $20 \text{ mg}\cdot\text{L}^{-1}$ BAP with Multiple Incisions	1.00 ± 0.00	2.00 ± 0.00	16.33 ± 0.57^a	34.47 ± 1.01^a	65.40 ± 2.10^a	137.60 ± 3.85^a	256.27 ± 12.45^a
T2 - $30 \text{ mg}\cdot\text{L}^{-1}$ BAP with Multiple Incisions	1.00 ± 0.00	2.00 ± 0.00	14.27 ± 0.49^b	29.40 ± 0.88^b	56.80 ± 1.76^b	109.67 ± 3.11^b	203.53 ± 10.98^b
p-value	1 ns	1 ns	0.0005 *	0.004*	0.002*	0.001*	0.0046*

ns = not significant ($p > 0.05$); * = significant at 5% level ($p \leq 0.05$)

Table 2 presents the effects of two concentrations of 6-benzylaminopurine (BAP) combined with the multiple incision technique on the shoot multiplication of tissue-cultured Lakatan banana (*Musa acuminata* Colla (AA)). During the initiation stage (0 and 1 month), no significant differences were observed between the two treatments ($p > 0.05$). Both treatments produced one successfully established explant at the start of culture and two developing shoots after one month. This indicates that increasing the BAP concentration from $20 \text{ mg}\cdot\text{L}^{-1}$ to $30 \text{ mg}\cdot\text{L}^{-1}$ did not influence explant establishment or the initial breaking of dormancy. The absence of significant differences at this stage suggests that the explants possessed comparable physiological conditions and that the multiple incision technique alone was sufficient to stimulate the initial activation of the shoot meristem.

Significant treatment effects became apparent beginning at the second month, when shoot protrusions started to develop. Explants cultured in $20 \text{ mg}\cdot\text{L}^{-1}$ BAP with multiple incisions produced significantly more protrusions (16.33) than those cultured in $30 \text{ mg}\cdot\text{L}^{-1}$ BAP (14.27) ($p = 0.0005$). This difference indicates that the lower BAP concentration provided a more favorable hormonal environment for the initiation of new shoot primordia. Although cytokinins are known to stimulate shoot proliferation, excessively high concentrations may inhibit cell differentiation and disrupt the balance between endogenous and exogenous plant growth regulators. Consequently, increasing the BAP concentration beyond the optimum level did not enhance shoot induction and instead reduced the regenerative response of the explants.

The superiority of the $20 \text{ mg}\cdot\text{L}^{-1}$ BAP treatment became more evident during the third month. Explants cultured in this treatment produced an average of 34.47 protrusions, significantly higher than the 29.40 protrusions recorded under $30 \text{ mg}\cdot\text{L}^{-1}$ BAP ($p = 0.004$). This result suggests that once shoot primordia were initiated, the lower BAP concentration was more effective in sustaining continuous meristematic activity and promoting the formation of additional shoot initials. In contrast, the higher BAP concentration appeared to suppress further proliferation, possibly because prolonged exposure to elevated cytokinin levels reduced the capacity of newly formed buds to continue developing.

As shoot development progressed, significant differences were also observed in the production of leaf-like structures. At four months, explants cultured in $20 \text{ mg}\cdot\text{L}^{-1}$ BAP produced 65.40 leaf-like structures, whereas those cultured in $30 \text{ mg}\cdot\text{L}^{-1}$ BAP produced only 56.80. By the fifth month, the number further increased to 137.60 and 109.67, respectively. The continuous increase in leaf-like structures demonstrates that shoots produced under the lower BAP concentration differentiated more efficiently into complete shoots capable of producing leaves. This observation indicates that $20 \text{ mg}\cdot\text{L}^{-1}$ BAP not only enhanced shoot initiation but also promoted

subsequent shoot development and differentiation. The reduced number of leaf-like structures under $30 \text{ mg}\cdot\text{L}^{-1}$ BAP suggests that excessive cytokinin may have limited normal shoot development despite the presence of actively dividing tissues.

The cumulative effect of these differences was reflected in the final number of regenerated plantlets after six months. Explants cultured in $20 \text{ mg}\cdot\text{L}^{-1}$ BAP with multiple incisions produced an average of 256.27 plantlets, which was significantly higher than the 203.53 plantlets obtained from $30 \text{ mg}\cdot\text{L}^{-1}$ BAP ($p = 0.0046$). This represents approximately a 26% increase in plantlet production, demonstrating that the lower BAP concentration was substantially more efficient for large-scale micropropagation of Lakatan banana. The results clearly indicate that increasing the BAP concentration beyond $20 \text{ mg}\cdot\text{L}^{-1}$ did not improve multiplication efficiency and instead reduced the overall regeneration capacity of the explants.

The superior performance of $20 \text{ mg}\cdot\text{L}^{-1}$ BAP observed in the present study is consistent with previous reports emphasizing that moderate cytokinin concentrations are more effective than excessively high concentrations for banana micropropagation. Cronauer and Krikorian (1984) reported that BAP is one of the most effective cytokinins for inducing multiple shoots in *Musa* species; however, they emphasized that shoot multiplication declines when cytokinin levels exceed the optimum concentration. Similarly, Vuylsteke (1989) observed that although BAP stimulates rapid shoot proliferation, excessive concentrations frequently result in compact shoot clumps, poor elongation, and reduced regeneration efficiency.

The findings also agree with those of Strosse et al. (2004), who explained that successful banana micropropagation depends on maintaining an appropriate balance between endogenous hormones and exogenously supplied cytokinins. Moderate BAP concentrations promote continuous cell division and shoot differentiation, whereas excessive cytokinin may interfere with normal morphogenesis and reduce multiplication efficiency. Likewise, Resmi and Nair (2007) demonstrated that increasing BAP concentration beyond the optimum level does not necessarily increase shoot production and may instead decrease the regenerative capacity of banana explants.

The high multiplication rates observed in both treatments can also be attributed to the use of the multiple incision technique. Multiple incisions disrupt apical dominance by injuring the apical meristem and exposing numerous dormant axillary meristems to the culture medium. This allows cytokinin to stimulate the development of multiple shoot initials simultaneously. Israeli et al. (1995) reported that destruction or weakening of apical dominance through shoot-tip manipulation markedly enhances axillary bud proliferation in banana. Similarly,

Pradeep et al. (2013) found that multiple incision or decapitation techniques significantly increased shoot multiplication by activating dormant meristematic tissues that would otherwise remain suppressed.

The present study demonstrates that the interaction between 20 mg·L⁻¹ BAP and the multiple incision technique provided the most favorable conditions for shoot multiplication of tissue-cultured Lakatan banana. While both treatments successfully initiated shoot development, the lower BAP concentration consistently produced significantly higher numbers of protrusions, leaf-like structures, and regenerated plantlets throughout the six-month culture period. These findings suggest that maintaining BAP at 20 mg·L⁻¹ is sufficient to maximize shoot proliferation while avoiding the inhibitory effects associated with excessive cytokinin concentrations. This protocol therefore offers an efficient and reliable approach for the rapid mass propagation of disease-free Lakatan banana planting materials.

CONCLUSION

This study found that shoot proliferation of tissue-cultured corms of *Musa acuminata* 'Lakatan' was significantly stimulated by using 20 mg·L⁻¹ of 6-benzylaminopurine (BAP) alongside multiple incisions compared to the control (no treatments) and 30 mg·L⁻¹ of BAP. Optimal results were achieved using lower concentrations of cytokinin for all phases of the development indicating that excess cytokinin may

promote inhibitors of shoot multiplication and development. These effects were most likely induced by hormonal interaction with wounding to produce greater stimulation of the meristematic zone leading to increased multiplication. As the protocol uses much lower amounts of hormone as well as a simple method to increase production of banana plantlets efficiently, this system is economically viable and readily scalable for any commercial plant tissue laboratories and use very little of hormones.

RECOMMENDATION

This study therefore suggests utilizing a 20 mg·L⁻¹ of BAP combined with multiple incisions as a standard propagation system for 'Lakatan' banana tissue culture and to develop a protocol in combination with other hormones, physiological, biochemical or molecular studies to be conducted in future to expand its' range to large scale and field.

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