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# Micropropagation of Banana via Organogenesis

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**Abstract**
Bananas (*Musa acuminata* cv. Berangan) face significant challenges in micropropagation due to microbial contamination and inconsistent growth in conventional cultivation. This study aimed to develop and optimize a micropropagation protocol via organogenesis by testing effective surface sterilization and plant growth regulator (PGR) conditions. Explants sourced from Seelong, Johor, were sterilized using varying bleach concentrations (0-80%), revealing that a 60% bleach concentration achieved 100% survival and eliminated contamination without damaging the tissues. Additionally, combinations of 6-Benzylaminopurine (BAP) and 1-naphthaleneacetic acid (NAA) were evaluated. The optimal combination of 5 mg/L BAP and 0.5 mg/L NAA significantly enhanced shoot proliferation (2.50 ± 0.34), elongation (7.01 ± 0.05 cm) and root formation (5.10 ± 0.23). These results demonstrate an efficient, reproducible *in vitro* method for large-scale production of healthy, disease-free banana plantlets. The protocol provides a practical solution for nurseries and contributes to sustainable banana cultivation.
**Keywords:** Banana micropropagation; organogenesis; surface sterilization; plant growth regulators; *Musa acuminata*

## Introduction

Bananas (*Musa spp*.) are among the world's most important fruit crops, serving as a staple food and income source for millions of people in tropical regions. However, global banana production faces significant challenges due to pests and diseases such as Panama disease and Banana Bunchy Top Virus (BBTV), which severely threaten yield and sustainability (Sharma et al., 2013). To address these threats, micropropagation has become a crucial method for producing large numbers of healthy, genetically uniform, disease-free planting materials (George et al., 2008).

Despite its advantages, successful micropropagation is often hindered by microbial contamination, which can drastically reduce explant survival and propagation efficiency (Kane, 2004). Effective surface sterilization is crucial for eliminating contaminants without damaging plant tissues. Sodium hypochlorite (commercial bleach) is widely used for this purpose, but its optimal concentration for balancing decontamination and tissue viability varies across cultivars (Kumar et al., 2020).

Additionally, the success of in vitro propagation depends heavily on the use of appropriate plant growth regulators (PGRs). Cytokinin, such as 6-Benzylaminopurine (BAP), and auxin, like 1-naphthaleneacetic acid (NAA), are commonly used to induce shoot and root formation (Tan et al., 2010; Gebeyehu, 2013). Optimizing their concentrations is critical for maximizing shoot proliferation and healthy root development in *Musa acuminata* cv. Berangan. This study aims to enhance the large-scale production of high-quality banana plantlets.

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**Materials and methods**

Sword suckers of *Musa acuminata* cv. Berangan were collected from a plantation in Seelong, Johor, and shoot apical meristems were prepared as explants for micropropagation. The explants were first washed under running tap water for 30 minutes, then soaked in 70% (v/v) ethanol for 1 minute with gentle agitation. Surface sterilization was performed using commercial bleach (sodium hypochlorite) at concentrations of 0%, 20%. 40%, 60% and 80% with 1-3 drops of Tween-20 added as a surfactant for 30 minutes. Explants were then rinsed three times with sterile distilled water to remove any residual sterilant.

The culture medium was prepared using Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962), supplemented with 30 g/L sucrose and solidified with 4 g/L agar, adjusted to a pH of 5.8, and autoclaved at 121°C for 20 minutes. Different combinations of 6-Benzylaminopurine (BAP) (5–15 mg/L) and 1-Naphthaleneacetic acid (NAA) (0.5–1.5 mg/L) were tested to evaluate shoot and root induction. Sterilized explants were inoculated onto media under aseptic conditions and incubated at 23–27°C under a 16-hour photoperiod with a light intensity of 2000 lux. Subculturing was performed after 2 months. A Completely Randomized Design (CRD) with 5 replications. Contamination rate and survival were recorded as percentages, while data on shoot number, shoot length, and root number were analyzed by one-way ANOVA with Tukey's HSD test ($p < 0.05$) using SPSS software.

**Results and discussion**

Table 1 showed that varying bleach concentrations had a clear impact on contamination rates and explant survival. Without bleach (0%), all explants were contaminated, confirming that sterilization is essential. Lower concentrations (20% and 40%) partially reduced contamination but did not fully eliminate it, consistent with Kumar et al. (2020). The use of Tween-20 as a surfactant and ethanol pretreatment likely enhanced sterilization effectiveness, supporting similar findings to those of Nasir et al. (2014) and Srinivasan et al. (2018). The 60% bleach treatment was optimal, achieving 0% contamination and 100% survival, aligning with Dewir et al. (2015), who reported that properly balanced sodium hypochlorite levels can sterilize without damaging tissues. In contrast, 80% bleach fully controlled contamination but reduced viability to 20% due to oxidative stress (El-Banna et al., 2021). These results confirm that 60% bleach is the most effective option for surface sterilization of banana explants, ensuring the production of healthy, disease-free plantlets for large-scale micropropagation.

**Table 1:** Effectiveness of surface sterilization using varying bleach concentrations

| Bleach Concentration (%) | Contamination Rate (%) | Survival Rate% |
|---|---|---|
| 0 | 100 | 0 |
| 20 | 80 | 20 |
| 40 | 40 | 60 |
| 60 | 0 | 100 |
| 80 | 80 | 20 |

The optimization of BAP concentrations showed a significant effect on shoot proliferation and shoot length of *Musa acuminata* cv. Berangan explants. The results indicated that 5 mg/L BAP produced the highest mean number of shoots (2.50 ± 0.34) and the greatest shoot length (7.01 ± 0.05 cm). higher concentrations of BAP (10-15 mg/L) resulted in reduced shoot proliferation and shorter shoots, suggesting that excessive cytokinin can suppress shoot elongation due to hormonal imbalance (Tan et al., 2010; Gabeyehu, 2013). These findings align with those of Singh and Gantait (2021), who also reported that moderate BAP levels enhance shoot bud initiation, while excessive levels may cause vitrification and abnormal growth. Figure 1 and Figure 2 illustrate these findings, showing that T2 significantly outperformed other treatments in both shoot number and length.

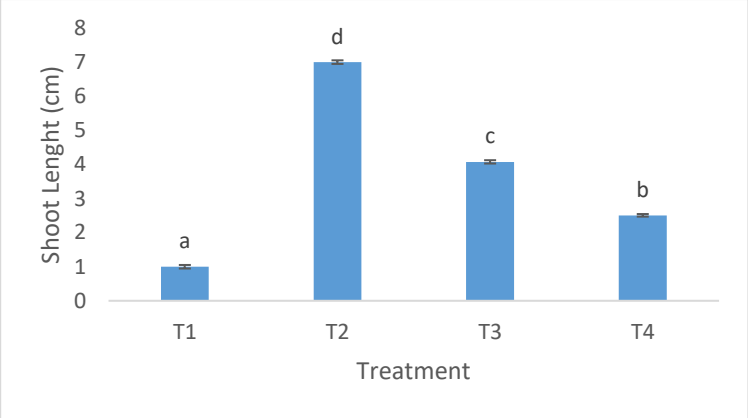


**Figure 1** Length of shoot per treatment in *Musa acuminata* cv. Berangan

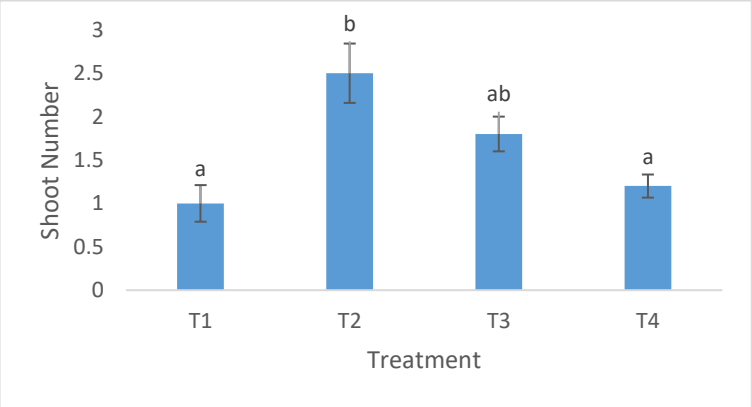


**Figure 2** Shoot number per treatment in *Musa acuminata* cv. Berangan

Evaluation of different NAA concentrations demonstrated that combining a low auxin level with optimal BAP enhanced root induction. Treatment with 0.5 mg/L NAA, combined with 5 mg/L BAP, resulted in the highest mean root number (5.10 ± 0.23). Increasing NAA concentration beyond 1.0 mg/L did not significantly improve rooting and, in some cases, reduced root length due to possible auxin overdose. Similar results were reported by Sharma et al. (2013), who emphasized that balanced auxin levels are crucial for the proper initiation of roots in banana micropropagation. Therefore, the combination of 5 mg/L BAP and 0.5 mg/L NAA was identified as the best treatment for simultaneous shoot and root development. Figure 3 illustrates the mean root number per treatment, with a standard error bar indicating variability.

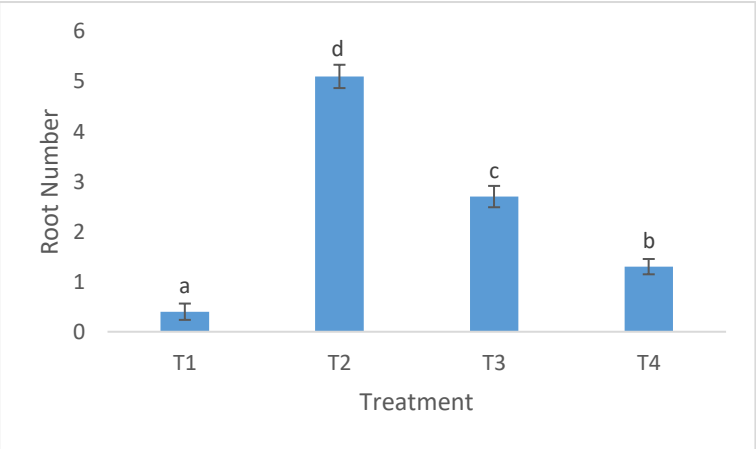


**Figure 3** Root number per treatment in *Musa acuminata* cv. Berangan

These results are summarized in Table 2, which presents the effects of BAP and NAA combinations on shoot and root formation.

**Table 2:** Effects of different BAP and NAA concentrations on shoot and root development in *Musa acuminata* cv. Berangan.

| Treatment Group | BAP (mg/L) | NAA (mg/L) | Shoot Number (Mean ± SE) | Shoot Length (cm) (Mean ± SE) | Root Number (Mean ± SE) |
|---|---|---|---|---|---|
| T1 | 0.0 | 0.0 | 1.00 ± 0.211$^{a}$ | 1.00 ± 0.052$^{a}$ | 0.40 ± 0.163$^{a}$ |
| T2 | 5.0 | 0.5 | 2.50 ± 0.342$^{b}$ | 7.01 ± 0.053$^{d}$ | 5.10 ± 0.233$^{d}$ |
| T3 | 10.0 | 1.0 | 1.80 ± 0.200$^{ab}$ | 4.08 ± 0.049$^{c}$ | 2.70 ± 0.213$^{c}$ |
| T4 | 15.0 | 1.5 | 1.20 ± 0.133$^{a}$ | 2.51 ± 0.038$^{b}$ | 1.30 ± 0.153$^{b}$ |

## Conclusion

In conclusion, this study established an effective micropropagation protocol for *Musa acuminata* cv. Berangan uses optimized surface sterilization and plant growth regulator treatments. The results confirmed that 60% bleach concentration eliminated contamination while maintaining explant viability. The combination of 5 mg/L BAP and 0.5 mg/L NAA produced the highest shoot proliferation and root formation. This protocol provides a reliable and reproducible method for large-scale production of healthy, disease-free banana plantlets, addressing key challenges in commercial banana propagation. Future research should explore alternative explant sources, additional plant growth regulators, omics-based approaches, improved acclimatization techniques and eco-friendly sterilization methods to further enhance micropropagation efficiency and sustainability for *Musa* spp.

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