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A Simple Protocol for Initial Micropropagation of *Musa acuminata* cv. Berangan (AAA)

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Abstract: This study presents an observational investigation into the *in vitro* propagation of *Musa acuminata* cv. Berangan (AAA), conducted during a 15-week industrial training program at the Plant Biotechnology Incubator Unit (PBIU), Universiti Malaya. Growth progression, contamination rates, and aseptic practices were evaluated during initiation and multiplication stages. Two culture batches, Batch 1 and Batch 2 were established on Murashige and Skoog (MS) basal medium supplemented with 6-benzylaminopurine (BAP), achieving clean culture rates of 89.6% and 98.6%, respectively. The findings highlight contamination sources and emphasize the importance of strict aseptic handling, accurate media preparation, and systematic monitoring to enhance micropropagation efficiency.

Keywords: Berangan, Banana, Plant tissue culture, Micropropagation, Contamination, Aseptic technique

Introduction

Musa acuminata cv. Berangan (AAA) is an economically important banana cultivar in Malaysia, belonging to the triploid AAA genomic group. It is widely cultivated due to its favorable fruit quality and high market acceptance. Although Berangan exhibits moderate tolerance to major diseases such as Fusarium wilt, conventional vegetative propagation through suckers limits large-scale multiplication and increases the risk of disease accumulation. Therefore, *in vitro* micropropagation has become a preferred approach for rapid production of uniform, disease-free planting materials suitable for commercial cultivation (Munusamy et al., 2019).

Plant tissue culture is an aseptic *in vitro* cultivation of plant cells, tissues, or organs on nutrient media under controlled environmental conditions, enabling rapid and uniform plant propagation (George et al., 2008). This technology encompasses techniques such as meristem culture, protoplast and cell suspension culture, organ culture, and anther or pollen culture (Thorpe, 2007). Widely applied in both research and commercial agriculture, plant tissue culture allows the production of high-quality, disease-free planting materials, supports germplasm conservation, and facilitates crop improvement and the development of stress-tolerant varieties (Bhojwani & Dantu, 2013; Kumar et al., 2024).

Despite the extensive application of micropropagation, most studies on *Musa* spp. focus on optimizing culture media or hormone combinations to maximize

multiplication rates. Limited observational data are available on routine growth progression, contamination dynamics, and aseptic practices during standard propagation procedures. Therefore, this study aimed to evaluate the growth and contamination rates of *Musa acuminata* cv. Berangan (AAA) under routine *in vitro* propagation conditions. Specifically, it focused on documenting explant developmental responses and identifying the frequency and severity of microbial contamination throughout the tissue culture process. This observational study provides baseline insights that are critical for improving laboratory protocols and ensuring high-efficiency micropropagation.

Methodology

Initiation stage

The banana suckers of *Musa acuminata* cv. Berangan were selected and trimmed approximately about 6 cm x 4 cm before surface sterilization in 100% chlorox solution (5.25% NaOCl) with a few drops of Tween-20 for 20 minutes to remove surface contaminants. This concentration and exposure duration were adopted based on established banana micropropagation protocols, which report effective microbial control while maintaining explant viability (Vuylsteke, 1989; Rahman et al., 2013).

After rinsing, explants were further trimmed to 5 cm x 3 cm and soaked in mixture of 50% chlorox solution and 50% distilled water for 10 minutes, it then trimmed to 4 cm x 2 cm under aseptic conditions in a laminar flow. Sterilized explants were initiated onto Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962) without growth regulators (MS0) and incubated under controlled culture conditions for initiation and observation. Surface sterilization with chlorox solution and Tween-20 is a standard practice in banana micropropagation to reduce microbial contamination, followed by placement of explant into MS medium for initiation.

Sterilized explants were initiated onto Murashige and Skoog (MS) basal medium without plant growth regulators (MS0) (Murashige & Skoog, 1962). Cultures were incubated at 25 ± 2℃ under a 16 hours light / 8 hours photoperiod, with a light intensity of approximately 40-50 µmol m^{-2} s^{-1} provided by cool white flourescent lamps. These conditions are widely used for banana tissue culture and support successful culture establishment and initial shoot development. (Strosse et al., 2006).

A total of 48 banana suckers were initiated in the first culture batch, while 46 suckers were initiated in the second batch on MS0 medium (Table 1).

Multiplication stage

After one month of initial culture, contamination-free and healthy explants were selected for multiplication. Explants were removed from MS0 and prepared for subculture in MS basal medium supplemented with 3 mg/L benzyl aminopurine (BAP) to promote shoot proliferation. The outer explant layers, which contain phenolic excretory were removed and trimmed before transfer to new media under sterile conditions. Prepared explants were placed onto MS + 3 mg/L BAP medium (Table 1), labeled and incubated under controlled conditions. During multiplication stage, cultures were monitored weekly to look for contamination and maintained the survival culture. The contaminated jars containing explants were autoclaved to prevent cross-contamination.

The multiplication cycle was continued on monthly basis for the next vegetative stage (V3), with explants subculture onto fresh medium according to their growth stage to ensure continuous shoot proliferation and healthy development.

Table 1: Medium used for growth stage of *Musa acuminata* cv.Berangan (AAA).

Growth Stage	Medium
V0	MS0
V1	MS + 3 mg/L BAP
V2	MS + 3 mg/L BAP
V3	MS + 5 mg/L BAP

Data were recorded at the end of the each month for two months for Batch 1, whereas data for Batch 2 were collected after one month of culture. Cultures that had reached the V2 stage were subsequently transferred to the next vegetative stage (V3).

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Rescue of contaminated culture

Contamination during early plant tissue culture requires prompt intervention to establish clean cultures. Before opening contaminated jars, surfaces were disinfected with 70% ethanol and lids briefly flamed. Explants were carefully removed and lightly flamed, and visibly contaminated, necrotic, or phenolic-rich tissues were excised using sterile instruments, which were re-sterilized between cuts.

Cleaned explants were transferred to fresh MS medium supplemented with 3 mg L^{-1} BAP to promote regeneration. Rescued cultures were labeled and incubated under standard growth room conditions for monitoring.

Results and Discussion

Culture survival and multiplication efficiency

The *in vitro* propagation of *Musa acuminata* cv. Berangan (AAA) showed high survival and multiplication efficiency. In Batch 1, 48 explants were initiated producing 82 clean cultures at V1 stage and successfully increased to 147 clean cultures at the end V2 stage. Similarly, Batch 2 produced 73 clean cultures at the V1 stage from 46 initiated explants. (Table 2). These results indicate a strong multiplication response following transfer to Murashige and Skoog (MS) medium supplemented with 6-benzylaminopurine (BAP).

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The effectiveness of BAP in promoting shoot proliferation observed in this study is consistent with previous reports describing its role in stimulationg axillary bud break and multiple shoot formation in AAA banana cultivars (Arinaitwe et al., 2000; Chhon et al., 2025). Cytokinins such as BAP are known to overcome apical dominance and enhance meristematic activity, resulting in increased shoot numbers during successive subculture cycles. The progressive increase in clean cultures from V1 to V2 in Batch 1 further highlights the cumulative effect of stage-wise subculturing on multiplication efficiency.

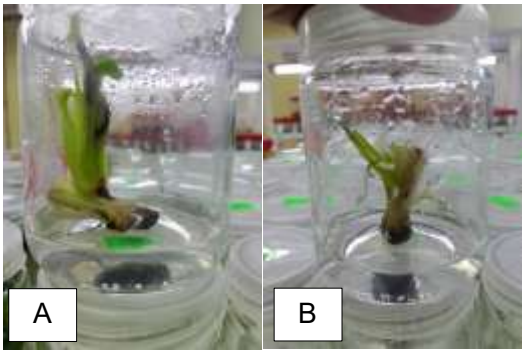

Figure 1: (A) Clean culture of V2 in Batch 1. (B) Clean culture of V1 in Batch 2.

Contamination incidence and culture cleanliness

Contamination rates differed between batches. Batch 1 recorded 10.4% contamination, whereas Batch 2 showed a substantially lower contamination rate of 1.4%, with 98.6% of cultures remaining clean. The reduction in contamination reflects improved aseptic handling and experience over time. Bacterial contamination was identified by milky white exudates and media discolouration, while fungal contamination appeared as white or green mycelial growth, consistent with common microbial contamination in banana tissue culture (Leifert & Cassells, 2001). Comparable studies on banana micropropagation have reported contamination rates ranging from 5% to over 20%, depending on explant source, sterilization protocol, and laboratory practices (Strosse et al., 2006; Rahman et al.,2013). The relatively low contamination rate achieved in Batch 2 therefore indicates effective surface sterilization and improved culture management.

Effect of stage-wise subculturing on shoot proliferation

Stage-wise subculturing is a key practice in *Musa* spp. micropropagation, as transferring explants onto fresh cytokinin-enriched medium at defined intervals enhances shoot proliferation by maintaining optimal nutrient and hormone availability and reducing physiological stress associated with prolonged culture on depleted media (Gerema, 2022). Cytokinin-enriched media such as MS medium with added BAP have been widely shown to promote multiple shoot formation, with regular subcultures increasing the number of shoots generated per explant. Moreover, continuous monitoring for microbial contamination throughout successive subculture cycles is essential to maintain culture health and prevent culture loss, as unchecked contamination can compromise explant viability and shoot development. Reassessment of explant response and contamination incidence ensures high-quality, uniform and disease-free plantlet production during banana shoot multiplication.

Table 2: Number of *Musa acuminata* cv. Berangan (AAA) explants.

Batch	Total explant initiated	Stage	Total Number of Explant (Jar)	Stage	Total Number of Explant (Jar)
1	48	V1	82	V2	147
2	46	V1	73	N/A	N/A

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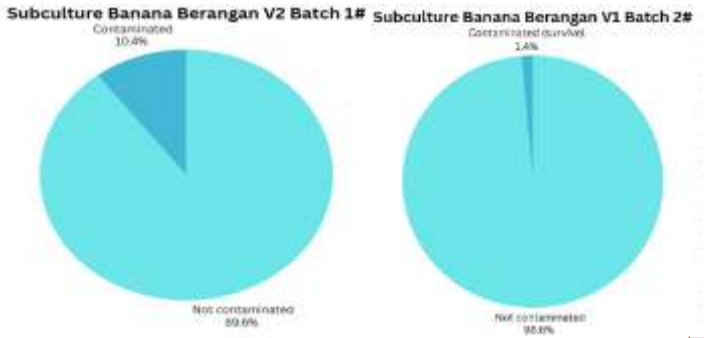

Figure 2: Pie Chart of Subculture *Musa acuminata* cv. Berangan (AAA).

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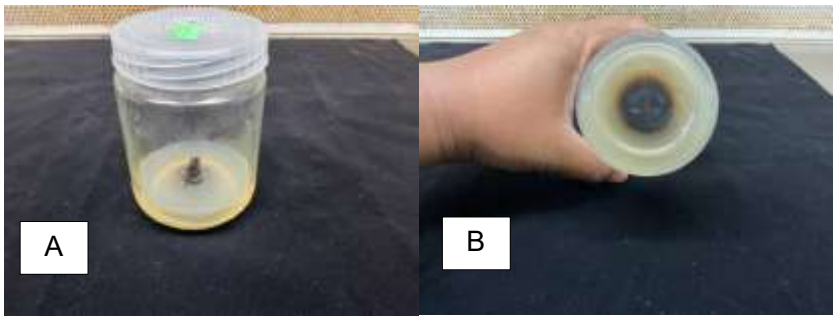
Rescue of contaminated cultures

Phenolic oxidation and microbial contamination reduced explant viability, sometimes indicating internal infection (Cassells, 2012). Contaminated vessels were identified and isolated, and rescue was performed under strict aseptic conditions in a laminar airflow cabinet (George et al., 2008).

Bacterial contamination appeared as milky white exudates and media discoloration, while fungal contamination showed white or green mycelial growth (Figure 3). A small proportion (1.4%) of cultures were rescued by excising infected tissues and transferring explants to fresh MS medium with BAP. Successful rescue was confirmed by renewed shoot growth; otherwise, cultures were discarded (Leifert & Cassells, 2001).

Early-stage contamination can be partially managed when the meristem remains intact, and improved aseptic technique is key to reducing contamination (Cassells, 2012).



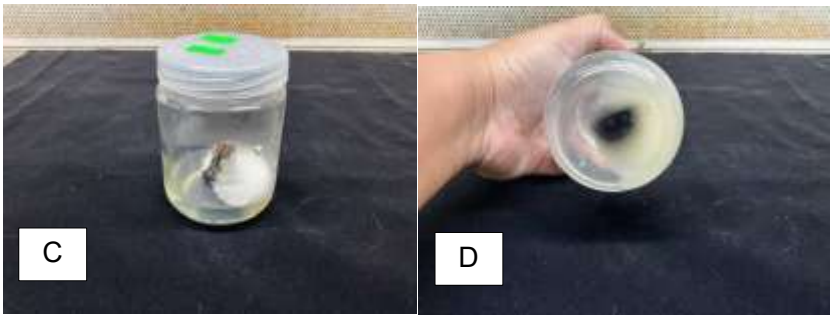


Figure 3: (A-D) Contaminated Explants of *Musa acuminata* cv. Berangan (Aaa). (A-B) Explant contaminated by bacteria; (C-D) Explant contaminated by fungus.

Limitations

Despite the positive outcomes, this study has certain limitations. The investigation was primarily observational and focused on short-term multiplication performance rather than long-term genetic stability or field performance of regenerated plantlets. Additionally, microbial contamination was categorized based on visual symptoms, and no molecular or microbiological identification of contaminants was conducted. Future studies incorporating pathogen identification, larger sample sizes, and assesment of somaclonal variation would further strengthen the reliability and applicability of the micropropagation protocol.

Conclusion

The successful *in vitro* propagation of *Musa acuminata* cv. Berangan (AAA) was achieved by using suitable protocol for sterilization and propagation was done by using Murashige and Skoog (MS) medium supplemented with optimized plant growth regulators. This resulted in high explant survival and the production of clean culture. This protocol is practical and reliable for banana micropropagation and supports the production of uniform, disease-free planting materials that could be used for further research and commercial use.

Future studies should focus on optimizing rooting and acclimatization stages, assessing the genetic fidelity of regenerated plantlets, and evaluating field performance under different agro-environmental conditions. Such investigations would further validate the protocol and support its large-scale application in banana improvement and production programs

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