

Research Article

Effect of 6-Benzylaminopurine (6-BAP) on Shoot Proliferation in Micropropagated Cavendish Banana (*Musa acuminata*)

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Plant development and growth are significantly influenced by plant hormones. 6-benzylaminopurine (6-BAP), a type of cytokinin hormone, is known to influence plant growth, development, and cell division. This study aims to determine the optimal concentration of 6-BAP for promoting shoot proliferation and growth under the tissue culture of Cavendish banana (*Musa acuminata*) in vitro. Suckers of 1 month old were used as explants, and two rounds of subculturing were conducted. The first subculture showed that banana explants treated with 3.0-mg/L 6-BAP produced an average of 4.17 shoots, significantly higher than other treatments under solid media conditions. The second subculture showed that the optimal concentration was 2.5 mg/L, yielding an average of 14.67 shoots per explant. For rooting, MS medium was supplemented with 1.0-mg/L indole-3-acetic acid (IAA), which significantly promoted rooting. The study demonstrates that 6-BAP significantly enhances shoot proliferation in Cavendish banana tissue culture, with the optimal concentration for initial shoot proliferation being 3.0 and 2.5 mg/L for multiple shoot generation. These findings hold significant implications for commercial banana propagation, as they can reduce the time and cost associated with plantlet production while ensuring uniformity and high multiplication rates, which are key factors in meeting the growing demand for disease-free and high-yielding planting material.

Keywords: 6-benzylaminopurine (6-BAP); Cavendish banana (*Musa acuminata*); cytokinin; micropropagation

1. Introduction

Cavendish banana (*Musa acuminata*) is a staple fruit crop globally, valued for its nutritional benefits and economic importance [1]. Bananas rank fourth among major food crops globally, after rice, wheat, and maize [1–3]. Between 2000 and 2017, global banana production increased annually by 3.2%, growing from 67 million to 114 million tons [4]. Cambodia is one of the Asian countries that produces large amounts of bananas, which are trending internationally in the region, especially in China [5, 6], and are also exported to

Korea, Japan, the Russian Federation, the United States, and European countries [4, 7–9]. Bananas are propagated using both in vitro (tissue culture) and traditional methods. However, traditional and conventional propagation methods are often insufficient to meet the demand due to their slow multiplication rate and susceptibility to diseases [10, 11]. Tissue culture techniques, specifically micropropagation, offer a promising alternative by enabling the rapid production of disease-free planting material [11–13].

Plant growth regulators (PGRs), particularly cytokinins, play a crucial role in the regulation of plant growth and

development [14–16]. Among these, 6-benzylaminopurine (6-BAP) is a synthetic cytokinin widely used in plant tissue culture due to its efficacy in promoting cell division, differentiation, and shoot proliferation [17, 18]. In the context of micropropagation, 6-BAP has been extensively studied for its ability to enhance shoot multiplication, which is essential for the large-scale propagation of many economically important crops [18–20].

The use of 6-BAP in banana tissue culture has shown significant potential for stimulating shoot proliferation. Previous studies have indicated that the application of 6-BAP can lead to a marked increase in shoot multiplication rates, thereby improving the efficiency of banana propagation [20–22]. However, the optimal concentration of 6-BAP for maximizing shoot production in *M. acuminata* remains to be precisely determined. This study aims to investigate the effect of different concentrations of 6-BAP on the shoot multiplication of Cavendish banana explants in vitro. By identifying the optimal conditions for 6-BAP application, this research seeks to enhance the efficiency of *M. acuminata* micropropagation, contributing to improved cultivation practices and greater availability of high-quality planting material.

2. Materials and Methods

Explants were collected from Mondulakiri Province, Cambodia (Figure 1). Twenty-three healthy banana suckers that were precisely 30 days old were selected for this study. These explants were chosen based on specific morphological criteria: (1) a height range of 0.40–0.80 cm, (2) the presence of 2–3 fully expanded leaves, and (3) visibly healthy tissue with no signs of damage, disease, or dehydration. The use of 1-month-old suckers was based on preliminary observations, indicating that plants at this stage exhibit stable growth patterns and optimal responsiveness to in vitro culture conditions. Subcultures were conducted to assess the impact of different concentrations of 6-BAP on shoot proliferation over a period of 30 days.

2.1. Explants Undergo Surface Sterilization. The 23 explants removed the outer sheaths of the sucker by keeping only suckers and cleaning with tap water to remove the soil residues. Explants measuring 8×15 cm were washed under running tap water for 15–20 min to remove small particles. The suckers running with tap water are immersed in 70% ethanol for 1 min, rinsed with sterile distilled water 3 times, and shaken with 2% sodium hypochlorite for 10 min, then rinsed with sterile distilled water 3 times to remove sodium hypochlorite. The twenty-three suckers were brought to laminar airflow to remove outer leaf sheaths, then only the central cylinder of rhizome (true stem) with a size of 2×2 cm was selected to culture on medium (Figure 2).

2.2. Initiation Stage. The 23 sterilized explants were inoculated in full-strength Murashige and Skoog (MS) medium containing 3% (w/v) and 0.4% (w/v) of agar gel for primary culture without adding any hormones (Figure 2) [23, 24]. After 30 days in primary culture, banana explants



FIGURE 1: Mapping of the sample size from Mondulakiri Province, Cambodia.



FIGURE 2: The central cylinder of a banana sucker was tipped on medium.

were ready to transfer for multiplication stages in secondary culture (Figure 3(b)).

2.3. Multiplication Stage and Rooting Stage. The secondary culture was cultured for two cycles, which were 24 explants for the first cycle and 36 explants for the second cycle. The first cycle included four treatments of 6-BAP concentrations: 0, 3.0, 5.0, and 7.0 mg/L, where each treatment condition was applied to six replicates. The second cycle included six treatments of 6-BAP concentrations: 0.0, 1.5, 2.5, 3.5, 4.5, and 5.5 mg/L, where each treatment condition was applied to six replicates. Multiple shoots were separated from the cluster as a single shoot and inoculated into MS medium supplemented with indole-3-acetic acid (IAA) at 1 mg/L for rooting. Each stage was cultivated for 30 days for data collection. To minimize fungal contamination and improve reproducibility, explants were first washed following the protocols in the Materials and Methods part. All procedures following the surface sterilization were carried out under a laminar flow hood, where instruments and the working area were presterilized with 70% ethanol and UV light exposure for 20 min. Moreover, the explant treatments were selected to be healthy and fresh. Though no additional antifungal agents were used, this combined approach effectively minimized fungal contamination.

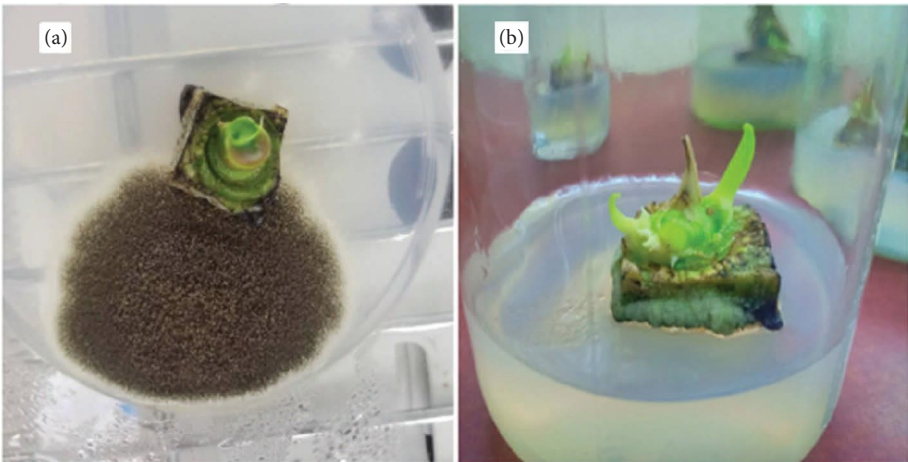


FIGURE 3: *M. acuminata* explants were contaminated by fungi. (a) Contaminated by fungi after 2 days of inoculation and (b) green buds appeared.

TABLE 1: The initiation stage of *M. acuminata*.

Full MS medium	Average	Standard deviation	Maximum	Minimum
Shoot numbers	1.64	1.86	8	1
Shoot height (cm)	2.21	1.38	5.5	1.00

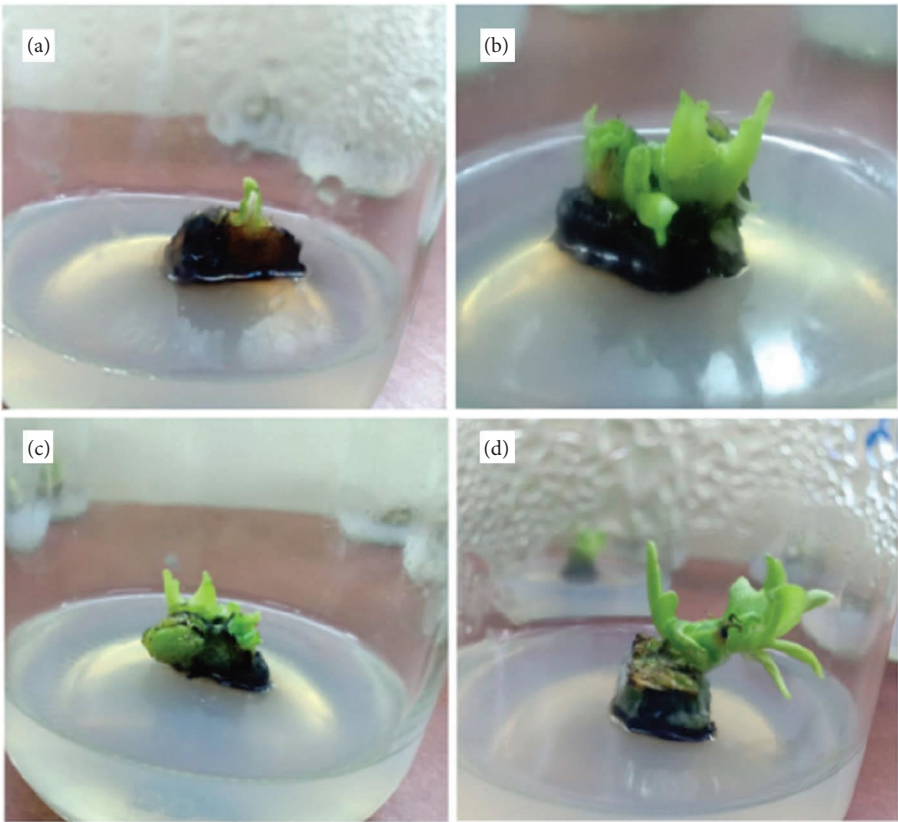


FIGURE 4: The appearance of *M. acuminata* growing under a concentration of 6-BAP for 30 days: (a) 0 mg/L, (b) 3 mg/L, (c) 5 mg/L, and (d) 7 mg/L.

TABLE 2: The 1st cycle of subcultured *M. acuminata* for 30 days.

6-BAP concentration (mg/L)	Shoot height (cm)	Shoot numbers	Leaf numbers	Root numbers
0	1.90 ± 0.66 ^a	1.33 ± 0.58 ^c	3.50 ± 0.71 ^a	0.00 ± 0.00 ^a
3	1.43 ± 0.71 ^a	4.17 ± 1.47 ^a	3.83 ± 1.72 ^a	0.00 ± 0.00 ^a
5	1.55 ± 0.34 ^a	3.83 ± 1.94 ^a	2.33 ± 0.58 ^c	0.00 ± 0.00 ^a
7	1.02 ± 0.23 ^a	2.83 ± 0.75 ^b	3.00 ± 1.41 ^b	0.00 ± 0.00 ^a

Note: Values (Mean ± SE) followed by the same notation in a column are not significantly different by Tukey's test at $p \leq 0.05$. Values in the same column with different superscript letters indicate a significant difference at $p \leq 0.05$.

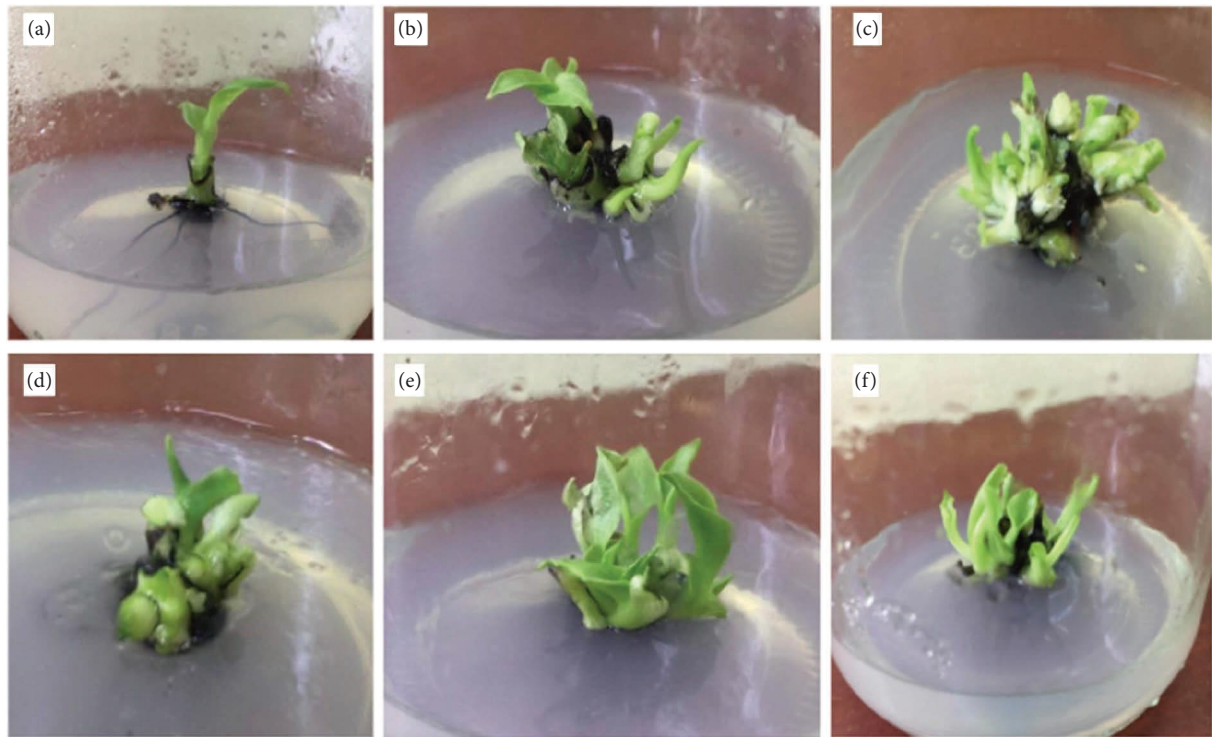


FIGURE 5: The appearance of *M. acuminata* growing under concentrations of 6-BAP for 30 days: (a) 0.0 mg/L, (b) 1.5 mg/L, (c) 2.5 mg/L, (d) 3.5 mg/L, (e) 4.5 mg/L, and (f) 5.5 mg/L.

TABLE 3: The 2nd cycle of subcultured *M. acuminata* for 30 days.

6-BAP concentration (mg/L)	Shoot height (cm)	Shoot numbers	Leaf numbers	Root numbers
0	2.28 ± 0.36 ^a	1.75 ± 0.75 ^c	3.67 ± 0.33 ^c	4.67 ± 0.67 ^a
1.5	1.62 ± 0.15 ^a	7.33 ± 1.23 ^b	3.60 ± 0.51 ^c	0.00 ± 0.00 ^b
2.5	1.28 ± 0.08 ^b	14.67 ± 0.49 ^a	2.75 ± 0.63 ^c	0.00 ± 0.00 ^b
3.5	1.57 ± 0.23 ^a	11.17 ± 3.83 ^a	4.20 ± 1.24 ^b	0.00 ± 0.00 ^b
4.5	1.58 ± 0.26 ^a	10.00 ± 2.02 ^a	6.00 ± 4.00 ^a	0.00 ± 0.00 ^b
5.5	2.00 ± 0.17 ^a	6.67 ± 0.99 ^b	3.25 ± 0.48 ^c	0.00 ± 0.00 ^b

Note: Values (Mean ± SE) followed by the same notation in a column are not significantly different by Tukey's test at $p \leq 0.05$. Values in the same column with different superscript letters indicate a significant difference at $p \leq 0.05$.

2.4. Acclimatization or Hardening. The number of plantlets that were acclimatized was 50. The treatments of organic medium, namely, husk charcoal, sand, and compost (1:1:2 ratio), were carried out using the pot tray with 50 individual cells, length 54 cm and width 28 cm. Each hole was 4.9 cm square and 4.5 cm deep, with drainage holes at the bottom of each cell to ensure proper water flow.

2.5. Data Collection and Analysis. The experiment was conducted over five stages of tissue culture, from Stage 0 to Stage 4. The subculture conducted two cycles. In each cycle, the following parameters were measured: shoot number, leaf number, shoot height (cm), and root number. The statistical analyses of the gathered data were performed using analysis of variance (ANOVA). These computations were done using

FIGURE 6: *M. acuminata* rooting stage for 30 days.TABLE 4: The rooting stage of *M. acuminata* for 30 days.

Full MS +1 mg/L IAA	Average	Standard deviation	Maximum	Minimum
Shoot height (cm)	5.22	0.85	6.50	3.00
Root number	4.28	0.93	7	3
Leaf number	3.88	1.22	7	2

the statistical software program (IBM SPSS Version 23). Tukey's honest significant difference test was used to compare means at the $p \leq 0.05$ level of significance.

3. Results and Discussion

The effect of different concentrations of 6-BAP on *M. acuminata* explants was investigated. The findings from this study will provide valuable insights into the optimal use of 6-BAP for banana micropropagation, with implications for both research and commercial production.

3.1. Initiation Stage. After 30 days in all types of media, the response, proliferation, and multiplication of explants were observed and recorded (Table 1). Within the first 2 days after inoculation, 9 (39.13%) of *M. acuminata* explants were contaminated by fungi (Figure 3(a)). Despite these contamination challenges, some explants remained healthy and showed initial signs of response and proliferation by the end of the second week. These results underscore the importance of stringent aseptic techniques and possibly the need for additional antifungal and antibacterial treatments in the inoculation protocol to improve the success rate of *M. acuminata* explant cultivation [25]. At the end of the second week, small green buds appeared on the true stem of

the Cavendish banana (Figure 3(b)). The explants are thriving in vitro on MS medium enriched with sucrose as the energy source [26, 27]. The average number of new shoots was 1.64, and the shoot height was 2.21 cm for the 23 shoot tips that were planted (Table 1).

3.2. Multiplication Stages. For the first cycle of the experiment, while media containing only the control medium generated individual shoots, it was shown that media containing only 6-BAP caused shoot bud multiplication. The observation that 6-BAP induces shoot bud proliferation aligns with the findings from various studies on plant tissue culture. For instance, Siamak et al. [28] indicated that cytokinin, particularly 6-BAP, plays a critical role in promoting shoot multiplication in numerous plant species, including bananas (*Musa* spp.). On the other hand, the use of high concentrations of 6-BAP (less than or equal to 10) inhibits the formation of shoots [29]. The lowest shoot number of 2.83 ± 0.75 was marked in medium, which supplied 7 mg/L of 6-BAP. The highest number of shoots was 4.17 ± 1.47 and was observed in a medium supplied with 3 mg/L of 6-BAP (Figure 4 and Table 2). This study provides a solid foundation for understanding the optimal concentrations of 6-BAP required for effective shoot proliferation in *M. acuminata* explants.



FIGURE 7: The appearance of the *M. acuminata* growth performance. (a) Rooting plants in vitro; (b) transplanting husk charcoal, sand, and compost (1:1:2); (c) healthy plantlet prepared to be placed in a plastic bag; and (d) plantlets placed in plastic bags after 35 days.

Second cycle: For the second cycle of the experiment, developed shoots of *M. acuminata* were cultured in MS medium with varying concentrations of 6-BAP. The results indicated that shoot multiplication was most effective in MS medium containing 2.5 mg/L of 6-BAP, with a notable response observed after 30 days (14.67 ± 0.49 shoots). According to Shalini et al. [30], the optimal concentration of 6-BAP for shoot proliferation in banana cultivars was found to be between 2 and 4 mg/L. Their research highlighted that lower concentrations facilitate better shoot growth, with significant increases in the number of shoots per explant. In contrast, shoots grown in MS medium, supplemented only with agar, demonstrated a better overall response in terms of root development, including the number of roots per shoot (4.67 ± 0.67) and root lengths. However, the response time for these shoots was longer compared to those grown in the presence of 6-BAP [29]. The shoot height was 2.28 ± 0.36 cm, and the leaf number averaged 3.67 ± 0.33 (Figure 5 and Table 3).

3.3. Rooting Stages. At this stage of root development, the forty single buds of *M. acuminata* are transferred to MS media supplemented with 1 mg/L IAA for 30 days. The result showed that the length of the banana plants was 5.22 cm, the root number was 4.28, and the leaf number was 3.88 for the rooting and elongation stage (Figure 6 and Table 4). The use of IAA in promoting root development in *M. acuminata* aligns with established findings in plant tissue culture

research. IAA, a common auxin, is known to enhance root induction and elongation when used in appropriate concentrations [21].

3.4. Acclimatization or Hardening. The 50 treatments from the rooting stage of single plantlets were transferred to a wet organic medium, namely, husk charcoal, sand, and compost (1:1:2 ratio). The healthy plantlet with a shoot height of 12 cm and 6 leaves survived 100% after 25 days. It was demonstrated that plantlets of banana varieties transferred to organic media mixtures show a high survival rate and growth [31]. After that, transfer the plantlets to black plastic bags (6 cm square and 16 cm high) with soil, husk charcoal, and dry cow dung (2:1:1 ratio) to maintain the growth rate for the field cultivation. After 35 days, the plantlet grows to a height of 25 cm with 6 leaves (Figure 7).

4. Conclusion

In conclusion, the ideal conditions for *M. acuminata* shoot multiplication were found to be 3 mg/L during the first cycle and 2.5 mg/L during the second cycle of 6-BAP. Conversely, without 6-BAP inhibiting shoot formation, the number of shoots was the lowest. Unlike previous studies that used a narrow range of 6-BAP concentrations or lacked sub-culture analysis, our two-cycle experimental design evaluated both the initiation and multiplication phases, identifying optimal concentrations for each stage with

robust statistical validation. These findings suggest that lower concentrations of 6-BAP facilitate better shoot proliferation and growth, providing a balanced approach to optimizing micropropagation protocols. During the rooting stage, using 1 mg/L of IAA significantly enhanced root development. Acclimatization showed a 100% survival rate in plantlets transferred to an organic medium, demonstrating a successful transition to conditions suitable for field cultivation. These findings provide valuable insights for optimizing banana micropropagation protocols, benefiting both research and commercial production.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

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