Research article

Effects of Benzyl Adenine and Thidiazuron on Shoot Regeneration of Cavendish Banana

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Keywords

Cavendish banana;
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Abstract

Banana (*Musa* spp.) is one of the most important fruit crops in the world in terms of consumption and production. Commonly, bananas are propagated using conventional and *in vitro* techniques. Conventional methods generally involve vegetative propagation by the use of suckers, which is a time-consuming technique and is prone to various diseases. To overcome these problems, tissue culture technique has played a major role. With this technique, plant growth regulators are used to help improve rapid propagation for large-scale production. The plant growth regulators, benzyl adenine (BA) and thidiazuron (TDZ), which are both cytokinins, have gained attention for their effects on plant growth and development in tissue culture. In the present study, the effects of BA and TDZ on the shoot multiplication of Cavendish bananas *in vitro* were investigated. There were two experiments performed due to their being two types of explants obtained: one-month-old banana plantlets and one-month-old banana clumps. Culture I used one-month-old plantlets, and Culture II used one-month-old clumps. The results revealed that there was no significant difference in shoot formation between BA and TDZ at any concentration. However, the optimum concentration of BA was found to be 3 ppm for plantlets (Culture I), which resulted in 1.60 new shoots, and 4 ppm for clumps (Culture II) producing 4.00 shoots. In the case of TDZ, 2 ppm which produced 2.60 shoots, was the optimum concentration for both types of explants. In addition, the study suggested that clumps were the most suitable choice for banana multiplication. Further investigation focusing on the effects of explant morphology and cutting technique on plant growth is recommended.

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1. Introduction

Banana (*Musa* spp.) is one of the most important fruit crops in the world in terms of consumption and production, and there are more than a thousand varieties of bananas that are produced and consumed locally around the world [1]. Available statistical data illustrates that between 2000 and 2017, there was a 3.2% annual rate of increase of global banana production, which was an increase from 67 million tons and 114 million tons [2]. According to the FAO report for 2023, there was a dramatic increase from 1.2 million tons to 19.1 million tons between 2021 and 2022 in global export quantities [3-5]. Approximately 5.6 million hectares of land were dedicated to plantain production globally in 2017 [6]. Asia, Latin America, and Africa are the predominant areas of bananas production and the largest producers are India and China, which accounted for 29 million tons per year and 11 million tons per year, respectively, over the period of 2010 and 2017 [2, 5, 7]. However, the production in these two massive Asian countries mostly serves domestic demand. Other large producers are the Philippines (7.5 million tons), Ecuador, and Brazil (7 million tons) [2-5], and Indonesia, Thailand, Mexico, Costa Rica, and Colombia [7, 8]. Banana ranks fourth on the world's list of major food crops after rice, wheat, and maize [8, 9]. The biggest banana exporters are Ecuador, the Philippines, Costa Rica, Guatemala, and Colombia [10]. In contrast, the biggest importers are European Union, United States, Russian Federation, Japan, and China [3-6].

Cavendish is the most commercialized banana cultivar and is dedicated to around half of the world's production. This cultivar is able to reach high yields per hectare and is less prone to damage from environmental influences such as storms due to its short stems [11]. In general, commercial Cavendish banana production yields between 40 and 50 tons per hectare, or 60 tons for well-established industries such as the Philippines and India. Meanwhile, smaller producers can produce only around 30 tons per hectare [2-5, 7].

Currently, Cambodia has exported Cavendish bananas to the Chinese market through a Vietnamese company (Hoang Anh Gia Lai). The company owns 1,000 hectares of banana farmland, and they have shipped about 240,000 tons of the product [12]. Moreover, in August 2018, Cambodia and China signed a protocol of phytosanitary requirements, which made way for yellow banana exports from the Kingdom to China [13, 14]. Commonly, bananas are cultivated using conventional techniques and *in vitro*. Conventionally, bananas propagate vegetatively through suckers (young banana shoots) [15]. However, using traditional methods is time-consuming and the produce is prone to various diseases [15, 16]. To illustrate, only 5 to 10 suckers can be produced per plant per year. To overcome these problems, tissue culture technique has played a major role, and plant growth regulators are used to help improve rapid propagation for large-scale production [16]. Two plant growth regulators, i.e. BA and TDZ, which are both cytokinins, have gained attention in the tissue culture field for their effects on plant growth and development [17-19]. Hence, this present study aimed to investigate *in vitro* effects of various concentrations of BA and TDZ applied individually on the shoot multiplication of Cavendish bananas.

2. Materials and Methods

2.1 Explants

There were two types of purchased explants used for Culture I and Culture II. For Culture 1, one-month-old plantlets being cultured *ex vitro,* and 1 to 1.5 cm of the basal part of the plants were cut and used (Figure 1a) and 1 × 1 cm in size of one-month-old clump explant was used for multiplication in Culture II (Figure 1b). The original medium was benzyl adenine (BA) at 4 ppm with a combination of indole-3-acetic acid (IAA) at 2 ppm [18, 19]. The explants were transferred

to the medium vertically. They were cultured in the culture room at 25°C for a photoperiod of 16 h under fluorescent light [20, 21] at approximately 2480 lux. Each treatment was done only once, with five replicates.

2.2 Culture and medium preparation

The 4.4 g/L of Murashige and Skoog medium, including vitamin (Duchefa Biochemie), and 30 g/L of sucrose (Kaset Phol Sugar Ltd.) [21]. The medium was supplemented with benzyl adenine (BA) at concentrations of 0, 2, 3, 4, and 5 ppm, and thidiazuron (TDZ) at 0, 0.2, 0.5, 1, and 2 ppm [22, 23]. All media were adjusted to pH 5.8 [21] either with 1N sodium hydroxide or 1N hydrochloric acid followed by the addition of gelrite (3 g/L) [20], and then transferred into 50 mL glasses (glass size, height: 12 cm, diameter: 8 cm) for autoclaving at 121°C with 0.22 MPa for 15 min. The control was prepared in the same manner, except for the exclusion of hormones.

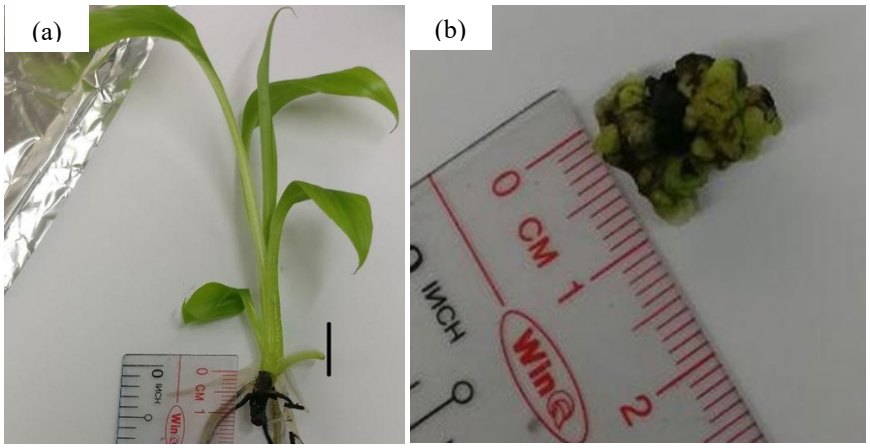

Figure 1. (a) 1-1.5 cm of shoots of Cavendish banana cut from the base; the bar indicates the base; (b) one-month-old clump explant 1×1 cm size

2.3 Data collection and analysis

After 1 month of culture, the following parameters were measured: shoot numbers, leaf number, highest shoot height (cm), and root height (cm). A shoot or leaf was counted when it was 0.5 cm long. Analysis of variance (ANOVA) was used for statistical analyses of the collected data. These computations were done using the statistical software program, IBM SPSS version 23. Tukey's honest significant difference test was used to compare means at $p \leq 0.05$ level of significance.

3. Results and Discussion

The results of Culture I and Culture II are illustrated together based on the parameters observed. The shoot numbers, leaf numbers, shoot heights, and root heights of Culture I and Culture II in response to the effect of BA and TDZ after one month of culturing are summarized in Tables 1-4. A study conducted by Backiyarani *et al*. [24] to investigate the effect of BA concentration at 1, 2, 3, 4, 5, and 6 ppm on shooting induction found that the optimum concentration of BA for shooting stimulation was 6 ppm. Another test was conducted to observe the effects on shoot multiplication

on three banana types (cultivar Alanya 5, Anamur 10, and Bozyaki 14) of BA at 1.2, 2.5, 4.5, and 6.75 ppm and TDZ at 0.01, 0.2, 0.4, and 0.6 ppm [25]. The study revealed that shoot proliferation and elongation were significantly greater with TDZ than BA for all three cultivars. In addition, BA below 4.5 ppm or TDZ below 0.2 ppm did not increase shoot proliferation [25]. A study on the effect of various levels of cytokines BA at 0, 1.5, 2.5, 3.5, 4.5, 5.5, and 6.5 ppm on shoot proliferation using the cultivars Grand Naine and Jahaji was conducted [21]. The result showed that 4.5 ppm of BA was the optimum concentration, which produced 7.2 buds (49.1 days, cultivar Grand Naine) and 7.05 buds (49.1 days, cultivar Jahaji) [21].

Table 1. The plantlets of Cavendish banana affected by BA after 1 month of culturing

Plantlets (Culture I)				
BA Concentration (ppm or mg/L)	**Shoot Numbers**	**Leaf Numbers**	**Shoot Height (cm)**	**Root Height (cm)**
0 ppm	1.20±0.20^{a}	4.40±0.40^{a}	11.40±2.40^{a}	7.20±0.58^{a}
2 ppm	0.80±0.20^{a}	3.80±0.97^{a}	4.40±1.12^{b}	6.40±2.11^{a}
3 ppm	1.60±0.60^{a}	4.65±1.08^{a}	4.40±1.25^{b}	5.40±1.43^{a}
4 ppm	0.65±0.25^{a}	2.00±0.84^{a}	2.40±0.98^{b}	2.60±0.68^{a}
5 ppm	1.40±0.40^{a}	4.60±0.60^{a}	4.60±0.75^{b}	4.80±1.07^{a}

Values (Mean±SE) followed by the same notation in a column are not significantly different by Tukey's test at $p \leq 0.05$

Table 2. The clumps of Cavendish banana affected by BA after 1 month of culturing

Clumps (Culture II)				
BA Concentration (ppm or mg/L)	**Shoot Numbers**	**Leaf Numbers**	**Shoot Height (cm)**	**Root Height (cm)**
0 ppm	3.20±0.37^{a}	10.20±1.77^{a}	3.20±0.98^{a}	10.60±2.50^{a}
2 ppm	3.20±0.49^{a}	5.80±1.24^{b}	1.20±0.20^{b}	0.00±0.00^{b}
3 ppm	3.60±0.93^{a}	8.40±2.16^{b}	1.20±0.34^{b}	0.60±0.60^{b}
4 ppm	4.00±0.76^{a}	7.20±0.86^{b}	1.20±0.26^{b}	0.00±0.00^{b}
5 ppm	3.60±0.93^{a}	7.80±1.80^{b}	1.66±0.33^{b}	1.40±1.40^{b}

Values (Mean±SE) followed by the same notation in a column are not significantly different by Tukey's test at $p \leq 0.05$

Table 3. The plantlets of Cavendish bananas affected by TDZ after 1 month of culturing

Plantlets (Culture I)				
TDZ Concentration (ppm or mg/L)	**Shoot Numbers**	**Leaf Numbers**	**Shoot Height (cm)**	**Root Height (cm)**
0 ppm	1.20±0.20^{a}	4.40±0.40^{a}	11.40±2.40^{a}	7.20±0.58^{a}
0.2 ppm	1.60±0.25^{a}	7.20±1.72^{a}	3.60±0.25^{b}	2.80±1.24^{ab}
0.5 ppm	1.60±0.68^{a}	3.20±0.86^{a}	1.70±0.58^{b}	1.60±0.81^{b}
1 ppm	1.20±0.37^{a}	3.40±1.03^{a}	2.60±1.21^{b}	1.20±1.20^{b}
2 ppm	2.20±0.97^{a}	4.80±1.46^{a}	2.80±1.07^{b}	3.22±1.38^{ab}

Values (Mean±SE) followed by the same notation in a column are not significantly different by Tukey's test at $p \leq 0.05$

Table 4. The clumps of Cavendish banana affected by TDZ after 1 month of culturing

Clumps (Culture II)				
TDZ Concentration (ppm or mg/L)	**Shoot Numbers**	**Leaf Numbers**	**Shoot Height (cm)**	**Root Height (cm)**
0 ppm	3.20±0.37^{a}	10.20±1.77^{a}	3.20±0.98^{a}	10.60±2.50^{a}
0.2 ppm	2.40±0.68^{ab}	4.00±1.67^{b}	1.30±0.46^{ab}	0.60±0.60^{b}
0.5 ppm	1.20±0.49^{ab}	1.80±0.86^{b}	0.50±0.22^{b}	0.00±0.00^{b}
1 ppm	0.80±0.37^{b}	1.00±0.45^{b}	0.60±0.25^{b}	0.00±0.00^{b}
2 ppm	2.60±0.68^{ab}	3.00±1.00^{b}	0.60±0.25^{b}	0.00±0.00^{b}

Values (Mean±SE) followed by the same notation in a column are not significantly different by Tukey's test at $p \leq 0.05$.

3.1 Shoot formation

The success factor in multiplication for both BA and TDZ was the appearance of small green buds on the side in the case of clump Culture II (Figures 2 and 3). The plant clump morphology may have a high density, which implies that the plant clumps are closely packed together, possibly with numerous plant cells or tissues [26]. Based on the result, 3 ppm of BA produced 1.60±0.60 shoots in Culture I, which seemed to be the optimum concentration. While in Culture II, 4 ppm of BA produce 4.00±0.78 shoots, and it was thus optimum. However, the mean shoot members for both Culture I and Culture II were not significantly different (Tables 1 and 2). For Culture I (plantlets), TDZ at 2 ppm induced the most shoots, which was 2.20±0.97 shoots, but there was no significant difference when compared to other concentrations. For Culture II (Clumps), there was a significant difference. The 0 ppm of TDZ induced the most shoots, which was 3.20±0.37, and 2 ppm induced 2.60±0.68, while 1 ppm produced only 0.80±0.37 shoots (Tables 3 and 4). These shoots, manifesting as small buds on the explants, signify the stage of acceptable multiplication in tissue culture, respectively [27].

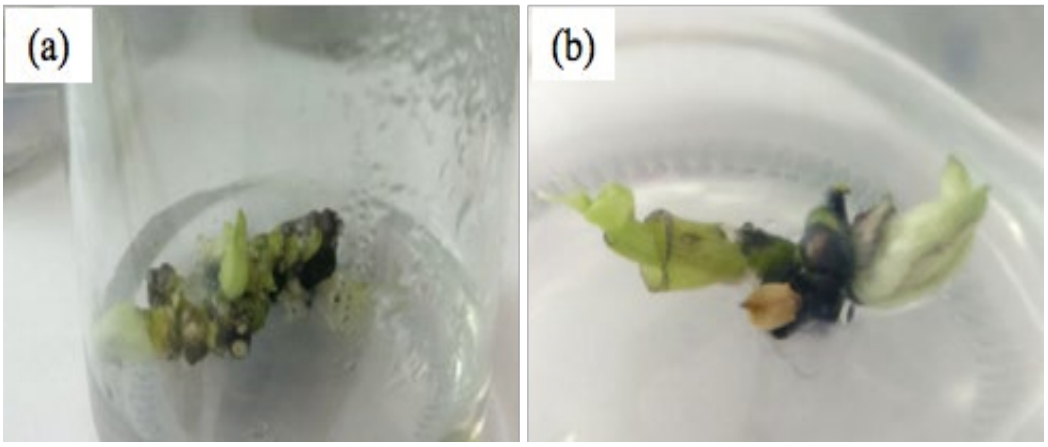

Figure 2. The appearance of the Cavendish banana in Culture I and Culture II.
(a) plantlets (Culture I); (b) Clumps (Culture II)

3.2 Leaf number

For leaf formation Culture I (plantlets), no significant difference was observed, and there were similarities in the leaf numbers for the concentration of 3 ppm, which was 4.65±1.08, and 4 ppm, which was 2.00±0.84 of BA. For Culture II, the trends were the same as for Culture I. There were 8.40±2.16 and 7.80±1.80 leaves for the treatments of 3 and 4 ppm of BA, respectively (Tables 1 and 2). Leaf formation with TDZ treatment was examined for Culture I and Culture II. For Culture I, no significant difference was found, and the observations revealed that the most notable leaf numbers were 7.20±1.72 for 0.2 ppm and 4.80±1.46 for 2 ppm. Culture II showed the same trend. There were 4.00±1.67 and 3.00±1.00 leaves produced by 0.2 ppm and 2 ppm, respectively. However, these findings were significantly lower than leaf number for 0 ppm TDZ, which produced 10.20±1.77 leaves (Table 3 and 4). This was in agreement with Dönmez *et al*. [28] who observed the greatest numbers of plant leaves in plant tissue cultured in full MS medium without a hormone supplement.

3.3 Shoot height

Based on the results, the difference between the treatments showed that there was a significant difference with the control for both Culture I and Culture II, which were 11.4± 2.40 and 3.20±0.98, respectively. However, there was 4.60±0.75 of shoot height observed with 5 ppm of BA, which seemed the optimum one but without any significant difference when compared to other concentrations. Culture II also showed a similar trend, which was a shoot height of 1.66±0.33 with 5 ppm of BA. The use of BA at a concentration of 5 ppm was proven to be effective in enhancing vegetative growth parameters, particularly in terms of shoot height [29]. For Culture I, 2 ppm TDZ gave the highest value of shoot height of 3.60±0.25 cm, but this was not significantly different to height observed for other concentrations of TDZ. Moreover, Culture II at 0.2 ppm TDZ produced the greatest shoot of 1.30±0.46 cm. However, there was no significant difference when compared to other concentrations.

3.4 Root height

The effects of BA on root height were also considered. For Culture I, 2 ppm BA induced longer roots (6.40±2.11 cm) than other concentrations, excluding the control. However, this result was not significantly different to that of other BA concentrations. For Culture II, the greatest root height observed was 1.40±1.40 cm at 5 ppm BA, while 2, 3, and 4 ppm of BA did not induce any roots at all, but all results were noy significantly different. For TDZ, the control of both Culture I and II performed the best, which were 7.20±0.58 cm and 10.60±2.50 cm, respectively. These results reveal the effects on root formation caused by the TDZ hormone. For both cultures, the control performed the best. In case of Culture I, 0.2 and 2 ppm TDZ were the best hormone treatments, producing roots of heights of 2.80±1.24 and 3.22±1.38 cm, respectively, which were not significantly different. For Culture II, 0.2 ppm TDZ induced a root height of 0.60±0.60 cm, which was not a significantly different result to that of the other concentrations which did not form any roots at all. These results strongly indicated that cytokines did not induce roots [29, 30], while roots were induced in the control. Therefore, as shown in Tables 1-4, the hormone-free medium produced the most shoot and leaf formation, and shoot, and root height. This was because the original media contained the hormones BA (4 ppm) and IAA (2 ppm), which were still present as essential nutrients in the clumps, [31]. These essential nutrients might be responsible for improved the growth performance of root height.

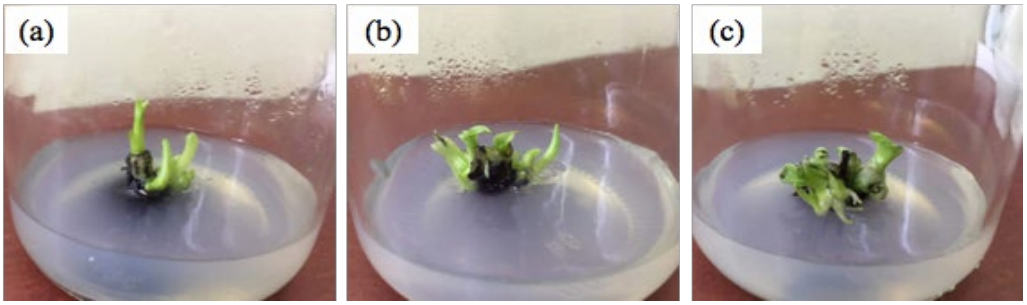

Figure 3. The appearance of the Cavendish banana.
(a) clumps in MS media; (b) clumps in TDZ media; (c) clumps in BA media

4. Conclusions

Based on the results obtained herein, there was no significant difference observed in the effects of each concentration of BA and TDZ on shoot formation, whereas full MS media had a positive effect on the shoot and leaf number, shoot, and root height, effectively. This important finding of the study will help minimize the cost of the plant tissue culture process. However, according to our observation of the explants of plantlets and clumps for each dose of BA or TDZ, the clumps are the most suitable choice for banana multiplication for further investigation. In addition, the selection of explant morphology and the technique of cutting may also affect plant growth.

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