

Effect of Thidiazuron Concentration on In Vitro Shoot Initiation and Multiplication of Banana cv. Robusta

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ABSTRACT

Banana (*Musa spp.*) is one of the most economically important fruit crops cultivated worldwide. The problem of availability of disease-free planting material is still a great challenge in sustainable banana production because of the presence of pests and pathogens and the low multiplication rate of conventional propagation techniques. Micropropagation is an efficient method for fast multiplication of elite cultivars. The present study was designed to assess the effect of various concentrations of thidiazuron (TDZ) on shoot initiation and multiplication of banana cv. Batisa. Under vitro conditions, Batisa is bathed. The shoot tip explants from healthy sword suckers were cultured on Murashige and Skoog (MS) medium containing TDZ at 0.1, 1.0, 2.0 and 3.0 mg L⁻¹. After 8 weeks of culture, various growth parameters such as explant survival, days to shoot initiation, number of shoots per explant, shoot length and fresh biomass were recorded. The highest survival percentage (95%) and maximum shoot length (5.50 cm) were obtained with 2.0 mg L⁻¹ TDZ while the highest shoot multiplication rate (4.50 shoots per explant) was obtained with 2.5 mg L⁻¹ TDZ among the tested concentrations. The shortest duration for shoot induction was observed at 3.0 mg L⁻¹ TDZ. The results suggest that the use of TDZ has a significant effect on shoot organogenesis in banana cv. Batisa and can be used effectively for large scale clonal propagation.

Keywords: Banana, Batisa, Tissue Culture, Thidiazuron, Micropropagation, Shoot Multiplication, *Musa spp.*

1. Introduction

Banana (*Musa spp.*) is one of the most widely grown fruit crops in the world and plays an important role in food security in many developing countries. India is the world's largest producer of bananas accounting for about 26%-28% of the world production [1]. The crop is very productive and is available throughout the year, which makes it an important part of rural livelihoods and agricultural economies [2]. Robusta is a locally important cultivar of banana which is grown in Bihar and adjoining areas due to its good fruit quality and adaptability [3]. But the large-scale cultivation of this cultivar is limited due to lack of healthy planting materials. The propagation by suckers is conventional and has a low multiplication rate, and often pathogens such as fungi, bacteria, viruses and nematodes are spread [4].

The use of plant tissue culture has become a very effective biotechnology for the rapid multiplication of elite cultivars [5,6]. The method allows for the production of large quantities of genetically identical and disease-free plantlets year-round. Several factors are important for successful micropropagation: explant type, culture medium composition, environmental conditions and plant growth regulators [7,8]. Thidiazuron (TDZ) is one of the plant growth regulators that has gained a lot of interest in tissue culture due to its high cytokinin-like activity [9]. TDZ is a relatively low concentration stimulator of cell division, shoot organogenesis and adventitious bud formation [10]. A number of studies have shown that TDZ is more effective than the conventional cytokinins, benzylaminopurine (BAP) and kinetin, in producing better shoot multiplication responses.

Although much work has been done on commercial banana cultivars, there is still a lack of information on the regeneration of local cultivars like Robusta using TDZ. Hence, the present study was conducted to find the best concentration of TDZ for efficient shoot initiation and multiplication of banana cv. Robusta

2. Materials and Methods

2.1 Experimental Site

The present study was conducted in Plant Tissue Culture Laboratory, Department of Botany, TPS College, Patna University, Patna, Bihar, India. The laboratory had a laminar airflow cabinet, autoclave, culture racks with fluorescent lights and temperature-controlled culture room facilities. To reduce microbial contamination, all culture manipulations were carried out under aseptic conditions.

2.2 Plant Material Collection

Sword suckers of banana (*Musa spp.*) cv. are healthy and disease free. Batisa were collected from the local banana growing areas of Bihar. Sword suckers with a weight of around 1.0-1.5 kg and with good growth were chosen as explants. The suckers selected were not showing any signs of fungal, bacterial or viral disease. Collected suckers were brought to the laboratory and processed within 24 h of collection. The outer leaf sheaths were gently peeled back to reveal the central shoot meristem. Explants of about 2-3 cm length with the apical meristematic area were cut and used for culture initiation.

2.3 Surface sterilization of explants

Surface sterilization is an important part of micropropagation of bananas as suckers grown in the field are easily contaminated with microorganisms [11]. The explants were first washed under running tap water for 30 min to wash away soil particles and debris. The explants were then placed in a solution of a few drops of liquid detergent and shaken gently for 10 min. The explants were then washed with distilled water and placed in a laminar airflow chamber for additional sterilization. Under aseptic conditions, explants were treated with 70% ethanol for 30 s followed by immersion in 1–2% sodium hypochlorite solution containing 2–3 drops of Tween-20 for 10–15 min. The explants were washed three to four times with sterile distilled water after sterilization to remove the traces of sterilizing agents. The outer oxidized tissues were cut off with sterile scalpels, and the explants were cut down to about 1-1.5 cm before being placed on the culture medium.

2.4 Preparation of Culture Medium

Murashige and Skoog (MS) basal medium was used for all culture experiments. The medium was composed of macro- and micronutrients, vitamins and organic supplements as per Murashige and Skoog (1962) formulation. The medium was supplemented with:

- Sucrose: 30 g L⁻¹
- Agar (HiMedia): 7–8 g L⁻¹
- TDZ: treated as per treatment

The pH of the medium was adjusted to 5.7 ± 0.1 with 0.1 N NaOH or 0.1 N HCl prior to the addition of agar. About 25–30 mL of medium was poured into the culture jars and then autoclaved at 121°C for 15 min at 15 psi. The culture vessels were then sterilized and left to cool and solidify under sterile conditions prior to inoculation.

2.5 Preparation of TDZ Stock Solution

Thidiazuron (TDZ) was used as the cytokinin source for shoot induction and multiplication [12]. A stock solution of TDZ (1 mg mL⁻¹) was prepared by dissolving the required quantity of TDZ in a small volume of dimethyl sulfoxide (DMSO) due to its limited solubility in water. The stock solution was filter sterilized with a 0.22 µm membrane filter and then aseptically added to the autoclaved media after cooling to about 45–50°C.

2.6 Experimental Treatments

To evaluate the effect of TDZ concentration on shoot initiation and multiplication, six treatments were established using different concentrations of TDZ:

Treatment	TDZ Concentration (mg L ⁻¹)
T1	0.1
T2	1.0
T3	1.5
T4	2.0
T5	2.5
T6	3.0

Each treatment consisted of multiple explants cultured individually in separate culture vessels.

2.7 Inoculation of Explants

Prior to inoculation, the culture jars and instruments were sterilized. The explants of the shoot tips were gently positioned vertically on the surface of the culture medium, ensuring good contact between the basal part of the explant and the medium. Inoculations were carried out in a laminar airflow cabinet which was disinfected with 70% ethanol and UV light prior to use. The culture vessels were then covered with parafilm to avoid contamination after inoculation.

2.8 Culture Conditions

The inoculated cultures were maintained in a controlled culture room under the following environmental conditions:

- Temperature: $25 \pm 2^{\circ}\text{C}$
- Relative Humidity: 60%–70%
- Photoperiod: 16 h light / 8 h dark
- Light Intensity: 2000–3000 lux provided by cool white, fluorescent lamps

Cultures were monitored regularly for contamination, shoot initiation, and growth response.

2.9 Subculturing

Once shoots were initiated, the cultures were moved to fresh medium of the same composition every 3–4 weeks to ensure the availability of nutrients and to encourage additional shoot multiplication. Subculturing also eliminated senescent tissues and minimized the buildup of phenolic compounds which might inhibit growth.

2.10 Data Recording

Observations were recorded after eight weeks of culture initiation. The following parameters were evaluated:

Survival Percentage (%)

The percentage of explants remaining viable and free from contamination was calculated using:

$$\text{Survival Percentage} = \frac{\text{Number of Survived Explants}}{\text{Total Number of Explants}} \times 100$$

Days to First Shoot Initiation

The number of days from inoculation to the appearance of the first visible shoot was recorded.

Number of Shoots per Explant

The total number of shoots produced by each explant was counted and averaged for each treatment.

Shoot Length (cm)

Shoot length was measured from the base of the shoot to the shoot apex using a digital Vernier caliper.

Fresh Weight (g)

Fresh biomass of regenerated shoots was measured using a precision electronic balance immediately after harvesting.

3. Results

3.1 Effect of TDZ on Explant Survival

During micropropagation, one of the most significant factors for successful establishment of culture is explant survival. Several factors are important in the survival of explants in vitro, such as the health of the explants, the efficiency of the sterilization process, the nutrient levels, and the concentration of plant growth regulators [13]. Cytokinins are essential for the early growth of culture establishment, for cell division, for stimulating metabolic activity and for maintaining tissue viability. Thidiazuron (TDZ), a phenylurea derived cytokinin, has been reported to have higher cytokinin activity than some of the traditional cytokinins and to improve shoot regeneration and explant establishment in several horticultural crops.

The present study showed that the survival rate of explants was significantly different depending on the different TDZ treatments, suggesting that the concentration of cytokinin has a significant effect on the establishment of banana cv. Batisa cultures. The survival percentage was between 72% and 95% as indicated in Table 1. The explants cultured on MS medium with 0.1 mg L⁻¹ TDZ had the lowest survival percentage (72%). This relatively low survival rate could be attributed to the lack of cytokinin, which leads to decreased cellular activity and adaptation of explants to in vitro conditions.

As the concentration of TDZ increased, a progressive increase in survival percentage was seen. Survival reached 80% and 90% at 1.0 and 1.5 mg L⁻¹ TDZ, respectively. The highest survival rate (95%) was achieved at 2.0 mg L⁻¹ TDZ concentration, indicating the most favorable hormonal environment for establishment and growth of the explants. Moderate concentrations of TDZ can increase endogenous cytokinin levels, stimulate cell division and increase nutrient utilization, which can improve the viability of the explants.

A slight decrease in survival percentage was noted at higher TDZ concentrations, however. The explants cultured on 2.5 and 3.0 mg L⁻¹ TDZ had survival rates of 87% and 85%, respectively. This reduction can be due to overproduction of cytokinins, which can cause hormonal imbalance, physiological stress, hyperhydricity, or abnormal tissue growth. Moderate cytokinin levels have been reported to be more effective than high levels in culture establishment in *Musa* species and other monocotyledonous crops.

The overall results showed that the use of TDZ significantly affects the survival of the explants during the initiation of culture, with 2.0 mg L⁻¹ TDZ being the most appropriate concentration for successful establishment of banana cv. Robusta explants.

Table 1. Effect of TDZ concentration on survival percentage and shoot initiation of banana cv. Robusta

TDZ Concentration (mg L ⁻¹)	Survival Percentage (%)	Days to First Shoot Initiation
0.1	72	55.80
1.0	80	69.70
1.5	90	54.43
2.0	95	55.50
2.5	87	52.50
3.0	85	52.00

As seen in Table 1, there was a clear trend that the higher the concentration of TDZ, the higher the percentage of survival, up to 2.0 mg L⁻¹, after which it decreased. This indicates that there is an optimum concentration of TDZ needed to obtain the highest viability of explants. The results also showed that the use of TDZ not only improved survival rates but also had an impact on the physiological processes involved in shoot initiation, which is crucial for successful culture establishment. The better survival rate achieved at 2.0 mg L⁻¹ TDZ is in agreement with previous reports on micropropagation of bananas, where moderate levels of cytokinin were found to favour explant establishment and shoot regeneration. Thus, the optimum concentration for the maximum survival of explants during the initiation stage of banana cv. can be recommended as 2.0 mg L⁻¹ TDZ. Robusta micropropagation.

3.2 Effect of TDZ on Shoot Multiplication

Shoot initiation is a crucial step in the micropropagation process because it is the first step that determines the ability of the cultured explants to regenerate and directly affects the efficiency of plantlet production. The endogenous plant hormones and externally provided growth regulators are required for successful shoot initiation. Thidiazuron (TDZ) is a cytokinin with a high cytokinin-like activity, which has been widely recognized for its exceptional ability to induce shoot organogenesis in tissue culture. TDZ stimulates development of shoot primordia by promoting cell division, activating dormant meristematic tissues and increasing the production of endogenous cytokinins.

Rapid shoot emergence is one of the most important stages in banana tissue culture as it shortens the culture period and enhances the overall multiplication efficiency. The present study showed that the concentration of TDZ significantly influenced the number of days taken for the first shoot to emerge in banana cv. Robusta.

The time to shoot initiation ranged from 52.00 to 69.70 days between treatments as shown in Table 2. The explants cultured on 1.0 mg L⁻¹ TDZ showed the longest period for shoot initiation (69.70 days). This lag time could be explained by a lack of cytokinin stimulation needed to stimulate cell division and shoot meristem development. In the same way, the shoot emergence time of explants cultured on 0.1 mg L⁻¹ TDZ was 55.80 days, which is relatively slower response to regeneration.

Generally, the higher the concentration of TDZ, the faster the shoot initiation. The shoots were initiated after 54.43 days and 55.50 days for explants cultured on 1.5 mg L⁻¹ and 2.0 mg L⁻¹ TDZ, respectively. The lowest time required for shoot initiation was recorded at 3.0 mg L⁻¹ TDZ with the appearance of shoots after 52.00 days from the establishment of culture. At 2.5 mg L⁻¹ TDZ, shoot initiation was recorded after 52.50 days, which was similar.

The shorter time required for shoot initiation at higher TDZ concentrations could be attributed to increased cytokinin activity that promotes DNA synthesis, cell differentiation and activation of dormant axillary meristems. TDZ has been reported to induce higher endogenous cytokinin levels and enhance the sensitivity of plant tissues to growth regulators, which helps to induce faster shoot induction. Rapid shoot formation in several banana cultivars such as Grand Naine, Dwarf Cavendish and Rasthali has also been reported with TDZ.

The findings of the present investigation indicate that the concentrations of TDZ (2.5-3.0 mg L⁻¹) are especially effective in reducing the time needed for the initiation of shoots in banana cv. Robusta. The ability to initiate shoots quickly is desirable for commercial micropropagation as it reduces the culture cycle and enhances the production of pathogen-free planting material.

Table 2. Effect of TDZ concentration on shoot initiation in banana cv. Robusta

TDZ Concentration (mg L ⁻¹)	Days to First Shoot Initiation
0.1	55.80
1.0	69.70
1.5	54.43
2.0	55.50
2.5	52.50
3.0	52.00

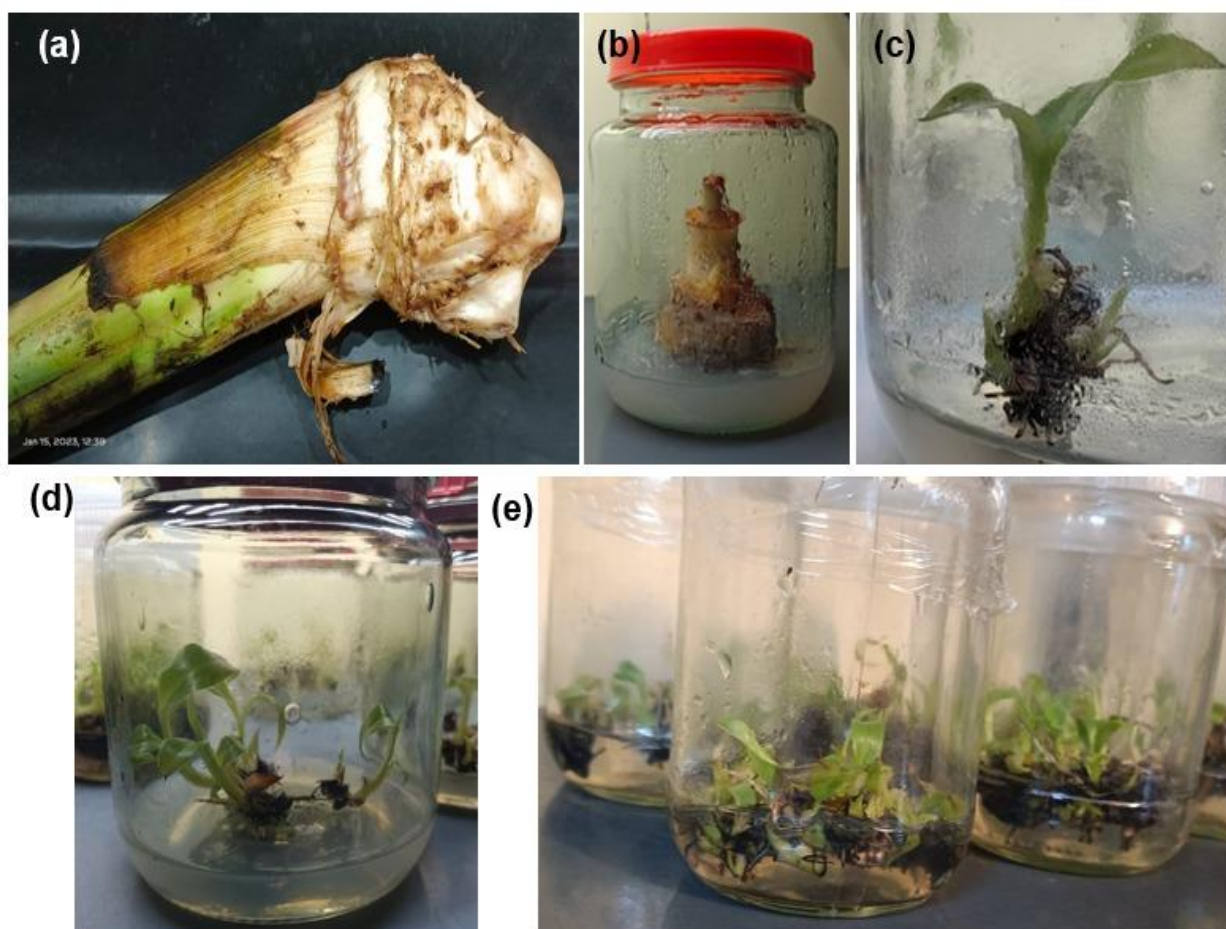


Figure 1: Shoot Initiation and Multiplication.

As shown in Table 2, the data clearly showed that the higher the concentration of TDZ, the fewer the number of days needed for shoot initiation. The highest cytokinin concentration (3.0 mg L⁻¹) gave the fastest response, indicating that higher cytokinin levels are more effective in stimulating meristematic activity and shoot bud differentiation. However, while 3.0 mg L⁻¹ TDZ resulted in the earliest shoot initiation, other growth parameters such as shoot multiplication and explant survival were optimized at slightly lower concentrations. Thus, an ideal shoot initiation and growth response should be considered in designing an efficient micropropagation protocol. The results agree with previous reports that TDZ is very effective in shoot organogenesis of *Musa* species and can shorten the culture establishment period. The improved shoot initiation response in the present study indicates the potential of TDZ as an important growth regulator for the rapid in vitro propagation of banana cv. Robusta.

4. Discussion

The present study clearly shows that TDZ has a significant effect on the in vitro regeneration response of banana cv. Robusta. The improved survival and shoot proliferation seen at intermediate TDZ concentrations could be due to the cytokinin-like activity of TDZ, which stimulates cell division and shoot meristem development. The highest percentage of survival (90%) was recorded at 2.0 mg L⁻¹ TDZ, suggesting that this concentration offers good physiological conditions for establishment of the explants. In *Musa* cultivars, moderate levels of cytokinin enhanced explant viability and regeneration frequency. The highest rate of shoot multiplication was obtained at 2.5 mg L⁻¹ TDZ, suggesting that higher cytokinin activity promoted axillary bud proliferation and adventitious shoot formation. TDZ has been reported to stimulate endogenous cytokinin production and to modify the auxin/cytokinin balance, which leads to increased morphogenic responses. The reduced shoot multiplication at 3.0 mg L⁻¹ TDZ suggests that excessive cytokinin concentrations may cause physiological stress or hormonal imbalance. High concentrations of TDZ have been shown to have inhibitory effects in a number of monocotyledonous and dicotyledonous species. TDZ's capacity to induce and multiply shoots quickly makes it a promising tool for large-scale propagation programmes for the conservation and commercialization of indigenous banana cultivars.

5. Conclusion

The present investigation revealed that TDZ is an effective cytokinin for micropropagation of banana cv. Robusta. The highest survival percentage and shoot elongation were obtained at 2.0 mg L⁻¹ TDZ, whereas the maximum shoot multiplication response was achieved at 2.5 mg L⁻¹ TDZ. The results obtained serve as a basis for the establishment of an effective micropropagation system for the mass production of pathogen-free planting material.

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