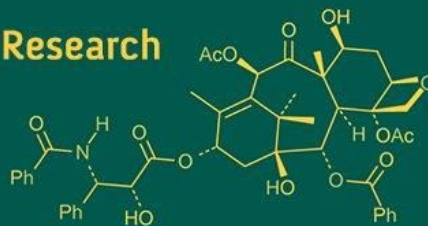
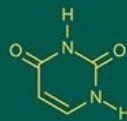
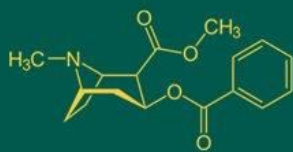


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## Effect of thidiazuron on *in vitro* regeneration of banana cv. rasthali (AAB)

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**Abstract**

Shoot tip explants of *Musa spp.* cv. Rasthali were cultured on Murashige and Skoog (MS) medium supplemented with varying concentrations of Thidiazuron (TDZ) to evaluate its effect on shoot proliferation. The maximum mean number of shoots per explant (3.3) was observed on MS medium containing 0.5 mg/l TDZ. Increasing the TDZ concentration beyond this level did not enhance shoot multiplication and instead resulted in the development of stunted and morphologically abnormal shoots. Elongated shoots were excised and successfully rooted on MS medium supplemented with 2.5 mg/l Indole-3-butyric acid (IBA). Rooted plantlets were acclimatized in plastic containers containing a substrate mixture of garden soil, farmyard manure, and sand in a 2:1:1 ratio. Following acclimatization, the plantlets were transferred to field conditions, where they exhibited a high survival rate (90%) and maintained morphological uniformity with the donor plants

**Keywords:** Banana, indole butyric acid (IAA), shoot tip, rasthali, thidiazuron (TDZ)

**Introduction**

Bananas are among the most widely consumed fruits worldwide, holding significant nutritional, economic, and cultural importance. Different varieties of bananas are cultivated for various purposes. Numerous varieties of bananas are cultivated for different purposes, with some prized for their taste, while others are favored for their processing qualities or resistance to pests and diseases (Madalla, 2021) [4]. Among these, the Rasthali banana (*Musa AAB*) is primarily grown in tropical and subtropical regions and is highly valued for its exceptional flavor, soft texture, and aromatic qualities. These attributes make Rasthali a preferred choice for both fresh consumption and culinary applications. Banana plants including Rasthali are normally propagated by vegetative means by using suckers which grow from lateral buds originating from corms. Suckers are commonly used for production of individual plants (Sivakumar and Visalakshi, 2021) [18]. However conventional plant materials are not the ideal propagule, because they carry pathogens and nematodes (Okolle *et al.*, 2025) [12]. The other disadvantages include low rate of multiplication, bulkiness and poor phytosanitary quality (Simbare *et al.*, 2023) [16]. The problem of emerging pest and diseases can be solved by propagating banana through micropropagation (Singh *et al.*, 2011; Sivakumar and Visalakshi 2021; Kumar *et al.*, 2024; Mekaunint *et al.*, 2024) [17, 18, 5, 8].

Cytokinins, such as Benzyl Amino Purine (BAP), are traditionally used for in various banana cultivars, owing to their effectiveness in promoting cell division and shoot proliferation (Khan *et al.*, 2021; Muhie and Teshome, 2023; Safitri *et al.*, 2023; Nugrahani, 2024) [4, 9, 14, 11]. However, recent advancements in tissue culture techniques have highlighted the potential of Thidiazuron (TDZ), a phenylurea-type cytokinin, as a potent growth regulator for enhancing shoot regeneration in banana (Lee, 2001; Bhaya and Al-RazzaqSalim 2019; Sivakumar and Visalakshi 2021; Alrazn *et al.*, 2023) [6, 2, 18, 1]. In the present study aimed to investigate the effect of Thidiazuron (TDZ) on the initiation and multiplication of shoots in Banana cultivar Rasthali.

**Materials and Methods**

The present investigation was carried out at Plant Tissue culture Laboratory of Dr.M.S.Swaminathan Agricultural College and Research Institute, Tamil Nadu Agricultural University, Eachangkottai, Thanjavur, Tamil Nadu during the period from February 2022 to

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November 2024. Rhizomes of Banana cv. Rasthali were sourced from the college orchard. The collected suckers were washed with running tap water to remove adhering soil residues. The older leaves and the outer leaves were trimmed off carefully by unwhorling of leaf sheath and a small portion of rhizome. The trimming was continued till the shoot tip measures 4cm long with a rhizome length of 3 cm and width of 2.5cm. The trimmed rhizome buds were stored for 15 minutes in antioxidant solution containing Ascorbic acid 100 mg/l and Citric acid 150 mg/l. The pre-treatment was done to overcome the problems of phenol exudation and resultant browning. The pre-treated explants were surface sterilized with 70% ethanol for 30 seconds and 2% sodium hypochlorite for seven minutes. The explants were washed several times with sterilized distilled water to remove the traces of sterilants. The explants were finally trimmed to remove the outermost whole of tissue exposed to sterilants. A vertical cut was given to arrest the apical dominance and to induce adventitious buds.

The sterilized individual explants were inoculated in MS (Murashige and Skoog, 1962) [10] medium containing different concentration of Thidiazuron (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) with 3 % sucrose and 4 g/l phytigel at 25 °C under a 16 h photoperiod. Multiple shoots were separated from the cluster and inoculated into MS medium supplemented with 2.5 mg/l of IBA. The rate of multiplication was calculated as the ratio of shoot number at the end of subculture to the initial number of shoots. The collected data also was analyzed by using statistical significance two ways analysis of variance (ANOVA).

## Results and Discussion

The present study investigated the effect of Thidiazuron (TDZ) on shoot initiation and multiplication in *Musa* spp. cv. Rasthali. The results revealed that the highest average number of shoots per explant (3.3) was obtained when shoot tip explants were cultured on Murashige and Skoog (MS) medium supplemented with 0.5 mg/l TDZ (Figure 1a; Table 1). Increasing the TDZ concentration beyond this optimal level did not enhance shoot proliferation. On the contrary, higher concentrations of TDZ led to a marked reduction in shoot quality and promoted the formation of stunted, abnormal, or vitrified shoots (Figures 1b and 1c).

These findings are consistent with earlier studies which demonstrated that TDZ, although a potent cytokinin-like compound, exhibits a narrow concentration window for optimal shoot regeneration in banana and other plant species. Lee (2001) [6] reported that the response to TDZ varies among different genomic groups of banana, with cultivars of the AAB and ABB groups showing greater sensitivity to higher concentrations of TDZ compared to those of the AAA group. Roels *et al.* (2005) [13] further observed that excessive TDZ application results in considerable morphological abnormalities, including hyperhydricity and reduced shoot elongation. Similarly, Shirani *et al.* (2009) [15] found that TDZ concentrations exceeding 5 µM significantly increased the abnormality index in several *Musa* cultivars, indicating a toxic effect at

higher dosages.

In the present study, lower concentrations of TDZ were more effective in promoting shoot induction, supporting the hypothesis that minimal exposure to TDZ is sufficient to trigger endogenous hormonal signaling for shoot organogenesis, while higher levels disrupt normal morphogenic pathways. Comparable results were reported by Bhaya and Al-RazzaqSalim (2019) [2], who noted that MS medium supplemented with 0.1 mg/l TDZ and 1.5 mg/l NAA yielded the highest shoot number and shoot length in *Musa* spp. cv. Cavendish.

However, these results contrast with findings from other studies, such as Khan *et al.* (2021) [4], Muhie and Teshome (2023) [9], Safitri *et al.* (2023) [14], and Nugrahani (2024) [11], who reported that Benzylaminopurine (BAP) was more effective than TDZ in promoting shoot initiation and multiplication across various banana cultivars. This divergence may be attributed to differences in genotype, explant type, culture conditions, or synergistic effects of auxins and cytokinins. Notably, Sivakumar and Visalakshi (2021) [18] observed that in *Musa* spp. cv. Poovan, a combination of higher BAP concentrations with lower TDZ levels yielded superior shoot proliferation rates, suggesting that cultivar-specific responses to growth regulators must be carefully optimized.

Elongated and healthy shoots obtained from the optimized TDZ treatment were excised and transferred to MS medium supplemented with 2.5 mg/l IBA for root induction. The IBA treatment proved effective, resulting in the formation of a well-developed root system within four weeks. Rooted plantlets were carefully removed from the culture vessels, and roots were gently washed under running tap water to eliminate residual medium.

For acclimatization, the plantlets were transferred to plastic containers containing a sterilized potting mixture of garden soil, farmyard manure, and sand in a 2:1:1 ratio. The hardening process was successfully completed under controlled humidity and light conditions. Following acclimatization, the plants were transplanted to field conditions, where they exhibited a high survival rate of 90%. The regenerated plants maintained normal morphology and phenotypic uniformity, closely resembling the donor (mother) plants, thereby confirming the genetic stability of the *in vitro* propagation protocol.

**Table 1:** Effects of different concentrations of TDZ for shoot induction and multiplication in banana cv. Rasthali

| Sl. No | TDZ (mg/l) | Number of shoots regenerated from explants |
|--------|------------|--------------------------------------------|
| 1.     | MS Basal   | -                                          |
| 2.     | 0.5        | 3.3 ±0.66                                  |
| 3.     | 1.0        | 2.6±0.33                                   |
| 4.     | 1.5        | 2.3±0.57                                   |
| 5.     | 2.0        | 2.3±0.57                                   |
| 6.     | 2.5        | 1.3±0.33                                   |
| 7.     | 3.0        | 1.3 ±0.33                                  |

Means in the same columns followed by different letters are significantly different ( $p \leq 0.05$ ) with DMRT.

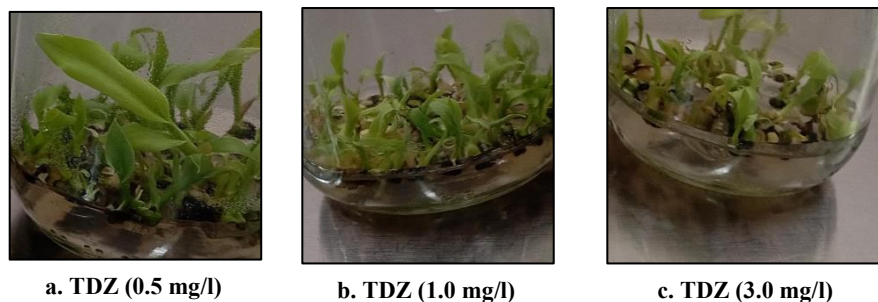


**Fig 1:** Effects of different concentrations of TDZ for multiple shoots induction in banana cv. Rasthali

## Conclusion

The present investigation evaluated the role of Thidiazuron (TDZ) in promoting shoot initiation and multiplication in *Musa* spp. cv. Rasthali. The results clearly demonstrated that MS medium supplemented with 0.5 mg/l TDZ induced the highest shoot proliferation rate, with an average of three shoots per explants. Increasing the TDZ concentration beyond 0.5 mg/l not only failed to improve shoot multiplication but also induced deleterious morphological effects, including shoot stunting, hyperhydricity, and abnormal tissue differentiation. These phenotypic aberrations are consistent with TDZ's known capacity to disrupt cellular homeostasis when used at supra-optimal levels, potentially due to excessive accumulation of endogenous cytokinins or auxin imbalance. The findings of this study are particularly significant for banana researchers and commercial tissue culture laboratories aiming to refine clonal propagation protocols. As banana cultivation increasingly relies on micropropagation to produce disease-free and uniform planting material, optimizing the use of TDZ for shoot multiplication can enhance the efficiency, cost-effectiveness, and scalability of propagation systems. Moreover, the protocol developed here could serve as a reference for other cultivars, pending genotype-specific adjustments.

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