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Research article

Micropropagation of Banana cv. Rasthali (AAB)

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Abstract

An efficient and effective *in vitro* regeneration protocol was developed and standardized for the banana cultivar Rasthali using shoot tip explants. The explants were placed on Murashige and Skoog (MS) medium enriched with different concentrations of benzyl aminopurine (BAP) and thidiazuron (TDZ) to promote shoot induction and proliferation, whereas indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) were utilized to induce rooting. Among the various treatments, the MS medium fortified with 3.5 mg/L BAP produced the highest shoot proliferation, with an average of 4.67 shoots per explant. This response was superior to that observed with TDZ at 0.2 mg/L, which produced an average of 3.33 shoots per explant. A synergistic effect was observed when BAP (3.5 mg/L) and TDZ (0.2 mg/L) were combined, resulting in up to 6.67 shoots per explant. Notable improvement in shoot elongation was observed in MS medium enriched with 0.2 mg/L GA_3, producing shoots reaching up to 9.4 cm in length. Rooting responses varied with auxin concentration, with the highest root number observed at 2.5 mg/L IBA. Rooted plantlets were successfully hardened in a soil mixture consisting of garden soil, farmyard manure, and sand in a 2:1:1 ratio, resulting in a survival rate of 90% under *ex vitro* conditions. The regenerated plants exhibited normal morphology, closely resembling the mother plants. This optimized protocol offers a reliable and scalable approach for the commercial micropropagation of banana cv. Rasthali.

Keywords: banana; plant growth regulators; Rasthali; regeneration; shoot tip

1. Introduction

Banana (*Musa* spp.) ranks as the second most significant fruit crop in India after mango and is often referred to as the "Apple of Paradise" due to its immense economic and nutritional importance. It takes up approximately 14% of the total area cultivation and contributes up to 32% of the total production of fruit crops in the country. India is the leading global producer of bananas, with major cultivation hubs in Andhra Pradesh, Maharashtra and Tamil Nadu. As of the most recent estimates, banana cultivation in India spans 9.24 lakh hectares, yielding over 33 million tons annually. Andhra Pradesh leads in overall yield, followed by Maharashtra and Tamil Nadu. Remarkably, Gujarat boasts the highest productivity at 66 tons per hectare,

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substantially above the national average of 38.25 tons per hectare. In 2024, Tamil Nadu alone recorded a production of 4.68 million tons, up from 4.52 million tons in 2023 (Kumar et al., 2024). Economically, the banana industry is projected to generate revenues of approximately USD 42.49 billion by 2025, with an anticipated annual growth rate of 6.94%. Market volume is also expected to grow by 4.9% by 2026. Banana is widely favored for its affordability and rich nutritional profile. It serves as a staple in both fresh and cooked forms, and is consumed ripe or unripe. Nutritionally, bananas are an excellent source of carbohydrates and are particularly rich in vitamin B complex. They also provide essential minerals such as potassium, phosphorus, calcium, and magnesium. Being low in fat and cholesterol-free, bananas are easily digestible and offer substantial health benefits (Ashokkumar et al. 2018). The varied agro-climatic zones of India facilitate the cultivation of numerous banana varieties, including Robusta, Rasthali, Poovan, Nendran, Red banana, Monthan, Malbhog, and Yelakki. Among these, Rasthali (AAB genome group) is one of the most sought-after cultivars, particularly valued in South India for its unique aroma, sweet taste, and culinary versatility (Pradeep et al., 2024). Rasthali bananas are not only delicious but are also associated with several health benefits, such as improved digestion, cardiovascular support, energy provision, and potential aid in weight management (Frederick & Mani, 2016). Traditionally, bananas are propagated through sword suckers, but the availability of high-quality suckers is often limited. Moreover, suckers may act as vectors for various destructive diseases like *Fusarium* wilt and Banana Bunchy Top Virus (BBTV), posing significant challenges to commercial cultivation (Kumar et al., 2024). *In vitro* propagation provides a strategic solution, facilitating the generation of genetically uniform, disease-free, and high-quality planting material at a commercial scale. It facilitates rapid multiplication, especially crucial for cultivars like Rasthali that are in high demand.

Several researchers have reported the regeneration of various banana cultivars using shoot tip explants (Mekonen et al*.,* 2021; Sivakumar & Visalakshi, 2021; Das et al*.,* 2025). However, the protocols specifically optimized for banana cv. Rasthali are limited (Govindaraju et al., 2012; Saraswathi et al., 2016; Kavitha et al., 2018; Nandhakumar et al., 2018; Uma et al., 2021). The low regeneration efficiency in Rasthali is largely attributed to its high phenolic content and its B-genome background, which is often more recalcitrant in tissue culture conditions (Suman, 2017; Justin et al., 2022). Most existing protocols report relatively low shoot multiplication rates, typically ranging from 1:3 to 1:4, which are insufficient for commercial-scale propagation (Sivakumar & Visalakshi, 2021). Hence, it is crucial to establish an enhanced, high-efficiency plant regeneration protocol for banana cv. Rasthali. This study aimed to investigate the impact of various plant growth regulators on shoot and root induction, with the objective of improving the overall micropropagation efficiency of this important cultivar.

2. Materials and Methods

2.1. Explant collection and surface sterilization

This study was carried out at the Plant Tissue Culture Laboratory of Dr. M.S. Swaminathan Agricultural College and Research Institute, Tamil Nadu Agricultural University (TNAU), Eachangkottai, Thanjavur, Tamil Nadu, India during the period from February 2022 to November 2024. Rhizomes of banana cv. Rasthali were sourced from the college orchard (Figure 1a). Collected suckers were initially washed with running tap water and removed adhered soil and debris. Subsequently, the suckers were trimmed and older and outer leaf



Figure 1. *In vitro* micropropagation of banana cv. Rasthali. a: explant, b: ideal size of the explants, c: culture initiation, d: shoot induction in MS + BAP (3.5 mg/L), e: shoot multiplication initiation in MS + BAP (3.5 mg/L) + TDZ (0.2 mg/L), f: shoot multiplication in MS + BAP (3.5 mg/L) + TDZ (0.2 mg/L), g: shoot multiplication in MS + BAP (3.5 mg/L) + TDZ (0.2 mg/L) (10 days old), h: *in vitro* rooting in IBA (2.5 mg/L), i: rooted plants, j: hardened plants, k: tissue cultured plants grown in field, l: established tissue culture plants in field

sheaths were removed by careful manual unwhorling. A portion of the rhizome was also excised to expose the shoot tip. Trimming was continued until the shoot tip measured approximately 4 cm in length, with the associated rhizome measuring 3 cm in length and 2.5 cm in diameter (Figure 1b).

For fungal decontamination, the trimmed suckers were immersed in a 1% Bavistin® (carbendazim) solution for 30 minutes. To mitigate oxidative browning caused by phenolic exudation, which is a common challenge in banana tissue culture, the decorated rhizome buds were pre-treated for 15 min by immersion in an antioxidant solution composed of 100 mg/L ascorbic acid and 150 mg/L citric acid. Surface sterilization of the pre-treated buds was performed by immersion for 15 min in 70% ethanol for 30 s, followed by immersion in 2% sodium hypochlorite solution for 7 min under aseptic conditions. The explants were then washed thoroughly 4 to 5 times with sterile distilled water to eliminate any residual sterilants. After sterilization, the outermost tissue layer directly exposed to sterilants was carefully trimmed away to eliminate any chemically damaged cells.

To overcome apical dominance and facilitate the induction of adventitious buds, a precise vertical incision was made through the apical region of the explants. This physical manipulation is known to disrupt apical dominance and promote lateral shoot proliferation, thereby enhancing the efficiency of shoot multiplication *in vitro*.

2.2. Culture initiation, induction of multiple shoots and elongation of shoots

Sterilized individual shoot tip explants of banana cv. Rasthali were aseptically cultured on Murashige and Skoog (MS) basal medium (sucrose concentration (3%) + solidifying agent (4 g/L agar)) (Murashige & Skoog, 1962) supplemented with different concentrations of cytokinins for shoot induction and multiplication (Figure 1c). The media were enriched with BAP at concentrations of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 mg/L, and TDZ at 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 mg/L, applied either separately or in combination (Sivakumar & Visalakshi, 2021). Gibberellic acid (GA_3) stock solution was prepared by first dissolving 5 mg of GA_3 powder in a small amount of alcohol and the volume was made up to 5 mL with distilled water. The GA_3 solution was filter-sterilized and added to the medium after autoclaving because it is heat-sensitive.

Each medium contained 3% (w/v) sucrose as the carbon source and was solidified with 4 g/L phytagel. The pH (5.8) was set to 5.8 before autoclaving at 121°C for 15 min. The inoculated cultures were then kept at 25±2°C under a 16-h photoperiod provided by cool white, fluorescent lamps with a light intensity of approximately 40-50 µmol m^{-2} s^{-1}. Relative humidity in the culture room was maintained at ~80% to support optimal growth conditions. Each treatment consisted of three replications, with ten explants per replication. Subculturing was carried out at 15-day intervals, with a total of five to six subcultures performed during the multiplication phase. The shoot count per explant was documented following each subculture to assess the response of explants to various hormonal treatments.

To promote elongation of stunted shoots, the shoots from both BAP and TDZ-containing media were moved into a shoot elongation medium containing MS basal salts supplemented with GA_3 at concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 mg/L. The shoots were kept in this elongation medium for an duration of two weeks before further analysis.

2.3. Root induction

Fully developed multiple shoots were gently separated from the shoot clusters and transferred to MS rooting medium supplemented with varying concentrations of auxins *viz*., IBA and NAA at 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg/L. The cultures were kept in standard growth conditions to facilitate root induction and development for a period of five weeks. Upon completion of the rooting phase, rooted plantlets (Figure 1i) were carefully removed from the culture vessels and gently rinsed under running tap water for approximately 10 minutes to remove any residual culture medium adhering to their root surfaces.

2.4. Acclimatization and hardening process

The rinsed plantlets were moved to plastic cups filled with a sterilized potting mix composed of garden soil, farmyard manure (FYM), and sand in a 2:1:1 ratio for initial hardening (Figure 1j). This stage was carried out under controlled environmental conditions of temperature: 25±2°C; and relative humidity: 80-85% for the duration of one month to facilitate acclimatization to non-axenic conditions. Following successful primary hardening, the acclimatized plantlets exhibiting vigorous growth were transplanted into polybags containing a 1:1 mixture of garden soil and FYM for secondary hardening. The plantlets were maintained under semi-controlled greenhouse conditions for an additional 35 days. This two stage hardening protocol ensured a high survival rate and robust establishment of micropropagated plants that were suitable for subsequent field transfer.

2.5 Field transfer and establishment

Tissue culture-derived plantlets of banana cv. Rasthali were successfully transplanted into the College Orchard under open field conditions at a spacing of 2.1 × 2.1 m (Figure 1k), a configuration strategically selected to optimize light interception across the canopy while maintaining sufficient inter-plant spacing to enhance airflow. This spatial arrangement is critical in minimizing localized humidity and microclimatic conditions that favor the development of foliar and soil-borne pathogens, thereby contributing to improved plant health and reduced disease incidence. To mitigate early pest threats, particularly from banana weevil (*Cosmopolites sordidus*) and plant parasitic nematodes, a pre-planting sucker pralinaging was performed using Carbofuran 3G. A dose of 40 g of the granular formulation was applied per planting pit to ensure systemic protection during the early establishment phase.

Nutritional management was achieved through a well-balanced fertilization regimen, delivering essential macronutrients to support optimal vegetative and reproductive development. Each plant received 200 g of nitrogen (N), 30 g of phosphorus (as P_2O_5), and 300 g of potassium (as K_2O) in the form of water-soluble fertilizers. These nutrients were applied in split doses to match the crop's dynamic growth requirements and minimize leaching losses. Irrigation was scheduled daily at a rate of 25 liters per plant to maintain consistent soil moisture levels, ensuring efficient nutrient uptake and sustained physiological activity throughout the crop growth cycle. This precise water management approach was critical in supporting high biomass accumulation and optimizing fruit yield under field conditions.

2.6 Data collection and analysis

Quantitative data were systematically recorded at specified intervals for key parameters, including the number of shoots per explant, shoot length (cm), number of roots per plantlet, root length (cm), and *ex vitro* survival rate (%) (calculated from three replicated experiments). The multiplication rate was determined by calculating the ratio of shoots produced at the end of each subculture cycle to the initial number of shoots at the start of that cycle. The experiment followed a completely randomized design (CRD) with three replications per treatment and ten explants per replication. All cultures were maintained under controlled *in vitro* conditions in a tissue culture growth room. The data were analyzed using a one-way ANOVA and treatment means (mean±SD) were compared using Tukey's multiple range test ($p \leq 0.05$) to assess the impact of varying concentrations of plant growth regulators and their interactions on the measured traits. All statistical analyses were performed using GraphPad Prism 10 software to ensure accuracy and reliability of the results.

3. Results and Discussion

This study was conducted to examine the impact of various plant growth regulators on the *in vitro* micropropagation of banana cv. Rasthali (AAB). Shoot and root induction were achieved by supplementing MS medium with different concentrations of cytokinins and auxins. A reliable protocol was established using shoot tip explants.

3.1. Effects of cytokinins for shoot initiation and multiplication

In this study, different concentrations of two cytokinins namely BAP and TDZ were evaluated for their effectiveness in inducing and multiplying shoots in *Musa* spp. cv. Rasthali. These growth regulators were tested individually to assess their effectiveness in shoot and root regeneration (Table 1).

Among the tested concentrations, MS medium enriched with 3.5 mg/L BAP showed the highest shoot proliferation, producing an average of 4.67 shoots per explant (Figure 1d). Elevated BAP levels (4.0, 4.5, and 5.0 mg/L) did not enhance shoot multiplication further, indicating a concentration-dependent response plateau for this genotype. These results were congruent with earlier studies that demonstrated the superior efficacy of BAP over other cytokinins in promoting shoot induction and multiplication in various banana cultivars (Arinaitwe et al*.,* 2000; Gubbuk & Pekmezci, 2004; Rahman et al., 2004; Roels et al*.,* 2005; Jafari et al. 2011, Ahmed et al., 2014; Khatun et al., 2017; Nair et al., 2018; Agbadje et al., 2021; Nugrahani et al., 2024). For instance, Hossain et al. (2016) observed maximum shoot regeneration in the cultivars Grand Naine, Amritasagar, and Sabri when using 5.0 mg/L BAP, while Khatun et al. (2017) observed the greatest explant response (84%) and shoot number (3.4) with the same concentration in banana cv. Sabri. In contrast, MS medium supplemented with TDZ showed a different response profile. The ideal concentration for shoot induction in Rasthali was found to be 0.2 mg/L TDZ, producing an average of 3.33 shoots per explant. The elevated concentrations of TDZ exceeding 0.2 mg/L resulted in a reduction in the number of shoots, suggesting a limited effective concentration range for optimal shoot proliferation. Previous studies reported that TDZ showed similar reduction effects on shoot elongation and increased occurrence of morphological abnormalities at higher concentrations (Arinaitwe et al., 2000;

Table 1. Effect of BAP and TDZ on shoot regeneration from shoot tip explants of banana cv. Rasthali

MS Medium Composition		Number of Shoots Regenerated from Explants	Shoot Lengths (cm)
BAP (mg/L)	TDZ (mg/L)		
-	-	0.00±0.00^{g}	0.00±0.00^{h}
0.5	-	1.67±0.33^{f}	1.93±0.10^{efg}
1.0	-	1.67±0.88^{f}	1.94±0.21^{efg}
1.5	-	2.00±0.58^{f}	2.21±0.15^{defg}
2.0	-	2.00±1.15^{f}	2.22±0.27^{defg}
2.5	-	3.33±0.67^{cde}	3.35±0.17^{bc}
3.0	-	4.00±1.00^{abc}	3.92±0.24^{ab}
3.5	-	4.67±0.88^{a}	4.50±0.21^{a}
4.0	-	4.33±0.33^{ab}	4.21±0.10^{a}
4.5	-	4.00±0.00^{abc}	3.93±0.60^{ab}
5.0	-	3.67±0.88^{bcd}	3.64±0.21^{abc}
-	0.2	3.33±1.76^{cde}	3.36±0.41^{bc}
-	0.4	3.00±0.00^{def}	3.07±0.16^{cd}
-	0.6	2.00±0.58^{f}	2.23±0.15^{defg}
-	0.8	2.33±0.88^{ef}	2.49±0.21^{cde}
-	1.0	2.67±0.67^{ef}	2.78±0.17^{cde}
-	1.2	2.00±0.58^{f}	2.24±0.15^{defg}
-	1.4	1.00±0.58^{f}	1.36±0.26^{fgh}
-	1.6	1.00±0.00^{f}	1.37±0.36^{fgh}
-	1.8	1.33±0.33^{f}	1.64±0.20^{fgh}
-	2.0	0.67±0.33^{f}	1.07±0.13^{gh}

Means (mean±SD) in the same column followed by different letters are significantly different ($p \leq 0.05$).

Lee, 2001; Gubbuk & Pekmezci, 2004; Roels et al., 2005). These findings suggest that TDZ, although effective in inducing shoot regeneration, may hinder subsequent shoot elongation and normal morphology if used in excess. The concentration sensitivity of TDZ observed in Rasthali can be attributed to its biochemical persistence in plant tissues. Unlike adenine-based cytokinins, TDZ is a urea-type cytokinin that is not degraded by cytokinin oxidase, resulting in prolonged hormonal activity. This can lead to cytokinin overaccumulation, disrupting normal morphogenesis and shoot elongation (Makara et al., 2010). Moreover, TDZ exposure has been shown to induce somaclonal variation and morphological deformities at higher concentrations, especially in Rasthali and other AAB cultivars, highlighting its concentration-dependent physiological effects (Bidabadi et al., 2010). Such prolonged cytokinin signaling can also cause a “carry-over effect” in subsequent subcultures, where excessive TDZ retained in tissues continues to influence shoot development and may suppress elongation unless transferred to hormone-free medium (Makara et al., 2010).

The response to TDZ also appears to be cultivar-specific. Lee (2001) reported significant variation in TDZ effectiveness between banana cultivars of the AAA and AAB genome groups. Similarly, Roels et al. (2005) found a higher incidence of shoot abnormalities in certain cultivars when TDZ was used at elevated concentrations. The findings presented in the study are supported by Gubbuk & Pekmezci (2004),

Venkatachalam et al. (2007), Strosse et al. (2008), and Bairu et al. (2008), who reported a decrease in shoot quality and quantity at elevated levels of TDZ. In this study, the highest shoot production of 6.67 shoots per explant was recorded on MS medium supplemented with 3.5 mg/L BAP and 0.2 mg/L TDZ (Table 2; Figure 1e-g). In contrast, a higer concentration of TDZ influenced the production of more number of shoots in Grain Nain hybrid cultivar of *Musa acumanata* (Alrazn et al., 2023). Recent studies have also explored synergistic and alternative cytokinin treatments. Budiyati and Ahmad (2024) demonstrated that a mixture of 6.0 mg/L BAP and 1.0 mg/L TDZ accelerated shoot emergence in banana cv. Barangan. Sorn et al. (2024) found no significant difference between BAP and TDZ at varying concentrations in Cavendish banana; however, optimal shoot formation occurred at 3 mg/L BAP and 2 mg/L TDZ, producing 2 to 4 shoots per explant. Hasmayaputra & Arifin (2024) reported enhanced shoot regeneration in Cavendish banana when explants were grown on MS medium enriched with 1.5 mg/L BAP and 150 mL/L coconut water. In another study, Chandana et al. (2024) achieved the highest regeneration efficiency of 5.02 shoots per explant, 5.20 cm shoot length, and a 73.33% success rate, in MS medium containing 2.5 mg/L BAP, 2.0 mg/L kinetin, and 200 mg/L ascorbic acid.

Collectively, the findings from this study and previous literature emphasize that the significance of fine-tuning of the type and concentration of cytokinins for particular cultivars is crucial to improving *in vitro* shoot proliferation, with BAP continuing to be the best cytokinin for shoot multiplication in banana cv. Rasthali, while TDZ, although capable of inducing shoot formation, should be used with caution due to its concentration-sensitive effects and potential to induce abnormal development.

3.2. Effect of GA_3 for shoot elongation

In the present study, micropropagated shoots regenerated on media containing BAP and TDZ exhibited stunted growth, a common response associated with high cytokinin concentrations, particularly TDZ. To overcome this limitation and promote shoot elongation, the shoots were moved to MS medium enriched with different concentrations of gibberellic acid (GA_3).

Among the concentrations tested, the maximum shoot elongation of 9.4 cm was observed in MS medium containing 0.2 mg/L GA_3 (Table 3). Increasing GA_3 concentration beyond this level did not result in any statistically significant improvement in shoot length, suggesting that 0.2 mg/L represents the optimal concentration for shoot elongation in banana cv. Rasthali under the conditions of this study. These findings are consistent with previous reports that demonstrated the positive influence of GA_3 on shoot elongation in banana cultivars. For instance, Sivakumar & Visalakshi (2021) reported considerable shoot elongation in banana cv. Poovan when GA_3 was mixed into the culture medium.

3.3. *In vitro* rooting and field establishment

Root initiation was observed within 45 days subsequent to the transfer of regenerated shoots to MS basal medium. Rooting efficiency was notably high, with 100% of the explants forming roots. The mean number of roots per shoot was 7.35, and the average root length reached 6.4 cm after six weeks of culture (Table 4). Root development was assessed at the end of this six-week period to evaluate the effectiveness of auxin treatments.

In this study, prompt and vigorous root induction occurred in MS medium supplemented with Indole-3-butyric acid (IBA). Of the various concentrations evaluated,

Table 2. Effect of BAP in combination with TDZ on shoot regeneration of shoot tip explants of banana cv. Rasthali

MS Medium Composition		Number of Shoots Regenerated from Explants	Shoot Lengths (cm)
BAP (mg/L)	TDZ (mg/L)		
-	-	0.00±0.00^{k}	0.00±0.00^{i}
0.5	0.2	2.00±0.58^{ijk}	1.93±0.13^{fgh}
1.0	0.2	3.33±1.76^{gh}	1.96±0.25^{fgh}
1.5	0.2	3.67±0.88^{fg}	2.26±0.19^{efgh}
2.0	0.2	4.00±1.00^{ef}	2.67±0.31^{def}
2.5	0.2	4.33±0.33^{def}	3.35±0.20^{bcd}
3.0	0.2	5.67±0.67^{b}	3.99±0.28^{abc}
3.5	0.2	6.67±0.88^{a}	4.50±0.25^{a}
4.0	0.2	5.00±0.58^{bcd}	4.21±0.13^{ab}
4.5	0.2	4.00±0.00^{ef}	3.92±0.06^{abc}
5.0	0.2	4.67±0.33^{ede}	3.64±0.25^{bcd}
0.5	0.4	2.00±1.15^{ijk}	3.35±0.45^{bcd}
1.0	0.4	3.00±0.58^{h}	3.07±0.06^{cde}
1.5	0.4	3.67±0.33^{fg}	2.16±0.19^{fgh}
2.0	0.4	3.00±0.00^{h}	2.49±0.25^{efg}
2.5	0.4	4.33±0.67^{def}	2.78±0.20^{def}
3.0	0.4	4.67±0.33^{cde}	2.21±0.19^{efgh}
3.5	0.4	5.33±0.88^{bc}	4.50±0.25^{a}
4.0	0.4	4.00±0.00^{ef}	3.17±0.06^{cde}
4.5	0.4	4.33±0.67^{def}	3.50±0.20^{bcd}
5.0	0.4	3.33±0.33^{gh}	2.50±0.13^{efg}
0.5	0.6	1.33±0.33^{jk}	0.50±0.13^{hi}
1.0	0.6	2.67±0.67^{hi}	1.84±0.20^{gh}
1.5	0.6	2.33±0.88^{ij}	1.50±0.25^{gh}
2.0	0.6	3.00±0.58^{h}	2.17±0.19^{fgh}
2.5	0.6	3.33±1.76^{gh}	2.51±0.45^{efg}
3.0	0.6	3.33±0.67^{gh}	2.52±0.20^{efg}
3.5	0.6	3.67±0.33^{fg}	2.84±0.13^{def}
4.0	0.6	3.33±0.33^{gh}	2.53±0.13^{efg}
4.5	0.6	3.00±0.00^{h}	2.18±0.09^{fgh}
5.0	0.6	3.00±0.58^{h}	2.19±0.19^{fgh}

Means (mean±SD) in the same columns followed by different letters are significantly different ($p \leq 0.05$).

Table 3. Effect of GA_3 on shoot elongation of banana cv. Rasthali cultured *in vitro*

MS Medium Composition GA_3 (mg/L)	Shoot Length (cm)
0	0.00±0.00^{c}
0.2	9.40±0.70^{a}
0.4	9.10±1.69^{a}
0.6	8.30±1.49^{a}
0.8	5.10±0.20^{b}
1.0	4.40±0.64^{b}

Means (mean±SD) in the same columns followed by different letters are significantly different ($p \leq 0.05$).

Table 4. Effect of IBA and NAA on root generation from regenerated shoots of banana cv. Rasthali

MS Medium Composition		Number of Roots per Explants	Root Length (Cm)
IBA (mg/L)	NAA (mg/L)		
-	-	0.00±0.00^{d}	0.00±0.00^{e}
0.5	-	4.59±0.35^{c}	4.20±0.13^{cd}
1.0	-	5.34±0.41^{c}	5.10±0.70^{abc}
1.5	-	5.22±0.97^{c}	5.60±0.48^{ab}
2.0	-	6.74±0.63^{ab}	5.80±0.71^{ab}
2.5	-	7.35±1.03^{a}	6.40±1.03^{a}
3.0	-	6.52±0.85^{b}	6.20±1.12^{a}
-	0.5	4.82±0.34^{c}	3.30±0.44^{d}
-	1.0	6.56±0.28^{b}	3.50±0.42^{d}
-	1.5	6.51±0.36^{b}	3.90±0.49^{d}
-	2.0	6.51±0.44^{b}	4.70±0.21^{bcd}
-	2.5	4.92±0.38^{c}	4.50±0.19^{bcd}
-	3.0	4.02±0.67^{c}	4.60±0.39^{bcd}

Means (mean±SD) in the same columns followed by different letters are significantly different ($p \leq 0.05$).

2.5 mg/L IBA proved to be the most effective, leading to strong root development and optimal root architecture (Figure 1h). This result aligns with the known physiological role of IBA in stimulating auxin-responsive gene expression, vascular differentiation, and lateral root initiation. Its higher stability and conversion efficiency compared to IAA make it ideal for sustained root induction in bananas (Bohra et al., 2016). These findings are consonant with those of Alrazn et al. (2023), who reported the successful use of IBA for root induction across various *Musa* cultivars. Similar observations were observed by Rahman et al. (2004) detected 1.0 mg/L IBA as the ideal concentration for root induction in banana cv. Agnishwar. However, cultivar-specific responses to auxins have been widely documented. For instance, Hossain et al. (2016) reported that Grand Naine responded more favorably to naphthaleneacetic acid (NAA), with the highest rooting performance recorded on MS medium enriched with 4.0 mg/L NAA. Following successful *in vitro* rooting, the regenerated plantlets were gradually acclimated before being transferred to field conditions. The hardened plantlets exhibited a 90% survival rate (Figures 1k and 1l), which suggested strong physiological integrity and efficient nutrient uptake due to well-developed root systems. Comparable high survival rates were reported in banana cultivars acclimatized from IBA-induced plantlets under controlled hardening conditions (Msogoya & Mwakisitu, 2014), confirming the morphological stability of the micropropagated banana cv. Rasthali. The standardized protocol for Rasthali is highly suitable for producing greater number of micropropagated plants for commercial production compared to existing protocols available for this cultivar.

4. Conclusions

The present study establishes a robust and highly reproducible regeneration protocol *in vitro* for the micropropagation of *Musa* spp. cv. Rasthali. Maximum shoot proliferation was achieved with MS medium enhanced with 3.5 mg/L BAP and 0.2 mg/L TDZ, resulting in an

average of 6.67 shoots per explant. The optimal shoot elongation of 9.4 cm was observed in MS medium supplemented with 0.2 mg/L GA_3. Root formation was most efficient on MS medium containing 2.5 mg/L IBA, yielding an average of 7.3 roots per explant with a mean root length of 6.52 cm. These findings demonstrate the robustness, reproducibility, and scalability of the protocol, indicating its strong potential for commercial-scale micropropagation of banana cv. Rasthali. The successful establishment of phenotypically uniform, morphologically normal, and phenotypically stable plantlets underscored the protocol's practical applicability in banana tissue culture industries and germplasm conservation programs. This optimized regeneration protocol can serve as a reliable foundation for large-scale commercial propagation and germplasm conservation of banana cv. Rasthali and can potentially be adapted for transgenic development against biotic and abiotic stresses.

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6. Authors' Contributions

P. Sivakumar designed and performed research; coordinated research; wrote the paper.
K. S. Vijai Selvaraj analyzed data; performed research; wrote the paper.

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7. Conflicts of Interest

The authors declare no conflicts of interest relevant to this article

References

Agbadje, E. T. A. E., Agbidinoukoun, A., Zandjanakou-Tachin, M., Cacaï, G. T. H., & Ahanhanzo, C. (2021). Mass production of bananas and plantains (*Musa* spp.) plantlets through in vitro tissue culture partway: A review. *European Journal of Biology and Biotechnology*, *2*(4), 1-8. https://doi.org/10.24018/ejbio.2021.2.4.229

Ahmed, S., Sharma, A., Bhushan, B., Wali, V. K., Bakshi, P., & Singh, A. K. (2014). Studies on hardening and acclimatization of micropropagated plantlets of banana cv. Grand Naine. *The Bioscan*, *9*(3), 965-968.

Alrazn, S. M. H., Alkhalifa, A. A. S., & Al-Sereh, E. A. (2023). Effect of cytokinin TDZ and auxin IBA on the succession of plants of the banana plant *Musa acuminata*, the Grand-Nain hybrid cultivar, using tissue culture technology. *Journal of Wildlife and Biodiversity*, *7*(Special Issue), 277-290. https://doi.org/10.5281/zenodo.10213198

Arinaitwe, G., Rubaihayo, P. R., & Magambo, M. J. S. (2000). Proliferation rate effects of cytokinins on banana (*Musa* spp.) cultivars. *Scientia Horticulturae*, *86*(1), 13-21. https://doi.org/10.1016/S0304-4238(00)00124-2

Ashokkumar, K., Elayabalan, S., Shobana, V. G., Sivakumar, P., & Pandiyan, M. (2018). Nutritional value of cultivars of banana (*Musa* spp.) and its future prospects. *Journal of Pharmacognosy and Phytochemistry*, *7*(3), 2972-2977.

Bairu, M. W., Strik, W. A., Doležal, K., & van Staden, J. (2008). The role of topolins in micropropagation and somaclonal variation of banana cultivars 'Williams' and Grand Naine (*Musa* spp. AAA). *Plant Cell, Tissue and Organ Culture*, *95*, 373-379. https://doi.org/10.1007/s11240-008-9451-4

Bidabadi, S., Meon, S., Wahab, Z., & Mahmood, M. (2010). Study of genetic and phenotypic variability among somaclones induced by BAP and TDZ in micropropagated shoot tips of banana (*Musa* spp.) using RAPD markers. *The Journal of Agricultural Science*, *2*(3), 49-60. https://doi.org/10.5539/jas.v2n3p49

Bohra, P., Waman, A., Sathyanarayana, B., Umesha, K., & Gowda, B. (2016). Influence of different growth regulators on in vitro multiplication of mixed diploid banana (*Musa* AB). *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *86*, 179-185. https://doi.org/10.1007/s40011-014-0435-3

Budiyati, I., & Ahmad, R. (2024). Combination of BAP and TDZ in inducing the growth of banana shooting explant cv. Barangan *in vitro*. *Russian Journal of Agricultural and Socio-Economic Sciences*, *145*(1), 86-94.

Chandana, M., Thippesha, D., Sadashiv, N., Nagaraja, M. S., & Shashikala, S. K. (2024). Influence of antioxidants and cytokinins on in vitro plantlet regeneration of banana cv. Ney Poovan (AB). *Journal of Advances in Biology and Biotechnology*, *27*(11), 1400-1407. https://doi.org/10.9734/jabb/2024/v27i111727

Das, S., Singh, B., Singh, S., Singh, A. K., Hazarika, B. N., & Deo, C. (2025). Unlocking the potential of shoot tip culture for rapid multiplication of *Musa acuminata* (AAA) cv. Amrit Sagar. *National Academy Science Letters*, *48*(2), 165-171. https://doi.org/10.1007/s40009-024-01472-7

Frederick, N., & Mani, J. (2016). Phytochemical screening and in-vitro antioxidant activity of different solvent extracts of *Musa rasthali* fruit. *International Journal of Chemical Sciences*, *5*(3), 54-70.

Govindaraju, S., Saravanan, J., Jayanthi, B., Nancy, D., & Arulselvi, P. I. (2012). In vitro propagation of banana (*Musa* sp. Rasthali variety) from sword suckers for its commercial production. *Research in Plant Biology*, *2*(5), 1-6.

Gubbuk, H., & Pekmezci, M. (2004). *In vitro* propagation of some new banana types (*Musa* spp.). *Turkish Journal of Agriculture and Forestry*, *28*, 355-361.

Hasmayaputra, H., Asnawati, & Arifin, N. (2024). Effect of coconut water and BAP concentration on the induction of Cavendish banana shoots in vitro. *Brazilian Journal of Development*, *10*(12), Article e75841. https://doi.org/10.34117/bjdv10n12-044

Hossain, M. A., Rubel, M. H., Nasiruddin, K. M., & Evamoni, F. Z. (2016). Influence of BAP and NAA on in vitro plantlet regeneration of local and exotic banana cultivars. *Journal of Bioscience and Agriculture Research, 6*(2), 553-564. https://doi.org/10.18801/jbar.060216.66

Jafari, N., Othman, R. Y., & Khalid, N. (2011). Effect of benzylaminopurine (BAP) pulsing on in vitro shoot multiplication of *Musa acuminata* (banana) cv. Berangan. *African Journal of Biotechnology*, *10*(13), 2446-2450.

Justine, A. K., Kaur, N., & Pati, P. K. (2022). Biotechnological interventions in banana: current knowledge and future prospects. *Heliyon*, *8*(11), Article e11636. https://doi.org/10.1016/j.heliyon.2022.e11636

Kavitha, N., Saraswathi, M. S., Sajith, K. P., Bathrinath, M., Kannan, G., Backiyarani, S., & Uma, S. (2018). Development of a direct regeneration protocol for mass multiplication of banana cultivar Rasthali (AAB, Silk) using immature floral hands as explants. *Acta Horticulturae, 1272*, 105-112. https://doi.org/10.17660/ActaHortic.2020.1272.13

Khatun, F., Hoque, M. E., Huq, H., Adil, M., Ashraf-Uz-Zaman, K., & Rabin, M. H. (2017). Effect of BAP and IBA on in vitro regeneration of local banana variety of Sabri. *Biotechnology Journal International*, *18*(1), 1-10. https://doi.org/10.9734/BJI/2017/31592

Kumar, R., Singh, R. K., Shah, A., Srivastava, A. K., Singh, U. P., & Agarwal, A. (2024). Banana cultivation and micropropagation in India: Addressing challenges and exploring future prospects. *Biosciences Biotechnology Research Asia*, *21*(4), 1383-1395. http://dx.doi.org/10.13005/bbra/3311

Lee, S. W. (2001). Thidiazuron in the improvement of banana micropropagation. *Acta Horticulturae*, *692*, 67-74. https://doi.org/10.17660/ActaHortic.2005.692.7

Makara, A. M., Rubaihayo, P. R., & Magambo, M. J. (2010). Carry-over effect of thidiazuron on banana in vitro proliferation at different culture cycles and light incubation conditions. *African Journal of Biotechnology*, *9*(21), 3079-3085.

Mekonen, G., Egigu, M. C., & Muthuswamy, M. (2021). In vitro propagation of banana (*Musa paradisiaca* L.) plant using shoot tip explant. *Turkish Journal of Agriculture–Food Science and Technology*, *9*(12), 2339-2346.

Msogoya, T., & Mwakisitu, J. (2014). Effect of thidiazuron on in vivo shoot proliferation of popular banana (*Musa* spp. L) cultivars in Tanzania. *Journal of Applied Biosciences*, *81*, 7214-7220. https://doi.org/10.4314/jab.v81i1.1

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, *15*, 473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Nair, A. R. G., Ravichandran, P., & Bejoy, M. (2018). Direct shoot regeneration from male immature flower buds of *Musa paradisiaca* Linn. cv. Poovan (AAB). *Plant Science Today*, *5*(4), 142-148. https://doi.org/10.14719/pst.2018.5.4.403

Nandhakumar, N., Kumar, K., Sudhakar, D., & Soorianathasundaram, K. (2018). Plant regeneration, developmental pattern and genetic fidelity of somatic embryogenesis derived *Musa* spp. *Journal of Genetic Engineering and Biotechnology*, *16*(2), 587-598. https://doi.org/10.1016/j.jgeb.2018.10.001

Nugrahani, P., Purnobasuki, H., Ansori, A. N. M., Anuchai, J., & Priyanto, A. D. (2024). Effect of different strengths of MS media and BAP on banana plantlet growth *in vitro*. *Sarhad Journal of Agriculture*, *40*(4), 1110-1117. https://doi.org/10.17582/journal.sja/2024/40.4.1110.1117

Pradeep, M., Srivignesh, S., Krishna, K. R., Kathiresan, S., Pravin, I. A., & Kumar, A. R. (2024). Enhancing yield and economics of banana cultivar Rasthali (Silk-AAB) through bunch spray of growth regulators and nutrients. *Journal of Environmental Biology*, *45*(6), 756-762. http://dx.doi.org/10.22438/jeb/45/6/MRN-5342

Rahman, M. Z., Nasiruddin, K. M., Amin, M. A., & Islam, M. N. (2004). In vitro response and shoot multiplication of banana with BAP and NAA. *Asian Journal of Plant Sciences*, *3*, 406-409. https://doi.org/10.3923/ajps.2004.406.409

Roels, S., Escalona, M., Cejas, I., Noceda, C., Rodriguez, R., Canal, M. J., Sandoval, J., & Debergh, P. (2005). Optimization of plantain (*Musa* AAB) micropropagation by temporary immersion system. *Plant Cell, Tissue and Organ Culture*, *82*, 57-66. https://doi.org/10.1007/s11240-004-6746-y

Saraswathi, M. S., Uma, S., Kannan, G., Selvasumathi, M., Mustaffa, M. M., & Backiyarani, S. (2016). Cost-effective tissue culture media for large-scale propagation of three commercial banana (*Musa* spp.) varieties. *The Journal of Horticultural Science and Biotechnology*, *91*(1), 23-29. https://doi.org/10.1080/14620316.2015.1117227

Sivakumar, P., & Visalakshi, M. (2021). *In vitro* micropropagation of banana cv. Poovan (AAB). *Journal of Applied Horticulture*, *23*(1), 37-41. https://doi.org/10.37855/jah.2021.v23i01.07

Sorn, V., Chhon, Y., & Kun, M. (2024). Effects of benzyl adenine and thidiazuron on shoot regeneration of Cavendish banana. *Current Applied Science and Technology*, *24*(5), Article e0259302. https://doi.org/10.55003/cast.2024.259302

Strosse, H., Andre, E., Sagi, L., Swennen, R., & Panis, B. (2008). Adventitious shoot formation is not inherent to micropropagation of banana as it is in maize. *Plant Cell, Tissue and Organ Culture*, *95*, 321-332. https://doi.org/10.1007/s11240-008-9446-1

Suman, S. (2017). Plant tissue culture: A promising tool of quality material production with special reference to micropropagation of banana. *Biochemical and Cellular Archives, 17*(1), 1-26.

Uma, S., Karthic, R., Kalpana, S., Backiyarani, S., & Saraswathi, M. S. (2021). A novel temporary immersion bioreactor system for large scale multiplication of banana (Rasthali AAB-Silk). *Scientific Reports, 11*(1), Article 20371. https://doi.org/10.1038/s41598-021-99923-4

Venkatachalam, L., Sreedhar, R. V., & Bhagyalakshmi, N. (2007). Micropropagation in banana using high levels of cytokinins does not involve any genetic changes as revealed by RAPD and ISSR markers. *Plant Growth Regulation, 51*, 192-205. https://doi.org/10.1007/s10725-006-9154-y