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# Thidiazuron-mediated *in vitro* clonal propagation of banana cultivar Grand Naine

**[1,4]Umme Salma, [2]Suprabuddha Kundu, [3]Nirmal Mandal, [4]Debadrita Giri and [4]Mohit Kumar Sinha**

[1]Department of Agricultural Biotechnology, School of Agricultural Sciences, Sister Nivedita University, New Town, Kolkata, West Bengal, India

[2]Department of Agriculture, Swami Vivekananda University, Telinipara, North 24 Parganas, West Bengal, India

[3]Department of Agricultural Biotechnology, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

[4]Debgiri Agro Products Private Limited, N.H-34, Noapara, Barasat, Kolkata, West Bengal, India

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**Corresponding Author:** Suprabuddha Kundu

**Abstract**

An improved micropropagation protocol was established from field grown sword suckers of Banana Grand Naine (*Musa acuminata* AAA). Optimization of surface sterilization of sprouted apical meristematic buds and pre-culture on Shoot Bud Induction Medium [MS + 4.0 $mgL^{-1}$ BA under dark conditions for 3 weeks] resulted ideal number of multiple shoot clusters (12.45±0.33). Subculture of these shoot clusters on Shoot Bud Multiplication Medium fortified with 0.5 $mgL^{-1}$ TDZ and 20 $mgL^{-1}$ AdS induced highest shoot buds (16.30±0.45) in 3 weeks and showed consistent till seven passages. The individual *in vitro* shoots upon transferring onto MS + 0.5 $mgL^{-1}$ TDZ + 1.0 $mgL^{-1}$ IBA + activated charcoal (3 $gL^{-1}$) resulted in elongated shoots and *in vitro* rooting after 3 weeks at a photoperiod of 16 h light and 8 h dark conditions. The present investigation facilitates the massive propagation (90% survival rate) of healthy and quality *in vitro* plantlets of banana.

**Keywords:** Banana grand Naine, growth effects, micropropagation, Thidiazuron

## Introduction

A significant fruit crop of the Musaceae family, the banana (Musa spp. AAA), is widely farmed in underdeveloped nations. After citrus, bananas produce the second-largest fruit output (Swennen and Rosales, 1994) [15]. Originally from Southeast Asia, the banana, Musa Spp. (Musaceae), is currently grown by millions of people in 150 different countries for agricultural production, processing, and marketing (Alagesan *et al*., 2019) [1]. The fourth-most important global food commodity after rice, wheat, and maize is the banana, which is the world's oldest staple food and a fruit that is widely shipped to various regions of the world. In terms of yearly banana production, India is third.

All cultivated bananas are triploid, seedless, or seed sterile, which necessitates clonal propagation of the banana. According to Cronauer-Mitra and Krikorian (1984) [3], conventional propagation methods like as corms, suckers, and sward suckers are not suitable since they might harbour diseases, nematodes, and viruses. In addition, they grow slowly, are bulky, and have poor phytosanitary qualities (Sagi *et al*. 1998) [9]. The ability of banana tissue culture propagation to deliver genetically homogeneous, pest and disease free planting material has recently attracted interest. Banana micropropagation through shoot tips has been successfully proven (Vuylsteke and De Langhe 1985, Ganapathi *et al*. 1995) [17, 4], while Madhulatha *et al*. (2004) [8] documented the influence of liquid pulse treatment with growth regulators on *in vitro* propagation of banana. Banana root initiation is most usually induced by the auxins NAA, IAA, or IBA (Vuylsteke, 1989) [18]. On basal media devoid of any growth regulators, rooting is also possible (Cronauer and Krikorian, 1984; Jarret *et al*., 1985) [3, 6]. The literature reports on the effect of active charcoal on roots. According to Cronauer and Krikorian (1984) [3], there was no difference in rooting when NAA, IAA, or IBA were introduced to the medium in the presence of 0.025% (w/v) active charcoal.

As a result, this work is the first of its type to describe the specific role of cytokinins during banana *in vitro* regeneration investigations, and it also attributes TDZ's involvement in enhancing micropropagated plantlets' acclimatization and field performance.

## Materials and Methods

The suckers of banana cultivar Grand Naine (AAA) from field-grown mother plants that were free of viruses and illnesses were chosen to be aseptically excised and surface sterilized meristem explants needed for *in vitro* culture establishment. The suckers were treated with tween 20 (a few drops) for 20 minutes before being thoroughly rinsed several times with distilled water. The suckers were properly cleaned under running tap water for 30 minutes. They were then given 1% (w/v) bavistin treatment for 20 minutes, followed by five rinses with sterile distilled water. The explants were exposed to sodium hypochlorite at 4%

(v/v) for 6 minutes. The cut ends of the surface-sterilized explants were aseptically clipped to make them smaller for placement on the culture media.

The nutrient medium consisted of MS salts and vitamins (Murashige and Skoog, 1962) [19] and 3% (w/v) sucrose was used in all the experiments. The medium was gelled with 8 $mgL^{-1}$ agar and the pH of the medium was adjusted to 5.7 before autoclaving at 121 ºC for 20 min. The cultures were maintained in a growth room at 25±2 ºC under a 16/8-h (light/dark) photoperiod with a light intensity of 50 l mol m-2 s) 1 provided by cool-white fluorescent lamps.

The nodal segments (NSs) were cultivated on MS medium with 6-benzyladenine (BA) and kinetin (Kin) at various doses (0, 1.0, 2.0, 3.0, 4.0, and 5.0 $mgL^{-1}$) separately or in combination with (0.1, 0.3, 0.5 and 0.7 $mgL^{-1}$) TDZ and 20 $mgL^{-1}$ AdS for shoot induction. All cultures were transferred to fresh medium in every 3 weeks. After 8 weeks of inoculation, the percentage of explants that formed shoots and the number of differentiated shoots per explant were counted.

Shoots that were 4-5 cm long were cut out and placed in MS medium with IAA or IBA (0.1, 0.5, 1 and 2 $mgL^{-1}$) supplements for rooting. After 4 weeks, the percentage of rooting and the average number of roots per shoot were noted. Regenerated plantlets were properly rinsed after roots before being placed in pots with sterile vermiculite. To maintain a high humidity level, the potted plants were covered with clear polyethylene bags. After two weeks, the polybags were unsealed to allow the plants to acclimatize to field conditions.

The surviving plants were moved to pots filled with garden soil and kept in a greenhouse for another two months. With 20 explants for each treatment, all tests were carried out three times. Chemical treatments served as the main plot of the experiment to determine the impact of TDZ concentration and exposure time, and days of exposure served as the sub-plot. Means were compared using Tukey's tests at the 5% level of significance and the data was analyzed using SPSS Version 10 (SPSS Inc., Chicago, USA).

## Results and Discussion

Explants surface sterilized with 0.1% $HgCl_2$ for 6 minutes had a 70% survival rate after inoculation in MS medium with BA (0.5 $mgL^{-1}$). After one day of inoculation, blackish and dead plant portions were produced by prolonged treatment with 0.1% $HgCl_2$.

For those plant species that are commercially valuable and challenging to mass propagate using traditional methods, micropropagation offers a reliable, quick way of multiplication. As a result of this investigation, a repeatable methodology for the *in vitro* growth of the superior banana variety Grand Naine was created. In the current *in vitro* shoot regeneration tests, mature, healthy sword sucker explants that have undergone surface sterilization were employed as explants. The effect of cytokinin on the rate of *in vitro* shoot multiplication of an explant summarizes in Table 1. Explants in MS medium without any cytokinin (control) demonstrated a reduced rate of germination (72.23%) and. With regard to the cytokinins examined the presence of 4.0 $mgL^{-1}$BA, which was much greater than other concentrations of BA and KN. The quantity and length of shoots were also maximum in the 4 $mgL^{-1}$BA supplemented medium after 21 days (12.45±0.33; 7.80±0.25 cm). Nevertheless, BA in combination with 0.5 $mgL^{-1}$ TDZ noticeably improved the percent regeneration (80.0±0.96%), number of shoots (16.30±0.45) and shoot length (8.20±0.30 cm). However, the Banana cultivar Bwara showed significant increases in shoot proliferation rates with increasing BAP concentrations and TDZ can be used at a much lower concentration than BAP. Numerous earlier investigations (Siddique and Anis, 2008; Saha *et al*., 2016; Thokchom and Maitra, 2017) [11, 10, 16] have amply demonstrated the superiority of BA. BA is the most generally occurring ribosides and nucleotides are comparably more stable than those of other cytokinins (Letham and Palni, 1983) [7].

After shifting to the root induction medium for one week, roots started to form from the basal cut end of the shoots. It was discovered that the presence of an auxin (IAA or IBA) in ½ MS medium was effective for rooting (Fig. 1e), and the best rooting (90.0±0.96%) was obtained in this medium supplemented with 1.0 $mgL^{-1}$IBA with noticeably superior root numbers (12.36±0.56) and root lengths per shoot (7.8±0.36 cm) (Table 2). Rooting frequency gradually increased over time and peaked after 21 days of culture. Other plant species, such as *Teucrium stocksianum* (Bouhouche and Ksiksi, 2007) [2], *Talinum triangulare* (Swarna and Ravindran, 2012) [14], and *Viola pilosa* (Soni and Kaur, 2014) [13], have also used optimal root production employing IBA.

**Table 1:** Effect of PGR on shoot regeneration from nodal segments of *E. alba* in MS medium

| BA (mg/L) | KIN (mg/L) | NAA (mg/L) | Regeneration (%) | No. of Shoot | Shoot length (cm) |
|---|---|---|---|---|---|
| 0 | 0 | 0 | 72.23±0.56 | 5.42±0.88 | 5.2±0.37 |
| 1.0 | 0 | 0 | 73.5±0.55 | 7.62±0.76 | 5.6±0.33 |
| 2.0 | 0 | 0 | 75.5±0.33 | 9.23±0.48 | 6.2±0.78 |
| 3.0 | 0 | 0 | 76.6±0.45 | 10.50±0.62 | 7.4±0.45 |
| 4.0 | 0 | 0 | 80.0±0.36 | 12.45±0.36 | 7.8±0.25 |
| 5.0 | 0 | 0 | 78.2±0.25 | 12.33±0.33 | 7.5±0.56 |
| 0 | 1.0 | 0 | 73.5±0.75 | 8.80±0.56 | 4.5±0.36 |
| 0 | 2.0 | 0 | 76.2±0.45 | 9.20±0.75 | 6.8±0.45 |
| 0 | 3.0 | 0 | 76.6±0.66 | 11.08±0.84 | 7.8±0.95 |
| 0 | 4.0 | 0 | 77.6±0.50 | 11.45±0.33 | 7.2±0.33 |
| 0 | 5.0 | 0 | 76.6±0.33 | 10.62±0.67 | 7.6±0.45 |
| 4.0 | 0 | 0.1 | 78.6±0.23 | 11.63±0.33 | 5.8±0.36 |
| 4.0 | 0 | 0.3 | 88.8±0.45 | 14.50±0.56 | 7.2±1.15 |
| 4.0 | 0 | 0.5 | 90.0±0.96 | 16.30±0.45 | 8.2±0.30 |
| 4.0 | 0 | 0.7 | 82.6±0.75 | 13.56±0.88 | 7.5±0.78 |

**Table 2:** Effect of auxins on root formation from micro shoots of *E. alba* in ½MS medium

| IAA (mg/L) | IBA (mg/L) | Frequency of explant inducing Root (%) | No. of Root/Shoot | Root length/Shoot (cm) |
|---|---|---|---|---|
| 0 | 0 | 64.6±0.54 | 6.5±0.34 | 4.5±0.98 |
| 0.5 | 0 | 76.5±0.22 | 7.7±0.33 | 6.8±0.58 |
| 1.0 | 0 | 86.5±0.35 | 8.2±0.68 | 7.6±0.67 |
| 1.5 | 0 | 88.5±0.74 | 13.5±0.86 | 7.2±0.66 |
| 2 | 0 | 72.6±0.33 | 11.2±0.33 | 8.8±0.52 |
| 0 | 0.5 | 68.5±0.74 | 6.8±0.36 | 7.2±0.36 |
| 0 | 1.0 | 90.0±0.96 | 12.36±0.56 | 7.8±0.25 |
| 0 | 1.5 | 80.0±0.55 | 11.22±0.24 | 6.7±0.36 |
| 0 | 2.0 | 78.6±0.78 | 10.2±1.15 | 5.7±0.76 |


**Fig 1:** *In vitro* culture establishment of Banana. (a) Explant on MS media; (b) Multiplication of *in vitro* culture; (d) Root induction in micro shoots; (e) Primary hardening; (f) Secondary hardening

## Conclusion

The present study reports an improved *in vitro* regeneration protocol of commercially valuable cultivar of banana (Grand Naine). TDZ was more effective in producing more and better quality shoots in *in-vitro* banana propagation with BA. This research article offers valuable insights into the propagation of the specific banana variety, supporting the local banana industry and ensuring sustainable crop production. The findings can be further applied in commercial settings, contributing to the local economy and the conservation of genetic resources.

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