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# Use of Tissue Culture for Propagation of Banana Variety Grand Naine (*Musa acuminata*)

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

The present experiment was conducted in sword suckers of the Grand Naine variety of bananas to estimate a perfect blend of different growth hormones used to initiate explants' proliferation and shoot growth in the micropropagation of bananas. In the experiment, we concluded that the explants survival rate increased when they were treated with 0.1 and 0.2 percent of $HgCl_2$ for a period of 8 and 6 minutes respectively. Six different compositions of growth media or treatments were used to initiate the proliferation of explants and further shoot growth. Among the six treatments, MS +6 mg/l BAP + 1 mg/l NAA ($T_5$) gave excellent results, comparatively. For rooting all the explants were treated with the same media composition of IAA at a concentration of 0.1mg/l and activated charcoal, which proved an adequate blend for root imitation. Primary and Secondary hardening processes are carried out in greenhouse using soil-rite as growth media and in the shade house by using press mud and sand in a 1:1 ratio as growth media. Plantlets were established in the field following the second hardening.

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## 1. INTRODUCTION

India is called the fruit basket of the world (APEDA, https://apeda.gov.in/); it is the largest producer and one of the major exporters of fruits in the world [1]. Among the long list of fruits produced in India, major fruits are Mangoes, Grapes, Apple, Apricots, Orange, Fresh Banana, Avocados, Guava, Litchi, Papaya, Sapodilla and Water Melons (APEDA) [2]. In the year 2020-21, India exported approximately 0.610 million MT of fruits (excluding grapes and mango) and earned a foreign exchange of 302.00 million USD.

Fresh Banana is one of the major fruit crops; a ripened banana is energy-rich; loaded with carbohydrates and few minerals and vitamins [3]. Besides several varieties of banana cultivated, Grand Naine is a newly introduced one, but gaining popularity very quickly in comparison to other varieties. Grand Naine belongs to the species *Musa acuminate*; and cultivar group Cavendish (AAA); of banana fruit. Bananas can be consumed directly (ripened fruit) and cooked (raw/plantain type). Grand Naine has a cylindrical bunch, and a thick stem and its height ranges between 2.0 to 2.5 meters. The fruits are slightly dcurved and pointed with an approximate size of 22 cm. The demand for Grand Naine has increased in the last few years, accordingly, its production needs to be increased too Jones and Daniells [4].

Production of banana plants is done in two ways, conventional or tissue culture. The former method is not feasible for large-scale banana production due to: the slow rate of multiplication (4-5 suckers per year); transportation of bulky suckers; and disease and pest infection. Micro-propagation overcomes all these insufficiencies of the conventional method in the following ways: planting material produced is at a higher rate, uniform and disease-free. It also makes the production process, season independent as with the help of micro-propagation, planting materials can be produced all year round. Different types of Tissue-culture techniques applicable to bananas are callus culture, somatic embryogenesis, cell suspension, shoot culture, meristem culture, and protoplast cultures.

This research paper is part of an experiment conducted on the Grand Naine variety of bananas by utilizing the micro-propagation method. The experiment was conducted to record the performance of shoot, root and effect of hardening on the sword suckers of Grand Naine variety at six different treatments.

## 2. MATERIALS AND METHODS

The experiment was conducted in the Tissue Culture Laboratory of the Department of Plant Molecular Biology and Genetic Engineering, ANDUAT Ayodhya, UP. The experimental material was Sword suckers of banana variety Grand Naine; obtained from the Mali nursery situated in Malihabad, Lucknow, UP.

The sword sucker was trimmed to 1 to 1.5 $cm^3$ in size of explant from the apical meristematic region. A total of 50 such explants were extracted and sterilized using different chemicals at different intervals of time *viz*., ethanol (70%), and mercuric chloride ($HgCl_2$) at concentrations of 0.1 and 0.2 %. The explants (preferably with 1 shoot initial) were carefully transferred to six different bottles with different concentrations of BAP (2, 3, 4, 5 and 6 mg/l); however, the concentration of MS media and NAA (1mg/l) used in each treatment was constant. The same combination of media was used for sub-culture after 1 month and two months. For root initiation, 0.1mg/l of IAA Kinetin and activated charcoal were used for each of the six treatments. For primary soil, rite was used and for secondary hardening press, mud was used to grow plantlets further in the greenhouse and shade house respectively.

The six treatments were replicated thrice; data collected from each treatment of three replications were subjected to ANOVA following Complete Randomized Design [5].

## 3. RESULTS AND DISCUSSION

ANOVA results of CRD gave significant results for all the treatments at a 1% level of significance (Table 1). Similar findings have been reported by Shaoo et al. [6].

### 3.1 Sterilization of Explants

Sterilization of explants was done using two sterilants 70% ethanol for 30 seconds and 1 minute; and mercuric chloride at two different concentrations of 0.1 and 0.2 % for 6 minutes, 8 minutes and 8-10 minutes, respectively. The results (Table 2) indicated that survival of explants was maximum when 0.1% of $HgCl_2$ was

used for 8 minutes and 0.2% of $HgCl_2$ was used for 6 minutes. However, explants showed blackening and no growth when sterilized with $HgCl_2$ for more than 8 minutes. Sterilizing explants with 70% ethanol did not give any satisfactory results as most of the explants died. We finally concluded that sterilizing explants with 0.2% of $HgCl_2$ for 6 minutes was best in overcoming fungal or bacterial growth and it did not cause blackening of explants also. So, for further studies, we used 0.2% of $HgCl_2$ for 6 minutes to sterilize 18 explants. However, [7] obtained satisfactory results by using 0.2 % of $HgCl_2$ for 10 minutes in their findings. Dharmapalan et al. [8] used 0.1% of $HgCl_2$ for sterilization of explants.

## 3.2 Regeneration of Shoot Form Explants

Regeneration of shoot-form explants was carried out using MS media, BAP and NAA. We have used different concentrations of BAP viz., 0, 2, 3, 4, 5 and 6 mg/l in 6 treatments; however, the concentration of MS and NAA was kept constant. Shoot regeneration from meristem culture not only provide disease free (bunchy top banana, cucumber mosaic virus and Panama wilt) plantlet [9] but also accelerate the rate of production of plantlets in comparison to the traditional method of banana propagation. The explants of 1 to 1.5 $cm^3$ were placed in 18 culture bottles. These explants turned to brownish colour after 5-6 days of planting in culture media; after 30-35 days of intervals, a hard globular greenish structure grew from the explants (Fig. 1). These greenish structures produced adventitious plantlets later. Maximum growth in shoot length was observed in $T_4$ followed by $T_5$. Similar findings have been observed in the work of Sahoo et al. [6].

**Table 1. Effect of different concentration of plant bioregulators in shoot proliferation during culture and subculture stages**

| | | 1 month after culturing | | After one month | | 1 month after second sub-culture | |
|---|---|---|---|---|---|---|---|
| **Treatments** | **Treatment composition** | **Proliferation of shoot from explants** | | **First Sub-culture** | | **Second Sub-culture** | |
| | | **No. of shoot multiplied** | **Length of shoot (cm)** | **No. of shoot multiplied** | **Length of shoot (cm)** | **No. of shoot multiplied** | **Length of shoot (cm)** |
| $T_0$ | MS media only | 1.06 | 0.57 | 1.02 | 0.52 | 0.51 | 1.29 |
| $T_1$ | MS +2 mg/l BAP + 1 mg/l NAA | 0.36 | 0.65 | 2.21 | 1.02 | 2.43 | 1.73 |
| $T_2$ | MS +3 mg/l BAP + 1 mg/l NAA | 3.09 | 1.11 | 2.55 | 1.44 | 2.95 | 2.00 |
| $T_3$ | MS +4 mg/l BAP + 1 mg/l NAA | 3.30 | 1.39 | 4.19 | 2.18 | 4.24 | 2.46 |
| $T_4$ | MS +5 mg/l BAP + 1 mg/l NAA | 4.24 | 1.54 | 5.57 | 2.47 | 6.31 | 4.05 |
| $T_5$ | MS +6 mg/l BAP + 1 mg/l NAA | 3.33 | 1.60 | 5.17 | 2.74 | 5.27 | 4.43 |
| | CV (%) | 0.22 | 3.87 | 3.72 | 4.9 | 7.79 | 3.87 |
| | S.E (m) | 0.07 | 0.06 | 0.07 | 0.05 | 0.16 | 0.06 |
| | CD | 0.18 | 0.18 | 0.23 | 0.15 | 0.50 | 0.18 |

**Fig. 1. Shoot initiation stage**

**Fig. 2. Root initiation stage**


**Fig. 3. Plantlets after secondary hardening**

**Table 2. Survival rate and performance of different sterilants at different concentrations and time period**

| Sl. No. | Sterilant | Time | Results | Time | Results | Time | Results |
|---|---|---|---|---|---|---|---|
| 1. | 70% Ethanol | 30second | Not effective | 1.0 minute | Not effective | -- | -- |
| 2a. | Mercuric chloride @ 0.1% | 6 minutes | Survival of explants were poor | 8 minutes | Maximum survival of explants Performed best during sub-culture | 8 to 10 minutes | Blackening of rhizome increased No growth observed |
| 2b. | Mercuric chloride @ 0.2% | 6 minutes | Maximum survival of explants | 8 minutes | Survival of explants were relatively good but the rhizomes suffered from blackening | 8 to 10 minutes | Blackening of rhizome increased Necrosis of shoot No growth |

### 3.3 Number of Shoots Developed from Explants and Root Induction

Three observations were recorded for several shoots and leaves developed after a month of culturing, followed by two subsequent sub-cultures. $T_4$ gave the best results in all three cases followed by $T_5$. Root initiation was carried out for all six treatments by applying IAA at a concentration of 0.1mg/l and activated charcoal (Fig. 2).

### 3.4 Hardening of Plantlets

Contrary to field conditions, micropropagated plantlets are grown in a controlled environment viz., nutrition, photo-period, temperature, humidity and aseptic; hence they are required to undergo hardening so that they can acclimatize to field conditions. The process of hardening was completed in two steps: primary hardening and secondary hardening and it was conducted in the five best plantlets; however, for $T_0$ no plantlets were chosen for hardening because of fewer shoots given by them and poor performance comparatively. Before replacing the plantlets in a soilrite medium containing poly-bags; the plantlets were thoroughly washed in running tap water and then kept in a fungicide solution (1g/l) for 15 minutes. During primary hardening, plantlets were carefully transferred to black poly-bags with soil rite in a greenhouse where high humidity of 60-70% and low light intensity were maintained for 50 days [10]. After 50 days secondary hardening process was initiated, in which well-developed plantlets were transferred to shade house to let the plants acquainted with sunlight and carry out photosynthesis in field conditions (Fig. 3).

## 4. CONCLUSION

Micropropagtion is an alternative and fast method to obtain banana plantlets, in comparison to traditional methods of banana propagation. The requirement of media composition differs with variety and it also affects the cost of production at a larger scale. Hence, a perfect combination of media formulation is necessary, in the present experiment we concluded that the mercuric chloride-treated explants exhibited less damage in case of biotic stress. 0.5g/l of BAP is adequate for the growth of explants into plantlets with a maximum number of leaves and shoot length during the initial and two subculture stages. Primary and Secondary hardening acclimatized the young plantlets to grow into a completely healthy plant in the field.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Kumar A. Enhancing Export Potential of Indian Fresh Farm Produce Through Logistics and Supply Chain Innovations. IUP Journal of Supply Chain Management. 2022;19(4).

2. Saxena R, Kumar A, Singh R, Paul RK, Raman MS, Kumar R, Khan MA, Agarwal P. Examining export advantages in Indian horticulture: an approach based on product mapping and seasonality. Journal of Agribusiness in Developing and Emerging Economies; 2022.
3. Singh R, Kaushik R, Gosewade S. Bananas as underutilized fruit having huge potential as raw materials for food and non-food processing industries: A brief review. The Pharma Innovation Journal. 2018;7(6):574-80.
4. Jones DR, Daniells JW. Introduction to banana, abacá and enset. InHandbook of diseases of banana, abacá and enset. Wallingford UK: CAB International. 2019:1-40.
5. Gomez KA. Gomez AA. Completely Randomized Design. In: Statistical procedures for Agricultural Research, 2nd Edn. John Wiley and Sons, New York. 1984;8-20.
6. Sahoo C, Beura S, Rout S, Beura R. High Frequency *in vitro* Cloning of Banana (*Musa acuminata*) cv. Grande Naine. International Journal of Agriculture, Environment and Biotechnology. 2015; 8(4):943-950.
7. Jha Z, Sharma SN, Sahu H, Chandel G, Sharma DK. Generation of true to type micropropagated banana cultivar 'grand naine. International Journal of Advanced Biotechnology Research. 2011;1(1):1-10.
8. Dharmapalan B, Renuka S, Jisha S. Varghese S. An efficient protocol for large scale plantlet production from the apical Meristem of *Musa paradisica* L.cv 'Nendran'. International Journal of Agriculture Environment and Bio-technology. 2013;6:787-792.
9. Al-Amin M, Karim MR, Amin MR, Rahman S, Mamum ANM. *In vitro* Micropropagation of Banana (*Musa Spp.*). Bangladesh Journal of Agricultural Research. 2009; 34(4):645-659.
10. Bhalsing SR, Teli NP, Pawar PK, Saindane PV, Baviskar MP, Maheshwari VL. Tissue Culture Grown Banana: A Cost-Effective Strategy for Hardening. Physiological and Molecular Biology of Plants. 2001;7(2): 185-189.



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