Short communication

Use of tissue culture for propagation of Banana variety Grand Naine(*Musa acuminata*)

Comment [U1]: Revise Titile

Abstract

The present experiment was conducted in sword suckers of Grand Naine variety of banana to estimate a perfect blend of different growth hormones used to initiate explants proliferation and shoot growth in micropropagtion of banana. In the experiment we concluded that 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 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 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 process carried out in green house using soilrite as growth media and in shade house by using pressmud and sand in 1:1 ratio as growth media. Plantlets were established in field following second hardening.

Key words: Hardening, media composition,micropropagation,Naine Grande variety of banana, primary and secondary hardening

Introduction

India is called as fruit basket of world (APEDA, https://apeda.gov.in/); it is largest producers and one of the major exporters of fruits in world. 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). In year 2020-21, India had exported approximately 0.610 million MT of fruits (excluding grapes and mango) and earned a foreign exchange of 302.00million USD.

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

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Production of banana plant is done in two ways, conventional or by tissue culture. The former method is not feasible for large scale banana production due to: 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 conventional method in following ways: planting material produced is at 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 banana are callus culture, somatic embryogenesis, cell suspension, shoot culture, meristem culture, and protoplast cultures.

This research paper is part of an experiment conducted in Grand Naine variety of banana by utilizing 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.

Materials and Methods

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

Comment [U3]: GPS location of field

The sword sucker was trimmed to 1 to 1.5 cm^3size 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 concentration of 0.1 and 0.2 %. The explants (preferably with 1 shoot initial) were carefully transferred to six different bottles with different concentration 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 an interval of 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 soilrite was used and for secondary hardeningpressmud wasused to grow plantlets further in greenhouse and shade house respectively.

The six treatments were replicated thrice; data collected from each treatmentof three replications were subjected to ANOVA following Complete Randomized Design (Gomez and Goemz, 1984).

Results and Discussion

ANOVA results of CRD gave significant results for all the treatment at 1% level of significance (**Table 1**). Similar findings have been reported by Shaoo*et al*. (2015).

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 were 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% of 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, Jha*et al*. (2011) have obtained satisfactory results by using 0.2 % of $HgCl_2$ for 10 minutes in their findings. Dharmapalan*et al*. (2013) used 0.1% of $HgCl_2$ for sterilization of explants.

Regeneration of shoot form explants

Regeneration of shoot form explants were carried out using MS media, BAP and NAA. We have used different concentration 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 form meristem culture not only provide disease free (bunchy top banana, cucumber mosaic virus and panama wilt) plantlet (Al-amin*et al*. 2009) but also accelerate the rate of production of plantlets in comparison to 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 color after 5-6 days of planting in culture media; after 30-35 days of interval, hard globular greenish structure grew from the explants (**Figure 1**). These greenish structures produced adventitious plantlets later. Maximum growth in shoot length was observedin T_4 followed by T_5. Similar findings have been observed in work of Sahoo*et al*. (2015).

Number of shoots developed from Explants and root induction

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

Hardening of plantlets

On contrary to field conditions, micropropagated plantlets are grown in controlled environment viz., nutrition, photo-period, temperature, humidity and aseptic;hence they are required to undergo hardening so that they can acclimatize field conditions. The process of hardening was completed in two steps: primary hardening and secondary hardening and it was conducted in five

best plantlet; however, for T_0 no plantlets were chosen for hardening in view of less shoots given by them and poor performance comparatively. Before replacing the plantlets in a soilrite medium containing poly-bags; the plantletswere 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 soilrite in a green house where high humidity of 60-70% and low light intensity was maintained for a period of 50 days (Bhalsing*et al*. 2001). 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 (**Figure 3**).

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 cost of production at 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 growth of explants into plantlets with maximum number of leaves and shoot length during initial and at two subculture stages. Primary and Secondary hardening acclimatized the young plantlets to grow into a complete healthy plant in field.

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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

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

		
Figure 1: Shoot initiation stage	**Figure 2: Root initiation stage**	**Figure 3:Plantlets after secondary hardening**