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Study on *In vitro* Establishment and Callus Induction in Banana cv. Grand Naine

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Authors' contributions

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

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ABSTRACT

Banana is conventionally vegetatively propagated through suckers, which grow from lateral buds originating from corms and suckers. This process is very slow as the rate of multiplication of suckers through conventional vegetative means has been found to express several negative impacts which include transmission of diseases, low production and poor conservation of original plant genetic material. The rapid proliferation obtained in tissue culture allows nursery men to meet unexpected demand for a particular variety. Sword Suckers of cultivar Grand Naine were used as explants in our study. Contamination is the most severe problem encountered *in vitro* culture establishment. Mercuric chloride alone and in combination with 70% ethanol at different time duration was used in the study for the establishment of the cultures. The contamination significantly decreased with increase in concentration of sterilants and their time of exposure. The percent of establishment of explants was recorded highest (70.0% ± 2.40) after four weeks of culturing when explants were treated with ethanol (70%) for 30 seconds + $HgCl_2$ (0.1%) for 25 mins. MS media supplemented with 2,4-D 2.0 mg /l+ NAA 0.5 mg/l was found most effective for maximum percentage of callus formation (70.0% ± 1.00). Finally, regeneration of plantlets was

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achieved on MS medium supplemented with 3.0 mg/l BAP and 1.0 mg/l NAA. Our results described various factors that influence the *in vitro* establishment and callus formation of banana cv. Grand Naine.

Keywords: Callus formation; micropropagation; banana; In vitro regeneration.

1. INTRODUCTION

Banana (*Musa paradisica*) is a vital source of food widely enjoyed around the world. It is one of the oldest fruits in the world [1]. The fruit is delicious and seedless and one of the most important fruit crops grown in India. It has a rare combination of protein, energy value, vitamins, tissue building elements and minerals. Banana can also be in a diet for high blood pressure as it contains potassium which helps to reduce and control high blood pressure. Banana serves as the staple food for approximately 500 million people worldwide [2,3]. Quality banana is a triploid derived from two diploid species *Musa acuminata* (Malaysia) and *Musa balbisiana* (India) [1]. Presently banana is grown in around 150 countries across the world on an area of 50.34 lakh million hectare producing 106.84 lakh metric tonnes [4]. India ranks first in area and production of banana in the world. Its annual production is 29.72 lakh metric tonnes from 80.25 lakh hectare area with national average of 37.0 tonnes per hectare. Tamil Nadu is the leading state in the area as well as banana production with the highest productivity of 47.9 tonnes per hectare (NHB Stats and Indian Horticulture Database 2014, Department of Agriculture & Cooperation). Bihar occupies an area of 34.31 thousand hectare under banana cultivation with an annual production of 1435.78 thousand metric tonnes and productivity 41.84 tonnes per hectare. (IHD, 2014). Major banana producing belts in Bihar are Vaishali, Bhagalpur, Khagaria, Katihar, Purnea and Samastipur districts. Recommended varieties of banana for cultivation under Bihar conditions are Dwarf Cavendish, Robusta, Grand Naine, Rasthali, Poovan, and Monthan. Harvesting season of banana in Bihar is mainly August to December, in which September to November is the peak season for banana harvesting [5].

Grand Naine (G-9) is a cultivar of *Musa acuminate* and a source of commercial Cavendish bananas. It is also known as the Chiquita banana because it is the main product of Chiquita brands. This group of banana is distinguished from other groups by their AAA genotype. The AAA genotype refers to the fact that this group is a triploid variant of the species *Musa acuminata.* There are 33 chromosomes present in the AAA cultivar and all produce seedless fruits through parthenocarpy. The Grand Naine produces large inflorescence which develops into the edible fruit. It is a popular commercial cultivar grown extensively for table and processing purpose in the states of Maharashtra, Gujrat, Bihar and West Bengal. The bunch size, the fruit length and size is quite good, The average bunch weight with 6-7 hands and with about 13 fruits per hand is about 15-25 Kg. The selection yields bunch weighing 60-70 Kg. Its characteristic medium height and large fruit yields make it ideal for commercial agriculture. The moderate height allows easy harvesting and some resistance to wind throw. The seedless quality of the fruits also increases its popularity because of its importance as a staple crop as well as a cash crop.

Banana is generally propagated vegetatively through suckers, This process is very slow as the rate of multiplication of suckers through conventional vegetative means has been found to express several negative impacts which include transmission of diseases, low production and poor preservation of original plant genetic material [6].

Micropropagation is an excellent option for producing low cost quality planting material. Banana plantlets produced through micropropagation have been found to establish faster, stronger and healthier with a shorter production cycle and higher yield than conventional methods [7] as millions of plants can be grown from a single part of the plant within a year [8] and multiplication of plant can be done throughout the year. Through tissue culture, large quantities of banana plantlets are produced within a short period.

2. MATERIALS AND METHODS

The experiment was conducted at Plant Tissue Culture Laboratory, Bihar Agricultural College, Sabour, Bhagalpur during 2016. Sword sucker was used as an explant for all the experiments.

3. RESULTS AND DISCUSSION

3.1 Effect of Sterilizing Agents on Explants

The efficiency of sterilizing agents was evaluated in terms of number of aseptic explants sprout. The rhizome shoot tips were at first rinsed with 1-2% solution (treatment uniform) of teepol detergent and then 0.1% bavistin treatments was given for half an hour. The pre-treated rhizome shoot tips were washed two times with sterilized distilled water in a laminar flow. Then surface sterilization was done with different time duration of sterilizing agents (Table 1). When no sterilant was used all the cultured explants were contaminated. The contamination of explants significantly decreased with the increase in the concentration of sterilants and their time of exposure. The per cent establishment of explants was recorded highest (70.0±2.40 per cent) with T_8 treatment after four weeks of culturing. Although the minimum contamination was observed (15.0±1.0 per cent) with T_9 treatment percent mortality was recorded highest (50.0±1.80 per cent) in this particular treatment. Overall T_8 was found the most effective treatment and showed maximum percent establishment (70.0±2.40 per cent), less mortality percent (11.0±1.80) and percent contamination was also recorded considerably low (19±1.70).

Mercuric chloride was found very effective in controlling the contamination of explants. The per cent establishment of explants was recorded highest (70.0 ± 2.4 per cent) followed by (60.0±1.3 per cent) with T_8 and T_4 treatments respectively i.e. when explants were treated with $HgCl_2$ (0.1%) for 20 minutes along with Ethanol (70%) for 30 seconds and with $HgCl_2$ (0.1%) for 20 minutes alone respectively. Exposure to a lower concentration of sterilants results the increase in contamination of explants where as exposure to higher concentrations for longer durations though reduced the contamination but the mortality of explants increased considerably. This indicates the deleterious effect of the sterilants at higher concentration. In all the experiments mercuric chloride proved good disinfectant, despite its toxicity to plant tissues. Jaisy and Ghai [9] worked on in vitro propagation of banana also found the treatment of explants with mercuric chloride most effective surface sterilization procedure registering maximum culture banana establishment with minimum contamination.

3.2 Callus Formation

This experiment was conducted to assess the effect of 2,4-D in combination with NAA for callus formation. Sword sucker was taken as explant from Grand Naine cultivar of banana for callus induction. Explants were cultured on MS media containing six concentrations, *viz*. 0, 0.5, 1.0, 1.5, 2.0 and 2.5 mg/l of 2,4-D in combination with 0.5 mg/l NAA. The explant changed from creamy white to green within two weeks after inoculation

Table 1. Effect of different treatment duration of sterilizing agents on explants of banana cv. Grand Naine

Trt.	Treatment and duration	Per cent contamination	Per cent mortality	Per cent shoot establishment
T_1	Control	100.0 ± 0.0	-	-
T_2	$HgCl_2$ (0.1%) for 10 min	70.0 ± 0.9	10.0 ± 0.7	20.0 ± 1.4
T_3	$HgCl_2$ (0.1%) for 15 min	50.0 ± 1.4	20.0 ± 1.4	30.0 ± 1.8
T_4	$HgCl_2$ (0.1%) for 20 min	30.0 ± 1.1	10.0 ± 2.3	60.0 ± 1.3
T_5	$HgCl_2$ (0.1%) for 25 min	24.0 ± 1.9	36.0 ± 2.2	40.0 ± 1.7
T_6	Ethanol (70%) for 30 sec+ $HgCl_2$ (0.1%) for 10 min	43.0 ± 1.1	25.0 ± 0.9	32.0 ± 1.0
T_7	Ethanol (70%) for 30 sec+ $HgCl_2$ (0.1%) for 15 min	35.0 ± 0.7	15.0 ± 0.7	50.0 ± 1.2
T_8	Ethanol (70%) for 30 sec+ $HgCl_2$ (0.1%) for 20 min	19.0 ± 1.7	11.0 ± 1.8	70.0 ± 2.4
T_9	Ethanol (70%) for 30 sec+ $HgCl_2$ (0.1%) for 25 min	15.0 ± 1.0	50.0 ± 1.8	35.0 ± 1.0
CD (0.05)		2.20	2.65	2.83

Table 2. Effect of 2,4-D and NAA on callus induction

Medium code	Treatments	Callus formation (%)
MS_0	Control	-
MS_1	2,4-D 0.5 mg/l + 0.5 mg/l NAA	10.0 ± 0.7
MS_2	2,4-D 1.0 mg/l + 0.5 mg/l NAA	25.0 ± 0.7
MS_3	2,4-D 1.5 mg/l + 0.5 mg/l NAA	45.0 ± 0.7
MS_4	2,4-D 2.0 mg/l + 0.5 mg/l NAA	70.0 ± 1.0
MS_5	2,4-D 2.5 mg/l + 0.5 mg/l NAA	60.0 ± 1.5
CD (0.05)		0.21

on MS media with different treatment durations. Sub-culturing was done after 21 days on same media to get the callus induction. Initially, the explants showed swelling at basal portion of explants followed by the greening of apical portion. Another second subculturing was done after 21 days on the same media after removal of a brown portion of explants. Third Subculturing was done after 24 days again on the same media and as a result callus formation was observed. Maximum percentage of callus formation (70.0 ± 1.00) was found when explant was cultured on MS medium supplemented with 2.0 mg/l + 0.5 mg/l NAA followed by 2.5 mg/l 2,4-D + 0.5 mg/l NAA respectively (Table 2). Significant differences in callus formation were observed when the concentration of 2,4-D was increased from 0.5-2.5 mg/l. Finally regeneration of plantlets was achieved on MS medium supplemented with 3.0 mg/l BAP and 1.0 mg/l NAA. Almost same observations of callogenesis were found on MS medium supplemented with 2,4-D from thin meristematic layers excised from proliferating shoot tip explants by Banerjee et al. [10] and Daniels et al. [11]. Somewhat similar results were also found by Nietsche et al. [12] who reported callus formation in MS medium supplemented with 20.0 µM BAP and 4.0 µM NAA or/and 6.0 µM TDZ, although the callus died.

4. CONCLUSION

Exposure to lower concentration of sterilants resulted in increased contamination of explants whereas exposure to higher concentrations for longer durations though reduced the contamination but the mortality of explants increased considerably which indicates the deleterious effect of the sterilants at higher concentration. Maximum percentage of callus formation (70.0± 1.0) was found when explant was cultured on MS medium supplemented with 2.0 mg/l 2,4-D and 0.5 mg/l NAA.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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