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Research Article

In Vitro Propagation of Banana via Shoot Tip

Rinku Rani* and S.K. Sehrawat

Department of Horticulture, COA, CCSHAU, Hissar, Haryana, India

Abstract

The present investigation was undertaken to develop *in vitro* protocol for production of quality plant material in banana cv. Grand Naine. The results showed that the sterilization with 0.4% citric acid + 0.2% ascorbic acid for 40 minutes, 0.2% bavistin + 0.1% streptocycline for 30 minutes and 0.1% mercuric chloride for 7 minutes optimum for maximum culture establishment with minimum contamination. MS media containing 4.0 mg/l BAP resulted in maximum establishment of cultures in lesser time. Maximum number shoots were obtained on MS media supplimented with 4.0 mg/l BAP and 500 mg/l Casein hydrolysate.

Keywords: *in vitro*, banana, MS media, sterilization, establishment, shoot multiplication

***Correspondence**

Author: Rinku Rani

Email: rinkusthr49@gmail.com

Introduction

Banana, a herbaceous fruit plant, is member of the family Musaceae. It is believed to be one of the oldest fruits which have originated from Malaysia through a complex hybridization process [1]. Cultivated banana is a triploid derived from two diploid species that are, *Musa acuminata (*Malaysia) and *Musa balbsiana* (India) [2]. It is most important fruit in the world, both as staple food as well as a major export commodity for many tropical and sub-tropical countries. India is larger producer of banana, area under banana cultivation is 802.6 thousand hectare and total production is 29724.6 thousand metric tonnes [3]. Banana is conventionally propagated by sword sucker. Although propagation by sucker retains all the characters of the parent but viral diseases are also transmitted through suckers especially when parent material is infected and also suckers are found in touch to soil therefore, they become infested with those diseases for which organisms are present in the soil around the banana plant. *Musa* crop suffers from several devasting diseases. Besides, sufficient number of suckers is not available at a time for sowing on a large scale. Hence, non availability of disease free planting material is barrier in its cultivation. Regeneration through *in vitro* culture has now become a viable and alternate method to conventional one to produce disease free planting material within a short time of period.

Materials and Methods

The present study on "*In vitro* propagation of banana cultivar Grand Naine" was conducted at Centre for Plant Biotechnology, Government of Haryana, CCS HAU Campus, Hisar during 2015-2016. The banana cultivar Grand Naine growing in the experimental polyhouse of Centre for Plant Biotechnology, Hisar, (Haryana) was selected as source of explants. Shoot tips of 3-4 cm length were excised. Explants were washed under running tap water. The excised explants were washed with detergent (teepol) followed by washing under running tap water. They were finally washed with distilled water and ready for surface sterilization. The explants were treated with citric acid, ascorbic acid, bavistin, streptocycline and 0.1 per cent mercuric chloride. Further, the explants were given 5-6 washing with sterilized water to remove the traces of sterilizing agent. After surface sterilization, explants (shoot tips) were aseptically cultured on MS media containing diffferent concentration of cytokinins and auxins under laminar air flow cabinet. After initial establishment, elongated shoot tips were transferred to different shoot multiplication media and sub cultured for 3-4 times after an interval of 20-25 days for their multiplication. The data of all the experiment recorded during the present investigation were subjected to statistical analysis using "Completely Randomized Design" by using software OP STAT.

Results and Discussion

Table 1 indicated that the maximum survival per cent was observed in explants treated with treatment ST_3. It appears that the ST_3 treatment was sufficient to control bacterial and fungal growth as shown in **Figure 1**. In ST_4 and ST_5,

mortality was observed due to browning, which may be due to longer exposure of explants in 0.1% $HgCl_2$. Similar results have been also reported by [4] and [5]. Significantly highest per cent regeneration (67.7) was observed in treatment EM_4 (MS media + 4.0 BAP). **Table 2** shown that as concentration of BAP increase regeneration per cent also increase upto a optimum concentration (4.0 mg/l) as shown in **Figure 2**. Further increase in BAP concentration above optimum, resulted in a decreased regeneration rate which indicating adverse effect of plant growth regulators beyond the optimal dose. The similar results have been also reported by [6] and [7]. BAP was more effective than KIN for shoot regeneration. The results are in consistent with that of [4] who found BAP alone best for establishment of banana cultivar Robusta instead of combinations of different cytokinins and auxins. Variation in the activity of different cytokinins can be explained by differences in the uptake rates reported in different genomes [8], varied translocation rates to meristematic regions, and metabolic processes in which cytokinin may be degraded or conjugated with sugars or amino acids to form biologically inert compounds as reported by [9].

Table 1 Effect of various treatments used for surface sterilization on contamination, browning and survival percentage of shoot tip explants of banana cultivar Grand Naine

Sterilization procedure	%Contamination	%Browning	%Survival
ST_0 Control (No sterilization)	100	0	0
ST_1 [(0.4% citric acid + 0.2% ascorbic acid) for 20 min. followed by (0.1% bavistin + 0.05% streptocycline) for 30 min. and then with 0.1% mercuric chloride for 6 min.]	40.0 ± 5.77	56.7 ± 12.02	3.3 ± 1.67
ST_2 [(0.4% citric acid + 0.2 % ascorbic acid) for 30 min. followed by (0.2% bavistin + 0.1% streptocycline) for 30 min. and then with 0.1% mercuric chloride for 6 min.]	33.3 ± 3.33	16.7 ± 1.67	50.0 ± 5.77
ST_3 [(0.4% citric acid + 0.2% ascorbic acid) for 40 min. followed by (0.2% bavistin + 0.1% streptocycline) for 30 min. and then with 0.1% mercuric chloride for 7 min.]	3.3 ± 3.33	0	96.7 ± 3.33
ST_4 [(0.4% citric acid + 0.2 % ascorbic acid) for 40 min. followed by (0.2% bavistin + 0.1% streptocycline) for 30 min. and then with 0.1% mercuric chloride for 8 min.]	0	63.3 ± 8.81	36.7 ± 6.01
ST_5 [(0.4% citric acid + 0.2% ascorbic acid) for 40 min. followed with (0.2% bavistin + 0.1% streptocycline) for 30 min. and then with 0.1% mercuric chloride for 9 min.]	0	93.3 ± 3.33	6.7 ± 1.67

ST = Sterilization treatment

Table 2 Effect of different concentrations of growth regulators on regeneration percentage and number of days taken for shoot initiation from shoot tip explants

Hormonal composition of media (mg/l)	Per cent regeneration			minimum days taken for shoot Initiation
	7th day	14th day	21st day	
EM_0 MS media (no growth regulators)	0	0	16.7 ± 2.03	19.0 ± 0.58
EM_1 (MS + 1.0 BAP)	0	10.0 ± 2.89	43.3 ± 3.33	17.8 ± 1.97
EM_2 (MS + 2.0 BAP)	0	13.3 ± 1.67	53.3 ± 1.76	15.7 ± 2.03
EM_3 (MS + 3.0 BAP)	10.0 ± 0.00	30.0 ± 0	60.0 ± 0.57	14.7 ± 1.45
EM_4 (MS + 4.0 BAP)	40.0 ± 2.89	70.0 ± 2.89	90.0 ± 1.15	7.5 ± 1.48
EM_5 (MS + 5.0 BAP)	0	23.3 ± 3.33	46.7 ± 3.33	15.0 ± 1.53
EM_6 (MS + 6.0 BAP)	0	0	43.3 ± 1.67	18.7 ± 0.67
EM_7 (MS + 1.0 BAP+ 0.1 NAA)	13.3 ± 3.33	20.0 ± 2.89	53.3 ± 2.02	14.8 ± 2.69
EM_8 (MS + 1.0 BAP + 0.1 IAA)	20.0 ± 0.00	30.0 ± 5.00	30.0 ± 1.73	12.1 ± 3.12
EM_9 (MS + 1.0 KIN + 0.1 NAA)	0	0	20.0 ± 1.00	17.7 ± 0.67
EM_{10} (MS + 1.0 KIN + 0.1 IAA)	0	13.3 ±1.76	36.7 ± 3.33	16.8 ± 1.36

EM = Establishment media

Treatment EM_4 (MS media + 4.0 BAP) took minimum number of days (7.5) for shoot initiation. It might be suitable concentration of BAP which played an important role for early shoot initiation. [10] also observed that MS media supplemented with BAP (4.0 mg/l) was produced maximum 3.8 buds per explant in cultivar Grand naine in 2.2 days. However, the maximum number of buds per explant was not so much different in other cases, but it showed slow response and took more number of days for bud formation. Maximum number shoots were obtained in treatment SMM_3 (MS media + 4.0 BAP + 500 Casein hydrolysate) followed by SMM_4 (MS media + 4.0 BAP + 750 Casein hydrolysate) as shown in **Table 3**. It was observed that BAP at 4.0 mg/l concentration in combination with 500mg/l casein hydrolysate most optimum for multiplications of shoots and by increase or decrease in its concentrations caused a decrease in multiplication rates. Maximum length (9.2 cm) of shoots and number of leaves per explants was recorded with treatment SMM_3 as shown in **Figure 3**. Casein hydrolysate is a protein, serve as source of organic nitrogen and used in tissue culture. [11] used casein hydrolysate for regeneration of shoots of banana cultivar Sabri. They were found that MS medium supplemented with 1 mg/l BA, 0.5 mg/l IAA and 500 mg/l casein hydrolysate best for regeneration of shoots. [12] observed that Casein hydrolysate in media led to increased callus proliferation and production of primary somatic embryo in banana cultivar Dwarf Brazilian.

Table 3 Effect of shoot multiplication media on multiple shoot formation, shoot lenght and number of leaves

Media composition (mg/l)	Multiplication of shoots			Shoot length (cm)			Number of leaves		
	7th day	14th day	21st day	7th day	14th day	21st day	7th day	14th day	21st day
SMM_0 (control, no growth regulators)	1.0 ± 0.00	1.2 ± 0.25	2.5 ± 0.14	4.8 ± 0.52	5.5 ± 0.31	5.6 ± 0.24	3.0 ± 0.36	3.6 ± 0.61	3.7 ± 0.60
SMM_1 (MS + 4.0 BAP)	1.0 ± 0.00	2.5 ± 0.09	3.1 ± 0.24	5.9 ± 0.03	6.6 ± 0.06	6.7 ± 0.14	3.7 ± 0.27	4.1 ± 0.24	4.4 ± 0.35
SMM_2 (MS + 4.0 BAP + 250 Casein hydrolysate)	1.5 ± 0.55	3.5 ± 0.09	5.2 ± 0.25	6.9 ± 0.07	7.1 ± 0.10	7.2 ± 0.14	4.5 ± 0.14	5.0 ± 0.19	5.2 ± 0.22
SMM_3 (MS + 4.0 BAP + 500 Casein hydrolysate)	5.4 ± 0.22	9.0 ± 0.58	10.6 ± 0.73	8.9 ± 0.06	9.1 ± 0.02	9.2 ± 0.03	5.5 ± 0.09	6.2 ± 0.09	6.4 ± 0.20
SMM_4 (MS + 4.0 BAP + 750 Casein hydrolysate)	1.5 ± 0.29	5.2 ± 0.31	6.8 ± 0.40	7.7 ± 0.09	8.1 ± 0.03	8.1 ± 0.03	4.8 ± 0.11	5.3 ± 0.19	5.4 ± 0.13
SMM_5 (MS + 2.0 BAP + 0.5 NAA+ 500 Casein hydrolysate)	2.9 ± 0.48	4.7 ± 0.31	4.8 ± 0.25	6.0 ± 0.22	6.5 ± 0.21	6.6 ± 0.13	4.5 ± 0.09	4.8 ± 0.11	4.9 ± 0.11

SMM= Shoot multiplication

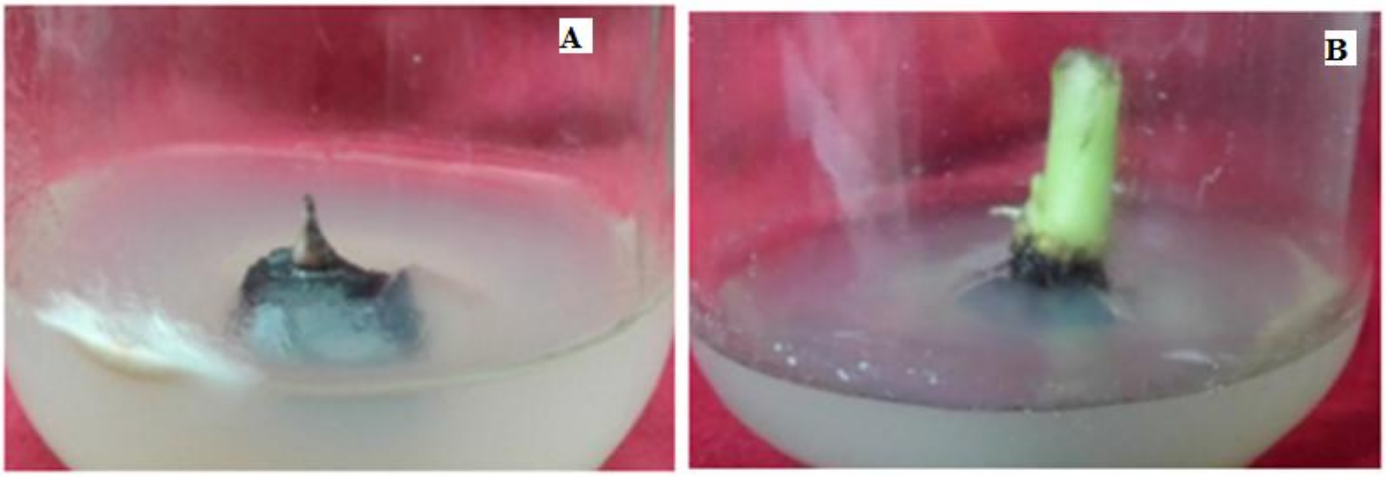

Figure 1 Effect of sterilization treatment on explant.
(A; Control (No sterilization), B; 0.4% citric acid + 0.2% ascorbic acid for 40 minutes and 0.2% bavistin + 0.1% streptocycline for 30 minutes and 0.1% mercuric chloride for 7)

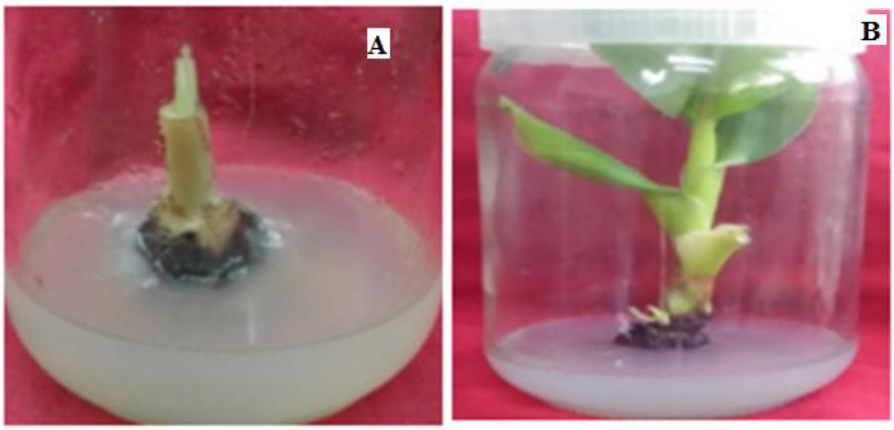

Figure 2 Effect of growth regulators on shoot initiation.
(A; No growth regulators, B; MS + 4.0 mg/l BAP)

Figure 3 Effect of growth regulators and casein hydrolysate on shoot multiplication.
(A; MS media (control), B; MS media + 4.0 mg/l BAP + 500 mg/l casein hydrolysate)

Conclusion

In the present investigation, attempts were made to develop an efficient protocol for *in vitro* propagation. Salient features of the results obtained in the present study are as given below :

The explant sterilization, 0.4% citric acid + 0.2% ascorbic acid for 40 minutes followed by 0.2% bavistin + 0.1% streptocycline for 30 minutes and then with 0.1% mercuric chloride for 7 minutes treatment, proved to be the best. MS media supplemented with 4.0 mg/l BAP produced highest regeneration percentage in minimum number of days. For shoot multiplication, MS media supplemented with 4.0 mg/l BAP and 500 mg/l casein hydrolysate proved to be the best.

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