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Reproducible Protocol on Morphogenesis and Growth Studies on Shoot Tips of Various Banana (*Musa Paradisiaca*) Genotypes Under Different Levels of Hormones *in Vitro*

Narwade A.V.[1*], Patel S.R.[2] Khatri R.T. [3], Manjushree Singh[4], Sanjay Pradhan[5], Sagar K Jadav[1], and Vivek N. Zinzala[1]

ABSTRACT: *The shoot tips four banana genotypes were treated with different sterilizing agents to know the best treatment combinations to avoid maximum contamination from the cultures. Total 17 combinations (with control) were used for shoot tip treatments. The treatment Bavistine 2.5mg/l and Chloromphenicol 500 mg/l (45 min.) + 1.0% $HgCl_2$ (10 min.) registered maximum culture establishment (68.02%) followed by 66.41% culture establishment in the treatment Bavistine 2.5mg/l + Straptomycin 500 mg/l (45 min.) + 1.0% $HgCl_2$ (10 min.). The genetic background of genotypes of banana are differed hence multiple shoots were obtained in different numbers from shoot tips. Total 16 combined treatments were studied for producing maximum multiple shoots using Murashige and Skoog (MS) medium. The basal media supplemented with 6-BAP (3 mg/l) + Adenine Sulphate (2 mg/l) in various genotypes of banana produced multiple shoots, G9 (2.54), Mahalaxmi (2.34) Shrimanti (2.14) and Basarai (2.23) followed by the treatment combination 6-BAP (4 mg/l) + Adenine Sulphate (1 mg/l) in G9 (2.42), Mahalaxmi (2.30) Shrimanti (2.09) and Basarai (2.19)* in vitro. *15 treatments (with control: only ½ MS medium) were investigated to study of root induction by using two auxins individually i.e. IBA and NAA. The highest number of roots per shoot and maximum root length (cm) were observed in the Half strength MS medium containing 1 mg/l IBA in different cultivars* viz., *G9 (12.2, 8.6 cm), Mahalaxmi (12.0, 8.4 cm), Shrimanti (11.7, 8.3 cm), and Basarai (11.3, 8.1 cm) respectively, followed by the treatment 0.75 mg/l IBA found most suitable for the induction of roots in all the genotypes. In the root studies, the highest number of roots per shoot and maximum root length (cm) were observed in the Half strength MS medium fortified with 1.25 mg/l NAA in different genotypes* viz., *G9 (11.0, 8.3 cm), Mahalaxmi (10.8, 8.1 cm), Shrimanti (10.4, 8.0 cm), and Basarai (10.1, 7.8 cm) respectively.*

Key words: *Sterilizing agents,* Banana *shoot tip, Micro-propagation, Multiple shoots, Rooting behavior*

INTRODUCTION

Banana (*Musa paradisiaca* L.) is a large herbaceous perennial monocotyledonous and monocarpic plant. Banana belongs to family Musaceae. "Apple of Paradise" i.e., Banana governs its antiquity in India sub-continent from the ancient periods. Banana is one of the popular fruit available to the common man. It is delicious, seedless and grown throughout the year. Banana and plantain are wonder berry, forming staple food of millions of people across the globe, providing a more balanced diet than any other fruit or vegetable. Basarai, Mahalxmi, and Grand Naine are mostly cultivated in Maharashtra and Gujarat. The productivity of banana in India is very low due to infestation of nematodes and other pests; infected suckers due to various diseases such as leaf spot, bacterial wilt and bunchy top; deficient in good planting material. Besides, banana propagation is through suckers is very low. In this context, tissue culture technique is elegant tool and becoming blissful to provide disease free and genetically true-to-type plantlets and huge numbers of shoots within short

[1] Dept. of Genetics & Plant Breeding, N. M. College of Agriculture, NAU, Navsari -396 450 (Gujarat)
[2] Dept. of Genetics & Plant Breeding, College of Agriculture, NAU, Maktampur, Bharuch- 390 012 (Gujarat)
[3] Soil & Water Management Research Unit, NAU, Navsari -396 450 (Gujarat)
[4] Dept. of Agricultural Engineering, N. M. College of Agriculture, NAU, Navsari -396 450 (Gujarat)
[5] Dept. of Animal Science, N. M. College of Agriculture, NAU, Navsari -396 450 (Gujarat)

period of time. Other benefits of tissue culture are improved crop yield, easier transportation and year round availability of planting materials whereas; such problems cannot be solved by conventional method. The uniformity in harvesting is yet another problem faced by the banana growers throughout the country. The harvesting period extends even up to four months hence, farmer cannot able to get benefit another crop.

MATERIALS AND METHODS

Robust, disease free and high yielding plants of banana were selected and tagged in the field. The suckers from such plants of four varieties namely, Grand Naine, Mahalaxmi, Shrimanti and Basarai were dug out and collected. They were cleaned thoroughly by repeated washing, then trimmed and used as explants. The MS medium was used as basal medium. All inoculations and manipulations involving sterile culture or media were carried out inside laminar airflow cabinet. The shoot tips were treated with different solutions and then washed with 10 per cent solution of detergent (Labonin) for 10 minutes. MS medium with various auxin(s) and cytokinin(s) singly or combination were used in the trial for *in vitro* shoot multiplication and root induction for their standardization. The observations like number of shoots proliferation, number of roots/shoot and root length in cm were recorded. The data analyzed in Completely Randomized Design (CRD) wherever necessary as prescribed by Panse and Sukhatme (1985).

RESULTS AND DISCUSSION

The results of the experiments on micropropagation of banana (Musa paradisiaca L.) conducted at Plant Tissue Culture Laboratory, Department of Genetics and Plant Breeding, N. M. College of Agriculture, Navsari Agricultural University, Navsari (Gujarat) are presented in this chapter.

1. **Effect of Surface Sterilization Treatments on Establishment of Shoot Tip Explants of Banana Cultivars:** For attaining good results in establishment of shoot tips, explant sterilization is one of leading step in plant tissue culture. *In vitro* technique of plant tissue culture is more beneficial for fast production of new varieties, uniform maturity, disease free materials, uniformity of shoots, short harvest interval, profuse multiplication rather than traditional propagation suggested by Vuylsteke, 1989, Daniells, 1991 and Arias, 1992. Three chemicals were used as a surface sterilization of shoot tips namely; Bavistine, Chloromphenicol and $HgCl_2$. Table 1 showed that 17 chemical combinations (with control) were used during shoot tip treatment. The treatment combination Bavistine 2.5 mg/l and Chloromphenicol 500 mg/l (45 min.) + 1.0% $HgCl_2$ (10 min.) registered maximum culture establishment (68.02%) followed by 66.41% culture establishment was noticed in the treatment Bavistine 2.5mg/l + Straptomycin 500 mg/l (45 min.) + 1.0% $HgCl_2$ (10 min.). Due to adverse effect of the chemicals on shoot tips, 12 per cent culture was died off and 20 per cent was contaminated in the experimentation. Minimum shoot tips were survived in the treatment i.e. Bavistin (2.5g/l) + Streptomycin (250g/l) except control. Hamill *et al.,* 1993 explained in his research that kinds of disinfection and their concentrations are different with differ in explant type and size (single or double sterilisation). For controlling bacterial contamination, Van Den Houwe and Swennen (2000) suggested the role of antibiotics in banana tissue cultures.

2. **Effect of Different Treatments on Shoot Multiplication in Banana Varieties *in Vitro*:** Due to differences in genetic background of various genotypes of banana, they responded differently for production of multiple shoots in the same treatments in table 2. MS medium containing 6-BAP (3 mg/l) + Adenine Sulphate (2 mg/l) showed highest rate multiple shoots in various varieties of banana namely, G9 (2.54), Mahalaxmi (2.34) Shrimanti (2.14) and Basarai (2.23) which was significantly superior than all other treatments followed by the treatment combination 6-BAP (4 mg/l) + Adenine Sulphate (1 mg/l) in G9 (2.42), Mahalaxmi (2.30) Shrimanti (2.09) and Basarai (2.19). The banana variety Grand Naine produced higher numbers of shoots in all the treatments followed by the genotype Mahalaxmi. For shoot multiplication, the individual treatment of 6-BAP as well as combined treatments of 6-BAP with Adenine Sulphate at lower concentrations (<3.0 mg/l 6-BAP) and at higher concentrations (>5.0 mg/l6-BAP) found ineffective. The lower concentration of BAP (2.0 mg/l) for

establishment and higher concentration of BAP (4.0 mg/l) with AS for multiplication of banana cv. Basrai responded better on solid medium (Babylatha *et al.*, 1997). In banana tissue culture several reports suggested that BAP is necessary for multiplication of shoot (Bannerjee and De Langhe, 1995; Daquinta, 2000; Nguyen *et al.*, 2001; Muhammad *et al.*, 2004; Acharjee *et al.*, 2004). Our result was also agreed with their consequences. Proliferation /multiplication of shoots was critically affected by genotype and other factors in banana have been reported by many scientists such as Bhagyalakshmi and Singh 1995; Oliveira *et al.*, 1999; Gubbuk and Pekmezci, 2004. They suggested that different genotypes of the banana responded differently to a fixed set of treatments. The difference may be attributed to the difference in the level of endogenous phytohormons, nutrients and metabolites, and interaction between various growth factors. Thorpe (1980) knew little about how hormones induced a particular pattern of morphogenesis. The interaction between various growth factors plays an important role in organogenesis (Skoog and Miller, 1957). The organogenesis in culture are more complex and solely influenced by organic and inorganic nutrients, plant hormones and osmotic concentration (Ammirato,1986).

3. **Effect of Auxins on *in Vitro* Rooting:** Roots formation is the third stage of micro-propagation of any plant spices. Rooting behaviour of micro shoots of banana was exclusively affected auxin concentration (Table 3). The study mentioned that rooting of *in vitro* banana shoots was not a problem. Cent per cent root formation was observed in all the treatments by using two auxins individually namely, IBA and NAA.

Significantly the highest number of roots per shoot and significantly maximum root length (cm) were observed in the Half strength MS medium containing 1 mg/l IBA in various genotypes *viz.*, Grand Naine (12.2, 8.6 cm), Mahalaxmi (12.0, 8.4 cm), Shrimanti (11.7, 8.3 cm), and Basarai (11.3, 8.1 cm) respectively, followed by the treatment 0.75 mg/l IBA found most suitable for the roots formation in all genotypes. The maximum number of roots per shoot and highest root length (cm) were noticed in the Half

Table 1
Effect of Surface Sterilization Treatments on Establishment of Shoot Tip Explants Of Banana Cultivars

Medium: MS — Incubation period: 25 days

Tr. No.	*Sterilization treatment*	*Duration (min.)*	*Contamination (%)*	*Death of culture (%)*	*Culture Establishment (%)*
T_1	Bavistin (2.5g/l) + Chloromphenicol (250g/l)	45	36.85	18.10	45.05
T_2	Bavistin (2.5g/l) + Chloromphenicol (500g/l)	45	33.81	15.01	51.18
T_3	Bavistin (2.5g/l) + Streptomycin (250g/l)	45	38.03	18.35	43.62
T_4	Bavistin (2.5g/l) + Streptomycin (500g/l)	45	35.64	16.29	48.07
T_5	Bavistin (5g/l) + Chloromphenicol (250g/l)	45	29.92	14.11	55.97
T_6	Bavistin (5g/l) + Chloromphenicol (500g/l)	45	27.95	12.84	59.21
T_7	Bavistin (5g/l) + Streptomycin (250g/l)	45	31.27	14.73	54.00
T_8	Bavistin (5g/l) + Streptomycin (500g/l)	45	29.31	13.94	56.75
T_9	Bavistin (2.5g/l) + Chloromphenicol (250g/l) + 0.5% $HgCl_2$	45 + 10	26.54	12.44	61.02
T_{10}	Bavistin (2.5g/l) + Chloromphenicol (500g/l) + 0.5% $HgCl_2$	45 + 10	24.33	11.43	64.24
T_{11}	Bavistin (2.5g/l) + Streptomycin (250g/l) + 0.5% $HgCl_2$	45 + 10	27.25	13.90	58.85
T_{12}	Bavistin (2.5g/l) + Streptomycin (500g/l) + 0.5% $HgCl_2$	45 + 10	25.06	11.22	63.72
T_{13}	Bavistin (2.5g/l) + Chloromphenicol (250g/l) + 1.0% $HgCl_2$	45 + 10	23.95	10.45	65.60
*T_{14}	Bavistin (2.5g/l) + Chloromphenicol (500g/l) + 1.0% $HgCl_2$	45 + 10	20.21	11.77	68.02
T_{15}	Bavistin (2.5g/l) + Streptomycin (250g/l) + 1.0% $HgCl_2$	45 + 10	22.71	11.45	65.84
*T_{16}	Bavistin (2.5g/l) + Streptomycin (500g/l) + 1.0% $HgCl_2$	45 + 10	21.09	12.50	66.41
T_{17}	Control	-	0.52	0.52	0.52
	SE.M±		0.57	1.54	0.54
	CD at 5%		1.61	4.35	1.54
	CV%		4.79	2.80	2.23

Table 2
Effect of BAP and Adenine Sulphate on Shoot Multiplication Rate of Banana Cultivars

Medium : MS — Incubation period : 30 days

Tr. No.	*Growth Regulators*		*Shoot Multiplication*			
	BA(mg/l)	*Adenine Sulphate (mg/L)*	*Grand Naine*	*Mahalaxmi*	*Shrimanti*	*Basarai*
T_1	1.0	-	1.73	1.58	1.22	1.48
T_2	2.0	-	1.87	1.81	1.48	1.64
T_3	3.0	-	2.12	2.07	1.73	1.89
T_4	4.0	-	2.23	2.14	1.81	2.02
T_5	5.0	-	2.17	2.07	1.67	1.93
T_6	1.0	1.0	1.76	1.67	1.26	1.51
T_7	2.0	1.0	1.99	1.89	1.54	1.73
T_8	3.0	1.0	2.30	2.19	1.89	2.09
*T_9	4.0	1.0	2.42	2.30	2.09	2.19
T_{10}	5.0	1.0	2.37	2.21	1.98	2.08
T_{11}	1.0	2.0	1.81	1.73	1.34	1.58
T_{12}	2.0	2.0	2.07	2.02	1.61	1.81
*T_{13}	3.0	2.0	2.54	2.34	2.14	2.23
T_{14}	4.0	2.0	2.36	2.23	1.99	2.12
T_{15}	5.0	2.0	2.11	2.09	1.77	1.87
T_{16}	Control		0.70	0.70	0.70	0.70
	SE.M±		0.01	0.01	0.01	0.01
	CD at 5%		0.038	0.030	0.042	0.046
	CV%		1.49	1.26	2.06	2.08

Table 3
Effect of Auxins (Iba/Naa) and Medium Strength on the Induction of Rooting of in *Vitro* Regenerated Shoots of Various Banana Cultivars

Medium : Ms — Incubation Period : 20 Days

Sr. No.	*Treatment*	*Grand Naine*		*Mahalaxmi*		*Shrimanti*		*Basarai*	
		No. of Roots per shoot	*Root Length (cm)*	*No. of Roots per shoot*	*Root Length (cm)*	*No. of Roots per shoot*	*Root Length (cm)*	*No. of Roots per shoot*	*Root Length (cm)*
T_1	½ MS + 0.25mg/l IBA	8.9	6.3	8.7	6.1	8.5	6.0	8.4	6.0
T_2	½ MS + 0.50mg/l IBA	10.6	7.0	10.3	6.9	10.1	6.7	10.0	6.4
*T_3	½ MS + 0.75mg/l IBA	11.5	7.4	11.3	7.2	11.1	7.1	10.8	7.0
*T_4	½ MS + 1.00mg/l IBA	12.2	8.6	12.0	8.4	11.7	8.3	11.3	8.1
T_5	½ MS + 1.25mg/l IBA	11.1	7.2	10.9	7.0	10.7	6.8	10.3	6.6
T_6	½ MS + 1.50mg/l IBA	9.2	6.8	9.0	6.6	8.8	6.4	8.5	6.3
T_7	½ MS + 1.75mg/l IBA	7.8	4.2	7.4	4.0	7.3	3.8	7.1	3.7
T_8	½ MS + 0.25mg/l NAA	8.7	6.1	8.6	6.0	8.4	5.8	8.2	5.7
T_9	½ MS + 0.50mg/l NAA	9.5	7.0	9.3	6.8	9.1	6.6	9.0	6.5
T_{10}	½ MS + 0.75mg/l NAA	10.4	7.0	10.2	6.9	10.1	6.6	10.0	6.5
T_{11}	½ MS + 1.00mg/l NAA	10.6	7.8	10.4	7.6	10.2	7.5	10.1	7.3
*T_{12}	½ MS + 1.25mg/l NAA	11.0	8.3	10.8	8.1	10.4	8.0	10.1	7.8
T_{13}	½ MS + 1.50mg/l NAA	8.6	6.5	8.4	6.3	8.3	6.1	8.1	6.0
T_{14}	½ MS + 1.75mg/l NAA	6.8	3.9	6.6	3.6	6.5	3.4	6.3	3.3
T_{15}	½ MS Control	4.1	2.2	4.0	2.1	3.9	2.2	4.0	2.0
	SE.M±	0.01	0.01	0.01	0.03	0.01	0.03	0.04	0.04
	CD at 5%	0.029	0.040	0.039	0.086	0.040	0.085	0.018	0.123
	CV%	0.72	1.20	0.99	2.66	1.05	2.65	3.07	3.89

strength MS medium containing 1.25 mg/l NAA in various genotypes *viz*., G9 (11.0, 8.3 cm), Mahalaxmi (10.8, 8.1 cm), Shrimanti (10.4, 8.0 cm), and Basarai (10.1, 7.8 cm) respectively. Rooting response in various genotypes were not responded in high / low concentrations of IBA and NAA.

Highest rooting was achieved in half strength MS media supplemented with 1.0 mg/l IBA (Babylatha, 1993). MS medium containing activated charcoal without auxin also induced best rooting (Gubbuk and Pekmemezci, 2004) while, root length and number of roots were higher only in medium fortified with auxin. Yoeman, 1986 suggested that concentration of auxin play significant role for initiating roots and inhibit caulogenesis.

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