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Low cost micropropagation package for Banana (*Musa paradisiaca* L.)

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Abstract

Banana is one of the important horticultural crops extensively cultivated in India. It has been conventionally propagated through suckers which is a time consuming method with lower rates of multiplication. To overcome this, the standardized protocols for shoot-tip culture have been commercially exploited by various tissue culture industries. The LBTM supplemented with different concentration of growth regulators the high shoot induction frequency was obtained in the combination of BAP 0.2 mg/l + IAA 0.1 mg/l (68.1±4.2) followed by KIN 0.2 mg/l + IAA 0.1 mg (65.2±2.8) respectively. The high multiple shoot induction frequency was obtained in the combination of BAP 2.0 mg/l + IAA 0.5mg/l combination (18.1±3.6) followed by the combination of KIN 2.0 mg/l + IBA 0.5 mg/l (13.2±0.8) respectively. In root induction different LBTM combination of IBA + GA_3. In this 2.0 mg/l IBA + 0.5 mg/l GA_3 produced more number of roots (12.5 ± 2.2) followed by other combination. The overall percentage of cost reduction was comparatively analysed for banana tissue culture propagation viz. LBTM (73.20%), Instruments (84.31%) and glassware's (93.4%) respectively.

Keywords: MS medium, micro-propagation, variety; banana; Hardening

Introduction

Banana is one of the most important major fruit crops grown in India. Botanically, banana is a monocotyledonous herbaceous plant belonging to the family Musaceae. Almost every part of the banana plant is used some way or the other and it is rightly called "poor man's apple". It is probably the cheapest fruit available throughout the year.

Commercial production of micropropagated banana is now common in many countries and it is estimated that 25 million plants are produced worldwide each year. This plant tissue culture technique provides a large number of uniform, high quality and disease-free planting material to meet demand in a short span of time on a year-round basis anywhere, irrespective of the season and weather (Anonymous, 2004). The most widely used MS medium (Murashige and Skoog, 1962) is used for commercial production of plantlets through shoot-apical meristem culture of banana. High cost of plantlet production through micropropagation technique is a major concern limiting its wide application, despite its obvious advantages. Due to the high cost of production, 32 out of 90 commercial micropropagation units were closed down in India after the tremendous growth in 1990s (Prakash, 2001 and Savangikar, 2004). In developed countries also this industry has undergone a pause, as it is difficult to

remain cost-effective (Govil and Gupta, 1997; Dhanalakshmi and Stephan, 2014).

The shoot multiplication rate obtained in the low cost options was not less when compared to the other *in vitro* multiplication trails. (Ganapathi *et al.,* 1995) used tap water, commercial grade sugar and reduced the salt components in medium for banana plantlet production and achieved a maximum cost reduction of 31.2 %. Use of the media, culture vessel and low cost substitutes for mass propagation was successful in several other species (Sujatha and Chandran, 1997; Varshney *et al*., 2000; Kodym and Arias, 2001 ;Kadota and Niim, 2004; Piatezak *et al.,*2005; Hung *et al.,* 2006).

In order to increase the tissue culture technology in banana farming, innovative approaches are needed to lower the cost of micropropagule production. Banana plant production via low cost technology in which cost reduction is achieved by improving process efficiency and better utilization of resources is reported by Savangikar (2002). Low cost options should lower the cost of production without compromising the quality of the micropropagules and plants (Prakash *et al.,* 2004). It is necessary to develop low cost technologies by improving the process efficiency and better utilization of resources. Keeping the above facts present study was aimed to reduce the cost of banana tissue culture by using alternative nutrient sources.

Materials and Methods

Plant materials

To develop a low cost tissue culture techniques in *Musa paradisiaca* L. var.Monthan (Fig-1).The sword sucker of this plant were collected from National Research Centre for Banana (NRCB), Thogamalai road, Tiruchirappalli, Tamil Nadu, India. And these varieties are maintained in the college garden, PG and Research Department of Botany, Government Arts College, Ariyalur and were used as a source of mother plant in this, the sword suckers were used as a source material for *in vitro* studies.

Low cost Banana Tissue culture Medium (LBTM) preparation

In the present study two tissue culture medium were used for the *in vitro* studies of *Musa paradisiaca* L. The first one was MS medium (Murashige and Skoog, 1962) and this medium composition was used as the control (Table- 1). Among the different low-cost alternative viz. macronutrients , micronutrients, iron source, vitamin source, carbon source, growth regulators and solidifying agent, The selected low-cost nutrients and their composition are standardized using sword sucker explants of Musa paradisiaca L. The standardized low cost nutrients are listed in the Table -1. This medium composition i.e LBTM (low cost banana tissue culture medium) was used throughout the banana micropropagation. (Table- 1).

Cost analysis (2015-2016)

The market price quotation were obtained for 2015-16 from the reputed companies for the conventional and alternative (low cost) sources of medium components, instruments and glasswares. Based on the quotation of the manufacture quantities used per liter of the medium, per unit cost differences were evaluated by the following formula. (Table- 2), For instruments and glassware's based on the Quotation comparative statement was used. (Table- 3,4).

$$\frac{\text{Amount used in the culture medium (g/l)} \times \text{Price of amount bought (Rs)}}{\text{Amount bought (g)}}$$

Sterilization of explants

Sword suckers of *Musa paradisiaca* L. was collected from the college garden and used as a explants source. The sword suckers with medium size were carefully removed from field grown banana plant. The older parts were excised with stainless steel knife. The shoot tips about 3-4 cm length were excised. They were washed thoroughly with a solution of Tween - 20. (2-3 drops in 500 ml water). All traces were removed by repeated washings under running tap water to remove dust particles for 30 min, and then treated with liquid detergent for 30 min, and finally rinsed three times with RO water. Then the explant was treated with an antimicrobial agent (Bavistin) for 1 min and again rinsed three times with distilled water. Further, sterilization treatments were done under a laminar-air flow chamber. The explants were then disinfected with 0.1% (w/v) Mercuric chloride (HgCl2) for 3 minutes under aseptic conditions. After these explants were thoroughly washed 3-4 times with sterilized distilled water to remove the traces of Mercuric chloride, then the explant was ready for inoculation.

Inoculation:

Inoculation was carried out in a sterile laminar airflow hood chamber. Surface sterilization was achieved through spraying 70% (v/v) ethanol. The sword sucker cuttings were dissected from four weeks-old *in vitro* plantlets using sterile blade and forceps. The single shoots cuttings were inoculated into a test tube containing 10 ml LBTM. All cultures were incubated at 25 $\pm 10^0$C with a 16-hr photoperiod (2000 lux). The materials were sub-cultured at 30 day interval in same medium to produce multiple shoots.

Effect of different growth regulators on micropropagation.

In *Musa paradisiaca* L. sword suckers explants var. Monthan are supplemented with different combinations of Auxin and Cytokinin. The Sword suckers explants were cultured on LBTM supplemented with various concentration of BAP and IBA (0.1, 0.2, 0.3, 0.4 and 0.5 mg/1) with 0.1 mg/l IAA for shoot induction (Table-5). The effect of growth regulators on the culture development response was studied and the effort was made to determine the optimal shoot growth combination.

Effect of different growth regulators on multiple shoot induction

The well-developed shoots were sub cultured on LBTM (Low cost Banana Tissue culture Medium), supplemented with various concentration of BAP (1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) with 0.5 mg/l KIN for shoot multiplication. The effect of growth regulators on the shoot multiplication response was studied and the effort was made to determine the optimal shoot multiplication combination. (Table- 6),

Root induction

Well-developed shoots were sub cultured into LBTM (Low cost Banana Tissue culture Medium), supplemented with different concentrations of IBA and GA_3 (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/l) for root induction (Table -7). Rooting was observed at 10 to 15 days.

Hardening

Plantlets with well-developed roots were removed from culture tubes and transferred to greenhouse after washing their roots in running tap water, and grown in the mixture of red soil, sand and saw dust in 1:1:1 ratio in the plastic cups for 10 days and subsequently transferred to pots. The plants need 90-100% humidity and they were covered with plastic bags with perforation or holes (Table -8). After 20 days the plantlets in the plastic cups were transferred into potting mix.

Statistical analysis

The well-developed cultures were examined periodically and morphological changes were recorded on the basis of visual observations. Whenever possible the effects of different treatments were quantified on the basis of percentage of cultures showing the responses per culture. The experimental design was completely randomized design (CRD) and factorial with Auxin and cytokinin as independent variables. All the experiments were repeated thrice. The data pertaining to frequencies of shoot induction, root induction, number of shoots was subjected to statistical analysis with Standard Deviations.

Results

In the present study the MS nutrient composition was replaced by low cost alternatives (Table -1) i.e called the LBTM (Low cost Banana Tissue culture Medium). The LBTM quantity per litre compostion was slightly increased, in compare with the convetinal MS medium composition (Table – 1).

Comparative cost analysis of low cost medium and conventional MS medium

In the present study the MS nutrient composition plus low cost nutrient composition (LBTM) was comparatively analyzed cost wise as per the price list of 2015-16 (Table -2). In comparison with the individual requirement per litre of conventional MS medium and LBTM composition was cost wise compared per litre conventional medium Rs. 152.48 was required for banana tissue culture propagation. In contrast using LBTM (Low cost Banana Tissue culture Medium) Rs. 40.86 is required per litre. The overall percentage of cost reduction for banana tissue culture propagation was 73.20% respectively (Table –2).

Comparative cost analysis of low cost instruments

In banana tissue culture laboratory setting purpose several high costs and low-cost instruments were analyzed. As per the comparative price list 2015-16,

Table: 1. Low cost medium and conventional medium composition

Conventional MS medium (Murashige&Skoog composition, 1962)	Amount (mg/l)	Low cost Banana tissue culture medium composition (LBTM)	Amount (mg/l)
Macro nutrients		***Macro nutrients***	
Ammonium Nitrate (NH_4NO_3)	1650	Ammonium nitrate fertilizer	2200
Calcium chloride ($CaCl_2$)	440	Calcium Chloride fertilizer	460
Potassium Nitrate (KNO_3)	1900	Potassium Nitrate fertilizer	2100
Magnesium Sulphate ($MgSO_4$)	370	Magnesium Sulphate fertilizer	420
Potassium dihydrogen Phosphate (KH_2PO_4)	170	Single super Phosphate	210
Micro nutrients		***Micro nutrients***	
Potassium iodide (KI)	0.83	Potassium Iodide(LR)	0.1
Boric oxide (H_3BO_3)	6.2	Power B-boran, Boric powder	15.0
Manganese Sulphate ($MnSO_4.4H_2O$)	22.3	Manganese Sulphate fertilizer	27.0
Zinc Sulphate ($ZnSO_4.7H_2O$)	8.6	Zinc Sulphate fertilizer	10.0
Sodium Molybdate ($Na_2\ MOO_4.2H_2O$)	0.25	Adbor powder	0.50
Copper Sulphate ($CuSO_4.5H_2O$)	0.025	Chelated fertilizer	0.025
Cobalt chloride ($COCl_2$)	0.025	Grandular/ powder	0.025
Iron Nutrient		***Iron Nutrient***	
Ethylene diamine tetra acetic acid (EDTA)	1.9	Ethylene diamine tetra acetic acid (EDTA)	2.1
Ferrous Sulphate ($FeSO_4.7H_2O$)	1.39	Ferrous Sulphate fertilizer	1.6
Vitamins		***Vitamins***	
Myo Inositol	100	BecosulesB-complex Tablets	15.0
Glycine	2	containing	
ThiamineHcl	0.1	Thiamine	
Nicotinic acid	0.5	Riboflavin	
Pyridoxine Hcl	0.5	Pyridoxine HCl	
		Ascorbic acid	
Growth regulators *		Biotin	
IAA	0.1	Folic acid	
2, 4-D	0.1	Calcium pantothenate	
NAA	0.1	Niacinamide	
IBA	0.1	***Growth regulators****	
Kinetin	0.1	IAA	0.1
BAP	0.1	2, 4-D	0.1
GA3	0.1	NAA	0.1
		IBA	0.1
Carbon source		Kinetin	0.1
Sucrose	30 (g)	BAP	0.1
		GA3	0.1
Solidifying agent		***Carbon source***	
Agar - Agar	8(g)	White refined sugar (Table sugar)	30 (g)
		Solidifying agent	
		Agar Agar (AR)	8 (g)

Table: -2 Comparative cost analysis of low cost Banana tissue culture medium (LBTM) and conventional medium composition as price quotation of 2015-16.

Conventional MS medium (Murashige & Skoog composition, 1962)	Amount (mg/l)	Cost in Rupees (Rs.)	Low cost Banana tissue culture medium composition (LBTM)	Amount (mg/l)	Cost in Rupees (Rs.)	% cost reduction
Macro nutrients			***Macro nutrients***			
Ammonium Nitrate (NH_4NO_3)	1650	7.2	Ammonium nitrate fertilizer	2200	0.1	98.6
Calcium chloride ($CaCl_2$)	440	1.9	Calcium Chloride	460	0.01	99.4
PotassiumNitrate (KNO_3)	1900	1.1	fertilizer			
Magnesium Sulphate			Potassium Nitrate	2100	0.6	45.4
($MgSO_4$)	370	1.3	fertilizer			
Potassium dihydrogen			Magnesium	420	0.02	98.4
Phosphate (KH_2PO_4)	170	1.8	Sulphate fertilizer			
Micro nutrients			Single super Phosphate	210	0.8	55.5
Potassium iodide (KI)	0.83	11.2	***Micro nutrients***			
Boric oxide (H_3BO_3)	6.2	3.7	Potassium Iodide(LR)	0.1	1.9	83.0
Manganese Sulphate			Power B-boran, Boric	15.0	2.8	24.3
($MnSO_4.4H_2O$)	22.3	1.4	powder			
Zinc Sulphate			Manganese Sulphate	27.0	4.0	66.6
($ZnSO_4.7H_2O$)	8.6	4.2	fertilizer			
Sodium Molybdate			Zinc Sulphate fertilizer	10.0	1.42	72.7
(Na_2 $MOO_4.2H_2O$)	0.25	1.1	Adbor powder	0.50	0.30	75
Copper Sulphate ($CuSO_4$.			Chelated fertilizer	0.025	0.01	70
$5H_2O$)	0.025	0.04	Grandular/ powder	0.025	0.06	63.7
Cobalt chloride ($COCl_2$)	0.025	0.2	***Iron Nutrient***			
Iron Nutrient			Ethylene diamine tetra			
Ethylene diamine tetra acetic	1.9	5.8	acetic acid (EDTA)	2.1	0.09	99.8
acid (EDTA)			Ferrous Sulphate	1.6	0.002	93.1
Ferrous Sulphate	1.39	1.1	fertilizer			
($FeSO_4.7H_2O$)			***Vitamins***			
Vitamins			Becosules B-complex	15.0	1.0	92
Myo Inositol	100	1.4	Tablets containing			
Glycine	2	4.8	Thiamine			
ThiamineHcl	0.1	1.7	Riboflavin			
Nicotinic acid	0.5	1.5	Pyridoxine HCl			
Pyridoxine Hcl	0.5	5.3	Ascorbic acid			
Growth regulators*			Biotin			
IAA	0.1	1.27	Folic acid			
2, 4-D	0.1	0.85	Calcium pantothenate			
NAA	0.1	2.16	Niacinamide			
IBA	0.1	1.9	***Growth regulators****			
Kinetin	0.1	4.8	IAA	0.1	0.10	97.6
BAP	0.1	6.3	2, 4-D	0.1	0.02	95.2
GA3	0.1	1.2	NAA	0.1	0.09	99.9
Carbon source			IBA	0.1	0.01	99.6
Sucrose	30 (g)	14.46	Kinetin	0.1	0.004	99.4
			BAP	0.1	0.023	75
Solidifying agent			GA3	0.1	0.007	
Agar - Agar	8(g)	64.80	***Carbon source***			
			White refined sugar	30 (g)	3.50	45.9
			(Table sugar)			
			Solidifying agent			
			Agar Agar (AR)	8 (g)	24.0	
Total		**152.48**			**40.86**	**73.20%**

***(This will reduce depends upon the requirement)**

high cost vs low cost instruments for banana tissue culture propagation using conventional (High cost) Rs.17,95,000 (Seventeen lakes ninety-five thousand rupees) was required. Similarly in comparison of low cost instruments Rs. 2,81,600 (Two lakes eighty-one thousand and six hundred rupees) was required. The overall cost percentage reduction for setting banana tissue culture laboratory 84.31 % respectively (Table –3).

Table -3: Comparative cost analysis of low cost and conventional instruments used for Banana tissue culture as per the price quotation of 2015-16.

Name of the instruments					% cost reduction
S.No	**Conventional (High cost) instruments**	**Cost in (2015-16)price Quotation (Rs.)**	**Equivalent Low cost instrument**	**Cost in (2015-16) price Quotation (Rs.)**	
1	Laminar air flow chamber with all accessories	1,0,0000	Laminar air flow champer with minimum accessories	50,000	50
2	pH meter in (Digital)	20,000	pH meter ordinary	10,000	50
3	Autoclave 1.Small size 2. Medium size 3. Large size	25,000 50,000 75,000	Pressure cooker (10 L)	5,000	80
4	Micro oven	20,000	Induction stove	1,500	92.5
5	Microbalance (0.01mg) (Sartorious)	1,25,000	Microbalance (0.1 mg)	10,000	92
6	Culture rack with light and timer	40,000	Ordinary culture rack with light	10,000	75
7	Double Distillation unit	25,000	Single distillation unit	10,000	60
8	1.Deep freezer (-20^{0}C) 2.Double Door Refrigerator	40,000 25,000	Refrigerator	10,000	75
9	Hot air oven with all accessories	25,000	Hot air oven with minimum accessories	10,000	60
10	Plant growth chamber with all accessories	10,00,000	Shade net with humidity	50,000	95
11	Working table with all racks and accessories	50,000	Ordinary table with accessories	50,000	-
12	Air conditioner (spilit AC)	30,000	Used spilt or window air conditioner	15,000	50
13	Glass bead sterilizer	10,000	Spirit lamb	100	99
14	Further with all necessary tissue culture room miscellaneous amenities	2,00,000	Minimum miscellaneous amenities	50,000	75
	Total	**17,95,000**	**Total**	**2,81,600**	**84.31 %**

Comparative cost analysis of low cost Glassware's

For the Banana tissue culture laboratory purpose several high cost and low cost glasswares were analyzed. As per the comparative price list 2015-16, the high cost vs low cost glasswares (Table-4) for the banana tissue culture propagation low cost glasswares Rs. 2790 (Two thousand seven hundred ninety rupees) was required. Similarly in high cost glassware Rs. 43,205 (Forty three thousand and two hundred five rupees) was required. The overall cost reduction was 93.4 % respectively (Table - 4).

Table 4: Comparative cost analysis of low cost and conventional glass ware used for Banana tissue culture as per the price quotation of 2015-16.

	Name of the Glassware's				
S.No	**Conventional (High cost) instruments**	**Cost in (2015-16) price Quotation (Rs.)**	**Equivalent Low cost instrument**	**Cost in (2015-16) price Quotation (Rs.)**	**% cost reduction**
1	Test tube (Borosil)	20.0	Test tube from local blowers	10.0	50
	Conical flasks (Borosil)				
2	1.250 ml	150.0			
	2. 500ml	200.0	Horlicks and Jam bottles	5.00	96.6
	Measuring cylinders				
3	(Borosil) 1. 10 ml	250	Measuring Cylinder Plastic*		
	2.100 ml	500	1. 10 ml	50	80
	3.500 ml	1000	2.100 ml	200	60
	4.1000ml	1500	3.500 ml	250	75
	Beakers		4.1000ml	250	83.3
4	1. 50ml	250	Beakers plastic		
	2.100 ml	500	1. 50ml	50	80
	3. 500 ml	1000	2. 100 ml	200	60
	4. 1000 ml	1500	3. 500 ml	250	75
	Culture bottle (Borosil)	60	4. 1000 ml	250	83.3
	with cap		Soda glass bottle with cap	25.0	58.3
			Hospital used glucose saline	20.0	
5	Micropipette 0.1 ml	10,000	bottle		66.6
	various size 0.5 ml	25,000			
			Glass pipettes *		
6	Vessels for micro oven		1. 0.5ml	250	97.5
	Aluminum foil roll	250	2. 1ml	250	97.5
			3. 2.5ml	250	97.5
			4.5ml	200	98
	Parafilm roll	500	5. 10ml	200	98
	Forceps	500	Used news paper	5.0	98
7					
	Surgical blade	25	News paper		
				5.0	99
8			Forceps	500	0
			Normal blade with holder	25	0
10					
11					
	Total	**43,205**	**Total**	**2790**	93.4%

Effect of different growth regulators on Shoot induction frequency of var. Monthan

The trimmed surface sterilized sword sucker explant of *Musa paradisiaca* L. var. monthan were subjected to LBTM composition with different growth regulators. Among the different concentration of growth regulators BAP and KIN (0.1, 0.2, 0.3, 0.4 and 0.5 mg/l) with 0.1 mg/l IBA and IAA in the LBTM composition. The high shoot induction frequency was obtained in the combination of BAP 0.2 mg/l + IAA 0.1 mg/l (68.1±4.2) followed by KIN 0.2 mg/l + IAA 0.1 mg (65.2±2.8) respectively (Table - 5)

Table : 5 Effect of BAP and KIN in combination of (0.1 mg/l) IAA and IBA supplemented with LBTM (low cost banana tissue culture medium) on shoot induction of *Musa paradisiaca*L. Var. Monthan using sword suckers explants.

Concentration of growth regulators (mg/l)	% of response	Shoot induction frequency
BAP+ IAA		
0.1+0.1	78.1±1.0	42.1±1.0
0.2+0.1	**85.6±6.1**	**60.6±6.8**
0.3+0.1	82.4±5.2	58.4±5.0
0.4 +0.1	81.1±6.4	45.1±2.8
0.5+0.1	72.5±2.8	40.5±2.8
BAP+ IBA		
0.1+0.1	70.0±6.0	60.0±2.0
0.2+0.1	75.6±3.8	40.6±3.2
0.3+0.1	**77.1±4.0**	**68.1±4.2**
0.4 +0.1	68.2±3.1	58.2±3.0
0.5+0.1	64.8±2.8	38.8±2.4
KIN +IAA		
0.1+0.1	80.4±1.2	35.4±1.6
0.2+0.1	**95.6±6.0**	**54.6±6.2**
0.3+0.1	84.8±1.4	42.8±1.0
0.4 +0.1	78.8±3.2	38.8±3.2
0.5+0.1	82.6±2.0	32.6±2.8
KIN +IBA		
0.1+0.1	88.8±6.7	28.8±6.4
0.2+0.1	72.4±7.2	55.8±7.0
0.3+0.1	87.5±1.9	32.4±1.8
0.4 +0.1	**90.2±3.4**	**65.2±2.8**
0.5+0.1	80.2±3.1	52.6±1.9

Data presented as the mean value ±standard error after 30 days of culture from four independent experiments each with 10 replicates..

Effect of different growth regulators on multiple shoot induction of var. Monthan

After 30 days old elongated shoot tip of banana are sub cultured in LBTM supplemented with different concentration of BAP and KIN (1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) with IAA and IBA 0.5 mg/l for multiple shoot induction. Among the different growth regulators in the BAP 2.0 mg/l + IAA 0.5mg/l combination produced high frequency of shoots (18.1±3.6) followed by the combination of KIN 2.0 mg/l + IBA 0.5 mg/l (13.2±0.8) respectively (Table -6).

Table : 6- Effect of BAP and KIN (0.5mg/l) in combination of (0.5mg/l) IAA and IBA supplemented with LBTM (low cost banana tissue culture medium) on multiple shoot induction of *Musa paradisiaca* L. Var. Monthan using sword suckers explants.

Concentration of Growth regulators (mg/l)	% of response	No. of Shoots
BAP+IAA		
1.0+0.5	85.8±0.6	12.8±0.3
1.5+0.5	86.8±3.8	12.5±1.2
2.0+0.5	**94.8±3.1**	**15.5±0.8**
2.5 +0.5	75.4±4.1	10.8±0.8
3.0+0.5	81.6±3.6	8.4±1.2
BAP +IBA		
1.0+0.5	82.9±0.8	12.7±0.8
1.5+0.5	85.8±3.1	14.2±1.2
2.0+0.5	**91.6±4.1**	**18.1±3.6**
2.5 +0.5	87.4±3.6	10.4±1.8
3.0+0.5	78.3±6.1	7.2±0.7
KIN +IAA		
1.0+0.5	81.9±0.2	12.1±0.2
1.5+0.5	**90.8±0.7**	**13.2±0.8**
2.0+0.5	84.2±3.3	8.2±1.6
2.5 +0.5	87.4±3.6	8.31±2.1
3.0+0.5	74.1±2.8	14.1±0.8
KIN +IBA		
1.0+0.5	82.3±0.2	12.9±0.3
1.5+0.5	71.3±1.8	13.1±0.8
2.0+0.5	**88.2±2.7**	**10.4±1.3**
2.5 +0.5	86.2±3.0	8.4±3.6
3.0+0.5	85.37±0	3.7±1.8

Data presented as the mean value ±standard error after 30 days of culture from four independent experiments each with 10 replicates..

Effect of different growth regulators on Root induction of var. Monthan

After 41 days of old multiple shoot culture were trimmed into single shoot tips using laminar air flow champers. This shoot tips were transferred to rooting medium containing LBTM supplemented with different concentration of IBA (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) with GA3 0.5 mg/l for root induction. Among the different concentration of IBA + GA3 combination the 2.0 mg/l IBA + 0.5 mg/l GA3 produced more number of roots (12.5 ± 2.2) followed by other combination. In all the IBA combination root was significantly produced in sub cultured banana shoot tips var. monthan (Table- 7).

Table :7. Effect of LBTM supplemented with different concentration of IBA with GA3 (0.5 mg/l) on root induction frequency of *Musa paradisiacal* L. Var. Monthan

Concentration of growth regulators (mg/l)	Root induction frequency of root (Mean ±SD	No. of roots (Mean ±SD)
IBA + GA3		
0.5+ 0.5	10.8±1.7	9.5±3.2
1.0+0.5	15.8±2.5	8.3±2.1
1.5+0.5	14.6±2.1	9.6±1.8
2.0+0.5	10.4± 3.6	8.9±2.6
2.5+0.5	**16.8±1.7**	**12.5±2.2**
3.0+0.5	10.8±2.5	6.3±2.1

Data indicate mean ± standard deviation. Ten replicates were used per treatment and experiment was repeated trice.

Hardening

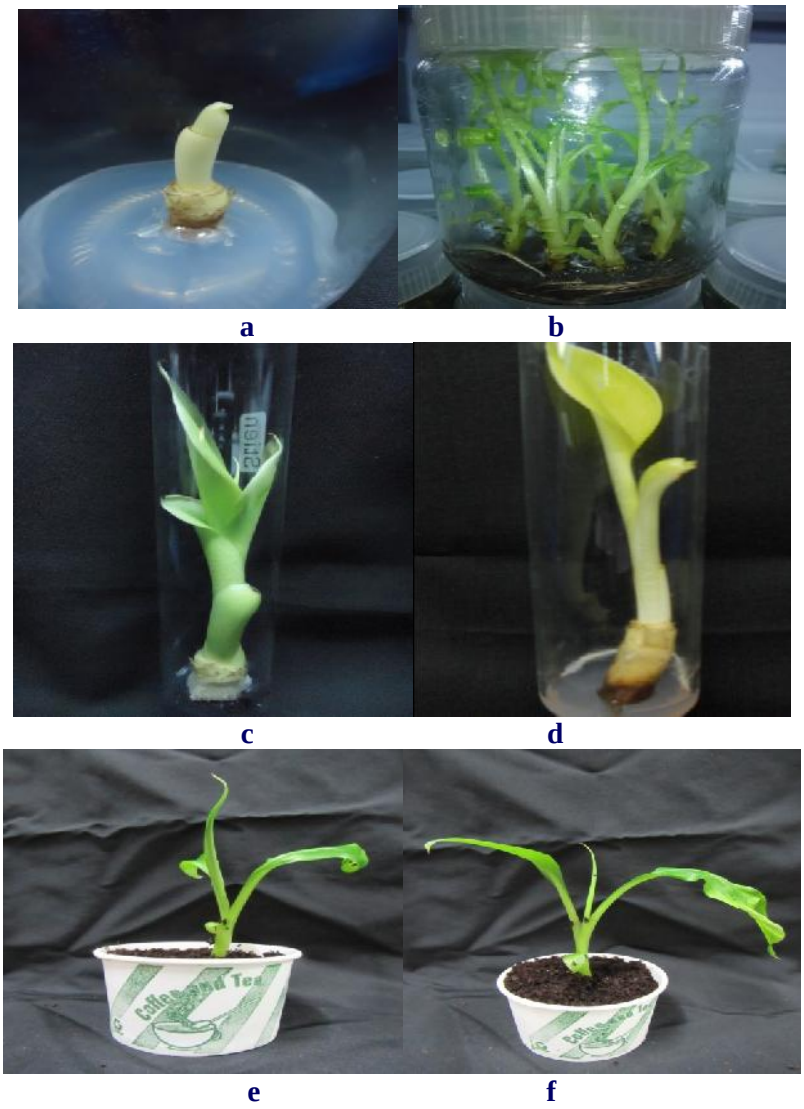
The plantlets having sufficient root and shoot system were taken out from the culture were washed under running tap water to remove the solidifying agent. The plantlets were transferred to poly cups containing autoclaved sand, soil and vermi compost (1:1:1) mixture and maintained in the green house conditions. Among the different low cost autoclaved compost mixture were also tried for hardening (Table – 8). Among the different composition mixture autoclaved sand + garden soil + vermi culture showed high survival percentage 78 ± 1.2 respectively (Table – 8).

Table –8. Effect of different low cost compost mixture for hardening on *Musa paradisiaca* L.

Compost mixture	Number of plantlets transferred	Survival (%)	Average shoot weight (cm)	Average Leaves plantlet
Autoclaved sand, Vermicompost +Vermiculate (1:1:1)	10	65 ±0.16	5.2 ±1.0	2.8 ±0.5
Autoclaved sand Garden soil +Vermiculate (1:1:1)	10	78 ±1.2	7.3 ±0.6	3.2 ± 0.4
Autoclaved sand Humus + Vermiculate (1:1:1)	10	85 ± 1.71	8.1 ±0.2	3.8 ±0.3

Data presented as the mean value ±standard error after 30 days of transfer.

Figure – 1 : Effect of LBTM supplemented with different concentration of growth regulators on micropropagation of *Musa paradisica* L. var. Monthan using sword sucker explants.



a- Shoots induction
b- Multiple shoot
c- Shoot elongation
d- Root induction
e, f- Hardening

Low cost banana tissue culture Package

In the present study main aim was to produce low cost micro propagation package in banana micropropagation using var. Monthan using sword sucker explants. To evaluate the factors contributing cost of tissue culture raised banana plants. In this several factors are identified for the production of tissue culture raised banana. They are cost of the instruments, chemicals glassware etc., Among the cost wise the low cost instruments, chemicals and glassware were standardized and this was used.

To evaluate the factors contributing medium composition for tissue culture raised propagation suitable alternative low cost nutrients were selected. Among the several instruments, high cost vs low cost was analyzed the following low-cost instruments are used throughout the study. They are 1. Laminar air flow chamber with minimum accessories 2.pH meter 3. Pressure cooker, 4. Induction stove, 5. Ordinary culture rack with light, 6.Single distillation unit 7. Hot air oven etc. (Table -3).Likewise, glasswares (Table -4) and medium alternative components were listed in (Table -2).

The overall cost analysis stated that *in vitro* Banana propagation package was cost wise in instruments. Rs. 2,41,600 (Two lakes forty one thousand and 600 hundred only) was needed and the percentage of 84.31 % respectively. For the glasswares, Rs.3745 (Three thousand seven hundred forty five rupees only), was needed and the comparative percentage cost reduction was 93.4 % respectively. In the medium components per litre production cost was Rs.40.86 (Fifty rupees and fifty six paisa only) the Overall comparative cost reduction analysis percentage was 73.20 % for LBTM medium preparation.

Discussion

Tissue culture plants are the major source of planting material, however the cost of production is higher than the conventional method of propagation by sword suckers in banana. In tissue culture industry the cost of media preparation chemicals and carbon source can account to 15-20 percent and the cost of energy can account up to 60 per cent of the production cost (Prakash and Savangikar, 2002).

The need for low-cost plant tissue culture systems, applicable for micro propagation and *in vitro* conservation of plant genetic resources, has been emphasized to allow the large-scale application and adaptability of such technology in developing countries (IAEA, 2004). Low cost option should lower the cost of production without compromising the quality of the micro propagation (Stephan and Dhanalakshmi, 2014). This problem has been addressed by inventing reliable cost effective tissue culture methods without compromising on quality of plants. Cost of chemicals, media, energy, labor and capital affects the production cost.

In the present investigation, stated the effect of different concentrations of BAP+IAA on shoot induction, and shoot multiplication were investigated using LBTM. Similar results achieved in Adenine based cytokinins are used in several Musa sp. For in vitro propagation, BAP is the most commonly prepared cytokine by Vulsteke, 1989. A part from the influence of genotypes, shoot proliferation rate and elongation are affected by cytokinin types and their concentration. The concentration of exogenous cytokine appears to be the main factor affecting multiplication of shoot.

Wong (1986) stated that when 11.1 µM BAP is supplemented in the medium, each of the explants produces as average of shoots, while increasing BAP concentration of 65.6 shoot induction frequency in per explants respectively. However, the optimum recommended BAP concentration is 20µM for banana micro propagation.

Root initiation and development was observed in all the media containing different concentrations of NAA, and also in the conventional medium and low cost media containing a combination of NAA and GA3. The superiority of NAA and IAA and IBA in the *in vitro* rooting of banana plantlets cv. William, Grande Naine by while Ahsan *et al.,* 1998 reported that the best response was achieved in hormone free MS media for table banana (*Musa sapientum*). In the present finding of root induction in hormonal combinations confirms the findings of Baby *et al.*,1997 who achieved 100% rooting in low cost media supplemented with various combination of NAA and GA_3.

The overall cost analysis stated that *in vitro* Banana propagation package was cost wise in instruments. Rs. 2,41,600 (Two lakes forty one thousand and 600 hundred only) was needed and the percentage of 84.31 % respectively. For the glasswares, Rs.3745 (Three thousand seven hundred forty five rupees only), was needed and the comparative percentage cost reduction was 93.4 % respectively. In the medium components per litre production cost was Rs.40.86 (Fifty rupees and fifty six paisa only) the Overall comparative cost reduction analysis percentage was 73.20 % for LBTM medium preparation.

Similar results achieved by Majuju Rakshi, *et al.*, 2017, reported 90% resource cost reduction in tissue culture of banana was achieved by replacing tissue culture grade sucrose and Gelrite in the medium with locally available commercial sugar and a starch/Gelrite mixture or sago 39.9% of agar cost, cotton fiber support 60.22% of agar cost, Starches of corn or potato could partially substitute for Gelrite and agar. Sugars of table sugar were suitable. AR grade sucrose by rock sugar 95.85% of sucrose cost and distilled water by aqua guard water 86.60% of double distilled water cost and by using sun light instead of artificial light.

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