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In Vitro Apical Culture of *Musa Paradisiaca* cv. Gaja Bantal

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ABSTRACT

Explants with apical domes have been reported to have higher rate of survival. In vitro propagation of banana (Musa spp.) from the apical explants isolated from the sword suckers is described here. Explants with apical domes were successfully cultured on Murashige and Skoog's medium containing 6-Benzyl Amino Purine , Kinetin , Indole -3-acetic acid , Naphathalene acetic acid and Adenine Sulphate. Multiple shoot initial could be produced from shoot tip explants in presence or absence of apical domes but the survival rates of explants were high when the apical domes were retained. From the in vitro apical culture of banana it was found out that 3 mg/lt BAP + 3 mg/lt KN + 1 mg/lt IAA + 150 mg/lt ADS induction medium and 3 mg/lt BAP + 1 mg/lt IAA + 0.25 mg/lt NAA + 75 mg/lt ADS multiplication medium shows remarkable results during different phases of in vitro culture.

Keywords: Apical domes, 6-Benzyl Amino Purine (**BAP**), Kinetin (**Kn**.), Indole -3-acetic acid (**IAA**), Naphathalene acetic acid (**NAA**) and Adenine Sulphate (**ADS**).

I. INTRODUCTION

Bananas and plantains (*Musa* spp.) are the world's fourth most important food crop after rice, wheat, and maize in terms of gross value of production, with the vast majority of the crop grown and consumed in the tropical and subtropical zones. Banana is generally propagated vegetatively through suckers. But the traditional method is laborious; time consuming and not very efficient as far as production of homogenous plant is concerned (**Banaerjee and De Langhe, 1985**). The extensive basic work on the *in vitro* propagation of banana (**Kodym and Zapata, 1999; Nandwani *et al*., 2000**) had led to the technological development of *in vitro* mass production of different cultivars.

Large number of uniform disease free plants can be produced from a single plant or even a small plant tissue (explants) showing good genetic potential in *in vitro* culture method (**Martin *et al*.,2006**) and plant multiplication can be continued throughout the year irrespective of seasonal variation (**Rahman *et al*., 2004**). Till date, protocols have been standardized for *in vitro* propagation of a wide range of *Musa* species and cultivars belonging to various ploidies and genomes (**Sathiamoorthy *et al.*, 1998**).

Tissue culture technique produce 39% higher yield than conventional sword suckers (**Farahani *et al*., 2008**).Shoot tip culture of banana has recently been studied in many laboratories (**Bower and Fraser, 1982; Cronauer and Krikorian, 1984; Ma and Shii, 1972**). The application of tissue culture and rapid propagation method for banana production continues to become more widely used in both developed and developing countries.

The goals of this study are (i) to examination the effect of different culture conditions on shoot tip culture, (ii) to find out the best plant growth regulators for shoot proliferation and multiplication of banana explants of *Musa paradisiaca* cv. Gaja Bantal with apical domes are culture on Murashige and Skoog's medium containing different concentration of phytohormones (Cytokinin and Auxin). The influence of the phytohormones on shoot proliferation and rooting of shoots is described through various experiments.

II. METHODS AND MATERIAL

Plant Materials

Gaja bantal is most widely cultivated plantain variety of Odisha that belong to *Musa paradisiaca.* It is a monocotyledonous, herbaceous, evergreen perennial ranging from 3-5 meters in height. Gaja bantal bear 4 – 6

numbers of hands in the inflorescence each containing 10 – 13 numbers of fingers in it. The banana mother block of R.P.R.C, Bhubaneswar is maintained properly to avoid all types of diseases and special care is taken for better growth and development of mother plants.

Suckers were collected from the banana mother block of Regional Plant Resource Centre, BBSR in January 2015 and washed thoroughly under running tap water for 10-15 min. The suckers were then chopped off about 5-6 cm in length and 3-4 cm in diameter.

Sucker Sterilization

Sucker collected from the mother block contain many contaminations like bacteria and fungus that are present in soil. Before inoculation in media they were treated with different chemicals for surface steriliztion. The following steps are carried out:

1. After processing suckers were washed in **liquid detergent (Labolene) for 2-3minutes**.
2. Explants were then dipped in **bavistin solution (1 %) for 30 minutes**.
3. After 30 minutes the suckers were washed with **autoclaved double distilled water** and transferred to **mercuric chloride solution (0.5 %) for 30 minutes**.
4. The suckers were washed in **70 % alcohol solution for 1 minute**.
5. Finally the suckers were washed 3- 4 times with autoclaved double distilled water to remove excess chemicals from the sucker surface.

Preparation of Culture Medium

The medium used for banana tissue culture was Murashige & Skoog Medium (MS) (**Murashige and Skoog, 1962**). The pH of the medium was adjusted 5.75 to 5.8 with 0.1N NaOH or 0.1N HCl. To one liter of medium 5.0gms of agar (Plant tissue Culture grade, Hi-Media, India) was added.

All the media were autoclaved at 15 psi and 121°C for 20 minutes. The autoclaved molten media were then dispensed into sterilized culture vessel inside a laminar air flow cabinet.

Table 1- Amount of phytohormones used in induction medium and multiplication medium along with **M.S** medium for in vitro apical culture.

Sl. No.	**Medium code**	**Induction medium (15 days) All hormone conc. Mg/Lt**			
		BAP	KN	IAA	ADS
1.	B1	6	0	1	150
2.	B2	4	0	1	150
3.	B3	2.5	0	1	150
4.	B4	3	3	1	150
5.	B5	0	6	1	150

Sl. No.	**Medium code**	**Multiplication medium (20 days for each subculture) All hormone conc. Mg/Lt**				
		BAP	KN	IAA	NAA	ADS
6	1a	3	0	1	0.25	75
7	1b	3	3	1	0.25	75
8	2a	3	0	1	0.25	75
9	2b	3	3	1	0.25	75
10	3a	3	0	1	0.25	75
11	3b	3	3	1	0.25	75
12	4a	3	0	1	0.25	75
13	4b	3	3	1	0.25	75
14	5a	3	0	1	0.25	75
15	5b	3	3	1	0.25	75

Sl. No.	**Medium code**	**Rooting medium (20 days) All hormone conc. Mg/Lt**			
		BAP	KN	IAA	ADS
16	A1	0	0	0.5	20
17	A2	0	0	1	20
18	A3	0	0	1.5	20

Aseptic Transfer of the Ex-Plant

The working area of the laminar airflow cabinet was first wiped with cotton moistened with ethanol and then irradiated with ultraviolet light for 30 minutes before inoculation.

The explants were surface sterilized as described earlier and cut aseptically by a sterile surgical scalpel to obtain the explants with apical domes. Then the explants were inoculated in the culture vessel containing induction medium.

Culture Condition

The culture vessel containing the explants on solid media was kept in culture rack in the culture room. The culture was maintained at 22°C to 25°C, 16 hr photo period of 35-50µEm-2s-1 intensity provided by cool white fluorescent tubes.

Analysis of Leaf Pigments

100 mg of fresh tissue samples of leafs were taken into a mortar and 10 ml of 80% acetone (acetone:water / 80:20 v:v)is added to it. Grind the tissue with a pestle till leaf homogenate completely.

Filter the leaf homogenate through the filter paper. The retentate is removed by the filter paper (and discarded) and the extract (or filtrate) is collected in a test-tube.

Clean cuvette for the spectrophotometer/colorimeter was taken and fills two-thirds full with 80% acetone; this is the blank. Wipe the cuvette with a tissue and put it into the spectrophotometer, then set the wavelength to 663 nm. Cover the cuvette chamber and set the spectrophotometer to 0 absorbance with the blank in place. Remove the blank and save for the next measurement.

Gently swirl your first extract in the test-tube and fill a second cuvette two-thirds full.

Wiped it clean, insert into the spectrophotometer, and close the hatch. The readout should give you the absorbance at 663 nm, the A663. Record this number, and repeat step 2 with the other extracts.

Change the wavelength to 645 nm. Reinsert the blank cuvette, and re-zero the spectrophotometer at the new wavelength. Remove the blank and insert a cuvette containing your first extract. Read and record A645. Repeat for the other extracts.

Calculations:

Use Arnon's equation (**Arnon, 1949**) to convert absorbance measurements to mg Chl g-1 leaf tissue:

$$\text{Chl a (mg g-1)} = \frac{[(12.7 \times A663) - (2.6 \times A645)] \times \text{ml acetone}}{\text{mg leaf tissue}}$$

$$\text{Chl b (mg g-1)} = \frac{[(22.9 \times A645) - (4.68 \times A663)] \times \text{ml acetone}}{\text{mg leaf tissue}}$$

$$\text{Total Chl} = \text{Chl a} + \text{Chl b.}$$

III. RESULTS AND DISCUSSION

Shoot Proliferation

Among the different combinations of medium tested, induction medium supplemented with BAP 3.0 mg/lt, KN 3.0 mg/lt, IAA 1.0 mg/lt and ADS 150mg/lt and multiplication medium supplemented with BAP 3.0 mg/lt along with IAA 1.0 mg/lt, NAA 0.25mg/lt and ADS 75mg/lt produced the highest number of plantlets.

Table 2- Number of shoot buds produced by apical culture of banana after 5 subcultures-

Sl. No.	No. of shoot buds in subcultures (Multiplication medium)					
		1st	2nd	3rd	4th	5th
1	1a	0	0	2	5	8
	1b	0	2	6	11	18
2	2a	0	1	5	13	16
	2b	1	3	8	15	24
3	3a	3	6	13	19	25
	3b	3	5	11	18	27
4	4a	3	7	18	27	41
	4b	4	5	16	25	39
5	5a	2	2	8	14	22
	5b	3	4	10	20	31

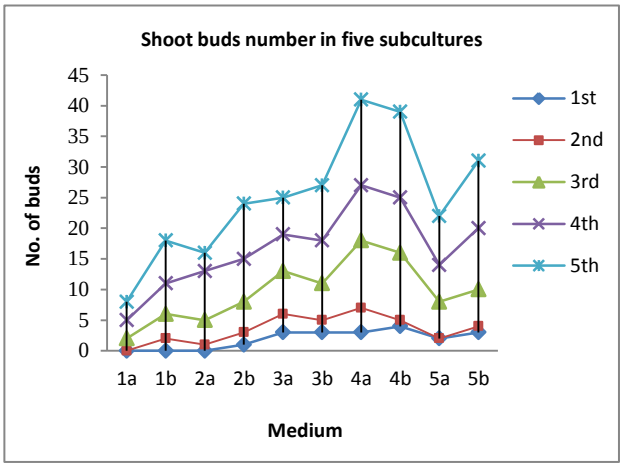
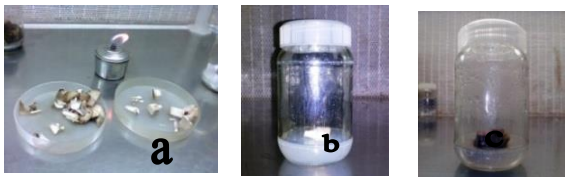


Figure 1: Shoot buds produced in different multiplication medium in five subcultures.

From the results it is clearly evident that efficiency of shoot bud regeneration from the rhizome explants depends on the concentration of cytokinins used in the medium. BAP shows better results in comparison to BAP + KN medium in shoot bud formation during the subculture of explants grown through *in vitro* apical culture of Gaja bantal.



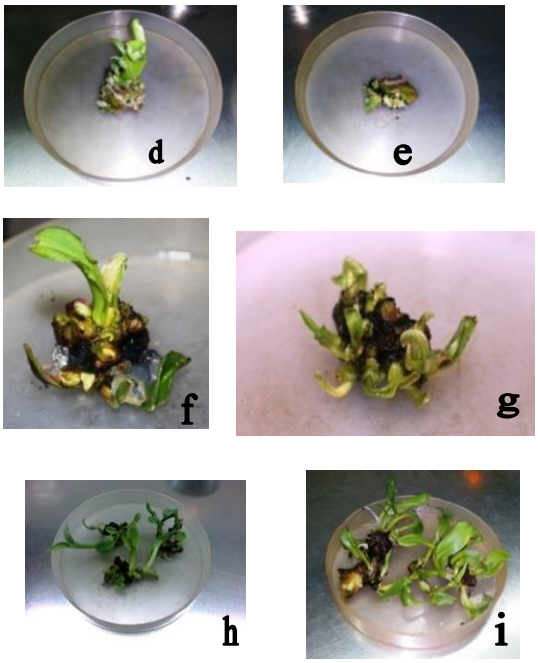


Figure 2: a. Apical explants from sucker, **b.** Inoculation of apical, **c.** Apical after 15 days, **d.** 1st subculture, **e.** 2nd subculture, **f.** 3rd subculture, **g.** 4th subculture, **h.** 5th subculture, **i.** Elongation.

After five cycles of subculture the shoot bud become fully developed into shoots with leaves. The explants are then transferred to elongation phase for the growth and elongation of shoots. The lengths of the shoots are measured at a given time interval for the comparative studies of the effect of phytohormones on shoots growth.

Table 3- Length of the shoots at different day's interval during elongation

Sl. No.	Medium	Length of the shoots (in Cm)		
		7th	14th	21st
1	1a	2.8 ± 0.18	3.6 ± 0.17	4.5 ± 0.12
	1b	3.3 ± 0.20	4.3 ± 0.19	5.0 ± 0.11
2	2a	3.0 ± 0.08	3.9 ± 0.11	4.7 ± 0.17
	2b	3.2 ± 0.13	3.7 ± 0.12	4.8 ± 0.08
3	3a	3.8 ± 0.18	4.2 ± 0.15	4.6 ± 0.16
	3b	3.6 ± 0.16	4.3 ± 0.20	5.0 ± 0.11
4	4a	3.7 ± 0.17	4.9 ± 0.08	5.5 ± 0.14
	4b	3.3 ± 0.14	4.6 ± 0.13	5.0 ± 0.12
5	5a	3.1 ± 0.12	4.3 ± 0.19	5.1 ± 0.13
	5b	3.4 ± 0.17	4.4 ± 0.07	5.3 ± 0.08

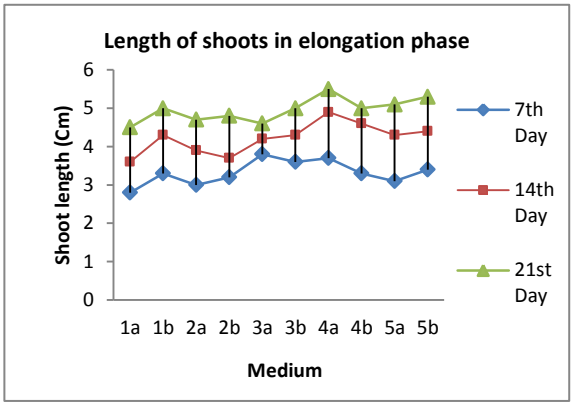


Figure 3: Length of shoot buds during the elongation phase.

Root Proliferation

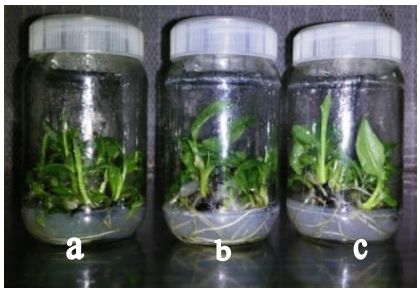


Figure 4: Root formation in a- **A1**, b- **A2** and c- **A3** medium.

Shoots produce roots after 10-15 days in medium containing IAA (0.5 mg/lt, 1 mg/lt, 1.5 mg/lt). From the above observation medium with 1 mg/lt IAA show high number of root per shoot and root length.

Table 4- Adventitious Root Induction-

Sl. No.	Medium	% of response	No. of root / shoot	Root length (in cm)
1	A1	70	3.6 ± 0.16	7.2 ± 0.21
2	A2	90	5.3 ± 0.15	10.3 ± 0.14
3	A3	80	4.7 ± 0.17	8.6 ± 0.29

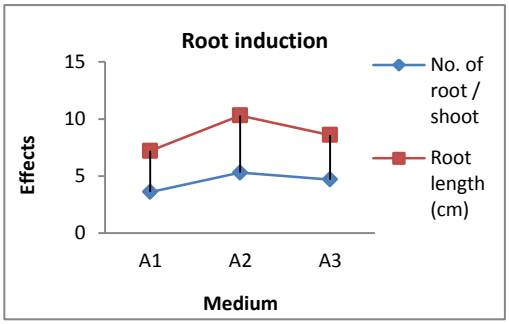


Figure 5: Root length (cm) and no. of roots per shoot in three different rooting medium

Chlorophyll Estimation

Fresh leaf sample was collected from the plantlets during elongation stage, were homogenized and filtered to collect the sample. The O.D of all the samples was measured at 663 nm and 645 nm.

Table 5- Photosynthetic pigments content-

Sl. No	Medium	Chl A (mg/g)	Chl B (mg/g)	Total Chl (mg/g)	Mean
1.	1a	1.31	0.97	2.28	2.28
		1.22	1.05	2.27	
	1b	1.41	1.23	2.64	2.54
		1.33	1.10	2.43	
2.	2a	0.91	0.73	1.64	2.07
		1.32	1.18	2.50	
	2b	1.30	1.02	2.32	2.16
		1.13	0.86	1.99	
3.	3a	1.31	1.17	2.48	2.39
		1.27	1.03	2.30	
	3b	1.45	1.17	2.62	2.75
		1.53	1.35	2.88	
4.	4a	1.71	1.40	3.11	3.01
		1.52	1.38	2.90	
	4b	1.62	1.34	2.96	2.56
		1.18	0.97	2.15	
5.	5a	1.32	1.02	2.34	2.27
		1.27	0.93	2.20	
	5b	1.43	1.11	2.54	2.50
		1.37	1.08	2.45	

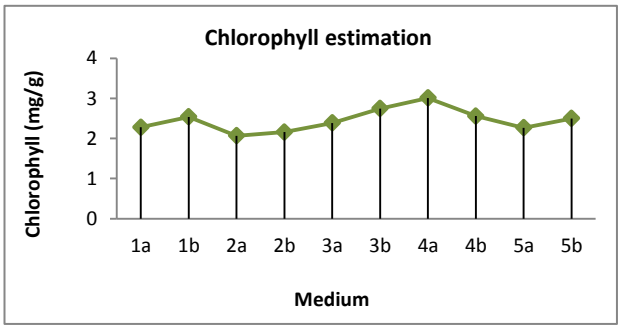


Figure 6: Chlorophyll content in different medium.

Analysis of leaf pigments was done from the leaf samples collected from different explants of elongation stages. In the present experiment the photosynthetic pigments (Chlorophyll-a, Chlorophyll-b, total Chlorophyll) were highest at the concentration of **BAP 3 mg/lt + KN 3 mg/lt + IAA 1 mg/lt + ADS 150 mg/lt** in initial medium and **BAP 3 mg/lt + IAA 1 mg/lt + NAA 0.25 mg/lt + ADS 75 mg/lt** in multiplication medium.

A considerable number of studies have indicated that the cultural conditions especially sucrose, growth regulators can influence the photosynthetic ability of in vitro grown plants (**Yadav *et al.*, 2010**).

Transplanting-

After the root formation the banana plantlets are send to nursery for primary hardening. The plantlets are taken out of culture vessel and the medium is removed by washing in water. Then the plantlets are arranged accordingly to their size. After primary hardening for 4-5 weeks the plantlets are transferred to polybags with soil and low cost minerals for secondary hardening.

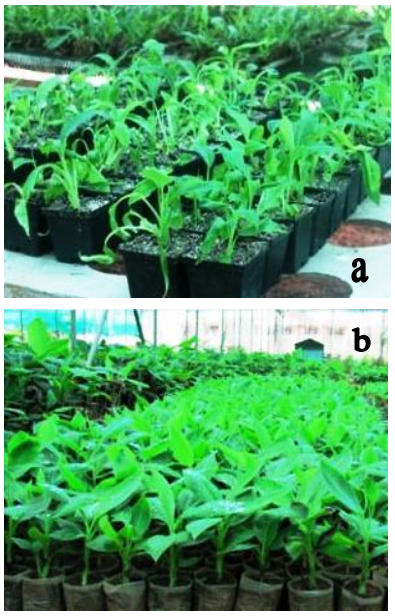


Figure 7: a- Primary hardening and **b**- Secondary hardening.

Ma & Shii (1972) reported that the destruction of apical dominance by removing the apical domes were essential for the production of multiple shoot-inductions in cv. Cavendish. Later several researches have been done to prove the production of multiple shoots from explants banana with intact apical dome. In the present experiment the effect of different concentrations of cytokinin (BAP and Kn) on shoot proliferation and different concentration of auxin (IAA) on root growth is observed.

Cytokinins play an important role in buds formation *in vitro*. However, buds proliferation *in vitro* is influenced by apical dominance which is controlled by various growth regulators (**Cline, 1994; Wickson and Thimann, 1958**). Cytokinins such as benzyl amino purine (BAP) and kinetin are known to reduce the apical meristem

dominance and induce both auxiliary and adventitious shoot formation from meristematic explants in banana (**Khalid, 2011**). 6-benzylaminopurine (BAP) is the most commonly preferred cytokinin (**Vuylsteke, 1989**). The others are isopentyladenine (2-ip), zeatin and kinetin (**De Langhe and Vuylstek, 1985**). Auxins and other growth regulator such as gibberellins play important roles in the growth and differentiation of cultured cells and tissues (**Bohidar *et al*., 2008**).

IV. CONCLUSION

From the above observation it was depicted that during *in vitro* apical culture of *Musa paradisiaca* cv. Gaja bantal highest number of shoot buds and length of shoots were observed in **BAP 3 mg/lt + KN 3 mg/lt + IAA 1 mg/lt + ADS 150 mg/lt** induction medium and **BAP 3 mg/lt + IAA 1 mg/lt + NAA 0.25 mg/lt + ADS 75 mg/lt** multiplication medium measured at different day's interval. In rooting medium **1 mg/lt IAA** along with MS medium have shown high percentage of response and root length (cm). By this study it was found that combination of two different cytokinins (BAP and KN) was more effective during initial culture where as BAP alone was found more effective during stimulation of shoot proliferation.

The evaluation of photosynthetic properties is essential for optimization of culture conditions in order to achieve efficient micropropagation. From chlorophyll estimation it was observed that the chlorophyll content in shoots grown on induction medium **B4** and **B3** and multiplication medium **4a** and **3b** is higher in comparison to other experimental mediums.

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