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In vitro plant regeneration in Banana (*Musa* sp.) cv. sabri

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Abstract

The establishment of a micro-propagation protocol for banana (*Musa* sp.) cv. Sabri, was carried out using meristematic stem cuttings explant. MS medium supplemented with BAP singly or in combination with auxin, IAA and coconut water was used for this purpose. Highest percentage of shoot regeneration (90%) and maximum number of shoots (10) per explant were observed when cultured on MS + 4.0 mg/1 BAP + 2.0 mg/1 IAA + 13% (v/v) coconut water. Best response towards root induction was achieved on half MS medium supplemented with 0.5 mg/1 IBA. The regenerated healthy rooted plantlets were transferred to small plastic pot containing garden soil and compost in a ratio of 2:1. Through this method, complete micro-propagated plantlets were obtained within three months.

Key words: *In vitro,* Micro-propagation, Meristematic, Banana, Auxin, Cytokinin

Introduction

Banana is one of the most important fruit crop grown all over the world. It provides a valuable source of income through local and international trade (Frison *et al.,* 1997). There are many cultivars of banana, such as Amritsagar, Sabri, Champa, Mehersagar, Dudsagar, Kabri, Agniswar, Genasundari, Kanaibashi, Basrai, Binisuta etc.

Sabri is a commercial variety. Fruits are medium-sized with a thin peel, ivory yellow in colour, firm in texture, sweet and tasty. Ripe bananas are one of the most rapidly digested foods. Eating several ripe bananas provides a readily available supply of hundreds of calories. It is a good source of carbohydrates, ascorbic acid (vit. C), vitamin B_6, potassium and iron. Sabri banana is free from substances that give rise tri-uric acid. Therefore, it is ideal for patients with gout or arthritis. Presently, world production of banana reaches to approximately 40 tons/ha (Anonymous, 2005). However, expansion of banana production is limited in Bangladesh, because of the shortage of healthy plant material availability to the farmers. The transmission of harmful insects, nematodes and viral disease by field-grown suckers has prompted interest in the use of aseptic culture techniques. Through this technique, such disorders are reducible and may be eliminated (Anonymous, 2005).

With the increasing demand and vast export potential coupled with the farmers desire to grow *in-vitro* propagated banana on a large area are becoming increasingly important in planting material for rapid multiplication of economically important commercial varieties (Roux *et al.,* 2001; Ray *et al.,* 2006). So *in-vitro* propagation, appears to be an attractive system for banana, which makes it possible to get plantlets free from insects, bacteria and other micro-organisms (Krikorian and Cronauer, 1984; Ma and Shii, 1972; Vuylsteke, 1998) to fulfill farmer's demands.

The present work, suggests a rapid multiplication protocol for banana cv. Sabri from meristematic stem tips using a medium with optimized concentration of the cytokinin (BAP), auxin (IAA) and coconut water. Findings of the present investigation may be helpful for the establishment of banana micro-propagation techniques to produce rapid and clean clones.

Materials and Methods

The experiment was conducted at Plant Tissue Culture Section, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhanmondi, Dhaka. Sword suckers of banana were collected from the experimental field of BCSIR. Shoot tips were prepared by removing a number of outer leaf sheaths from the suckers. The explants were surface sterilized with soft detergent for three times followed by washing with a few drops of Tween 20 and thoroughly washed in running tap water for 20-25 minutes. Then the explants were surface sterilized with 0.1% mercuric chloride

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($HgCl_2$) for 8 minutes. The sterilized explants were then rinsed 4-5 times with sterile distilled water inside the clean bench to remove all traces of $HgCl_2$. The sterilized explants were excised in the laminar airflow cabinet aseptically using a fine sterile forcep and scalpel. The excised explants were then inoculated on MS medium supplemented with BAP (1.0, 2.0, 3.0, 4.0, 5.0 mg/l) alone or in combination with IAA (0.5, 1.0, 2.0 mg/l) and coconut water (10%, 13%, 15%) for shoot regeneration and multiplication. The pH of the medium was adjusted to 5.8 ± 0.1. The cultures were maintained at the temperature set on 25 ± 2°C with a light intensity of ~2000 lux from fluorescent lamps. The maintained photoperiod was 18 hours light and 6 hours dark (18 L/6 D).

The established cultures on shoot induction medium were routinely transferred after every 3-4 weeks. The percentage of explants induced shoot, days to shoot initiation and number of shoots per explant have been recorded after three weeks of culture. *In vitro* shoots of banana were cultured on ½ MS medium supplemented with different concentrations of IBA (0.1, 0.3, 0.5, 1.0, 2.0 mg/l) and NAA (0.1, 0.3, 0.5, 1.0, 2.0 mg/l) for root initiation. The well rooted plantlets were then kept in room temperature for 2-3 days and transferred to plastic pot containing garden soil and compost in ratio of 2:1 and moist them adequately for proper hardening.

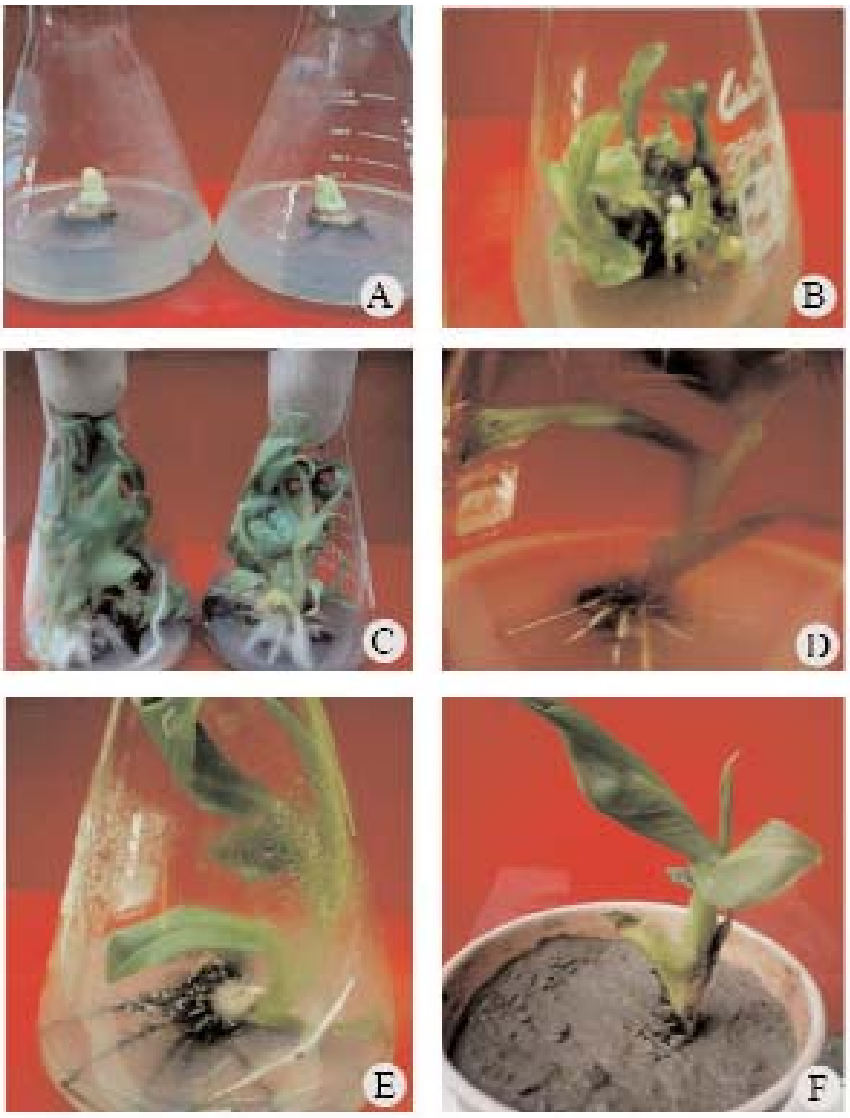
Results and Discussion

The effect of cytokinin, BAP singly or in combination with auxin, IAA and coconut water on shoot induction and proliferation from meristematic shoot tip explants of Sabri banana was investigated. The isolated explants were cultured on MS semi-solid medium supplemented with five different concentrations (1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) of BAP singly or in combination with BAP + IAA and BAP + IAA + Coconut water (Table I, Fig. A). The regeneration of shoots varied with the concentrations of supplements used. Among different concentrations, MS + 4 mg/l BAP + 2 mg/l IAA + 13% coconut water showed higher percentage of shoot induction (90%) and a maximum of 10 shoots per explant was produced, (Fig. B). It took about 10 days for shoot induction. The second highest response (75% shoot induction) was obtained when the explants were cultured on MS medium containing 5 mg/l BAP + 2 mg/l IAA + 13% coconut water and in this case the number of shoots per explants was 9 (Fig. C). Step-by-step protocol for *in-vitro* plant regeneration of the present working material has been presented below.

Table I: Effect of different concentrations of BAP, IAA and coconut water on shoot induction and proliferation from meristematic shoot tip explants of banana (*Musa* sp.) cv. Sabri

Growth Regulators (mg/l)	Shoot induction (%)	Days to shoot initiation	Shoot number/ explant
BAP			
1.0	0	0	0
2.0	0	0	0
3.0	0	0	0
4.0	0	0	0
5.0	0	0	0
BAP+IAA			
2.0+0.5	0	0	0
3.0+0.5	0	0	0
4.0+0.5	0	0	0
5.0+0.5	0	0	0
2.0+1.0	0	0	0
3.0+1.0	0	0	0
4.0+1.0	0	0	0
5.0+1.0	0	0	0
2.0+2.0	0	0	0
3.0+2.0	0	0	0
4.0+2.0	10	17	3
5.0+2.0	5	16	3
BAP+IAA+ Coconut water			
2.0+1.0+10%	0	0	0
3.0+1.0+10%	0	0	0
4.0+1.0+10%	25	16	4
5.0+1.0+10%	25	17	4
4.0+2.0+10%	50	12	6
5.0+2.0+10%	50	11	7
4.0+2.0+13%	90	10	10
5.0+2.0+13%	75	10	9
4.0+2.0+15%	50	12	8
5.0+2.0+15%	50	12	8

Explant
↓
Shoot induction medium
(MS + 4 mg/l BAP + 2 mg/l IAA + 13% Coconut water)
3 weeks
↓
Shoot multiplication medium
(MS + 4 mg/l BAP + 2 mg/l IAA + 13% Coconut water)
4 weeks
↓
Root induction medium
(½ MS + 0.5 mg/l IBA)
3 weeks
↓
Plants transferred into plastic pot
1 week



Figs. (A-F): ***In-vitro*** **propagation of banana (*Musa* sp.) cv. Sabri. A Inoculation of explant. B Multiplication on MS + 4 mg/1 BAP + 2 mg/1 IAA + 13% coconut water. C. Multiplic ion on MS + 5 mg/1 BAP + 2 mg/ IAA+14% coconut water. D. Root formation on ½ MS 0.3 mg/1 NAA E. Root formation on ½ MS + MS + 0.5 mg/1 IBA F. Transplantation in earthen po**

Different auxins, IBA and NAA were used in different concentrations in half strength of MS basal medium for root induction (Table II). The effectiveness of half-strength MS medium supplemented with auxin on root induction has been reported in many plants (Ahamed *et al.,* 2005; Huda *et al.,* 2003). Among the two auxins IBA showed best response towards root induction. The highest percentage of root formation (100%) and number of roots per explant (11) were found on ½ MS + 0.5 mg/1 IBA (Fig. E) which was significantly different from other treatments. In case of NAA maximum response (30%) of root induction was observed when the proliferated shoots were cultured on ½ MS medium containing 0.3 mg/l NAA (Fig. D). Use of auxins singly or in combination for rooting was also reported by different authors (Sahoo and Chand, 1998; Ajithkumar and Seeni, 1998; Rai, 2002; Sivakumar and Krishnamurthy, 2000; Hassan and Roy, 2004; Baksha *et al.,* 2007; Hassan, 2008).

The regenerated healthy rooted plantlets were carefully removed from the culture vessels. Roots were thoroughly washed under tap water to remove agar. The rooted plantlets were successfully transferred to plastic pot containing garden

Table II: Effect of different concentrations (mg/1) of IBA and NAA in ½ MS medium on root induction from *in vitro* grown shoots of banana (*Musa* sp.) cv. Sabri

Growth Regulators (mg/l)	Shoot induction (%)	Days to shoot initiation	Shoot number/ explant
IBA			
0.1	20	12	5
0.3	60	10	7
0.5	100	8	11
1.0	70	9	8
2.0	40	10	7
NAA			
0.1	0	0	0
0.3	30	17	7
0.5	20	15	6
1.0	0	0	0
2.0	0	0	0

soil and compost in ratio of 2:1 (Fig. F). The pots were immediately covered with polythene bag to prevent desiccation. About 90% plantlets were successfully established to the garden soil.

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