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In vitro Shoot Multiplication and Rooting of a Dessert Banana (*Musa* sp cv. Anupom)

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Abstract: The effects of various cytokinins (viz., BAP and KIN), auxins (viz., IBA, NAA and IAA) and coconut water (CW) alone or in combinations on shoot multiplication of banana cv. Anupom were investigated. The rate of multiplication varied in different treatments. Among the treatments, MS medium supplemented with 5.0 mg L^{-1} each of BAP + KIN and 13% coconut water produced the highest number of shoot per explant (5.8±0.154). The number of shoots responded for rooting and their survivability were found higher with IBA than NAA and IAA. MS medium supplemented with 1.0 mg L^{-1} IBA produced the highest number of roots per shoot (7.0±0.245). Plantlets grown without any auxin in the medium gave the least number of roots/shoot (2.60±0.219). The rooted plantlets were successfully transferred in the polybags and finally were well established in the field.

Key words: Banana, *in vitro* shoot multiplication, cytokinin, auxin

INTRODUCTION

Banana is the most widely consumed fruit and have a great economic importance in Bangladesh. It is the most important starch rich horticultural crop of Bangladesh cultivated over an area of more than 105835 acres with annual production of 606100 m tons[1]. It is also a rich source of vitamins, minerals and carbohydrates which are assimilates easily and provide energy (104 kcal/100 g) for human body[2].

Most of banana are either seedless or seed sterile and are therefore multiplied vegetatively using suckers of various sizes or pieces of the corm. However, this method of propagation is laborious, time consuming with low rate of multiplication and planting stock remain infested with various systematic diseases such as bunchy to virus, sigatoka, panama and also by weevils and nematodes. As a result banana productivity decreases remarkably. *In vitro* propagation through shoot culture may overcome these problems. So, the prevalence of disease problems and the need for generating clean planting material in large quantities has stimulated presently a surge of interest in the production of clonal material of bananas by the use of aseptic micropropagation techniques applying shoot tip culture[3-9]. Using this method, a large quantity of uniform and disease free plants with good genetic potential can be produced within a short time from a single explant[10] and plant multiplication can be continued throughout the year irrespective of the season[11]. Tissue culture plants grow rapidly, establish more quickly and take a shorter time to bunch emergence and harvest[12,13]. It is also reported that tissue cultured plant performed better and produce 39% higher yield than plants grown from conventional sword suckers[14,15].

Banana farmers in our country used to cultivate mainly two cultivars, namely Sagar (Amritsagar, Mehersagar and Ranginsagar) and Anupom for commercial purposes. Among these, Anupom was stated to be the second best, entirely seedless, superior table banana in Bangladesh and its taste even better than Amritsagar. Anupom is semi-dwarf clones and the fruit is very tasty. But this cultivar is threatened by the *Fusarium wilt*, which has limited its cultivation. It is therefore, a burning need to develop a tissue culture technique for rapid clonal propagation of disease free plant and that holds tremendous potentiality for commercial exploitation. This study focuses attention on efficacy of *in vitro* propagation of Anupom, a dessert cultivar of Bangladesh, through excised shoot tip, required various culture media for shoot multiplication and root induction.

MATERIALS AND METHODS

The experiment was conducted to find out a suitable media formulation for *in vitro* shoot multiplication as well as rooting of a dessert banana (cv. Anupom) in the Biotechnology Laboratory of Institute of Biological Sciences of Rajshahi University, Bangladesh, during the years of 2003-2004. Banana (*Musa* sp.) cultivar Anupom growing in the cultivated field in puthia upazila under

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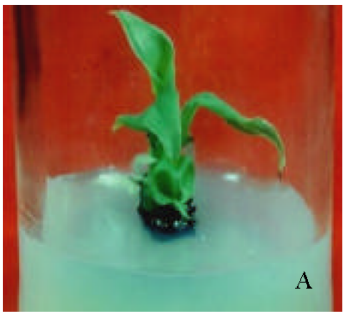

Fig. 1A: Single shoot formed by elongation of initial shoot apex of shoot tip explants at MS+5.0 mg L^{-1} BAP

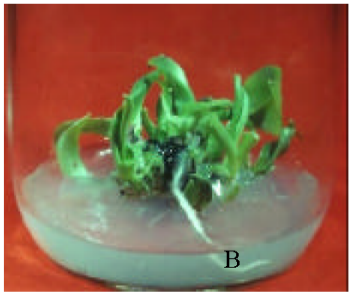

Fig. 1B: Multiple plantlets production from shoot tip cultured on MS+5.0 mg/BAP+5.0 mg L^{-1} KIN+13% CW

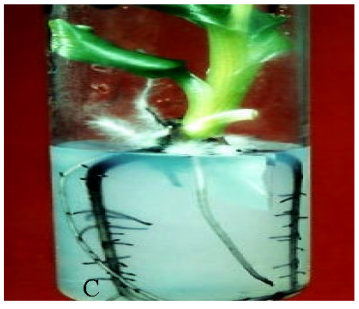

Fig. 1C: Root induction on ½ MS medium supplemented with 1.0 mg L^{-1} IBA

Fig. 1D: Banana plantlets established in polybag

Rajshahi District was utilized as the source material for obtaining shoot tips. Small healthy sword suckers were carefully collected from field grown banana then the older leaves and extraneous corm tissue were carefully removed with a stainless knife. Shoot tips containing several sheathing leaf bases enclosing the auxiliary buds and subjacent corm tissue and measuring about 5-6 cm in length, were isolated. These shoot tips were firstly washed in running tap water for 40 min and then with teepol for 15-20 min. All traces of teepol were removed by washing several times with autoclaved distilled water.

Finally, the explants were placed on inoculation chamber and treatment with 0.1% $MgCl_2$ for 14 min followed by washing several times with sterile distilled water to wash out any trace of $MgCl_2$. After surface sterilization they were trimmed 2-3 mm from both ends and the outer sheath was peeled off gradually. Intact shoot apex and one or more pairs of leaf primordia together with 2-3 mm in size including rhizomatous base were selected for inoculation.

The individual explants were cultured in agar gelled MS[16] nutrient medium containing different concentrations and combinations of cytokinins auxins and Coconut Water (CW). For rooting half strength MS medium with auxin was used. Three percent sucrose was used in all the media. The media were adjusted to pH 5.8 and autoclaved for 20 min at 121°C under 1.1 kg cm^{-2} pressures. Cultures were incubated at 25±1°C under 16 h photoperiod with light intensity of 2000-4000 lux.

RESULTS AND DISCUSSION

Shoot tips of banana (*Musa* sp. cv. Anupom) were isolated aseptically and cultured on MS medium supplemented with cytokinins, auxins and coconut water for initiating vegetative growth and inducing a maximum number of plantlets.

Establishment of cultures: When isolated shoot tips (creamy white) placed in solid medium, they became green within 10 days and in 25 days, a small shoot rarely multiple shoots was visible to the naked eye. On sub-culturing these shoots went on to produce clusters of multiple shoots within 3 to 4 weeks. The different concentrations of BAP and KIN were used singly and the best results were obtained in MS+5.0 mg L^{-1} BAP (Table 1) for the primary establishment of shoot tip culture (Fig. 1A). For induction of shoot-bud proliferation under in vitro condition, BAP is the cytokinin of choice. In regeneration of banana, BAP is superior to KIN was also reported by other researchers[6,17-20]. The results of the present investigation indicated that the multiplication rate

Table 1: Effect of different concentrations of BAP and KIN in MS medium on primary establishment of shoot tips of banana *in vitro*. Each treatment consisted of 12 explants and data represented as mean±SE

Growth regulators (mgL^{-1})	% of shoot regeneration	Average No. of shoot per explant after		Average length of largest shoot (cm) 35 days
		25 days	35 days	
BAP				
1	16.67	1.00±0.00	1.00±0.000	1.30±0.071
2	25.00	1.00±0.00	1.66±0.272	1.23±0.072
3	33.33	1.25±0.217	1.75±0.217	1.77±0.108
4	58.33	1.28±0.171	1.85±0.241	1.85±0.166
5	75.00	1.66±0.157	2.22±0.304	1.98±0.134
7	66.67	1.37±0.143	1.75±0.234	1.43±0.088
KIN				
1	8.33	1.00±0.00	1.00±0.000	0.70±0.000
2	25.00	1.00±0.00	1.00±0.000	0.86±0.054
3	33.33	1.00±0.000	1.25±0.112	0.97±0.052
4	50.00	1.16±0.152	1.50±0.204	1.06±0.045
5	58.00	1.28±0.171	1.71±0.171	1.18±0.087
7	41.67	1.20±0.179	1.20±0.179	0.96±0.046

Table 2: Effect of different combinations and concentrations of cytokinins, auxins and coconut water (cw) on the production of multiple shoots in MS medium. Data collected after 45 days of culture

Hormonal supplements ($mg\ L^{-1}$)	No. of explant inoculated	% of response	Average No. of multiple shoots per explant	Average length of highest shoot (cm)
BAP+IAA+IBA				
2.0+1.0+1.0	12	25.00	2.00±0.271	2.10±0.170
3.0+2.0+2.0	12	33.33	3.00±0.354	2.27±0.198
4.0+1.0+1.0	12	58.33	3.42±0.275	2.80±0.245
4.0+2.0+2.0	12	83.33	4.60±0.290	3.80±0.191
5.0+2.0+2.0	12	75.00	4.22±0.262	3.47±0.189
6.0+2.0+2.0	12	41.67	3.00±0.283	2.30±0.139
BAP+KIN+IAA				
3.0+1.0+1.0	12	16.67	1.50±0.254	2.25±0.106
4.0+2.0+1.0	12	41.67	2.33±0.272	2.70±0.181
4.0+2.0+2.0	12	66.67	3.00±0.283	3.35±0.141
5.0+1.0+1.0	12	50.00	3.75±0.293	2.38±0.104
5.0+2.0+2.0	12	75.00	4.44±0.355	3.78±0.200
6.0+2.0+2.0	12	8.33	2.00±0.000	1.95±0.106
BAP+KIN+CW				
5.0+5.0+10%	12	91.67	4.1±0.094	2.66±0.072
5.0+5.0+13%	12	91.67	5.8±0.154	3.70±0.075
5.0+5.0+20%	12	91.67	4.6±0.120	3.12±0.058

in banana under *in vitro* condition is a function of BAP concentration.

Shoot multiplication: For induction and multiplication of shoots, the single shoots were cultured on MS nutrient medium supplemented with different concentrations and combinations of cytokinins, auxins and coconut water. The multiple shoots were produced successfully on MS medium supplemented with 4.0 mg L^{-1} BAP + 2.0 mg L^{-1} IAA + 2.0 mg L^{-1} IBA, 5.0 mg L^{-1} BAP+ 2.0 mg L^{-1} KIN + 2.0 mg L^{-1} IAA and 5.0 mg L^{-1} BAP + 5.0 mg L^{-1} KIN + 13% CW (Table 2). The results of the present study clearly show that shoot tips explant from selected suckers are capable of producing multiple shoots *in vitro* which can be rooted to form complete plantlets. Superiority of BAP over other cytokinins for multiple shoot formation in banana has been reported[17,18,21,22]. Habiba *et al.*[23] reported that MS medium in combination with 4.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA + 13% CW was optimum for highest number of shoot regeneration in banana whereas Azad and Amin[24] developed a medium for regeneration of banana excised floral apices which was MS + 2.0 mg L^{-1} BAP + 1.0 mg L^{-1} KIN + 1.0 mg L^{-1} IAA + 15% CW. In present experiment it was observed that MS + 5.0 mg L^{-1} BAP + 5.0 mg L^{-1} KIN + 13% CW were most optimum for maximum number (5.8±0.154) of shoot regeneration and highest length (3.7±0.075) of shoots from sucker explants (Fig. 1B). The different results might be due to differences of genotypes and explants used.

Rooting and hardening: The rooting response differed according to different concentrations and combination of auxins used (Table 3). Among the three types of auxins, IBA was found to be the best for root induction and 1.0 mg L^{-1} IBA was found most suitable in which 100% shoot rooted within 10-15 days with 7.0±0.245 roots per shoot (Fig. 1C). The average length of root in this medium was 4.84±0.257 cm.

Table 3: Effect of different concentrations of auxins in ½ MS medium on root induction from *in vitro* grown plantlets of banana. In each treatment 10 explants were used. Data collected after 28 days of culture

Auxins (mg L^{-1})	% of shoot rooted	Average No. of roots per shoots	Average length of longest root (cm)
IBA			
Control (0)	40	2.60±0.219	1.50±0.165
½ MS+0.5	80	4.00±0.250	2.23±0.205
½ MS+1.0	100	7.00±0.254	4.84±0.257
½ MS+1.5	100	6.20±0.352	4.49±0.204
½ MS+2.5	90	5.10±0.386	3.18±0.189
½ MS+4.0	70	4.14±0.314	3.05±0.255
NAA			
Control (0)	40	2.60±0.219	1.50±0.165
½ MS+0.5	80	3.13±0.275	1.73±0.148
½ MS+1.0	100	5.60±0.429	3.54±0.142
½ MS+1.5	100	5.10±0.359	3.29±0.153
½ MS+2.5	80	4.00±0.314	2.86±0.204
½ MS+4.0	60	3.16±0.282	1.55±0.056
IAA			
Control (0)	40	2.60±0.219	1.50±0.165
½ MS+0.5	60	3.16±0.281	2.06±0.216
½ MS+1.0	80	4.25±0.342	3.08±0.164
½ MS+2.0	100	4.60±0.253	3.35±0.131
½ MS+3.0	70	3.42±0.341	2.20±0.170
½ MS+4.0	50	2.80±0.179	1.84±0.201

For the induction of roots from *in vitro* raised shoots of banana, De Langhe[25] used half MS + 1.0 mg L^{-1} IBA whereas, Cronauer and Krikorian[17] used auxin free MS medium for rooting of banana microshoots. On the other hand, Banerjee and De Langhe[8] obtained rooted banana shoots in ½ MS + 0.2 mg L^{-1} IBA. In the present study, it was observed 1.0 mg L^{-1} IBA in half strength MS medium was found most suitable for root induction. Atique *et al.*[26] also found similar results in banana cv. Sagar.

For acclimatization, rooted shoots were planted in small polythene bags containing sterile sand, soil and humus (1:2:1). The plantlets were covered by transparent polythene sheet to maintain high humidity and within 15-20 days new leaves emerged out from about 80% of the plantlets that resumed new growth (Fig. 1D). After 60-70 days the plants were transplanted in the open field where 90% plants survived and grew satisfactory. The protocol, that has been established, holds great promise for commercial cultivation of disease free planting materials for the popular banana cultivar of Anupom.

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