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High Frequency Plant Regeneration of a Dessert Banana Cv. Mehersagar for Commercial Exploitation

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Abstract: Banana cultivar 'Mehersagar' is a dessert banana clones cultivated all over the country. For the purpose of the production of disease free plantlets suitable for large-scale cultivation in the field, a micropropagation protocol based on multiple shoot regeneration has been established. Highly proliferative cluster of multiple shoots are obtained by culturing shoot tips from ex vitro grown healthy suckers on MS medium with different concentrations and combinations of cytokinins (viz., BAP and KIN), auxins (viz., IBA, NAA and IAA) and coconut water (cw). The efficacy of these supplements on the rate of shoot multiplication was tested. Best results were obtained on MS+0.5 mg L^{-1} BAP+0.5 mg L^{-1} KIN+13% cw, where highest average number of shoots per explant was 8.2±0.236. When individual shootlets were separated and implanted in half strength MS medium with various concentrations of auxins, the better root formation were noted at 1.0 mg L^{-1} IBA with 6.10±0.359 roots per shoot. Then the rooted plantlets were transferred to polybags and about 90% of the acclimatized plantlets were survived when transferred to open field.

Key words: Dessert banana, micropropagation, callus, regeneration

INTRODUCTION

Banana plantains (*Musa* sp.) are a staple food in many tropical and subtropical countries that ranks fourth among fruits produced in the world. Bangladesh produced about 606100 M. tons of banana from 105835 acres of land (Anonymous, 2001). Banana provides a major food, income and employment to many Bangladeshi people. Most of the bananas are either seedless or seed sterile and are therefore multiplied vegetatively using suckers of various sizes or pieces of the corm. Since forcing buds under field condition is a tedious and time consuming procedure, the amassing of a sufficient amount of suitable planting material is slow at best (Baker, 1959; Simmonds, 1966). This is a problem when a new clone is being generated to replace older planting or when a large amount of planting material of a specific pathogen tolerant clone is needed for field-testing and assessment. Because, the banana propagules were infected by the various systematic diseases such as bunchy top virus, Sigatoka, Panama and also by weevils and nematodes. The prevalence of disease problems and the need for generating clean planting stock in large quantities has stimulated recently a surge of interest in the production of clonal material of both cooking and dessert bananas by the use of aseptic micropropagation techniques i.e, shoot tip culture (Krokorian and Cronauer, 1984; Wong, 1986; Fitchet and Winnaar, 1988; Huang and Chi, 1988; Eckstein and Robinson, 1995). Using this technology, from a single shoot tip, a large quantity of uniform and disease free plants with good genetic potential can be produced within a short time (Vuylsteke and De Langhe, 1985). Tissue cultured plants grow vigorously, establish more quickly and take a shorter time to bunch emergence and harvest (Vuylsteke and Qritz, 1996). It is also reported that microplantlets performed better and produce 39% higher yield than plants from conventional sword suckers (Pradeep *et al*., 1992; Faisal *et al*., 1998). This study focuses attention on *in vitro* propagation of Mersagar, a high yielding banana cultivar in Bangladesh, through excised shoot tip using different culture medium for shoot multiplication and root induction.

"Mehersagar" is an important banana cultivar, particularly in the northern part of Bangladesh. The farmers cultivate this cultivar for its semi-dwarf in size and for high yield. The commercial cultivation over the area is done by conventional method of suckers, which acts as the potential carriers of systematic diseases. It is therefore

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a burning need to develop a tissue culture protocol for rapid clonal propagation of diseases free plants and that holds tremendous potentiality for commercial exploitation. Therefore, the present study was undertaken to formulate the appropriate protocol for better shoot multiplication as well as rooting of banana cv. Mehersagar.

MATERIALS AND METHODS

Banana (*Musa* sp.) cultivar "Mehersagar" growing in the cultivated field in Puthia upazila under Rajshahi District of Bangladesh was used as the source material for obtaining shoot tips and research has been done in the year 2003-2004 at Biotechnology Laboratory, Institute of Biological Sciences, Rajshahi University, Bangladesh. Small healthy sword suckers were carefully removed from field-grown banana. Then the older leaves and extraneous corm tissue were carefully removed with a stainless knife. Shoot tips containing several sheathing leave bases enclosing the auxiliary bud 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 treated with 0.1% $MgCl_2$ for 14 min. followed by washing several times with sterile distilled water to wash out any trace or $MgCl_2$. 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 nutrient medium containing different concentrations and combination of cytokinins, auxins and coconut water. For rooting, half strength MS with auxins 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±10°C under 16 h photoperiod with light intensity of 2000-4000 lux.

RESULTS AND DISCUSSION

Shot tips of banana (*Musa* sp. cv. Mehersagar) 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 creamy white shoot tips were placed in agar gelled medium, they became green within 10 days and in 25 days, a small shoot rarely multiple shoots was visible to the naked eye. These shoots went on to produce clusters of multiple shoots, which could be subcultured. 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. 1). For induction of shoot-bud proliferation under *in vitro* condition, BAP is the cytokinin of high promise. In regeneration of banana BAP is superior to KIN was also reported by other workers (Cronauer and Krokorian, 1984; Zamora *et al*., 1986). The results of the present investigation that the multiplication rates in banana under *in vitro* condition are a function of BAP concentration.

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

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 0.5 mg L^{-1} BAP+0.5 mg L^{-1} KIN+13% cw (Table 2).

The results of the present study clearly showed that shoot tip explants 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 shoots formation in banana has been reported (Cronauer and Krokotian, 1984; Abdullah *et al*., 1998; Mohammed *et al*., 2000). Habiba *et al*. (2002) 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

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

		Mean No. of shoots/explants after		Mean length of the largest shoot (cm) after
Growth regulator (mg L^{-1})	Shoot regeneration (%)	25 days	35 days	35 days
BAP				
1.0	50.00	1.00±0.00	1.50±0.204	1.25±0.163
2.0	66.67	1.25±0.153	1.63±0.246	1.33±0.135
3.0	75.00	1.33±0.157	1.67±0.222	1.38±0.177
4.0	91.67	1.55±0.198	1.91±0.201	1.86±0.140
5.0	91.67	1.91±0.271	2.09±0.271	2.01±0.166
7.0	75.00	1.44±0.166	1.67±0.222	1.32±0.171
10.0	75.00	1.33±0.157	1.44±0.166	1.26±0.131
KIN				
1.0	16.67	1.00±0.000	1.00±0.000	0.90±0.071
2.0	16.67	1.00±0.000	1.00±0.000	0.95±0.177
3.0	33.38	1.00±0.000	1.25±0.217	0.98±0.074
4.0	66.67	1.25±0.153	1.58±0.171	1.09±0.072
5.0	75.00	1.44±0.166	1.91±0.222	1.26±0.090
7.0	41.67	1.20±0.179	1.40±0.219	1.04±0.104
10.0	41.67	1.00±0.000	1.33±0.272	0.93±0.109

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

Hormonal supplements (mg L^{-1})				
BAP+IAA+IBA	No. of single shoots inoculated	Response (%)	Average No. of multiple shoots/explant	Average length of highest shoot (cm)
2.0+2.0+2.0	12	33.33	3.25±0.217	2.65±0.251
3.0+2.0+2.0	12	41.67	3.60±0.219	2.78±0.184
4.0+1.0+1.0	12	66.67	4.13±0.328	2.88±0.155
4.0+2.0+2.0	12	91.67	6.18±0.527	4.45±0.389
5.0+2.0+2.0	12	91.67	5.46±0.433	3.73±0.166
6.0+2.0+1.012	12	33.33	3.50±0.250	2.45±0.238
BAP+KIN+IAA				
3.0+1.0+10.	12	16.67	2.50±0.352	2.25±0.181
4.0+1.0+1.0	12	33.33	3.00±0.353	2.58±0.191
4.0+2.0+1.0	12	58.33	3.70±0.261	2.87±0.122
4.0+2.0+2.0	12	75.00	4.78±0.262	3.76±0.211
5.0+1.0+1.0	12	41.67	3.60±0.222	2.76±0.101
5.0+2.0+2.0	12	83.33	5.50±0.353	4.04±0.200
6.0+2.0+2.0	12	25.00	2.67±0.271	2.13±0.152
BAP+KIN+CW(%)				
0.5+0.5+10	10	91.67	6.20±0.194	4.59±0.110
0.5+0.5+13	10	91.67	8.20±0.236	5.50±0.095
0.5+0.5+10	10	91.67	7.00±0.173	4.75±0.108

Fig. 2: Multiple plantlets production from shoot tip cultured on MS+0.5 mg L^{-1} BAP+0.5 mg L^{-1} KIN+13% cw after 7 weeks of culture on proliferation medium

Azad and Amin (2001) developed a medium for regeneration of banana from excised floral apices which was MS+2.0 mg L^{-1} BAP+1.0 mg L^{-1} KIN+15% cw. In our experiment it was observed that MS+0.5 mg L^{-1} BAP+0.5 mg L^{-1} KIN+13% cw were most optimum for average number (8.2±0.236) of shoot regeneration (Fig. 2) and highest length (5.5±0.095) of shoots from sucker explants. The different results obtained by different authors might be due to differences of genotypes and explants used.

Rooting and hardening: The rooting response differed according to different concentrations and combinations 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%

Table 3: Effect of different concentrations of auxins in 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})	Shoot rooted (%)	Mean No. of roots/shoots	Mean length of longest root (cm)
A			
Control (0)	50	2.20±0.335	2.00±0.152
½ MS+0.5	90	3.89±0.246	2.97±0.166
½ MS+1.0	100	6.10±0.359	4.06±0.258
½ MS+1.5	100	5.30±0.285	3.79±0.224
½ MS+2.5	90	4.56±0.319	3.24±0.192
½ MS+4.0	70	4.13±0.241	2.81±0.191
½ MS+5.0	60	3.67±0.304	2.25±0.208
NAA			
Control (0)	50	2.20±0.335	2.00±0.152
½ MS+0.5	90	3.56±0.355	2.31±0.159
½ MS+1.0	100	5.90±0.465	3.15±0.191
½ MS+1.5	100	5.00±0.346	2.73±0.195
½ MS+2.5	100	4.50±0.324	2.61±0.171
½ MS+4.0	70	4.00±0.404	2.32±0.137
½ MS+5.0	40	3.25±0.545	1.68±0.119
IAA			
Control (0)	50	2.20±0.335	2.00±0.152
½ MS+0.5	70	2.71±0.265	2.38±0.149
½ MS+1.0	90	3.56±0.277	2.90±0.215
½ MS+1.5	100	4.70±0.348	3.30±0.236
½ MS+2.5	70	3.57±0.397	2.54±0.210
½ MS+4.0	60	3.16±0.281	2.11±0.202
½ MS+5.0	60	2.83±0.282	1.91±0.148

Fig. 3: Root induction on ½ MS medium supplemented with 1.0 mg L^{-1} IBA

shoot was found rooted within 10-15 days with 6.20±0.395 roots per shoot (Fig. 3) The average length of root in this medium was 4.01±0.210 cm.

For the induction of roots from *in vitro* raised shoots of banana, De Langhe (1985) used half MS+1.0 mg L^{-1} IBA whereas Cronaur and Krokorian (1984) used auxin free MS medium for rooting of banana microshoots. On the other hand, Azad and Amin (2001) obtained

Fig. 4: Banana plantlets established in polybag

rooted banana shoots in MS+0.2 mg L^{-1} IBA. In the present study, we observed 1.0 mg L^{-1} IBA in half strength MS agar gelled medium was optimal for root induction. Akber *et al.* (2003) also found similar results in banana cv. Sagar.

Acclimatized rooted shoots were planted in small polythene bags or pots (Fig. 4) 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 leave emerged from about 90% of the plantlets that resumed new growth. 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 cultivar of "Mehersagar"

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