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In vitro Rooting and *Ex vitro* Plantlet Establishment of BARI Banana 1 (*Musa* sp.) as Influenced by Different Concentration of IBA (Indole 3-butyric Acid)

M.M.H. Molla, M. Dilafroza Khanam, M.M. Khatun, M. Al-Amin and [1]M.A. Malek
Biotechnology Division, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh
[1]Plant Genetic Resources Centre, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh

Abstract: Shoot tip culture of BARI banana 1 (*Musa* sp.) were cultured on MS medium supplemented with 5.0 mg L^{-1} BAP for shoot proliferation. Well-developed shoots were used for rooting. Among the six different concentrations of IBA (0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg L^{-1}) with half strength MS medium, a good number of healthy roots were produced on half MS+0.5 mg L^{-1} IBA (7.86) followed by half MS+0.6 mg L^{-1} IBA (6.89) and half MS+0.4 mg L^{-1} IBA (6.31) with the weight of 0.85, 0.83 and 0.77 g $plantlet^{-1}$, respectively. However, 95-100% plantlets were survived when they were transferred to small plastic pots after 15-20 days *in vitro* culture on half MS medium supplemented with 0.4–0.6 mg L^{-1} IBA and 7 days hardening at room temperature.

Key words: Banana, *in vitro* rooting, hardening, IBA concentration

INTRODUCTION

Banana (*Musa* sp.) is a most important fruit crop in Bangladesh. The plants are propagated vegetatively by means of sucker, which is a slow process. Moreover, suckers are sometimes infected with banana bunchy top virus as a symptomless carrier, Sigatoka and Panama diseases. Application of tissue culture technique is, therefore, a tool to produce large number of true to type disease free plants in limited period of time and space[1]. Apical meristem culture offers an efficient method for rapid clonal propagation, production of pathogen free material and germplasm preservation in plants[2-10]. Shoot tip culture is a well established adequate and relatively simple *in vitro* method for the rapid propagation of selected *musa* materials and the clean planting material[11]. A wide range of plant tissue culture techniques is increasingly being used as an enabling and enhancing technology for the handling of *musa* germplasms[12]. But *in vitro* propagation of different cultivar required different culture media for shoot proliferation and root differentiation[13]. However, there is still lack of information on *in vitro* rooting of banana. Now a day, the plant growth regulators are widely used in modern agriculture to promote rooting. Widiastoety and Soebijanto[14] reported that good rooting and the best survival (96.6%) were obtained with IBA treatment in *Hibiscus rosa* sinensis. Kundu *et al.*[15] reported that indole 3-butyric acid (IBA) had a highly significant effect on the percentage success of rooting, number of root and length of root production in *Ixora coccinia*. Percentage of rooting, number of roots and length of roots were markedly increased with IBA treatment in seedless lemon[16]. Hence, the present study was undertaken to standardize IBA concentration and days for *in vitro* rooting with 7 days hardening at room temperature for better plant survival of BARI banana-1 in *ex vitro* condition.

MATERIALS AND METHODS

BARI Banana 1 (*Musa sp.*) is a high yielding and newly released banana variety in Bangladesh. Shoot tips were collected from the virus free sword sucker of the variety. Roots and outer layer of tissues of the suckers were removed and the remaining portions were washed with tap water using detergent. The explant then surface sterilized in 0.1% mercuric chloride ($HgCl_2$) with a few drops of Tween 20 for 30 min. After washing 4 times with sterilized distilled water, the shoot tips were further cut to a size of approximately 5x8 mm portion containing an intact apex under clean bench. The explants were placed on MS solid medium[17] with 5.0 mg L^{-1} BAP. All cultures were incubated at 25±1°C with a 16 h photoperiod (2000 lux) provided by cool white fluorescent tubes. The pH of the medium was adjusted to 5.8 prior to autoclaving. The materials were sub-cultured at 30 days interval in the same medium to produce multiple shoots. Well-developed shoots were transferred to rooting medium containing half MS medium supplemented with different concentrations

Corresponding Author: M.M.H. Molla, Scientific Officer, Biotechnology Division, BARI, Joydebpur, Gazipur-1701, Bangladesh E-mail: mhmolla@hotmail.com

of IBA (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg L^{-1}). Each treatment contained ten replicate with six shoots/replicate. Every five days interval plantlets from different concentrations, transferred at room temperature for seven days hardening. After that, the plantlets were washed in water carefully to remove the media and transferred to small plastic pots containing a mixture of sterilized soil and sand for five days where high humidity was maintained by covering the plantlets with polyethylene sheet. The plantlets were then planted in poly bags (12.5x17.5 cm) containing soil, sand and cowdung (1:1:1) and were kept in nethouse. After incubation in rooting medium every 5 days interval, number of roots were recorded. After 15 days *in vitro* culture with 7 days hardening at room temperature average number of roots, length of roots, fresh weight of roots, number of leaves and length of shoots were recorded.

RESULTS AND DISCUSSION

Shoot proliferation and growth patterns of cultured explants are shown in Fig. 1(a-d). Rooting initiation was started within 3-4 days after incubation in half MS medium supplemented with 0.5 mg L^{-1} IBA where 4-5 days were needed in half MS medium supplemented with 0.1, 0.2, 0.3 and 0.4 mg L^{-1} IBA. Five to six days were needed in half MS medium supplemented with 0.6 mg L^{-1} IBA and in control treatment (Table 1). Khanam *et al.*[1] observed root initiation within 3-4 days with 2 μM L^{-1} IBA in banana cv. Amritsagar. Number of roots on different concentration of IBA (0.0, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg L^{-1}) are given in Table 1. In every concentration, number of roots was increased with increasing the duration of incubation in half MS supplemented with IBA medium.

Half MS medium without IBA also produced roots (2.44) but their growth was very poor. Half MS + 0.5 mg L^{-1} IBA produced highest number (8.28) of roots followed by half MS + 0.6 mg L^{-1} IBA medium (7.33) and half MS+0.4 mg L^{-1} IBA (6.33). Half MS+0.1 mg L^{-1} IBA, half MS+0.2 mg L^{-1} IBA and half MS+0.3 mg L^{-1} IBA were produced more or less similar number of roots such as 3.89 and 3.97, respectively (Table 1). After 25 days of *in vitro* culture, the initial roots were changed in colour and new roots initiation was stopped except secondary and tertiary root initiation.

Length of root was not increased with the increasing of IBA concentration in half MS medium and the range was 2.60–5.67 cm (Table 2). But fresh weight of roots/plantlet was increased with the increasing of IBA concentration in half MS medium. Highest fresh weight of roots were recorded on half MS+0.5 mg L^{-1} IBA concentration (0.85 g) followed by half MS +0.6 mg L^{-1} IBA concentration (0.83) and half MS+0.4 mg L^{-1} IBA concentration (0.77 g). Lowest fresh weight of roots/plantlet was recorded in half MS medium without IBA (0.23 g). On the other hand, half MS medium with 0.1, 0.2 and 0.3 mg L^{-1} IBA concentration produced fresh wt. of roots/plant 0.30, 0.31 and 0.35 g, respectively where as poor developed or unsatisfactory fresh weighted roots were produced on 0.0-0.3 mg L^{-1} IBA supplemented half MS media. Good rooting was observed on 0.4-0.6 mg L^{-1} IBA concentration on half MS medium. Number of leaves

Table 1: Effect of different concentrations of IBA and duration of *in vitro* culture on rooting of BARI Banana 1

		Average number of roots at				
Concentration	Days to root initiation	5 days	10days	15 days	20 days	25 days
Control	5-6	0.67	1.89	2.42	2.44	2.44
0.1	4-5	1.42	3.42	3.79	3.87	3.89
0.2	4-5	1.22	3.22	3.54	3.71	3.89
0.3	4-5	0.87	3.24	3.77	3.89	3.97
0.4	4-5	1.12	3.86	5.70	6.24	6.33
0.5	3-4	1.78	4.37	5.81	7.04	8.28
0.6	5-6	0.11	4.05	5.79	6.81	7.33

Table 2: Growth and development of plantlets as influenced by IBA at 15 days *in vitro* culture with 7 days hardening

Concentration	No. of explant	Average No. of roots/plant	Length of roots (cm)/plantlet	No. of leaves/ plantlet	Length of shoots/plantlet	Fresh wt. of roots/plantlet (g)
Control	20	2.44	2.60±1.53	5.50±0.53	8.30±0.30	0.23
0.1	20	3.88	3.86±1.56	5.20±0.42	8.12±0.16	0.30
0.2	20	4.00	4.50±1.30	5.50±0.54	8.20±0.20	0.31
0.3	20	4.60	5.67±1.92	6.40±0.52	8.35±0.32	0.35
0.4	20	6.31	4.96±1.37	6.00±0.57	8.25±0.26	0.77
0.5	20	7.86	4.64±1.51	5.80±0.58	8.30±0.35	0.85
0.6	20	6.89	3.59±1.07	6.00±0.50	8.14±0.17	0.83

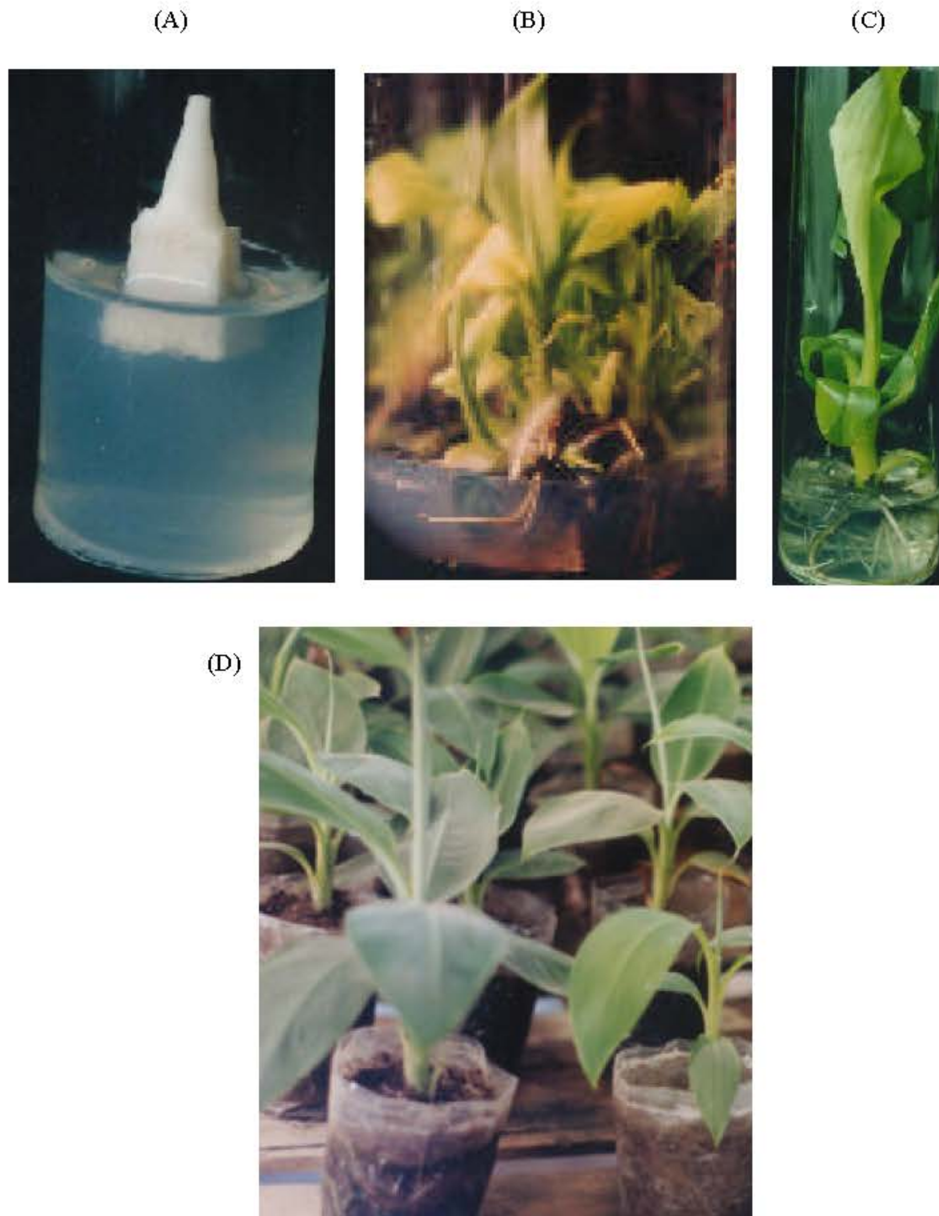

Fig. 1(A-D): Different stages of *in vitro* regeneration and rooting of banana
(A) Explant initiation (B) Multiple shoot (C) Rooted plantlet (D) Established plantlets in plastic bags

Table 3: Days to *in vitro* culture with different conc. of IBA and 7 days hardening at room temp. on survival percentage of BARI banana 1 plantlets

Concentration of IBA	Survival %				
	5 days	10 days	15 days	20 days	25 days
0.1	0.0	0.0	27.66	38.67	41.63
0.2	0.0	0.0	33.67	47.00	49.10
0.3	0.0	12.33	48.37	58.66	62.67
0.4	0.0	25.66	95.13	96.68	96.33
0.5	0.0	29.13	98.33	99.67	99.67
0.6	0.0	29.66	99.79	99.87	99.89

and length of shoots were varied from 5.20 – 6.40 and 8.12-8.35 cm, respectively.

Plantlets transferred to plastic pots after 10, 15, 20, 25 and 30 days *in vitro* culture with different concentrations of IBA and 7 days hardening are shown in Table 3. Results indicated that 95-100% plantlets survived when they were transferred after 15 -20 days *in vitro* culture on 0.4-0.6 mg L^{-1} IBA concentration with 7 days hardening at room temperature.

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