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In vitro Root Development of Banana (*Musa* spp.) As Affected by Different Concentrations of BAP and NAA

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Abstract: The experiments were conducted at the Biotechnology Laboratory, Bangladesh Agricultural Research Institute, Gazipur, during the period from September 2004 to June 2005 to investigate the effect of different concentrations of BAP, NAA, IAA and IBA on regeneration, on shoot proliferation and root formation in Banana (*Musa* spp). Meristems were collected from about four months old banana suckers were used on explants. In experiment 1, BAP (0.0, 2.5, 5.0, 7.5 and 10.0 mg L^{-1}) and NAA (0.0, 0.5, 1.0, 1.5 and 2.0 mg L^{-1}) were used as treatments. It was observed that the highest number of root was produced by 0.5 mg L^{-1} IAA + 0.5 mg L^{-1} IBA (3.50, 4.50 and 6.50 per explant respectively), which was statistically significant than other treatment. The highest length was observed at 10, 20 and 30 DAI in the treatment concentrations 0.5 mg L^{-1} IAA and IBA (2.93, 4.63 and 5.88 cm respectively) which was statistically significant. However, the meristem having no or lowest concentration of growth regulators showed the lowest root development.

Key words: *Musa spp*. Callus % Rooting % Growth regulators % Regeneration

INTRODUCTION

The banana and plantains (*Musae spp*.) belonging to the family *Musacae* are one the world's most important subsistence crops. It is originated in Malaysia through a complex hybridization process [1]. Banana ranks first in terms of production and second in terms of area, among the fruit crops and so has commercial value in Bangladesh. It comprises nearly 42% of the total fruit production of the country. It occupies an area of 43 thousand hectares of land with total production of 606 thousand metric tonnes with an average yield of 14.16 t ha^{-1} [2]. This yield is quite low compared to other banana growing countries of the world like Argentina (34 t ha^{-1}) and Costa Rica (33 t ha^{-1}) [3].

The low yield and production of banana is influenced by many natural and field factors, problem of virus free planting materials being the major among them. The traditional clonal propagation method appears unable to satisfy the increase in demand for disease free and healthy planting materials of banana. The productivity of vegetative propagated banana crops, such as banana and plantain is greatly reduced by virus disease [4].

To minimize the above mentioned problems, micropropagation could be an alternative for propagation of planting materials for banana. In this method, over a million of plant can be grown from a small or even a microscopic piece of plant tissue within a year [5]. Moreover the shoot multiplication cycle is very short (2-6 weeks), each cycle resulting in an exponential increase in the number of shoots and plant multiplication. Plant growth regulators are the essential part for *in vitro* regeneration of crop plants grown in any artificial medium. Generally, cytokinin helps in shoot proliferation and auxins helps in rooting of proliferated shoots. However, the requirement of cytokinin and auxins depends on the variety of banana and culture conditions [6]. BARI-1 banana variety plays a vital role in our national economy due to their popularity and acceptability to marginal and commercial farmers. To obtain virus and disease free healthy planting materials, development of a protocol for meristem culture of banana cv. BARI-1 are of prime importance. Therefore, considering the above facts the present study was undertaken to determine the effect of IBA and IAA growth regulators and their concentration required for banana *in vitro* root development.

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MATERIALS AND METHODS

The study was conducted at the Biotechnology laboratory, Biotechnology Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, Bangladesh during the period of_@September, 2004 to June, 2005. The planting materials of banana cv. BARI-1 were collected from Biotechnology Division, BARI, The meristem used for establishment of culture was prepared under the microscope from the collected suckers through dissection and removal of leaf sheath.

Murashige and Skoog [7] medium (MS medium) supplemented with different growth regulators were used as culture medium for shoot induction and shoot multiplication. For the preparation of media, stock solutions were prepared at the beginning and stored at 9±1°C temperature. The respective media were prepared from the stock solutions. Separate stock solutions for macronutrients, micronutrients, irons, vitamins, growth regulators etc were prepared and used. To prepare a stock solution of any of these growth regulators, 10 mg of the growth regulator was taken on a clean watch glass then dissolved in 1 ml measuring cylinder and the volume was made up to 1000 ml with distilled water. The solution was then poured into a clean glass container and stored at 9±1°C and used for a maximum period of two months. The culture tubes with media were then autoclaved at 1.06 kg cmG^{2} pressure at 121°C for 25 minutes. The surface was sterilized properly.

The initial explant of banana was prepared under stereomicroscope by removal of outer tissue of meristem with the help of sterile scalpel, which was about 5×5 mm in size.The isolated and surface sterilized explants, meristems were collected carefully under the stereomicroscope through maintaining aseptic condition inside the laminar air flow cabinet, to use those as explants. The individual meristems were directly inoculated to each of the culture tube containing 20 ml of MS medium supplemented with different concentration of hormones as per treatment covered with alumnium foil. The culture tubes were transferred to growth chamber maintaining the temperature at 25 ± 1°C under the light intensity of 2000 lux for16 hours.

There were 3 levels of IAA (0.0, 0.5 and 1.0 mg LG^{1}) and 4 levels of IBA (0.0, 0.5, 1.0 and 1.5 mg LG^{1}) were used as treatments. The experiments were arranged in Completely Randomized Design (CRD) with 4 replications. Each of replications consisted of 10 culture tubes.

Excised shoots were used for root induction. The percentage of shoots induced roots were calculated using the following formula.

$$\% \text{ shoot induced root} = \frac{\text{Number of shoot induced root}}{\text{Total number of shoots inoculated}} \times 100$$

Average number of roots per plantlet was counted at 10 day's interval up to one month of the culture and the mean was calculated. Root length was measured in centimeter from the base to the tip of the roots at 10 days interval up to one month of the culture. Average length of the root was calculated using the formula as mentioned earlier. The data was analyzed using MSTAT-C [8] programme.

RESULTS AND DISCUSSION

The regenerated shoots were collected from *in vitro* grown plants. Then it was subcultured on half strength MS medium supplemented with different levels of IBA (0, 0.5, 1.0 and 1.50 mg LG^{1}) and IAA (0, 0.5 and 1.0 mg LG^{1}) in order to allow root formation. Root numbers varied with different concentrations of IBA and IAA.

Number of Root per Explant: The effect of IAA and IBA on the number of root per explant produced by different combination at 10, 20 and 30 DAI was shown in the Table 5 showed significant variation. The highest number of root was produced by 0.5 mg LG^{1}IAA + 0.5 mg LG^{1} IBA (3.50, 4.50 and 6.50 per explant respectively) which was statistically significant than other treatment (Table 1). 0.5 mg LG^{1} IAA + 1.0 mg LG^{1} IBA produced 6.0 roots per explant at 30 DAI but at 20 DAI 3.50 roots was produced per explant. The lowest number of root was produced by control treatment. Vigorous roots of *in vitro* grown plantlet on MS media supplemented with 0.5 mg LG^{1} IAA + 0.5 mg LG^{1}IBA was shown in Plate 1. The present results are similar with the findings of Gubbuk and Pekmezci [9].

Molla *et al*. [10] obtained 8.28 number of roots/plantlet on 0.5 mg LG^{1} IBA followed by 6.33 roots, 0.6 mg LG^{1} BA. They also observed 3.89 and 3.97 number of roots in 0.2 mg LG^{1} IBA and 0.3 mg LG^{1} IBA respectively. Present results in similar to the results obtained by Molla *et al*. [10].

The results of the present experiment similar with the findings of Khanam *et al*. [11]. Rahman *et al*. [12] obtained highest roots (2.88/explant) at 30 DAI by 3.0 mg LG^{1} NAA where as not respond on control.

Length of Root: The length of roots developed by the plantlets was influenced considerably by different concentration of IAA and IBA used in the experiments and the results have been presented in Table 2. The result

Table 1: Effect of different concentrations of IAA and IBA on root number of multiplied shoot of banana cv. BARI-1 at different days after inoculation

Treatments			Number of root		
IAA (mg LG^{1})	IBA (mg LG^{1})	Vigour of regenerated root	10 DAI	20 DAI	30 DAI
0	0.0	+	0.00 e	0.00 e	0.00 f
	0.5	+	1.50 cd	2.00 d	3.25 e
	1.0	++	2.25 bc	2.25 d	3.50 de
	1.5	++	2.25 bc	2.50 cd	3.50 de
0.5	0.0	+++	2.75 ab	2.25 d	3.25 e
	0.5	+++	3.50 a	4.50 a	6.50 a
	1.0	++	3.25 ab	3.50 abc	6.00 ab
	1.5	++	3.25 ab	4.00 ab	5.00 bc
1.0	0.0	+	1.00 de	2.50 d	3.25 e
	0.5	+	2.75 ab	2.75 cd	3.75 de
	1.0	++	1.50 cd	3.00 bcd	4.00 cde
	1.5	+	1.25 cd	3.50 abc	4.50 cd
LSD value (0.01)			1.03	1.16	1.16
CV (%)			25.36	22.19	15.51

+= Less vigorous growth

++= Good growth and vigour

+++= Best growth and vigour

Table 2: Effect of different concentrations of IAA and IBA on root length of multiplied shoot of banana cv. BARI-1 at different days after inoculation

Treatments		Root length (cm)		
IAA (mg LG^{1})	IBA (mg LG^{1})	10 DAI	20 DAI	30 DAI
0	0.0	0.00 e	2.00 ef	2.00 f
	0.5	1.08 d	1.88 f	2.30 e
	1.0	1.08 d	2.30 de	3.15 d
	1.5	1.13 d	2.45 d	3.08 d
0.5	0.0	1.23 d	1.60 f	2.08 e
	0.5	2.93 a	4.63 a	5.88 a
	1.0	2.55 ab	3.88 b	4.83 b
	1.5	3.03 a	3.85 b	4.88 b
1.0	0.0	1.80 c	2.33 de	3.45 cd
	0.5	1.80 c	2.35 de	3.48 cd
	1.0	2.08 bc	3.15 c	3.75 c
	1.5	2.15 bc	2.70 d	3.70 c
LSD value (0.01)		0.49	0.42	0.55
CV (%)		16.28	7.95	7.57

indicated that there was a sharpe increasing trends in root length at different DAI (10, 20 and 30 DAI) which is significant at 1% level.

The root length of plantlets after 30 DAI was remarkably highest which is Auxin was essential for successful root induction of banana and has been reported by Raut and Lokhand [13]. The highest length was observed at 10, 20 and 30 DAI in the treatment concentration 0.5 mg LG^{1} IAA and IBA (2.93, 4.63 and 5.88 cm) which was statistically significant. The second highest result (3.03, 3.85 and 4.88 cm at 10, 20 and 30 DAI) was observed with 0.5 mg LG^{1} IAA and 1.5 mg LG^{1} IBA and the lowest (0.00, 2.00 and 2.00 cm at 10, 20 and 30 DAI) value obtained with control treatment. Similar results were obtained by Molla *et al*. [10] where they got 2.60-5.67 cm range of root length in 0.5 mg LG^{1} IBA.

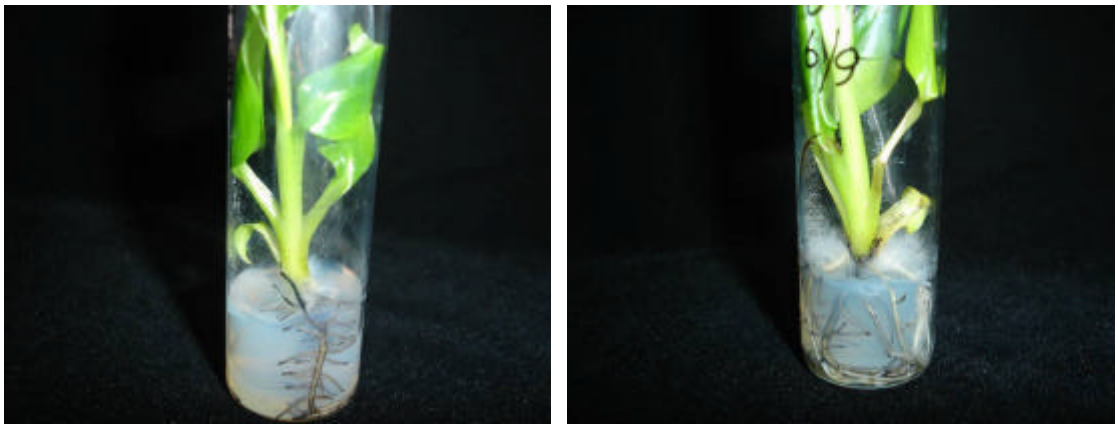

Plate 1: Vigorous roots of banana cv. BARI-1 grown on MS media supplemented with 0.5 mg L^{-1} IAA + 0.5 mg L^{-1} IBA

Plate 2: Well established meristern derives plantlets Banana BARI-1 in poly bags

Habiba[14], Khanam *et al*. [11] and Ali [15] also got more or less same observation. Therefore, the presented result partially agrees with the findings of Gubbuk and Pekmezci [9] who reported that 1.0 µM IBA per litre with MS medium. Meristem derived plantlet transferred to poly bags containing 1:1 (ground soil: cowdung) mixture after 7 days hardening in room temperature (28-30°C). A good established plantlet was shown in plate 2, which is ready for planting.

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