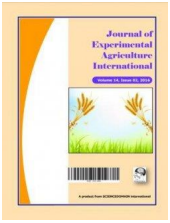

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Effect of Different Concentrations of IAA (*Indole acetic acid*) and IBA (*Indole butyric acid*) on Multiple Root Regeneration of Banana (*Musa* spp.) cv. Giant Cavendish from Meristem Derived Explants

Adane Gebeyehu[1*]

[1]National Agricultural Biotechnology Research Center, Ethiopia.

Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

The present study was conducted at the Tissue culture Laboratory of Amhara Region Agricultural Research Institute (ARARI), Bahir Dar - Ethiopia during the period from May to June 2012 to investigate the effect of different concentrations of IAA and IBA on root regeneration of banana cv. Giant Cavendish. For rooting, individual shoots (3-6 cm) with 5-6 leaves were transferred to the MS medium supplemented with (0.0, 0.5 and 1.0 mg/l) IAA and (0.0, 0.5 and 1.0 mg/l) IBA for 4 weeks. Among the different concentrations, highest number of roots was produced by 0.5 mg/l IAA + 0.5 mg/l IBA (3.00, 4.67, and 6.67) 10, 20, and 30 DAI respectively. The longest roots observed was (2.83, 4.60, and 5.87 cm) at 10, 20, and 30 DAI respectively in concentration 0.5 mg/l IAA + 0.5mg/l IBA, which was statistically significant ($P<0.05$) at 10, 20, and 30 DAI.

Keywords: Banana; regeneration; micro propagation.

**Corresponding author: E-mail: adyamrot@gmail.com;*

1. INTRODUCTION

Bananas are perennial herbaceous monocots which belong to the *Musa* genus of the *Musa*ceae family originated from the South East Asian region, where greatest diversity of edible bananas found [1]. Bananas account for approximately 22% of the fresh fruit production and are ranked as the second most important fruit crop [2]. The different types of banana have not been fully exploited since Cavendish types mainly dominate the market place globally.

Concerning the introduction of banana to Ethiopia, no one knows for sure when it entered to the country. All sorts of varieties (dessert, cooking, brewing) are grown throughout Ethiopia but not studied well [3].

Unfortunately, expansion of banana production is frequently limited by costly high quality planting materials. The farmer-produced suckers are good transmitters of insect pests and diseases [4], [5]. This has prompted interest in the use of *in vitro* tissue culture technique. Through meristem micro propagation, pathogen free clones are obtained. Micro propagation of banana is highly efficient, allowing a large turnover of plants in a very short period of time within very little space [6,7]. Therefore, considering the above fact; the present study was undertaken with the following major objective:

- To study the effect of IAA (*Indole acetic acid*) and IBA (*Indole butyric acid*) on multiple root regeneration of banana (*Musa* spp.) Cv. Giant Cavendish from meristem derived explants.

2. MATERIALS AND METHODS

The present study was conducted at the Tissue culture Laboratory, Amhara Region Agricultural Research Institute (ARARI), Bahir Dar - Ethiopia during the period from May to June 2012 to investigate the effect of different concentrations of IAA and IBA on multiple root regeneration of banana (*Musa* spp.) Cv. Giant Cavendish from meristem derived explants. Three levels of IBA (0.0, 0.5, and 1.0 mg/l) and three levels of IAA (0.0, 0.5, and 1.0 mg/l) were used as treatments. The experiments were arranged in completely randomized design (CRD) with 4 replications. Each treatment consisted of 5 culture tubes per replication. Data on number of roots per clump and length of roots per plantlets were collected. Statistical analysis of quantitative data on root number and root length (cm) at 10, 20 and 30 days after inoculation (DAI) were collected and analyzed using ANOVA by SAS version 9.2 Software and Microsoft Office Excel 2010 spread sheet. Least Significant Difference (LSD) at probability level of $p < 0.05$ was considered significant for all analysis.

2.1 Stock Solution and Media Preparation

The media namely MS [8] were prepared by dissolving the appropriate amount of macro and micro nutrient; and organic supplements. Similarly, growth regulators (IBA and IAA) stock solutions were prepared using the proportion of 1 mg: 1 ml and stored in refrigerator at 4^0C. The MS culture media were prepared from its respective stock solutions using the appropriate amount of sucrose, plant growth regulators and agar (7 g/l) and were used as culture medium for root regeneration. All combinations of both growth regulators at three levels of IBA and three levels of IAA were added separately to the media to study its effect on root regeneration. The jars with media were then dispensed 35 ml each and autoclaved at 121°C for 25 minutes after adjusting the pH to 5.7 with 1 N NaOH and/or 1 N HCl.

2.2 Root Regeneration

Individual shoot or shoot clumps were transferred to a nutrient medium which does not promote further root formation. The cytokinin in the regeneration medium was completely omitted. To initiate root rhizogenesis IAA (0.0, 0.5 and 1.0 mg/l) and IBA (0.0, 0.5 and 1.0 mg/l) were added in the medium. After rooting, plants were hardened *in vitro* for 2-4 extra weeks on the regeneration/rooting medium prior to transplantation to soil.

3. RESULTS AND DISCUSSION

3.1 Analysis of variance (ANOVA) of Giant Cavendish Root Number at Different DAI

Analysis of variance (ANOVA) of Banana cv. Giant Cavendish for single factor complete block design revealed significant difference ($P<0.01$) due to the main effect of different concentrations of IAA and IBA for the means of number of roots per clump at all days after inoculation (Table 1). These findings were in agreement with the results obtained by Vuylsteke [9].

3.2 Analysis of Variance (ANOVA) of Giant Cavendish Root Length (cm) at Different DAI

Analysis of variance (ANOVA) of Banana cv. Giant Cavendish for single factor complete block design revealed significant difference (P<0.01) due to the main effect of different concentrations of IAA and IBA for the means of root length (cm) at all days after inoculation (Table 2). Therefore, the present result agreed with the findings of [10] who got 2.60-5.67 cm root length in 0.5 mg/l IBA.

3.3 Effect of Different Concentrations of IAA and IBA on Root Number per Explants of banana cv. Giant Cavendish

The effect of IAA and IBA on number of roots showed significant variation at 10, 20, and 30 DAI at (P<0.05). The highest number of roots was produced by 0.5 mg/l IAA + 0.5 mg/l IBA (3.00, 4.67, and 6.67) 10, 20, and 30 DAI respectively, which was statistically significant than other treatments (Table 3). Vigorous roots of *in vitro* grown plantlet on MS media supplemented with 0.5 mg/l IAA + 0.5 mg/l IBA. The present results are similar with the findings of [11] who obtained 8.28 roots per plantlet on 0.5 mg/l IBA.

3.4 Effect of Different Concentrations of IAA and IBA on Root Length (cm) Per Explant of Banana cv. Giant Cavendish

The length of roots developed by the plantlets was influenced considerably by different concentrations of IAA and IBA. The result indicated that there was an increasing trend in root length at different DAI, which is significant at (P<0.05). The highest length observed was (2.83, 4.60, and 5.87 cm) at 10, 20, and 30 DAI respectively at a concentration of 0.5 mg/l IAA + 0.5mg/l IBA, which was statistically significant (Table 4). Similar result was obtained by Vuylsteke [9] where they got 2.60-5.67 cm root length in 0.5 mg/l IBA. Therefore, the present result agreed with the findings of [12].

3.5 Mean Value Comparison of Banana cv. Giant Cavendish on Rooting

The effect of IAA and IBA on number and length of roots were statistically significant at 10, 20 and 30 DAI. The highest number of roots was produced by MS medium supplemented with 0.5 mg/l IAA + 0.5 mg/l IBA. The number of roots was 3.00, 4.67 and 6.67 at 10, 20 and 30 DAI respectively (Fig. 1).

The effect of IAA and IBA on length of roots (cm) were statistically significant (P<0.05) at 10, 20 and 30 DAI. The highest length of roots were produced by 0.5 mg/l IAA + 0.5 mg/l IBA. The length of roots were 2.83 cm, 4.60 cm and 5.87 cm 10, 20 and 30 DAI respectively (Fig. 2).

3.6 Correlation Analysis between Rooting of Giant Cavendish

Correlation coefficients among parameters of Banana cv. Giant Cavendish was shown in (Table 5). As expected, all parameters were positively and significantly correlated to each other (P<0.01) indicating that as number of shoots increases, probability of getting longest leaves became increased at 10, 20 and 30 DAI.

Table 1. ANOVA of root number of Banana cv. Giant Cavendish at different 10, 20 and 30 DAI respectively

Sources of variation	*df*	SS	MS	F *cal*	$F_{5\%}$	$F_{1\%}$
Treatments	8	3428.9	428.6	3.71	2.48	3.63
Error	19	2002.1	105.4			

Table 2. ANOVA of root length (cm) of Banana cv. Giant Cavendish at different 10, 20 and 30 DAI respectively

Sources of variation	*df*	SS	MS	F *cal*	$F_{5\%}$	$F_{1\%}$
Treatments	8	3435.6	429.5	4.05	2.48	3.63
Error	19	2009.4	105.8			

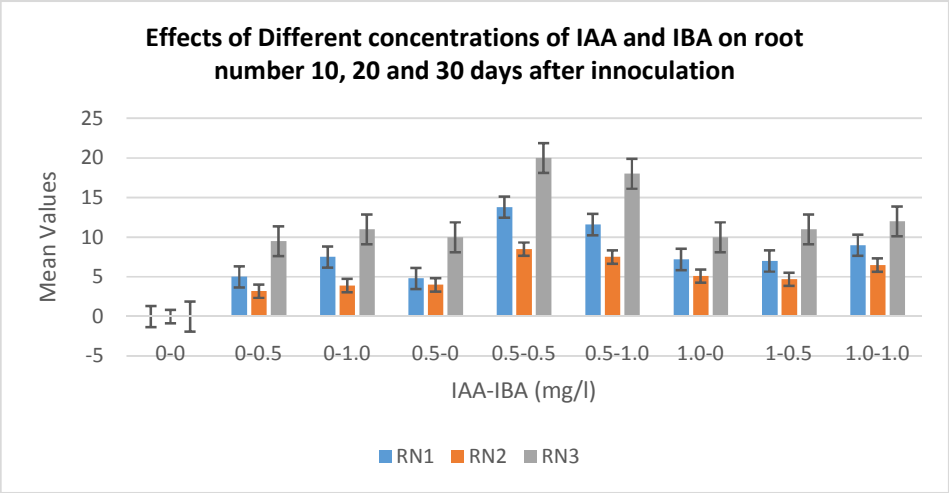

Fig. 1. Effects of different concentrations of IAA and IBA on root number 10 DAI (RN1), 20 DAI (RN2) and 30 DAI (RN3)

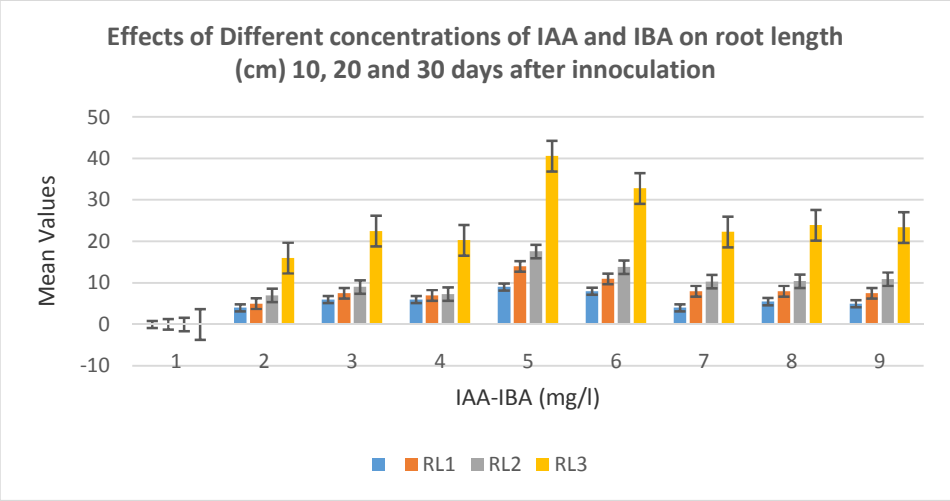

Fig. 2. Effects of different concentrations of IAA and IBA on root length (cm) 10 DAI (RN1), 20 DAI (RN2) and 30 DAI (RN3)

3.7 Acclimatization of Banana cv. Giant Cavendish

In vitro developed plantlets were fragile till the physiological adjustments fit to the surrounding environment. A total of 29 Giant Cavendish plantlets were acclimatized in the growth room on a sterile soil mix containing 50% compost and 50% sand on acclimatization trays (7 cm length and 4 cm width) covered with cheese cloth to prevent desiccation. After a week in the growth room the plastic bags were removed and plantlets with approximate height of 7-8 cm, with well-developed roots of 4-5 cm length, were taken from the culture jar using forceps and transferred to small acclimatization pots to a greenhouse with 80% humidity, at a temperature of 25± 2^0C and 16 hrs light and 8 hrs of dark. After a week in the greenhouse, the survival rates were recorded and the survival rates were found to be 96.6%. After a week in the greenhouse the survival rates were recorded (Table 6) and the survival rates were found to be 96.6%. There were no aberrant phenotypes for all the varieties grown in the green house. A good number of established plantlets were shown in plate, which were ready for planting (Table 6).

Table 3. Mean effects of IAA and IBA combinations on root number of Banana cv. Giant Cavendish at different days after inoculation

Treatments	Parameters		
	RN1	RN2	RN3
IAA-IBA (mg/l)			
0-0	0.00d	0.00d	0.00c
0-0.5	1.33c	1.67c	3.17b
0-1	2.00bc	2.50bc	3.67b
0.5-0	2.00bc	2.33c	3.33b
0.5-0.5	3.00a	4.67a	6.67a
0.5-1	2.67ab	3.67ab	6.00a
1-0	1.33c	2.67bc	3.33b
1-0.5	1.83bc	2.67bc	3.67b
1-1	1.67c	2.50bc	4.00b
LSD	0.94	1.25	1.12
CV (%)	31.00	28.72	17.22

*Means followed by the same letter(s) are not significantly different at P > 0.05. **= significant at (P<0.01); RN1 = number of roots per clump 10 days after inoculation; RN2 = number of roots per clump 20 days after inoculation; RN3 = number of roots per clump 30 days after inoculation.*

Table 4. Mean effects of IAA and IBA combinations on root length of Banana cv. Giant Cavendish at different days after inoculation

Treatments	Parameters		
	RL1 (cm)	RL2 (cm)	RL3 (cm)
IAA- IBA(mg/l)			
0-0	0.00c	0.00e	0.00e
0-0.5	1.07b	1.67cd	2.33d
0-1	1.30b	2.50b	3.00cd
0.5-0	1.33b	1.60d	2.43d
0.5-0.5	2.83a	4.60a	5.87a
0.5-1	2.50a	3.87a	4.6b
1-0	1.70b	2.40bc	3.43c
1-0.5	1.60b	2.33bcd	3.47c
1-1	2.17b	3.00b	3.63bc
LSD	0.68	0.78	0.98
CV (%)	24.39	18.59	17.65

*Means followed by the same letter(s) are not significantly different at P > 0.05; **= significant at (P<0.01); RL1 = length of roots per plantlet (cm) 10 days after inoculation; RL2 = length of roots per plantlet (cm) 20 days after inoculation; RL3 length of roots per plantlet (cm) 30 days after inoculation.*

Table 5. Simple correlation coefficients (r) among rooting of Banana cv. Giant Cavendish at different days after inoculation (DAI)

Parameter	RN1	RL1	RN2	L2	RN3	RL3
RN1	-	0.85**	0.92**	0.84*	0.92**	0.86**
RL1		-	0.87**	0.95**	0.89**	0.95**
RN2			-	0.88**	0.95**	0.91**
RL2				-	0.93**	0.98**
RN3					-	0.93**
RL3						-

**significantly different at P > 0.05; **= significant at (P<0.01); RN1 = number of roots per clump10 DAI, RN2 = number of roots per clump 20 DAI, RN3 = number of roots per clump 30 DAI, RL1 = length of roots per plantlet (cm) 10 DAI, RL2 = length of roots per plantlet (cm) 20 DAI, RL3 length of roots per plantlet (cm) 30 DAI.*

Table 6. Survival rate of acclimatized plantlets in the growth room (GR) and green house (GH)

Variety	Survival rate				
	Plantlets transferred to GR	Plantlets survived in GR (%)	Transferred to GH	Survived in GH (%)	Plantlets Died
Giant Cavendish	29	100	29	96.55	1

4. CONCLUSIONS

The study highlighted the importance of banana in Ethiopia, and its difficulties in traditional propagation. The present study also allows for conservation of banana clones, rapid propagation of selected disease-free planting materials and breeding procedures efficiency. According to the findings of the present study root regeneration of banana cv. Giant Cavendish, were best on MS medium supplemented with 0.5 mg/l IAA + 0.5 mg/l IBA. The experimental attempt might also be used for the massive in vitro production of banana plantlets. Studies were conducted for different banana and plantain cultivars and limited to banana tissue culture

experiments. The surveys should be carried out in different parts of the country to conserve the germplasm, which in due course, serve as a source of genetic material (gene pool). The future research needs to optimize protocols for each variety of banana using different tissue culture techniques in the region. It is recommended that the study could be used as a resource for researchers, students and companies engaged on banana research worldwide.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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