STUDY ON DIFFERENT CONCENTRATIONS OF IBA AFFECTING NUMBER OF LEAVES OF BANANA PLANTLETS AT SPECIFIC TIME INTERVALS

By

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ABSTRACT
Study was conducted to explore the effect of eight different concentrations of IBA (0.1 to 0.7mg/l + ½ MS including 0.00 as control) on number of leaves of banana plantlets which were produced through shoot tip culture in the Department of Biotechnology, Faculty of Crop Production, Sindh Agriculture University Tandojam during the year 2004. The effect of concentrations in terms of number of leaves was recorded after 10, 20, 30 and 40 days of plantlet initiation. ANOVA from statistical analysis revealed highly significant effects of different concentrations of IBA on number of leaves at all the above mentioned four time intervals under study. Numerically, IBA concentration of 0.3mg/l produced more number of leaves (3.90, 4.52, 5.75 and 7.80) in banana plantlet at all the four time intervals i.e. 10, 20, 30 and 40 days respectively followed by concentration 0.4mg/l which produced 3.00, 4.20, 5.10 and 7.40 number of leaves after 10, 20, 30 and 40 days respectively after initiation of banana plantlet. However, control treatment with zero IBA concentration produced the lowest number of leaves at all the four time intervals followed by the concentration with 0.1mg/l which produced 2.0, 2.88, 3.98 and 5.95 leaves at 10, 20, 30 and 40 days respectively. Considering the results of present study, it is recommended that IBA concentrations 0.3 and 0.4mg/l may be used while producing healthy and vigorous plantlets of banana for further multiplication commercially.

KEYWORDS: Banana (Musa cavendishii L.), tissue culture, micro propagation, BAP

INTRODUCTION
Banana belongs to the family Musaceae. There are only two genera viz Ensete and Musa. The edible cultivated parthenocarpic banana originated from Musa acuminate and Musa balbisiana (Khader, et al. 1990). Mostly, cultivated banana is triploid (2n = 3x = 33) and it has been shown that triploid plants are more vigorous and their fruits grow faster than diploids (Simmonds, 1996).

The total cultivated area, under banana in Pakistan, is about 29.7 thousand hectares, with an annual production of 142.9 thousand tonnes. Among the four provinces, Sindh is the leading banana producing province with an area of 25.4 thousand hectares, producing 112.9 thousand tones, while banana is cultivated on negligible area in rest of three provinces; Punjab, NWFP and Balochistan (Pakistan statistical year book 2004).
In Sindh during 1988-89 when the bunchy top disease was first observed and later on (1991) identified as "Banana Bunchy Top Disease" BBTD caused by BBTV (Khalid et al. 1993). The virus has been spreading through infected suckers as well as by insect vector (the black banana aphid *pentalonia nigronervosa*). However, presence of the aphid was confirmed in 1993 (Soomro and Khalid 1994). The disease attained the form of an epidemic and devastated the crop by causing losses upto 100% in some areas (Soomro et al. 1992).

Banana plants are propagated vegetatively by means of suckers, which is a slow process. Moreover, suckers are some times infected with banana bunchy top virus as a symptoms carrier, sigatoka and panama diseases (Molla et al. 2004). 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 (Khanam et al. 1996). Apical meristem culture offers an efficient method for rapid clonal propagation, production of pathogen free material and germ plasm preservation in plants, 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 (Vuylsteke and Swennen 1993).

A wide range of plant tissue culture techniques are increasingly being used as an enabling and enhancing technology for the handling of musa germplams (Vuylsteke, 1989), but *in vitro* propagation of different cultivars, required different culture media for shoot proliferation and root differentiation (Dore et al. 1983). Molla et al. (2004) found that among six different concentrations (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg/l) with half strength of MS medium, a good number of healthy roots were produced on half MS + 0.5 mg/l IBA (7.86) followed by half MS + 0.6 mg/l, IBA (6.89) and half MS + 0.4 mg/l IBA (6.31). However, 95-100% plantlets were survived when they were transferred to small plastic plots after 15-20 days *in vitro* culture on half MS medium supplemented with 0.4-0.6 mg/l IBA and 7 days hardening at room temperature.

Babylatha et al. (1997) stated that the establishment of banana (cv. Basrai) shoot tips was best in Murashige and Skoog (MS) medium supplemented with 2.0 mg benzyladenine/litre and shoot proliferation was best in MS medium supplemented with 4.0 mg benzyladenine/litre. Hundred per cent rooting was achieved on full strength MS medium supplemented with various combinations of IBA (1.0-10.0 mg), NAA (1.0-5.0 mg/litre), IAA (1.0-2.0 mg/litre) and/or activated charcoal (0.05%).

Wargantiwar et al. (1997) explored the possibility of micro propagation of banana through shoot tip culture. Maximum multiple shoot formation was given by Murashige & Skoog MS basal medium supplemented with 7mg BAP [benzyladenine]/litre or 5mg BAP/litre + 15% coconut milk. Rooting of the multiple shoots was best in MS medium + 2mg IBA/litre + 0.1% charcoal.

Dilip et al. (2000) described a protocol for large-scale multiplication and *ex vitro* survival of banana var. 'Basrai' through tissue culture. Multiple shoot cultures from shoot-tip ex-plants of 'Basrai' were established on modified Murashige and Skoog MS medium. Maximum 28 shoots could be achieved on MS medium containing Ascorbic acid (50 mg/l), Adenine hemisulphate (100 mg/l), Benzyladenine (100 mg/l), Kinetin (3.0 mg/l) and Indole acetic acid (0.5 mg/l). Maximum 90% rooting were achieved in well-developed shoots on MS medium supplemented with Indolebutyric acid (1.0 mg/l). More than 25,000 plantlets produced through tissue culture were transferred to field with 90% survival rate.

The present study was undertaken to explore the effect of different concentrations of IBA + ½ MS on the number of leaves in banana plantlets at specific time intervals.

**MATERIAL AND METHODS**

Banana explants were taken from the field. Suckers were excised and surface sterilized with aqueous calcium hypochlorite for 10 minutes. Explants were then washed three times with double distilled water. Shoot apices of 1 cm bearing 2-4 leaf primordial were excised aseptically from the sterilized tissue and cultured on the MS medium. The culture media used for this purpose contained the essential salts and nutrients as given below:
**Ingredient** | **Chemical composition** | **Weigh in mg/l**
---|---|---
**MICRO NUTRIENTS** |  | 
Manganese sulphate & $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ & 22.2
Zinc Sulphate & $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ & 8.2
Boric Acid & $\text{H}_3\text{BO}_3$ & 6.2
Potassium Iodide & $\text{KI}$ & 0.83
Sodium Molybdate & $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ & 0.025
Copper sulphate & $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ & 0.025
Cobalt Chloride & $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ & 0.025
**IRON** |  | 
Iron Sulphate & $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ & 27.8
Sodium EDTA & (Sodium Ethylene diamine tetra acetic acid) & $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ & 37.26
**VITAMINS** |  | 
Pyridoxol Hydrochloride & $\text{C}_8\text{H}_{12}\text{CINO}_3$ & 0.5
Thiamine HCl & $\text{C}_12\text{H}_6\text{N}_4\text{OSCl}$ & 0.5
Glycine & $\text{NH}_2\cdot\text{CH}_2\cdot\text{COOH}$ & 2.0
Nicotinic acid & $\text{C}_6\text{H}_5\text{NO}_2$ & 0.5
Myo-inositol & & 0.1gm/l
**MACRO-NUTRIENTS** |  | 
Potassium nitrate & $\text{KNO}_3$ & 1.90gm/l
Ammonium nitrate & $\text{NH}_4\text{NO}_3$ & 1.65gm/l
Calcium chloride & $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ & 0.44gm/l
Potassium phosphate & $\text{KH}_2\text{PO}_4$ & 0.17gm/l
Magnesium sulphate & $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ & 0.37gm/l
Sugar & $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ & 25.00gm/l
Casein & & 2.00gm/l
Agar & & 8.00gm/l
pH & & 5.7

**Procedure**
MS media salt mixture is commercially available in powder or it may be prepared according to the composition.

1. Take approximately 400 ml DDH$_2$O to a liter beaker, weigh and dissolve each of micro nutrients salt given in the above table.
2. From each of stock solution previously prepared, add through pipette the required quantity of the nutrient to the solution, stock solutions are prepared because micro nutrients which are required in small quantity can not be measured straight away and added in the medium.
3. Weigh 100 mg Myo-Inositol and dissolve in medium.
4. Add the hormones and vitamins in the solution according to the procedure.
5. Add DDH$_2$O until the total volume is about 1000ml.
6. The pH of media has to be adjusted to 5.7 before the later have been solidified with the agar.
7. Weigh 25.00 gm/liter of sugar and 8.00 gm/liter agar (According to the specification) and add into medium and warm the medium until the agar is dissolved.
8. Pipette 5 ml medium into the transparent and sterilized bottles according to the requirement and then seal with aluminum foil or screw cap.
9. Autoclave the culture bottle containing medium for 20-30 minutes under 15 lbs pressure at 121°C.
10. Remove the medium or culture bottles and move in deep freezer before culture time.

**Surface sterilization**
Explants must be free from micro organisms when placed on nutrient media and this was achieved by surface sterilization with 10% sodium hypochlorite supplemented with “tween-20”. After 10 minutes, explant material was rinsed thrice with double distilled water (DDH$_2$O). All these practices were done in sterilized area.
Preparation of laminar flow cabinet
First of all, laminar flow cabinet is to be started and swept with 70% alcohol. The entire instruments/materials e.g knife, forceps, scissors, medium containing bottles, sterilized plastic containing petridish, ring rubbers; spirit lamp is to be placed in the laminar flow cabinet. The UV lamp is started for about 10 minutes before working, which is mostly used for killing the micro organisms.

Meristem tip culture
Apical meristems, particularly of shoot apical meristem were excised and cultured which is known as meristem culture. For culturing the shoot apical meristems, banana suckers, which were used as explants were taken from the field and washed with running tap water. Excised explant material was surface sterilized in 10% Sodium hypochlorite for about 10 minutes and rinsed thrice with DDH₂O in pre-sterilized area. Explant material again excised and shoot apical meristems were placed in medium in culture bottles. Those bottles were covered with pre- sterilized transparent plastic sheets, tightly held with the help of ring rubber. Cultured bottles were incubated in growth room at 21°C –23°C in daylight.

Protocol for banana shoots apex isolation
1. Young and healthy looking banana plant or sucker is obtained from the field.
2. Cut leaves and Pseudo stem about 30cm above soil level.
3. Remove all soil and cut off roots to expose the corm.
4. Peel off outer sheathing from leaf base of the Pseudo stem one at a time until they become too small, the leaf sheath may be removed carefully by hand.
5. Remove the last remaining leaves using a dissecting microscope and a scalpel.
6. Open excised shoot with a scalpel.
7. Sterilize in 1% commercial bleach (0.0525% NaOCL) and “Tween-20” for 5 minutes swirling occasionally.
8. Wash 4 times with sterilized distilled water.
9. Place 5ml culture medium.
10. Transfer to fresh medium after three weeks.

Plant regeneration
Within 21 days of inoculation of shoot apices into the shoot growth medium, the leaf primordia changed from white or creamy white to green.

Sub-culture
After 21 days, banana plantlets were longitudinally cut into two equal halves and transfer into fresh medium containing culture bottles. Later on younger leaf primordia grew out through the unrolled outer leaf, transfer the material in fresh medium every week for three times. Plantlet was then ready for weaning process.

Protocol for stimulation of multiple shoot formation
1. Begin with an aseptically cultured banana shoot or sucker 21 days after explantation.
2. Make a transverse cut to separate leaves; this will yield a section of Pseudo stem approximately 3cm long including an intact vegetative bulb.
3. Trim the lower part of explant to remove darkened or necrotic tissue.
4. Cut Pseudo stem explant longitudinally in two equal halves through the apex.
5. Transfer cut halves to culture medium in bottles or jars.
6. After 4–7 days, remove from culture bottles or jars. Trim off outer most leaves and blackened base of explant.
7. Transfer to fresh medium.
8. After 2-3 weeks, use a scalpel to cut the multiple shoots that have formed. Transfer shoots thus separated to fresh growth medium.
9. Maintain the multiple shoot cultures by transferring to fresh culture medium and separating the multiple shoots in the same way every 3-4 weeks.
Weaning process
The *invitro* regenerated plantlets obtained through shoot tip culture are ready to be transferred from the aseptic containers to pots; humidity is maintained at about 100%, 95% and 90% each for one week. After maintaining the humidity, plants produced *invitro* were shifted to the soil. Sterilizing the soil mixture eliminates serious infection problems.

Transplantation to the field
The plants, which survived after the weaning process, were transferred in the field which was already prepared for this purpose.

RESULTS AND DISCUSSION
The genetic improvement of banana by conventional breeding is hampered because of sterility and parthenocarpic development of fruit. For the renaissance of this crop, new strategies, including implementation of biotechnology may be a suitable approach to achieve the goal. Biotechnology techniques allow the manipulation of genetic material to import desirable traits with greater accuracy in a much shorter time than is possible with conventional breeding methods.

To establish the effect of 1/2 MS supplemented with different concentrations of IBA on number of leaves of banana plantlet after time intervals of 10, 20, 30 and 40 days; mean squares from ANOVA are presented in Table-1 demonstrating highly significant effect of different concentrations on number of leaves for all the four time intervals under study. The mean number of leaves/plantlet of banana *invitro* propagated at specific time intervals (10, 20, 30 and 40 days) are presented in the Table-2. It can be observed that number of leaves/plantlet (recorded after 10, 20, 30 and 40 days of rooting) different significantly among the various concentrations of IBA. However, after 10 days of rooting, maximum number of leaves were recorded in case of 0.30 mg of IBA (3.90/plantlet), followed by 0.4 mg of IBA (3.00/plantlet), while no application of IBA or application of 0.1 mg IBA gave equally poor number of leaves (1.25 and 2.00/plantlet). After 20 days of rooting, a similar trend of leaves/plantlet was observed, although the number of leaves increased when compared to 10 days of rooting. Maximum number of leaves was recorded under 0.3mg of IBA (4.52/plantlet), while lowest under controlled culture (1.96/plantlet). Results further indicated that after 30 days of rooting, all the concentrations of IBA enhanced number of leaves/plantlet when compared to 20 days of rooting. Comparatively, application of 0.3 mg IBA produced greater number of leaves (5.75/plantlets) closely followed by 0.4 (5.10 / plantlet) and 0.5mg/l (4.92/plantlet) respectively. After 40 days of rooting, all concentrations of IBA improved number of leaves/plantlet, as compared to 30 days of rooting. The best dose was found to be 0.3mg of IBA (7.80/plantlet), the next best was 0.4 and 0.5mg (6.92 and 6.50 /plantlet) respectively. On an average, the maximum number of leaves (5.49/plantlet) was recorded in case of 0.3mg of IBA. The results further showed that number of leaves/Plantlet recorded after 10, 20, 30 and 40 days of rooting affected significantly by the various levels of IBA. It was found (Table-2) that during each interval application of 0.3mg IBA proved superior in producing leaves/plantlet, while further increase in the concentration levels of IBA did not prove sustainable. Present results are in accordance with those of Molla *et al.* (2004) who found six different concentrations (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg/l) with half strength of MS medium as beneficial. Babylatha *et al.* (1997) also reported similar results as of ours and opined that the establishment of banana (cv. Basrai) shoot tips was best in Murashige and Skoog (MS) medium supplemented with 2.0 mg benzyladenine/litre and shoot proliferation was best in MS medium supplemented with 4.0 mg benzyladenine/litre. Our results also coincide with the results of Wargantiwar *et al.* (1997) who explored the possibility of micro propagation of banana through shoot tip culture and reported that maximum multiple shoot formation was given by Murashige & Skoog MS basal medium supplemented with 7mg BAP [benzyladenine]/ litre or 5mg BAP/ litre + 15% coconut milk. Rooting of the multiple shoots was best in MS medium + 2mg IBA/ litre + o.1% charcoal. The similarity of our results is also established with those of Dilip *et al.* (2000) who described a protocol for large-scale multiplication and *ex vitro* survival of banana var. 'Basrai' through tissue culture and reported that maximum 90% rooting were achieved in well-developed shoots on MS medium supplemented with Indolebutyric acid (1.0 mg/l).
Table-1: Mean squares from ANOVA for number of leaves of banana plantlet at specific time intervals under different concentrations of IBA

<table>
<thead>
<tr>
<th>Source</th>
<th>D. F.</th>
<th>10 days</th>
<th>20 days</th>
<th>30 days</th>
<th>40 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>3</td>
<td>0.003 NS</td>
<td>0.011 NS</td>
<td>0.002 NS</td>
<td>0.039 NS</td>
</tr>
<tr>
<td>Concentrations</td>
<td>7</td>
<td>2.348**</td>
<td>2.753**</td>
<td>3.195**</td>
<td>8.282**</td>
</tr>
<tr>
<td>Residual</td>
<td>21</td>
<td>0.004</td>
<td>0.004</td>
<td>0.003</td>
<td>0.052</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** Significant at P < 0.01
NS = Non-significant

Table-2: Number of leaves of banana plantlet after specific time interval under different concentrations of IBA.

<table>
<thead>
<tr>
<th>Concentrations: ½ MS + IBA (mg/l)</th>
<th>No. of Culture</th>
<th>Number of leaves of banana plantlet after</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10 days</td>
</tr>
<tr>
<td>0.0 (control)</td>
<td>10</td>
<td>1.25 e</td>
</tr>
<tr>
<td>0.1</td>
<td>10</td>
<td>2.00 e</td>
</tr>
<tr>
<td>0.2</td>
<td>10</td>
<td>2.38 d</td>
</tr>
<tr>
<td>0.3</td>
<td>10</td>
<td>3.90 a</td>
</tr>
<tr>
<td>0.4</td>
<td>10</td>
<td>3.00 b</td>
</tr>
<tr>
<td>0.5</td>
<td>10</td>
<td>2.75 c</td>
</tr>
<tr>
<td>0.6</td>
<td>10</td>
<td>2.45 d</td>
</tr>
<tr>
<td>0.7</td>
<td>10</td>
<td>2.40 d</td>
</tr>
</tbody>
</table>

S.E 0.0214 0.0335 0.0202 0.0803
L.S.D. at P< 0.05 0.0930 0.09300 0.08054 0.3353
L.S.D. at P< 0.01 0.1266 0.1266 0.10970 0.4565

Means followed by similar letters do not differ significantly according to DMR test
REFERENCES


