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Standardization of protocol for *in vitro* propagation of banana (*Musa sapientum*)

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Abstract

An experiment was conducted at the Plant Biotechnology Laboratory, Department of Horticulture, Patuakhali Science and Technology University during the period from August 2016 to April 2017 to standardize the protocol for *in vitro* propagation of banana. The experiment was laid out in completely randomized design with four replications. Three to four months aged field grown rhizome attached shoots were used as explants and cultured on MS medium with different concentrations and combinations of BAP (6-benzylamino purine), BAP + KIN (Kinetin) + NAA (Naphthalene Acetic Acid) and IBA (Indole-3- Butyric Acid) + IAA (Indole-3- Acetic Acid) to observe their efficacy on single shoot initiation, shoot multiplication and root formation respectively. Minimum number of days required for shoot initiation (9.07) with highest shoot initiation percentage (91.14) and the longest shoot (2.23 cm) was found in MS medium supplemented with 5.0 mg/L BAP. On the other hand, highest shoot multiplication percentage (80.99) with maximum number of shoots per explant (4.47), the highest length of shoots (4.17 cm) and maximum number of leaves (4.04)was observed in MS medium supplemented with 4.0 mg/L BAP + 2.0 mg/L KIN + 2.0 mg/L NAA. In case of root regeneration, the best results on days required for root initiation (9.00), the highest root initiation percentage (85.05), maximum number of roots per plantlet (5.83) and the highest length of roots (4.17 cm) was obtained in MS medium supplemented with1.5 mg/L IBA + 0.5 mg/L IAA. After 5-7 days of hardening in room temperature, established plantlets were ready for planting.

Introduction

Banana is a perennial herbaceous monocot which is originated from Malaysia through a complex hybridization process (Novak, 1992). It is now cultivated in over 100 countries under tropical and sub-tropical zones. Banana supports livelihood of million people with total production of 774,000 metric tonnes from 50,000 hectares during 2015–16 (Anonymous, 2016). Banana is highly nutritious fruit which provides energy (104 cal./100g) in human body (Simmonds, 1996). Banana is also a good fiber yielding plant and its corm is mostly exploited as animal feed (Uma *et al.*, 2005). In Bangladesh, average yield of banana is 14.16 t/ha (BBS, 2015). Banana is generally propagated through sucker but it is laborious, time consuming, only 5 to 10 uniform size obtained from a plant per year (Bohra *et al.*, 2013). Every year in our country, a large number of banana fields are destroyed due to natural hazards like flood, heavy rainfall, storm etc. Furthermore, the productivity of vegetatively propagated banana is greatly reduced by virus disease (Lepoivre, 2000). High yielding varieties are unavailable and the traditional clonal propagation method appears to be unable to supply the increasing demand for disease free and healthy planting materials of banana and it is also season dependent (Hanumantharaya *et al.*, 2009).

To overcome this problem, tissue culture or *in vitro* propagation offers an alternative over traditional propagation methods facilitating, large scale production of disease-free planting materials all year round keeping, physiological uniformity of the plants from using only a few explants (Abeyaratne and Lathiff, 2002; Waman *et al.*, 2014). Meristem culture offers an efficient method for rapid clonal propagation with production of virus free materials (Helliot *et al.*, 2002). Moreover, the shoot multiplication cycle is very short (2–6 weeks) and plants multiplication can be continued throughout the year irrespective of the season (Razdan, 1993).

The apical meristem or shoot-tip culture is very efficient for rapid clonal micropopagation in banana (Lalrinsanga *et al.*, 2013). Tissue culture technique of banana produce 39% higher yield than conventional sword suckers (Farahani*et al.*, 2008). Tissue culture protocol with ascertained field performance of *in vitro* has been developed for banana plantlets (Jalil *et al.*, 2006; Resmi and Nair, 2007). Therefore, the present study was undertaken to evaluate the propagation rate of *M. sapientum* under the influence of PGRs as well as large scale production of banana.

Materials and Methods

Young, healthy, disease free, 3–4 old months amritasagar plants were collected from Germplasm Center, Department of Horticulture, PSTU. The rhizome attached shoot of amritasagar was used as plant material in this research work. Outer leaves, roots, dust, other

debris and outer tissues were removed until the explant measured about 2.0–3.0 cm in length and 2.0 cm width with the help of sharp knife. Then the explants washed with distilled water until complete removal of all traces. Surface sterilization of explant was done under laminar airflow cabinet with a few drops of savlon, tween 20, 0.1% mercuric chloride and 70% ethyl alcohol respectively. Then the explants rinsed with sterile distilled water three to four times.

All the plant growth regulators, stock solutions were prepared before preparing the culture media. The media were adjusted to pH 5.8 with 1 N NaOH or 1 N HCl and autoclaved at 121°C for 45 minutes at 15 psi pressure. The isolated and surface sterilized explants were directly inoculated individually to each of the culture tube containing 25 ml of MS medium supplemented with different concentration and combinations of auxins (IAA, IBA, NAA) and cytokinins (BAP and KIN) to conduct three experiments. Five different concentration of BAP (1.0, 3.0, 5.0, 7.0, 9.0) mg/L was used for single shoot regeneration. After raising several growing points, the *in vitro* grown shoot-tips were cultured on MS medium supplemented with BAP (1.0, 2.0, 3.0, 4.0, 5.0) mg/L, KIN (2.0 mg/L) and NAA (2.0 mg/L) for shoot multiplication. When the regenerated shoots became 2–3 cm in length with 3–6 well developed leaves, they were rescued aseptically from the culture tubes. After separating from each other, they were again cultured on freshly prepared medium containing different concentrations and combinations of IBA (1.0, 1.5) mg/L and IAA (0.5, 1.0, 1.5) mg/L for root initiation.

The cultures were incubated at 25±2°C by an air conditioner with light intensity varied from 2000-3000 lux under 16 hours light and 8 hours dark. When the regenerated plantlets produced 3–5 cm roots with vigorous shoot, they were transferred in soil. Plantlets were planted in poly bags containing garden soil + compost + sand (1:1:1). The experiments were arranged in completely randomized design (CRD) with 4 replications. Each treatment consisted of 10 culture tubes per replication. Data were collected on the effect of different treatments on shoot proliferation and rooting. The significance among the means of treatments was evaluated at 5% level of significance by Duncan's Multiple Range Test (DMRT, Gomez and Gomez, 1984)by using MSTAT-C statistical package (Steel *et al.* 1997).

Results and Discussion

The research activities of *in vitro* propagation of amritasagar banana *(Musa sapientum)* using different growth regulators were developed under three experiments that were done separately.

In vitro shoot formation

In vitro shoot formation of *Musa sapientum* under the influence of different concentrations of BAP was investigated and data were presented in Table 1.

Table 1. Effect of different concentrations BAP on *in vitro* shoot formation of banana plantlets

Treatments BAP (mg/L)	Days required for single shoot initiation	Percentage of shoot initiation	Length of single shoot (cm)
1.0	18.07 a	63.01 e	1.19 e
3.0	15.94 b	75.01 c	1.35 d
5.0	9.07 e	91.14 a	2.23 a
7.0	11.03 d	81.90 b	1.98 b
9.0	13.11 c	69.81 d	1.66 c
Level of sig.	*	*	*
$LSD_{0.05}$	0.76	0.76	0.13
CV (%)	3.73	0.66	5.38

In a column, difference letter(s) indicates a significant difference at 5% level as per DMRT

Explants when cultured on 5.0 mg/L BAP required minimum days for shoot initiation (9.07) and gave the highest shoot regeneration percentage (91.14) with the longest length of shoot per explant (2.23 cm) which was superior to other treatments and 1.0 mg/L BAP show lowest results (Table 1). It may be considered that 5.0 mg/L BAP accelerate cell division and elongation than other treatments. Ferdous *et al.* (2015) obtained the longest shoot (2.64 cm) from 0.5 mg/L BAP. Sholi *et al.* (2009) suggested that *Musa* cultivars require different levels of plant growth regulators and BAP is more effective in shoot generation. In another experiment, Bhuiyan (2007) observed shoot initiation after 7 days with 91.23% shoot regeneration in MS medium supplemented with 5.0 mg/L BAP.

In vitro shoot multiplication

In vitro grown shoot-lets were used for shoot multiplication.

From the above result, it is evident that 4.0 mg/L BAP+ 2.0 mg/L KIN + 2.0 mg/L NAA gave the highest shoot multiplication percentage (80.99) with the highest number of shoots per plantlets (4.48) where 1.0 mg/L BAP + 2.0 mg/L KIN + 2.0 mg/L NAA shows the lowest results among the treatments (Table 2). Lalringanga *et al.* (2013) got 100% response in MS + 1.0 mg/L NAA + 0.2 mg/L BAP. Earlier studied have shown that the use of 5.0 mg/L BAP + 5.0 mg/L KIN was found sufficient for *in vitro* highest number of shoots (3.11 ± 0.66) production at 28 DAI (Rabbani, 1996).

Varying response was observed in all the media for shoot length and number of leaves. The longest micro shoots (4.17 cm) was observed in the media 4.0 mg/L BAP+2.0 mg/L KIN+ 2.0 mg/L NAA. The same treatments also produced maximum number of leaves (4.04) than other treatments (Table 2). In another experiment, Al-Amin *et al.* (2009) obtained maximum number of leaves (7.0 leaves) in MS+ 7.5 mg/L BAP + 0.5 mg/L NAA.

Table 2. Effect of different concentrations and combinations of BAP, KIN and NAA on *in vitro* shoot multiplication of banana plantlets

Treatments (mg/L)			% of shoot multipli-cation	No. of shoot multipli-cation	Length of shoot (cm)	Number of leaves
BAP	KIN	NAA				
1.0	2.0	2.0	61.94 e	3.49e	2.82 d	2.85 e
2.0	2.0	2.0	66.05 d	3.75d	3.17 c	3.15 d
3.0	2.0	2.0	70.98 c	4.02c	3.84 b	3.72 d
4.0	2.0	2.0	80.99 a	4.48a	4.17a	4.04 a
5.0	2.0	2.0	75.12 b	4.29b	3.61 b	3.45 c
Level of sig.			*	*	*	*
$LSD_{0.05}$			0.83	0.13	0.31	0.14
CV (%)			0.78	2.01	5.74	2.81

In a column, difference letter(s) indicates a significant difference at 5% level as per DMRT

***In vitro* root regeneration**

For induction of healthy root system from the regenerated shoots is an essential part of successful development of plantlets.

Table 3. Effect of different concentration and combinations of IBA and IAA on *in vitro* root initiation of banana plantlets

Treatments (mg/L)		Days required to root initiation	Percentage of root initiation	Number of roots	Root length (cm)
IBA	IAA				
1.0	0.5	21.37 a	68.98 f	3.85f	2.91 e
1.0	1.0	19.11 b	72.91 e	4.13e	3.19 d
1.0	1.5	13.09 d	79.04 c	4.56d	3.80 b
1.5	0.5	9.04 f	85.05 a	5.83 a	4.17 a
1.5	1.0	11.46 e	81.94 b	4.92c	3.69 b
1.5	1.5	16.98 c	76.01 d	5.32b	3.40c
Level of sig.		*	*	*	*
$LSD_{0.05}$		0.77	0.12	0.14	0.12
CV (%)		3.42	2.36	2.02	2.63

In a column, difference letter(s) indicates a significant difference at 5% level as per DMRT

From the Table 3, it is observed that, minimum days (9.04) for root initiation was observed from medium containing 1.5 mg/L IBA+0.5 mg/L IAA with the highest regeneration percentage (85.05). These treatment may be optimum for banana plantlets. In another experiment, Rahman *et al.* (2013) observed that IBA at a concentration of 1.0 mg/L showed lowest days for root initiation with highest response (96%) in rooting. Maximum number (5.83) of roots with longest root length (4.17 cm) was obtained from 1.5 mg/L IBA + 0.5 mg/L IAA where1.0 mg/L IBA + 0.5 mg/L IAA gave the lowest result (Table 3). These results was supported by the findings of Al-amin *et al.* (2009) who obtained 6.50 number of root with the longest 5.88 cm root at 0.5 mg/L IBA+ 0.5 mg/L IAA.

When the plantlets produced 3–5 cm length roots with vigorous shoot, they were kept in room temperature for hardening. After 5–7 days hardening in room temperature (28–30°C), the successfully survived plantlets were then transferred in the field under natural condition. In fields, plantlets were planted in poly bags containing garden soil + compost + sand (1:1:1) mixtures. Uzaribara *et al.* (2015) also used the similar media for acclimatization of tissue cultured banana plantlets.

Fig. 1. Shoot initiation from rhizome attached shoot of banana on MS medium supplemented with 5.0 mg/LBAP

Fig. 2. Shoot multiplication of banana MS medium supplemented with 4.0 mg/L BAP+ 2.0 mg/L KIN + 2.0 mg/L NAA

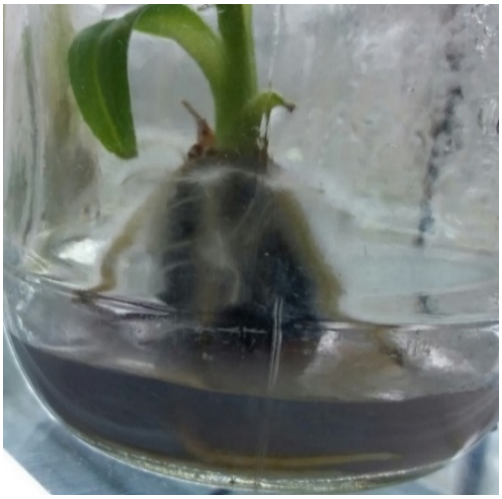

Fig. 3. Root initiation on of banana MS medium supplemented with 1.5 mg/L IBA+0.5 mg/L IAA

Fig. 4. Well established amritasagar plantlet from rhizome attached shoot in polybag

Conclusion

Tissue culture technologies are applicable for the mass propagation of Amritasagar to get true to type plantlets. Amritasagar shows better response in *in vitro* condition with plant growth regulators. Further comprehensive study is needed to test the performance of tissue culture derived plantlets.

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