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Rapid and Efficient *In vitro* Propagation Protocol of Banana (*Musa paradisiaca*) Using Sucker Explants

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Abstract

Micropropagation of fruit crops is essential for producing disease free plants, ensuring rapid multiplication, and preserving the genetic integrity of the original plant materials. *Musa paradisiaca*, a widely cultivated fruit crop in Bangladesh is commonly propagated on a large-scale using sucker explants. An efficient *in vitro* micropropagation protocol was developed using healthy suckers of *Musa paradisiaca* cultured on MS medium supplemented with 4.0 mg/l BAP, 0.5 mg/l IAA and 20 mg/l adenine sulphate, this combination resulted in the formation of highly proliferative multiple shoots. For shoot elongation, MS medium containing 4.0 mg/l BAP, 0.5 mg/l IAA, and 13% coconut water produced the greatest average shoot length of 8.4 cm. Rooting was most successful on half-strength MS medium supplemented with 1.0 mg/l IBA. The regenerated plantlets were successfully acclimatized and established in field conditions, achieving a 100% survival rate. These findings demonstrate an optimized protocol for the large-scale production of genetically uniform and healthy banana plants through *in vitro* culture.

Introduction

Banana (*Musa* sp.) belongs to the Musaceae family. It is consumed as one of the most important nutritious fruits in the world (Padam et al. 2014). At present, global banana production increased from 115 million tons in 2017-2019 to 135 million tons in 2022-2023 (FAO 2025). In Bangladesh banana is one of the highly cultivated and consumed food. It ranks second in terms of cultivation among the fruits comprising of 42% of the total fruits production and its financial return is higher compared to other fruits and field crops Haque, M. A. (1988). Although, the production of banana fluctuated noticeably in recent years, in 2017, its production in Bangladesh was about 807104 tonnes (Knoema 2017). Banana not only contributes directly to food security but also as a source of income to farmers. An increase in mass production of banana can play an important role in the cultivation in Bangladesh. It has been reported that the average yield of banana is 2.64%

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when compared with the neighboring country, India (Knoema 2017). Therefore, there is an urgent need for developing the production of banana in Bangladesh.

Banana is usually propagated by vegetative means by using suckers grown from lateral buds of corms for the production of individual plants. This conventional vegetative process is monotonous and time consuming. Moreover, several negative impacts, including poor preservation of original plant genetic material, transmission of diseases and low production is observed (Hussein 2012). Therefore, *in vitro* propagation of banana can lead to the development of *in vitro* mass production of different cultivars (Kodym and Zapata 1999, Nandwani et al. 2000, Ali et al. 2011). It not only offers mass propagation (sucker propagation) of banana but also increases production with respect to optimal uniformity, yield and disease free plant type. Also, mass multiplication of tissue culture plants could be done in a short period of time and transportation of planting material is cheaper. In addition, bananas produced using the micropropagation technology are reported to be more vigorous, high yielding and produce better quality fruits than those produced by conventional means (Hwan et al. 1976). Based on this, the main objective of the study is to identify the most effective plant growth regulators for shoot proliferation and multiplication of Musa cv. Paradisiaca, determine the optimal hormonal conditions for root induction, and develop a micropropagation system suitable for large scale production and commercial supply of banana plants.

Materials and Methods

Fresh banana suckers of *Musa paradisiaca* were collected from the field of the Plant Biotechnology Division, National Institute of Biotechnology, Savar, Dhaka. Explant preparation, sterilization, and culture were carried out following previously established protocols (Nasrin et al. 2010, Sen et al. 2013, 2014, Nasrin et al. 2014, Yesmin et al. 2018). Generally, suckers (3-5 cm) were excised from banana plants and washed under running tap water for 15 min. Shoot tips were prepared by trimming the corm and outer sheaths, rinsed under tap water, and treated with detergent, Tween-20 (Sigma, USA), and distilled water. The tips were divided into four pieces and surface sterilized with 0.1% (w/v) $HgCl_2$ (Sigma, USA) for 5, 10 or 15 min and then rinsed by three times with sterile distilled water.

Explants were cultured on MS medium (Sigma USA) supplemented with BAP (2, 4 or 5 mg/l) and IAA (0.5, 1 or 1.5 mg/l (Duchefa, Netherlands). For enhanced shoot proliferation, the optimal BAP-IAA combination was further tested with adenine sulphate (10-40 mg/l) or 13% coconut water (freshly collected from the NIB garden). The medium pH was adjusted to 5.8 before autoclaving at 121°C for 20 min (1.16 kg/cm^2). Cultures were maintained at 23 ± 2°C under a 16h photoperiod (approximately 2000 lux, cool white fluorescent light) and sub-cultured every 4 weeks, beginning with proliferation medium followed by elongation media.

Regenerated plantlets were transferred to half strength MS medium supplemented with IBA or IAA (0.2, 0.5 or 1 mg/l) for root induction. Once the rooting system was

sufficiently developed, it was transferred into the soil pots for hardening. Rooted plantlets were carefully removed from culture vessels and hardened in pots containing soil sand: compost (1: 1) as described by Sen et al. (2013) and Nasrin et al. (2014). Pots were covered with polythene bags to prevent desiccation, and humidity was maintained by spraying water inside the bags every 24 hrs. The plantlets were gradually exposed to the external environment by perforating the polythene bags, which were fully removed after seven days. Once the plantlets got optimized with the external environment, they were finally transferred to the field. Fertilizers were applied as needed until fruiting.

Results and Discussion

Micropropagation enables the regeneration of new plantlets from different cells or tissues when cultured on suitable media (Vasil et al. 1982, Nasrin et al. 2010, Sen et al. 2013a 2014, Nasrin et al. 2014, Yesmin et al. 2015, 2018). Unsuccessful plantlet development is often associated with improper sterilization, suboptimal culture media, or limited technical expertise (Johri 1982). In banana, micropropagation is commonly achieved using actively growing stem segments (micro-cuttings) under varying concentrations of cytokinins and auxins (Vuylsteke and Ortiz 1996, Wojtania and Gabryszewska 2001). In the present study, sucker explants were used for the induction and proliferation of multiple shoots from shoot tips followed by rooting and plantlet regeneration.

Sterilization of explants is a prerequisite for *in vitro* propagation, as plant tissues are often contaminated with fungi and other microbes (Sen et al. 2013a, b). To ensure aseptic culture, suckers were treated with 0.1% w/v) $HgCl_2$ for 5, 10 or 15 min. Contamination was lowest when suckers were treated for 15 min (Table 1). This result is consistent with earlier reports supporting the use of $HgCl_2$ for surface sterilization (Ramakrishna et al. 1991, Sen et al. 2013). The sterilized, contamination free explants were subsequently inoculated into tissue culture flasks (Fig. 1a).

Table 1. Sterilization of explants of *Musa paradisiaca* using 0.1% (w/v) $HgCl_2$ at different time period.

Chemicals used	Time period (min.)	Contamination (%)
$HgCl_2$	5	67.20
	10	24.55
	15	0

Different concentrations of cytokinin and auxin were tested with MS medium to optimize shoot proliferation, elongation, and root induction, following earlier reports (Sen et al. 2014, Nasrin et al. 2014). For Musa cv. Paradisiaca, MS medium was supplemented with BAP (2.0, 4.0 or 5.0 mg/L) in combination with IAA (0.5, 1.0 or 1.5 mg/l). Shoot proliferation efficiency under these treatments ranged from 18 to 83%. The highest proliferation rate (83%) was achieved with 4.0 mg/l BAP and 0.5 mg/l IAA, while 4.0 mg/l BAP alone resulted in 80% proliferation. In contrast, the lowest proliferation rate (18%) was observed with 2.0 mg/l BAP and 0.5 mg/l IAA (Table 2, Fig. 1b, c).

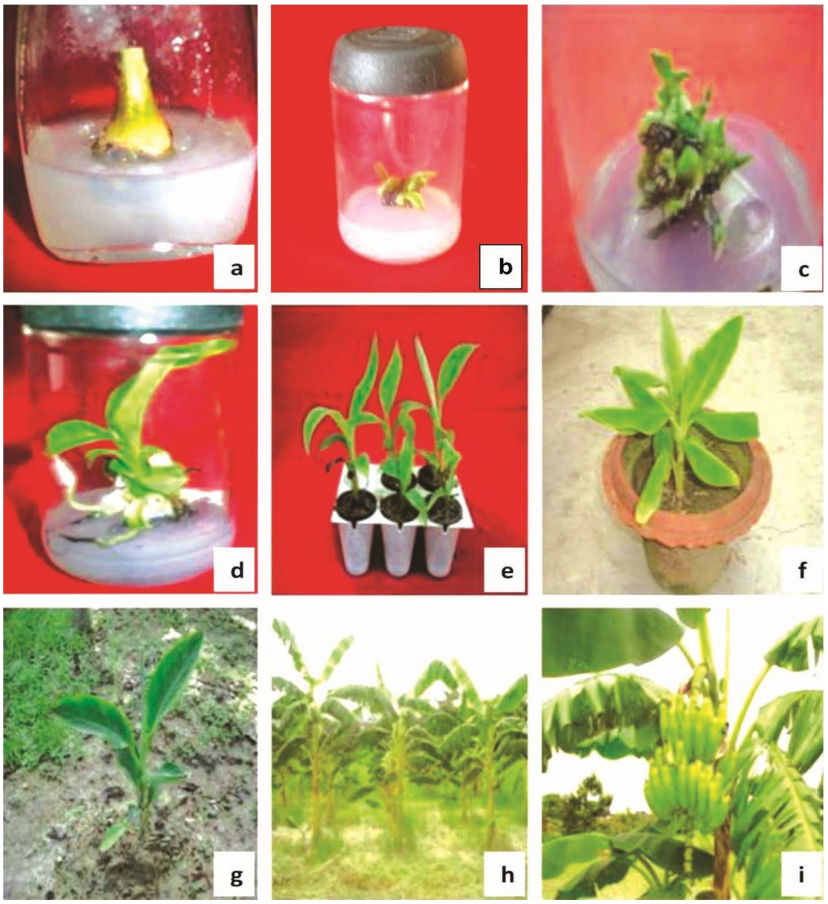

Fig. 1. Micropropagation of *Musa paradisiaca*: (a) inoculation of sucker explant, (b) initiation of the shoots, (c) multiplication of the shoot tips, (d) development of roots from the shoots, (e) plantlets transferred to the pots, (f) plantlets transferred to the earthen pot, (g) plants transferred to the field, (h) mass production of Banana plants, and (i) plant with fruits.

Previous studies have reported various hormonal combinations for banana regeneration from sucker explants, including MS medium with 0.5 mg/l BAP + 0.05 mg/l NAA + 10% (v/v) CW (Akbar et al. 2003) and MS medium with 4.0 mg/l BAP + 2.0 mg/l NAA + 13% CW (Habiba and Reza 2002). Regeneration has also been achieved from excised floral apices using MS medium supplemented with 2.0 mg/l BAP, 1.0 mg/l Kn, 1.0 mg/l IAA, and 15% CW (Azad and Amin 2001). In the present study, supplementation of adenine sulphate to BAP and IAA markedly enhanced shoot proliferation, reaching approximately 90%. MS medium containing 4.0 mg/l BAP, IAA (0.5, 1.0 or 1.5 mg/l) and adenine sulphate (10-40 mg/l) resulted in proliferation rates ranging from 67 to 96%. The highest proliferation (96%) and maximum shoot length (2.4 cm) were obtained with 4.0 mg/l BAP, 0.5 mg/l IAA, and 20 mg/l adenine sulphate, whereas other combinations produced shoots averaging <2.0 cm in length (Table 3, Fig. 1c).

Table 2. Effects of different concentrations of BAP and auxin on shoot proliferation in *Musa paradisiaca*.

Hormones Conc. (mg/l)		Shoot proliferation (%)	No. of explants	No. of Shoot (explants)	Shoot length (cm)
BAP	IAA				
2	0	24	25	1.4	1.2
	0.5	18	25	2.1	1.1
	1.0	22	25	1.3	1.3
	1.5	19	25	1.7	1.4
4	0	80	25	4.6	1.4
	0.5	83	25	5.0	2.3
	1.0	79	25	4.7	2.0
	1.5	69	25	4.2	1.9
5	0	46	25	3.7	1.7
	0.5	61	25	3.1	1.6
	1.0	55	25	3.9	1.4
	1.5	52	25	3.2	1.7

Table 3. Effects of different concentrations of BAP, auxin and adenine sulphate for shoot proliferation in *Musa paradisiaca* after 3 weeks.

Hormones Conc. (mg/l)		Shoot proliferation (%)	No. of explants	No. of Shoot (explants)	Shoot length (cm)
BAP + IAA (mg/l)	Adenine sulphate (mg/l)				
4 + 0.5	10	86	25	5.9	1.3
	20	96	25	6.2	2.4
	30	84	25	6.0	2.3
	40	83	25	5.9	2.2
4 + 1.0	10	69	25	5.8	2.0
	20	67	25	5.7	2.1
	30	70	25	5.9	1.9
	40	67	25	5.4	2.2
4 + 1.5	10	74	25	5.6	1.9
	20	76	25	5.5	2.0
	30	69	25	5.7	2.1
	40	73	25	5.4	2.0

Proliferated shoot tips from banana suckers were transferred to elongation media consisting of MS medium supplemented with 4.0 mg/l BAP, varying concentrations of IAA (0.5, 1.0 and 1.5 mg/l) and 13% coconut water. Shoot length was recorded after 6 weeks and ranged from 7.2 to 8.4 cm. The maximum elongation (8.4 cm) was achieved with 4.0 mg/l BAP, 0.5 mg/l IAA and 13% coconut water whereas the minimum (7.2 cm) occurred with 4.0 mg/l BAP alone (Table 4, Fig. 1c).

Table 4. Effects of different concentrations of BAP and auxin along with 13% coconut water for shoot elongation in *Musa paradisiaca* after 6 weeks.

Hormones Conc. (mg/l)		No. of Proliferated shoots	Shoot Length (cm)
BAP	IAA		
4	0	20	7.4
	0.5	20	8.4
	1.0	20	7.6
	1.5	20	7.5
4	0	20	7.2
	0.5	20	7.6
	1.0	20	7.5
	1.5	20	7.7
4	0	20	7.8
	0.5	20	7.9
	1.0	20	7.4
	1.5	20	7.8

Various hormonal treatments were applied to half strength MS medium to optimize root induction. Previous studies reported that NAA (0.2 mg/l) induced rooting in Eucalyptus citriodora (Gupta et al. 1981) whereas IBA was most effective in grape (Chakravorty et al. 1986. Chesick et al. (1991) reported that *Pinus strobus* explants treated with 50 μM IBA for 8 days, followed by culture on half-strength MS medium with 3% sucrose, achieved a 50% rooting rate after 3 months. In banana, half-strength MS with 1.0 mg/l IBA has been used for root induction (De Langhe 1985), though auxin free MS medium can also support rooting (Cronauer and Krikorian 1984).

Table 5. Effects of different concentrations of IBA and IAA of rooting in *Musa paradisiaca* after 2 weeks.

Growth regulators (GR)	Conc. of GR (mg/l)	% of shoots forming roots	No. of roots	Root length
IBA	0.2	86	7.4	3.4
	0.5	84	8.4	3.1
	1.0	96	8.6	3.8
IAA	0.2	84	7.6	2.9
	0.5	89	7.5	3.0
	1.0	86	7.7	3.5

In this study, regenerated shoots of Musa cv. Paradisiaca were transferred to half strength MS media supplemented with IBA or IAA (0.2, 0.5 and 1 mg/l). Rooting exceeded 85% under all treatments. The highest rooting efficiency and root length (3.8 cm) were observed with 1.0 mg/l IBA, which also produced the greatest average number of roots per explant. The shortest roots (2.9 cm) were observed with 0.5 mg/l IAA (Table 5, Fig. 1d). Overall 1.0 mg/l of IBA was ideal for the development of root in banana cv. Paradisiaca.

Rooted plantlets were washed and transplanted into pots covered with polythene bags for acclimatization under ambient light and temperature (Fig. 1e). Gradual exposure to outdoor conditions resulted in 100% survival (Fig. 1f-h), and their growth under these conditions was satisfactory, which is similar a finding consistent with Sharma and Thorpe, 1990. All the transferred plants to the field successfully produced banana fruits in large quantities (Fig. 1i).

Micropropagation provides a reliable and efficient method for the *in vitro* production of disease free plantlets on a large scale. In this study, a complete protocol has been optimized for Musa cv. Paradisiaca, covering all stages from shoot initiation to field transfer. The recommended media compositions are: (1) MS medium with 4.0 mg/l BAP, 0.5 mg/l IAA and 20 mg/l adenine sulphate for direct shoot proliferation from shoot tips, (2) MS medium with 4.0 mg/l BAP, 0.5 mg/l IAA and 13% coconut milk for effective shoot elongation, and (3) half-strength MS medium supplemented with 1.0 mg/l IBA for successful root induction. This protocol enables the rapid and large-scale production of healthy, genetically uniform, and disease free banana plantlets, suitable for commercial cultivation and market distribution within a short period.

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