



Effect of Banana Extract on Growth and Development of Protocorm Like Bodies in *Dendrobium* sp. orchid

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Abstract

An experiment was carried out in Plant Tissue Culture Laboratory, Department of Crop Botany, Bangladesh Agricultural University, Mymensingh to investigate the effect of banana extract on micropropagation of *Dendrobium* sp. var. Sonia orchid through PLBs. The experiment was conducted during July 2012 to October 2013. Half-Murashige and Skoog (1/2MS) medium were used as basal medium and the medium was supplemented with banana extract at 12.5, 25, 50, 100, and 200 ml L⁻¹ with a control, where no banana extract was supplemented. The cultures were done in 100 ml conical flasks and maintain at 25°C with 30μ mol m⁻² S⁻¹ lighting provided by florescent tubes for 16 hours per day. Banana extract showed significant effect on growth and development of PLBs. Among the treatments, 100 ml L⁻¹ banana extract enhanced new PLBs regeneration from explanted PLBs and growth and development of PLBs. Present research indicated that nutrient requirement for PLBs multiplication and plantlets growth of *Dendrobium* orchid is quantitatively different *in vitro*. Finally, 100 ml L⁻¹ and 25 ml L⁻¹ of banana extract may be recommended as supplement into 1/2MS medium for PLBs multiplication and plantlet regeneration, respectively *in vitro*.

Keywords: Banana extract, dendrobium orchid, protocorm like bodies, regeneration

1. Introduction

Orchids, the most fascinating and beautiful flower producing plant species belong to the family Orchidaceae. *Dendrobium* is one of the important orchid species which has long been grown in tropical countries like Thailand, Singapore etc. In many countries orchid industry plays an important role as a source of foreign exchange. Thailand alone exports orchids worth more than 11.4 million US dollar to other countries. The global export value of flower in 1985 was \$1.79 billion but the value at present is more than \$ 25 billion. The environmental

condition required for the survival and culture of orchids are adequately available throughout the year in Bangladesh. Therefore, it has a great scope of large scale production of dendrobium orchids to meet the internal demands as well as to earn foreign currency by export. Generally, orchids are propagated through both vegetative and sexual means but the conventional processes are slow and uncertain. Most of the cultivated orchids are found to be self-sterile. The traditional asexual orchid production is extremely slow which can give rise to 2-4 plants per year (Nasiruddin *et al.*, 2003). Asexually, through embryo culture, thousands of plants can

be propagated within short time in orchid. For mass propagation of orchid in commercial exploitation, millions of plantlets are produced by tissue culture techniques using different plant parts as explants. In tissue culture medium, different growth regulators and some organic compounds (coconut water, tomato and orange juice etc) are frequently used which have regulatory effects on growth and development (Islam and Ichihashi, 1999).

Studies on PLB derived plantlet, production through embryogenesis and the subsequent growth and development of plantlets on medium supplemented with different organic extracts in *Dendrobium* sp. orchid are scarcely available. A few reports on use of complex organic additives in medium for the growth of PLBs and plantlets regeneration of *Phalaenopsis* sp. is reported by Islam and Ichihashi (1999). Therefore, the present experiment was undertaken to investigate the effect of BE as supplement into culture medium on regeneration of new PLB, growth of PLBs and PLB derived plantlets of *Dendrobium* sp. (cv. Sonia) in vitro. The ultimate goal is to enhance growth and development of PLB derived plantlets of *Dendrobium* sp. orchid to support commercial production. Determination of suitable concentration of extract as supplement into medium and indirectly reduction of cost of the medium were also the objectives of this experiment.

2. Materials and Methods

2.1. Plant materials

Protocorm like bodies (PLBs) of *Dendrobium* sp. var. Sonia developed earlier from meristem culture and has been maintained by subculturing bimonthly on fresh medium in the Plant Tissue Culture Laboratory, Department of Crop Botany, Bangladesh Agricultural University (BAU), Mymensingh, were used as plant materials (Figure 1). This experiment was conducted during July 2012 to October 2013 in the same laboratory.

2.2. Treatments

Several cultures of PLBs and PLBs derived plantlets were conducted in the experiment. To study the effect on growth and development of PLBs and plantlets 0, 25, 50, 100 and 200 ml L⁻¹ of banana extract were supplemented into culture medium. The experiment was laid out in a Completely Randomized Design (CRD) and each treatment was replicated five times.

2.3. Culture medium

Murashige and Skoog (MS, 1962) medium was diluted to half strength (only macro elements) and was used as basal medium for PLB proliferation, plantlet initiation and the subsequent growth and development of initiated plantlets. Five stock solutions namely, macronutrients, micronutrients, Fe-EDTA, vitamins/organics and plant growth regulators were prepared prior to culture medium preparation.

2.4. Preparation of stock solution of nutrients

Stock solution of macro-nutrients was prepared 5 times higher than the concentration of medium used in experiment. Five times higher quantity of salt ingredients were taken in 1.0 L conical flask and dissolved in distilled water, serially by using a magnetic stirrer. Finally, distilled water was added up to the mark. Prepared solution was preserved in a refrigerator at 4°C for later use. Similarly, stock solution of micro nutrients (100 times), Fe-EDTA (100 times) and vitamins/organics (100 times) were prepared and stored.



Figure 1. PLBs of *Dendrobium* sp. var. Sonia. (Scale bar = 1.0 cm)

2.5. Preparation of banana extract

The ripe banana (cv. Sagar) was peeled and cut into 1.0 cm in size and 500 g of the diced fresh material was boiled for 30 minutes with 500 ml of distilled water and the hot supernatant was filtered through steel mesh (55 mm^{-1}) and adjusted up to 500 ml by adding distilled water.

2.6. Preparation of medium

To prepare 1 L of culture medium sucrose of 20.0 g, macro-nutrients 100 ml, micro-nutrients 10 ml, Fe-EDTA 10 ml and vitamins 10 ml were mixed in a conical flask with 500 ml distilled water. Myoinocitol 100 mg, dissolved in 100 ml distilled water was added to mixture. Different concentration of supplements as mentioned in Tables were added to the solution and mixed thoroughly. The mixture was then made near to 1000 ml by adding distilled water. The pH of the medium was adjusted to 5.8 by adding of 0.1 N NaOH or 0.1 HCL and made up the volume to 1 L. After adjusting the pH, 3.0 g gelrite was added and dissolved by heating in microwave oven. After dispensing the medium the conical were covered with aluminum foils and autoclaved at 121°C for 20 minutes under 1.15 kg cm^{-2} of pressure. After cooling at room temperature, the medium was used for culture.

2.7. Culture of PLBs and plantlets

The PLBs (100 mg) of similar size were cultured on 30 ml fresh medium supplemented with different concentration of banana extracts following treatments. After 90 days, culture data on new PLBs regeneration and their growth and development were recorded. PLB-derived plantlets were again cultured on same fresh medium supplemented with 1 g L^{-1} of charcoal and after 3 months data were collected. The culture flasks were kept in a growth room and allowed to grow at 25±1°C under 16 hours photoperiod illuminated with fluorescent tube of 30 $\mu \text{mole m}^{-2} \text{S}^{-1}$. For acclimatization and transplanting in pot, the plantlets were cultured for more than 3.0 months in same fresh medium.

2.8. Data collection and analysis

The data on average number of newly regenerated PLBs per flask, number of PLBs, weight per PLB, number of plantlet initiation, weight of plantlets, number of leaf and root per plantlets etc. were recorded after culture. The means were calculated and analyses of variance for all the characters were performed by F test. The significance of the difference among the pair of means was evaluated by Duncan's Multiple Range Test (DMRT) at 5% level of significance using MSTAT-C computer programmed.

3. Results and Discussion

3.1. Effects of banana extract (BE) on growth and development of PLBs

In culture, PLBs regenerated new PLBs and initiated PLB-derived plantlets both in control (free of BE) and banana extract supplemented medium. In control, both PLBs regeneration and plantlet initiation were lower in comparison with that in banana extract supplemented medium. The number of initiated new PLBs was only 86.6 per culture in control condition, where as the numbers were 206.6, 228.6, 554.3 and 514.3, respectively at 25, 50, 100, and 200 mL^{-1} of banana extract supplemented medium (Table 1). Average weight per PLB was significantly higher in control compared to that derived on 100 and 200 mL^{-1} banana extract supplemented medium.

The total weights of initiated new PLBs were higher with gradual increase of banana extract supplement up to 100 ml L^{-1} into the medium and then decreased. The number of PLB-derived shoots initiated in banana extract supplemented medium was significantly higher over control. Average PLB-derived shoots in 0.0, 25, 50, 100 and 200 mL^{-1} of banana extract supplemented medium were 41.6, 56.6, 63.3, 144.0 and 136.0, respectively. The numbers of shoots in banana extract supplemented medium were significantly higher over control. The highest number of shoot

was obtained in medium supplemented with 100 ml L⁻¹ of banana extract. Therefore, 100 ml L⁻¹ banana extract as supplement in to medium might be recommend for highest shoot regeneration from PLB in *Dendrobium orchid* var. Sonia (Table 1 & Figures 2, 3) *in vitro*.

3.2. Effects of banana extract on growth and development of shoots

Growth of shoot *in vitro* was significantly influenced by banana extract (Table 2, Figures 3, 4, 5). Different concentration of banana extract enhanced the length of shoots differently. The

tallest shoots was 2.8 cm found in medium supplemented with 25 mL⁻¹ of banana extract into BM, followed by 1.93 cm at 50 ml L⁻¹ and the shortest shoot was 1.03 cm found at 200 ml L⁻¹. Number of leaf per plantlet was also the highest at 25 mL⁻¹ of banana extract supplemented into basal medium and followed by control. Similarly, length of leaf, width of leaf, fresh and dry weight of shoots were enhanced significantly over control at 25 mL⁻¹ of banana extract into BM where the higher concentration of banana extract inhibited the growth of leaves (Table 2).

Table 1. Effects of BE on growth and development of PLBs after 90 days culture

Concent. of BE (ml L ⁻¹) supplemented	Avg. number of new PLBs culture ⁻¹	Avg. wt. (g) per new PLBs	Total wt. (g) of new PLBs culture ⁻¹	Avg. num. of PLB-derived shoots culture ⁻¹	Avg. wt. (g) of PLB-derived shoot culture ⁻¹	Total wt. (g) of shoot flask ⁻¹ (g)
Cont. (0.0)	86.6e	0.019a	1.70c	41.6e	0.029a	1.1c
25	206.6d	0.016a	2.94b	56.6d	0.025b	1.4b
50	228.6c	0.014ab	2.73b	63.3c	0.025b	1.5b
100	554.3a	0.008b	4.48a	144.0a	0.029a	4.0a
200	514.3b	0.008b	4.19a	136.0b	0.028a	3.9a
LSD _{0.05}	10.41	0.005	0.481	4.88	0.002	0.128

In a column different letter indicate significant difference at 5% level of significance.

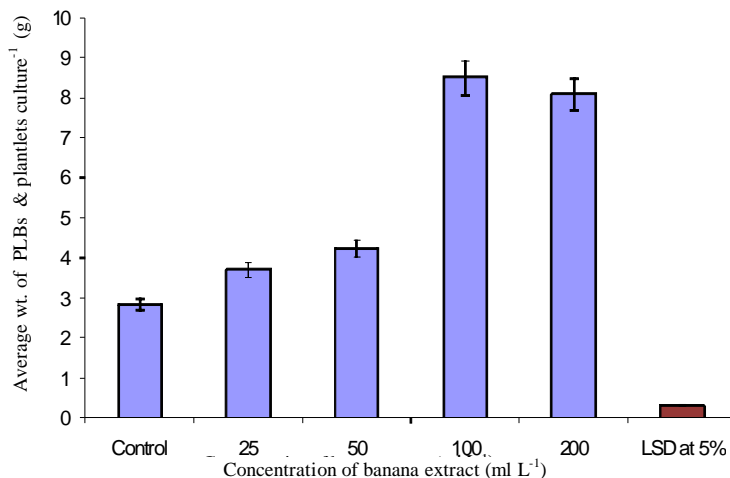


Figure 2. Effects of banana extract in culture medium on growth of PLBs after 90 days (Narrow vertical bars at top of each bar represent STD values)

Table 2. Effects of BE on growth and development of shoots after 90 days culture

Con. of BE in medium (mlL ⁻¹)	Length of shoot (cm)	Number of leaf plantlet ⁻¹	Length of leaf (cm)	Width of leaf (cm)	Fresh weight of shoot (g)	Dry weight of shoot (g)
Control	1.8 b	3.6bc	1.3c	0.66c	0.28bc	0.014ab
12.5	2.6 a	4.3ab	1.6b	1.03b	0.31ab	0.013ab
25	2.8 a	5.3 a	2.1a	1.20a	0.34a	0.029a
50	1.9 b	3.3bcd	1.2cd	0.50d	0.25c	0.012ab
100	1.3 c	2.6cd	1.0de	0.36de	0.18d	0.011ab
200	1.0 c	2.3d	0.8e	0.26e	0.06e	0.007b
LSD _{0.05}	0.463	1.02	0.269	0.137	0.056	0.017

In a column different letter indicates significant difference at 5% level of significance.

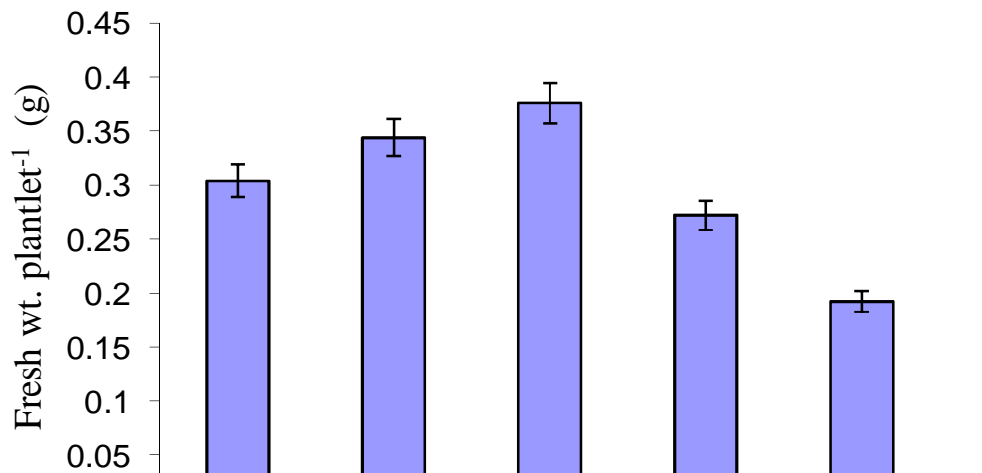


Figure 3. Effects of BE supplemented into culture medium on growth of plantlets after 90 days culture (Narrow vertical bars at top of each bar represent STD values)

3.3. Effects of BE on growth and development of roots

Growth of root *in vitro* was stimulated with BE. The highest number of root was 7.0 plantlet⁻¹ recorded at 25 ml L⁻¹ of BE supplemented into BM and the lowest number of root was 2.00 plantlet⁻¹ at 200 ml L⁻¹ (Table 3). Similar trend for the length of roots was recorded. Among the treatments, 25 ml L⁻¹ of BE supplemented into BM showed the longest root of 1.8 cm and the shortest roots of 0.6 cm was found at 200 ml L⁻¹. Higher or lower concentration of extract in

medium decreased the length. Diameter of roots was also enhanced by BE supplemented into BM. The maximum diameter of roots were 0.16 and 0.08 cm at 25 ml L⁻¹ and 200 ml L⁻¹ of BE, respectively. Similarly, BE supplemented into basal medium also influenced the average fresh and dry weight of roots. The maximum fresh and dry weight of root plantlet⁻¹ was recorded at 0.03 cm and 0.008 cm, respectively at 25 ml L⁻¹ BE. The lowest fresh weight and dry weight were 0.01 cm and 0.001 cm, respectively at 200 ml L⁻¹ of BE supplement.

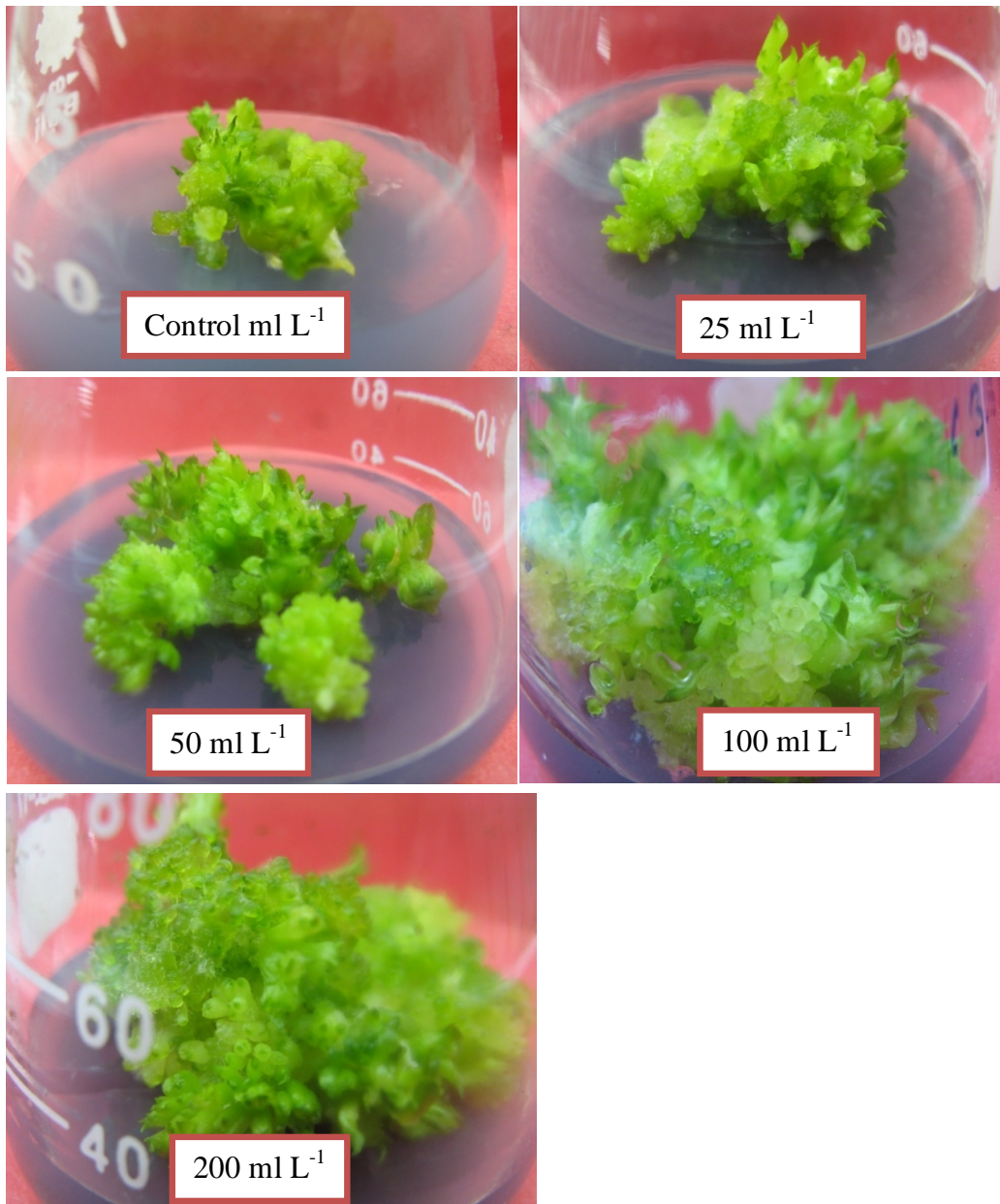


Figure 4. The growth and development of PLBs of Dendrobium orchid var. Sonia in medium supplemented with banana extract at 0.0 ml L⁻¹, 25 ml L⁻¹, 50 ml L⁻¹, 100 ml L⁻¹ and 200 ml L⁻¹ after 90 days culture. Scale bar = 1.0 cm.



Figure 5. Effects of BE (25 ml L^{-1}), on the growth of plantlets after 90 days culture.

Table 3. Effects of BE on growth and development of roots after 90 days culture

Concentration of BE (mgL^{-1})	Number of roots plantlet ⁻¹	Length of roots plantlet ⁻¹	Diameter of a root (cm)	Fresh wt. of root (g) plantlet ⁻¹	Dry wt. of roots (g) plantlet ⁻¹
Control	5.00 c	1.60 b	0.133 b	0.018 ab	0.006 ab
12.5	6.00 b	1.73 ab	0.143 ab	0.028 ab	0.007 ab
25	7.00 a	1.83 a	0.160 a	0.030 a	0.008 a
50	3.66 d	1.40 c	0.126 bc	0.016 ab	0.005 ab
100	3.00 e	0.90 d	0.103 cd	0.012 ab	0.003 ab
200	2.00 f	0.60 e	0.080 d	0.010 b	0.001 b
LSD _{0.05}	0.530	0.159	0.025	0.017	0.005

In a column different letter indicate significant difference at 5% level of significance.

In the present experiment, number and weight of new PLBs and plantlet initiation per culture were enhanced with banana extract supplement in medium compared to control. Indicating the beneficial effect of banana extract as supplement into medium on growth and differentiation of PLBs and PLB-derived plantlets *in vitro*. Similar reports were stated by Chen and Chang (2001) and Arditti (1979). An efficient concentration of 100 mL^{-1} in 1/2MS medium was found to be the best as supplement for growth and development of PLBs in terms of new PLBs regeneration. The positive and enhancing effects of banana homogenate (BH) on new PLBs regeneration and

development of healthy shoot system (long and robust shoots) from PLBs were attributed earlier by Aktar *et al.* (2008) in *Dendrobium* orchid. Similarly, 10% banana homogenate enhanced leaf size of *Spathoglottis kimbali* *lianaii* (Minea *et al.*, 2004). Banana homogenate significantly increased the number of leaves in *Dendrobium nobile* cultures (Sudeep *et al.*, 1997). Similar result was also reported by Saranjeet and Bhutani (2012). In the present experiment, a higher concentration of BE 200 mL^{-1} was inhibitory to regeneration and growth of PLBs and thus indicated supra-optimal. Similarly 25 mL^{-1} was optimum for plantlets growth over higher

concentration. This result is in agreement to the report of Lekha, *et al.* (2005) in *Dendrobium* hybrid.

4. Conclusions

Organic extracts is a complex substance containing different levels of carbohydrate, amino acids, proteins, vitamins, phenolic acids and many alkaloid compounds. Any of these component(s) could be responsible for promoting growth and development of the cultures. Which ingredient or ingredients were supportive for growth and development of PLBs or plantlets in vitro are to be clarified by further detail study. However, the present study indicates that nutritional requirement is different in PLBs and PLB-derived plantlets. Finally, it might be concluded that banana extract could help in building an organic medium for in vitro production of *Dendrobium* orchids.

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