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# *In vitro* Regeneration and Rapid Multiplication of *Dendrobium bensoniae,* an Indigenous Ornamental Orchid

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**Abstract**

An experiment was conducted on *in vitro* regeneration and multiplication of *Dendrobium bensoniae*. Different concentrations of BA and IBA alone or combination of both hormones were used as treatment for regeneration. It was revealed that shoot regeneration from node was the best at 2.0 mg/l BA supplemented to MS medium. It gave better responses than all other concentrations and combinations of BA and BA+IBA, used in the present study. The highest number of shoots and leaves were found when 1.0 mg/l BA with 1.5 mg/l IBA was supplemented into MS medium. For rooting, 0.5 mg/l BA with 1.0 mg/l IBA was found to be the most effective. The well-rooted plantlets were successfully acclimatized under 70-80% humidity and planted in pots and transferred to the shade house for establishment. Around 85% of plantlets survived in the field. From the present result, it may be recommended that MS medium supplemented with 2.0 mg/l BA may be used for rapid shoot induction and regeneration of *D. bensoniae*.

***Keywords:*** *Dendrobium* orchid, *in vitro* regeneration, phytohormone.

## 1. Introduction

Orchid represents the most evolved and one of the largest groups among the angiosperm. Orchidaceae is the most diverse family of flowering plants, consisting of 30,000 - 35,000 species belonging to 600 - 800 genera (Akter, 2007). Orchids are also widely used as medicine all around the world. Herbal extracts of orchids help to reduce or prevent diseases such as hypertension, migraine, allergies, headache, and cramps. Essential oil of orchid is very popular in aroma therapy (Bose, 1999). The climate of Bangladesh is suited for orchid cultivation. Orchid can widely be cultivated in Bangladesh (Kabir, 2012).

The genus *Dendrobium* is the third largest family of Orchidaceae, comprising of about 1184 species around worldwide (Kukulczanka, 1983). Among the genera, *Dendrobium* is one of the most popular orchids all over the world including Bangladesh. The variety *Dendrobium bensoniae* is also known as Lady Benson's *Dendrobium*. It is a popular ornamental orchid which mainly grows in Asian subcontinent. It has large flowers with a lip which has a characteristic golden disk and two large, purple spots. Plant requires warm to hot temperatures and medium amounts of light to grow well plant should keep moist and fertilize during growth season. *Dendrobium* is one of the most popular orchids all over the world including Bangladesh.

Beauty of the flower, year round production and long lasting of the flower stalk are the advantages of *Dendrobium.*

Plant tissue culture is widely used to produce clones from small parts of a plant in a method known as micropropagation. Various parts of orchids are used as explants for *in vitro* regeneration. Explants may be shoot tip, leaf segment, stem nodal segment, rhizome segment, root segment, flower bud segment, etc. Tissue culture techniques for plantlet regeneration of orchids are well known for their exploitation as a major trade in recent years in developed countries. Rapid multiplication of orchid in commercial exploitation, millions of plantlets is produced by tissue culture techniques. Among different plant growth hormone so far studied, indole butyric acid (IBA) is considered to be more important for *in vitro* root induction of orchids (Nongdam and Nirmala, 2009).

Use of diverse explants, medium composition and hormonal combination may influence *in vitro* regeneration and multiplication efficiency of orchid. The rapid multiplication rate may depends on a number of factors such as type and source of explants, orientation of explants in the culture medium, concentration and combinations of plant growth regulators. Seed viability of orchids are very less and propagation through stem cutting also not economical due to shortage of sufficient planting materials Therefore tissue culture technique can be alternate approach for overcome the natural barrier of orchid production. Hence, the present experiment was planned to investigate the effect of different plant growth regulators on in vitro regeneration of orchid.

## 2. Materials and Methods

### *2.1 Collection and surface sterilization of explant*

Disease free shoot nodes of *D. bensoniae* were used as explants (Fig. 1a) and the materials were collected from Horticulture Research Centre (HRC), Bangladesh Agricultural Research Institute, Gazipur (BARI). The trimmed shoot nodes of 1-2 cm long were washed thoroughly under running tap water followed by sterilized distilled water for several times. Subsequently, the explants were treated with 70% ethanol for 1-2 minute in an aseptic condition. They were then rinsed with sterile distilled water for 3-4 times. After ethanol treatment, they were immersed in 0.1% $HgCl_2$ and added 3-4 drops of Tween-20 for about 4-5 minutes with constant shaking. Then explants were further washed 3-4 times with sterile distilled water to make the materials free from chemical. Thus the explant was ready for culturing in culture medium.

### *2.2 Culture medium and inoculation*

Readymade complete composition of MS medium (Murashige and Skoog, 1962) (Duchafa Biochemie, The Netherland) was used for carrying out the experiment. The explants were cultured on MS nutrient medium supplemented with different concentrations of BA alone and combination of BA and IBA. Subculture was done with newly regenerated shoots. Newly formed adventitious shoots with adequate length of 2-3 cm were excised individually from the culture vial and subculture on rooting medium for induction of roots. The observations on shoot and root initiation were made throughout the entire culture period.

### *2.3 Culture environment*

The bottles were kept to the culture racks and allowed to grow in controlled environment. The cultures were maintained at 25±2 °C with light intensity varied from 2000–3000 lux (23 W white bulbs). White fluorescent lamps were used for growth of the culture. The photoperiod was generally 14 hours light and 10 hours dark having 70% relative humidity (RH).

### *2.4 Medium supplements and treatments*

Explants were inoculated onto media composed of basal MS (Murashige and Skoog, 1962) medium supplemented with BA and IBA hormone. It was added separately to different media according to the requirements. To do so, stock solutions of hormones were prepared

ahead of media preparation and stored at $4^{o}C$ temperature. For shoot and root proliferation BA and IBA were applied in concentration of 0.5, 1.0, 1.5 and 2.0 mg/l, respectively.

***2.5 Plantlets acclimatization***

After 60 days of culture on rooting medium, the plantlets were taken out from culture vial with the help of forceps with utmost care to prevent any damage to newly formed roots and dipped in water to remove agar medium from root zone for acclimatization. Plastic pots (6×6 cm) were kept ready filled with coconut husk fiber, fir bark, hardwood charcoal in 4:1:1 proportion respectively. Immediately after removing medium from roots, the plantlets were transplanted into the pots. The photoperiod was generally 14 hours light and 10 hours dark, 2000-3000 Lux and 70% RH for 7 days with consecutive irrigation in two times per day. The plants were shifted to shade house with less humidity and indirect sunlight. The orchid pots were grew at $25^{0}C$- $27^{0}C$.

***2.6 Data recording and analysis***

Data recorded at 15 days onward after inoculation. Data were recorded on shoot induction %, number of shoots per explant, average number of leaves per explant, length of leaves/plantlet, root induction %, number of roots per explants and average length of root per plant. Data were statistically analyzed by analysis of variance (ANOVA) technique and at 5% probability level using statistical program.

**3. Results and Discussion**

***3.1 Effect of BA on shoot regeneration***

The percentage of shoot induction from explants was varied significantly with different concentration of BA, supplemented into the medium. The highest percentage (80%) of shoot induction was observed at 2.0 mg/l BA supplement into the medium and the lowest one (20%) was in control (hormone free medium) in *D. bensoniae* (Table 1). Kim (2003) reported that presence of BA in the culture medium is necessary for shoot regeneration.

**3.1.1 Number of shoots per explants**

There was a significant influence of different concentrations of BA found in the number of shoots per explant initiated in this experiment (Table 1). It was observed that MS medium supplemented with 2.0 mg/l BA showed highest number (4.66 ± 0.57) of shoot induction at 30 days after inoculation (Figure 1b), whereas the lowest number of shoots (0.49 ± 0.57) at 30 days was found with hormone free medium in *D. bensoniae* (Table 1). The importance of BA in stimulating shoot elongation has been highlighted in *Vanilla planifolia*, *Dendrobium formosum* (Talukder *et al*., 2003). In the present study, MS medium with 2.0 mg/1 BA was found to be most effective for shoot multiplication (Figure 1c). This result was also supported by previous work of several researchers on *Dendrobium densiflorum* (Roy, 2003).

**3.1.2 Number of leaves per explant**

The number of leaves was recorded at 60 days after inoculation. The number of leaves per explant was significantly different due to the different concentrations of BA in to medium. The highest number of leaves per explant (9.33 ± 1.15) was noticed from 2.0 mg/l BA, whereas the lowest one was (1.40 ± 0.0) in control treatment. The mean leaf number varied from 4.00 to 6.85 observed after four weeks.

**3.1.3 Length of leaves/plantlet**

The length of leaves was recorded at 60 days after inoculation. The mean value of the data shows the average length of leaves/plantlet. The highest length of leaf was found 1.16 ± 0.21 cm at 1.5 mg/l BA. The lowest one was found (0.40 ± 0.0 cm) at control. These findings are in agreement with the investigation of Malabadi *et al.*2005), where the highest length of leaf (1.29 ± 0.16 cm) was obtained at 1.5 mg/L of BA supplemented into medium in *Dendrobium barisanum.*

***3.2. Combined effect of BA and IBA on shoot regeneration of D. bensoniae***

**3.2.1 Number of shoots per explant**

There was significant influence of different concentrations of BA and IBA on the number of shoots per explant after 30 days of inoculation. The results presented in Table 2. The treatment 1.0 BA+1.5 IBA (mg/l) gave the highest number of shoots (3.67 ± 0.57) after 30 days of inoculation (Figure 2b), whereas the lowest number of shoots (0.95 ± 0.0) was found in. The result shows that the combination of BA and IBA was suitable for shoot multiplication. The previous also showed that the high concentration of BA and low concentration of IBA was selectively favorable for the induction of multiple shoots. Vij and Kaur (1998) reported similar results where BA-enriched medium in combination with IBA favoured multiple shoot bud formation in *Dendrobium bensoniae.*

**3.2.2 Number of leaves per explant**

The number of leaves increased with days after inoculation. Maximum number of leaves was obtained at 60 DAI from these treatments compared to control. The highest number of leaves per explant (9.33 ± 0.57) was noticed at 1.0 BA+2.0 IBA (mg/l), whereas the lowest one was (1.23 ± 1.00) in control.

**Table 1.** Efficacy of BA on shoots and leaves induction in *D. bensoniae*

| Concentration of BA supplement (mg/l) | Explants induced shoot (%) | Number of shoots per explants ±SD (30 DAI) | Number of leaves per explants ± SD (60 DAI) | Avg. length of leaves after 60 days (cm) |
|---|---|---|---|---|
| MS (Control) | 20 | 0.49±0.57 | 1.40±0.00 | 0.40±0.00 |
| 0.5 | 50 | 4.00± 0.57 | 6.33±1.00 | 0.96±0.21 |
| 1.0 | 65 | 4.33±1.00 | 8.00±0.57 | 0.96±0.38 |
| 1.5 | 75 | 3.00±1.00 | 8.33±0.57 | 1.16±0.21 |
| 2.0 | 80 | 4.66±0.57 | 9.33±1.15 | 1.13±0.10 |
| SE | | 1.20 | 0.60 | 0.04 |
| LSD | | 1.99 | 1.40 | 0.38 |
| Level of Significance | | (*P ≤0.05*) | (*P≤0.05*) | (*P ≤0.05*) |

DAI= Days after inoculation

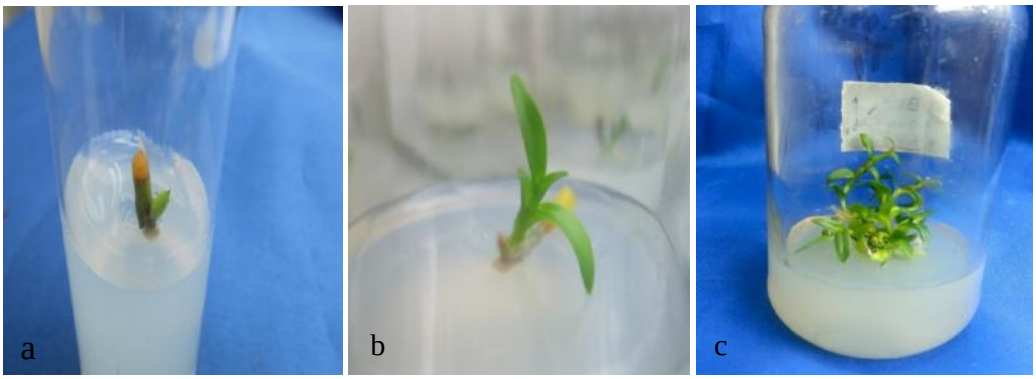


**Figure 1.** Shoot initiation, plantlet regeneration and multiplication of *D. bensoniae* on MS medium supplemented with 2.0 mg/l BA. *D. bensoniae* at (a) 15 DAI (b) 30 DAI (c) 45 DAI

### 3.2.3 Rooting with BA and IBA in vitro

The minimum rooting percentage was recorded in the control as compared to other treatments. The highest percentage (90%) of root induction was found at 1.0 mg/l BA +1.5 mg/l IBA and the lowest one (50%) was induced in control.

Hormonal concentration has significant level of variation on days for root induction. The maximum 40 days to root induction was observed in media lack of growth regulator. Minimum 21 days is required in case of 0.5 mg/l of BA+1.0 mg/l of IBA in case of *D. bensoniae* (Figure 3a).

**Table 2.** Combined effect of BA and IBA on shoot regeneration of *D. bensoniae*

| Hormonal (BA+IBA) concentration (mg/l) | Number of shoots per explants at 30 days ±SD | Number of leaves per explants after 60 days ± SD |
|---|---|---|
| MS (Control) | 0.95±0.0 | 1.23±1.00 |
| 0.5 + 0.5 | 3.00±1.15 | 6.35±0.00 |
| 0.5 + 1.0 | 3.34±1.00 | 8.00±0.57 |
| 1.0 + 1.5 | 3.67±0.57 | 8.25±0.57 |
| 1.0+ 2.0 | 2.65±0.57 | 9.33±0.57 |
| SE | 0.60 | 0.60 |
| LSD | 1.40 | 1.41 |
| Level of Significance | (*P ≤0.05*) | (*P ≤0.05*) |

DAI= Days after inoculation

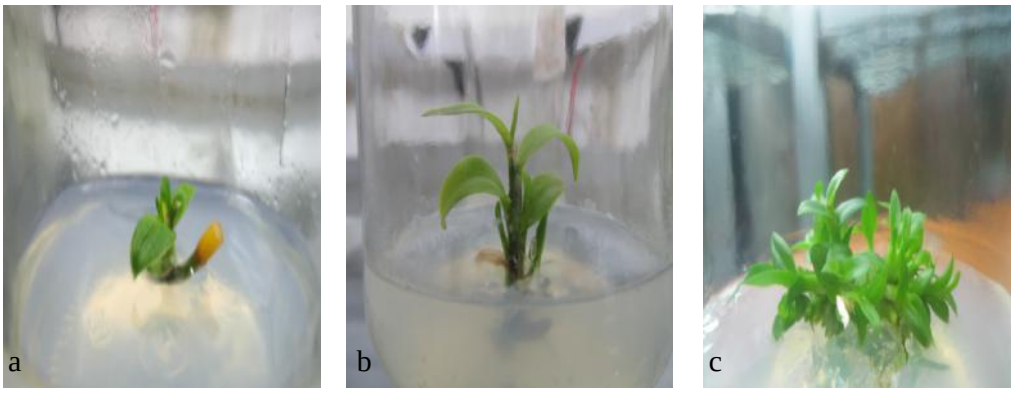


**Figure 2.** Shoot induction, regeneration and multiplication of *D. bensoniae* on MS media supplemented with 1.0 mg/l BA + 1.5 mg/l IBA. *D. bensoniae* at (a) 15 DAI (b) 30 DAI (c) 45 DAI

**Table 3.** Effect of BA and IBA on root induction and growth *in vitro*

| Concentration of BA and IBA in MS medium (mg/l) | No. of explants inoculated | % of explants initiated root | Average number of roots per explants ±SD | Average length (cm) of roots per explants ±SD |
|---|---|---|---|---|
| | | | 60 DAI | 60 DAI |
| Control | 10 | 50 | 3.95±0.08 | 0.63±0.07 |
| 0.5+0.5 | 10 | 70 | 6.68±0.15 | 0.83±0.08 |
| 0.5+1.0 | 10 | 80 | 10.35±0.07 | 1.35±0.15 |
| 1.0+1.5 | 10 | 90 | 7.00±0.10 | 1.20±0.25 |
| 1.0+2.0 | 10 | 70 | 9.35±0.20 | 1.19±0.15 |
| SE | | | 0.80 | 0.20 |
| LSD | | | 1.62 | 0.27 |
| Level of Significance | | | (*P≤0.05*) | (*P ≤0.05*) |

DAI= Days after inoculation

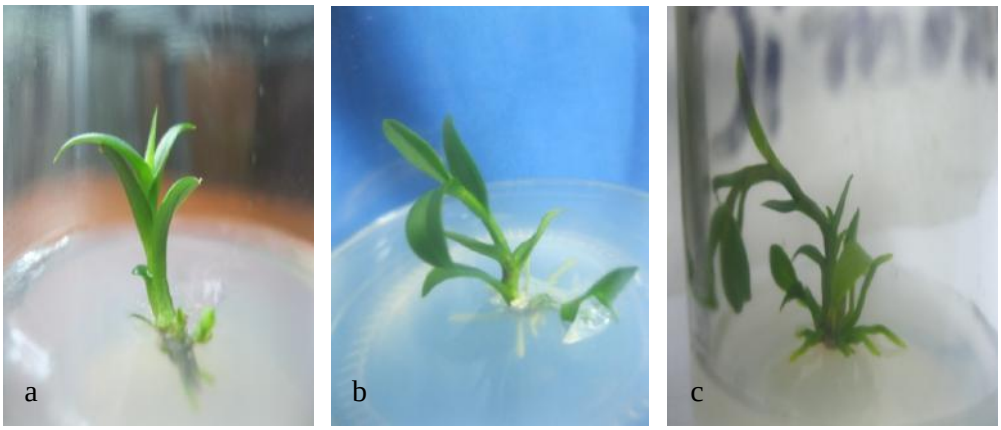


**Figure 3.** Root induction and elongation of *D. bensoniae* on MS media supplemented with 0.5 mg/l BA + 1.0 mg/l IBA. *D. bensoniae* at (a) 30 DAI (b) 45 DAI (c) 60 DAI

**Table 4.** Survival rate of *in vitro* regenerated plantlet of *D. bensoniae*

| Acclimatization | No. of plants transplanted | Duration of observation | No. of plants survived | Survival rate (%) |
|---|---|---|---|---|
| In culture room | 20 | 7days | 14 | 70 |
| In shade house | 14 | 14 days | 13 | 85 |

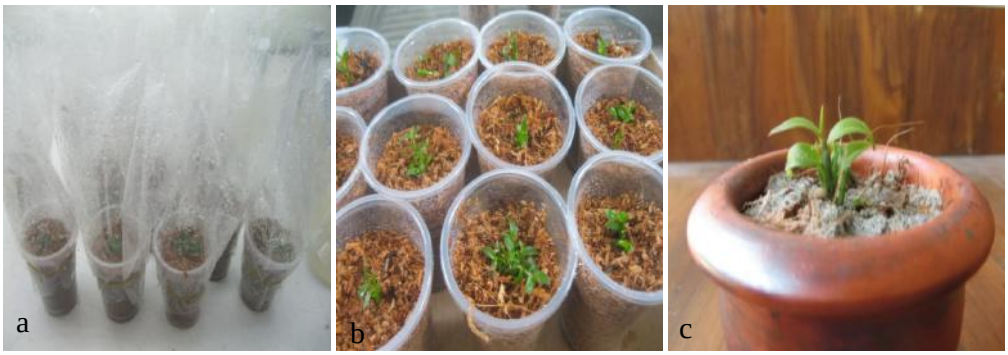


**Figure 4.** Acclimatization of regenerated plantlet of *D. bensoniae (*a) in culture room covered with transparent plastic sheet (b) in culture room without transparent plastic sheet (c) in shade house

***3.3 Effect of BA and IBA on rooting***

Rooting with BA and IBA varied significantly in vitro. The highest number of roots (10.35±0.07) per explant was recorded at 0.5 mg/l BA+1.0 mg/l IBA obtained after 60 DAI (Table 3, Figure 3c) and the minimum number of roots (3.95±0.08) was in control. Martin (2006) reported that the number of root per explant was 1.93 in *Dendrobium aphyllum* at 2.5 mg/l BAP and 0.5 mg/l NAA after 30 DAI.

**3.3.1 Length of root (cm)**

Root length was significantly influenced by growth regulators. The length of roots per explant (cm) was increased with combined effects of BA and IBA concentration and then decreased. . The maximum average root length (1.35±0.15 cm) was obtained at 0.5 mg/l of BA + 1.0 mg/l of IBA and the minimum (0.63±0.07 cm) length was in control. Khatun (2010) reported that, the root length was significantly high (0.916 cm) at 2.0 mg/l of BAP + 1.0 mg/l of IBA after 6 week of culture.

***3.4 Acclimatization and transplantation***

The results of acclimatization showed that 70% of plantlets were survived to the culture room (Figure 4a and 4b) and 85% of the plantlets survived in shade house (Figure 4c). It was also revealed that regenerated plants were morphologically similar to the mother plant. A micropropagation system can be deemed beneficial only by the successful transfer of plantlets from tissue-culture vessels to the ambient conditions found in *ex vitro* (Hajong, 2013) and Devi *et al* (1998) reported that *in vitro* acclimatization of plantlets prior to their *ex vitro* transplantation is important in producing healthy plantlets.

**4. Conclusions**

The nodal segments of *D. bensoniae* showed various responses on MS medium supplemented with different BA and IBA concentrations either separately or in combinations. These results showed that 2.0 mg/l BA supplemented to MS medium gives better response than all other combinations of BA and IBA. The combined effect of BA and IBA showed average responses than individual BA treatment. The combined treatments 0.5 mg/l BA and 1.0 mg/l IBA gave better response than all other combinations for root induction in *D. bensoniae.*

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