

## Organogenesis of *Dendrobium* Orchid Using Traditional Media and Organic Extracts

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### ABSTRACT

The study was undertaken with a view to investigate the effects of different basal media and organic extracts on organogenesis of *Dendrobium* orchid. Protocorm like bodies (PLBs) were cultured in Knudson C (KC), Vacin and Went (VW), half strength Murashige and Skoog ( $\frac{1}{2}$ MS) and New Phalaenopsis (NP) media supplemented with Sabri banana pulp (Sb), Charcoal (C) and Coconut water (Cw) organic extracts. KC, VW,  $\frac{1}{2}$ MS and NP media significantly influenced the plantlet regeneration of *Dendrobium* orchid. Among the media,  $\frac{1}{2}$ MS showed better performance on fresh weight, number PLBs, shoots and leaves per plantlet. The longest shoots were found in KC medium and maximum length of leaves was found in both KC and  $\frac{1}{2}$ MS media. In case of different organic extracts Sb showed significantly better performance on fresh weights of PLBs, number of shoot and leaves per explant, length of shoots and leaves.

**Key words:** Organogenesis, *Dendrobium*, media, organic extracts.

### INTRODUCTION

*Dendrobium* orchids are the most popular cut flowers in the orchid floral trade, especially in the Southeast Asian countries. Its mainly requires dry warm and humid environment with abundant sun light for optimum growth and flowering, that are prevailing in Bangladesh. Rapid growth, easiness in plantlet regeneration, year round production under controlled flowering, beauty of flower and long lasting of flower stalk are the advantages in orchids especially in *Dendrobium*. The stem of *Dendrobium* species are used in making baskets in the Philippines, Indonesia and New Guinea and pseudobulbs of *D. takai* are used as oral contraceptives (Bose and Bhaltacharjee, 1999).

Orchids grow in a wide range of media and the main components of most plant tissue culture media are mineral salts and sugar as carbon source and water. In addition, orchids depend on the media for mechanical support. The media and its formulation are extremely important to maximize orchid's vigor. A number of media are used for plant tissue culture and these are extensively reviewed by George *et al.* (1987). Some tissues respond much better on solid media, while others on liquid media. A large number of complex additives like coconut water, banana pulp, peptone, tomato juice, slap honey and beef extract can be very effective in providing undefined mixture of organic nutrients and growth factors. For *in vitro* growth of PLBs and seedlings some complex organic additives reported satisfactory while some have unsatisfactory and even inhibitory (Arditti, 1967).

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Many studies on micropropagation methods have already been conducted to establish a suitable method. Rotor (1949) for the first time tried to propagate *Phalaenopsis* clonally using flower stalk buds. Other research reports on the micropropagation of orchids through tissue culture of leaf (Tanaka 1987); root tips (Tanka *et al.* 1976) internodal section of flower stalk (Homma and Asahira 1985; Lin 1986) and lateral buds from young flower stalks (Ichihashi 1992) are available but none of these methods proved to be effective commercially in producing lots of plantlets in a short period because of low rate of PLB (Protocorm Like Body) formation, low viability of PLB, consuming long time for obtaining PLB and different responses among PLB and hybrids (Tokuhara and Mii 1993).

Considering the above idea in mind the present study was undertaken to investigate the effects of different media and organic additives on plantlet regeneration from PLB and its subsequent growth in *Dendrobium*.

## MATERIALS AND METHODS

The experiment was carried out at the USDA Biotechnology Laboratory, Bangladesh Agricultural University, Mymensingh during the period from February to April, 2006 to investigate the effect of different media and organic extracts on organogenesis of *Dendrobium* orchid. *In vitro* multiple PLBs were cultured on the Vacin and Went (Vacin and Went 1949), Knudson C (Knudson 1946), half strength Murashige and Skoog (Murashige and Skoog 1962) and New *Phalaenopsis* (New *Phalaenopsis*) media supplemented with charcoal (0.1% w/v), Sabri banana pulp (10% w/v) and coconut water (10% v/v). After preparing the media P<sup>H</sup> was adjusted to 5.8 with digital P<sup>H</sup> meter adding 0.1 N NaOH or 0.1 N HCl. Agar powder (10 g/l) was added to solidify the media. The culture vials containing the media were autoclaved with 1.16 kg/cm<sup>2</sup> of pressure at 121°C for 20 minutes. Thirty six glass vials were labeled and set up under twelve treatments with three replications. Four *in vitro* multiple PLBs were placed in each vial. All the culture vials were placed in a growth room and allowed to grow at 25±1°C under 16 hour photoperiod illuminated with fluorescent tube of 2000-3000 lux. On the basis of media and organic extract the experiment considered two factors (i) Media (ii) organic extract. The experiment was laid out in Completely Randomized Design (CRD). The data were collected and recorded at 20 days interval up to 60 days on fresh weight of PLBs explant<sup>-1</sup>, number of PLBs explant<sup>-1</sup>, number of shoots explant<sup>-1</sup>, length of shoots, number of leaves plantlet<sup>-1</sup> and length of leaves. The treatment means were compared based on Least Significant Difference (LSD), Zaman *et al.* (1983).

## RESULTS AND DISCUSSION

The results obtained from the present study are presented and discussed with figures and tables under the following headings.

### Fresh weight of PLBs

Different media showed a significant effect on fresh weight of PLBs at 20 DAI but not at 40 and 60 DAI. At 20 DAI, the fresh weight of PLBs in ½MS media was significantly higher (0.33 g) than those of other media. No significant difference of the fresh weight of PLBs was found between KC and VW media. At 40 DAI, the highest (0.39 g) and lowest (0.33 g) fresh weight of PLBs was obtained from NP and KC media, respectively. At 60 DAI, the highest (0.57 g) and lowest (0.49 g) of fresh weight of PLBs was from ½MS and VW media, respectively (Fig. 1). It appears from the present study that ½MS medium showed superiority in fresh weight of PLBs over others at 20 and 60 DAIs. It might be due to higher nitrate and relatively lower sulphate and phosphate content of ½MS medium than other media. Haque (1996) found similar effect in garlic (*Allium Sativum*). Khatun (2005) found 0.24 g and 0.19 g fresh weight of PLBs from KC and VW media, respectively at 60 DAI in *Dendrobium* orchid.

Different organic extracts showed highly significant effect on the fresh weight of PLBs at all DAIs. At 20 DAI, the highest (0.31g) fresh weight and lowest (0.16 g) fresh weight of PLBs were obtained from Sabri banana pulpe and Charcoal. At 40 DAI, the fresh weight of PLBs on Sabri

banana pulpe was significantly higher (0.47 g) than those on other organic extracts. No significant difference of fresh weight of PLBS was observed between Charcal and Coconut water. Similar trends were found at 60 DAI (Table.1).It appears from the present study that Sabri banana had superiority in fresh weight of PLBs over other extracts at all DAIs. Higher concentrations of sucrose and other nutrients in Sabri banana pulp had a promotive effect on fresh weight of PLBs, Singh (1995).

### Number of PLBs

Different media showed a significant effect on number of PLBs at 20, 40 and 60 DAI. At 20 and 40 DAI, the number of PLBs in ½MS was significantly higher than those on other media. At 40 DAI, no significant variation of the number of PLBs was found between VW and NP media. At 60 DAI, the highest (33.57/explant) and lowest (17.92/explant) number of PLBs were obtained from ½MS and VW media, respectively. No significant variation of the number of PLBs per explants was found between KC and NP media at 60 DAI (Fig. 2). The highest number of PLBs on ½MS medium in this study might be due higher nitrate and relatively lower sulphate and phosphate content of ½MS medium than other media. Haque (1996) found similar effect in garlic (*Allium Sativum*). Lakshmana *et al.* (1995) observed average of 13.5 PLBs in VW medium. Rahman *et al.* (2004) reported that NP medium enhanced the subsequent growth of mini plantlet of *Doritacenopsis* orchid. Shyamal and Pinik (2004) showed the best regenerated shoots of *Vanda ters* when grown on VW medium containing 10% coconut water.

The number of PLBs was significantly influenced by different organic extracts at all DAIs. At 20 DAI, the number of PLBs in Sabri banan pulp was significantly higher (11.85 /explants) than those in other organic extracts. No significant variation of the number of PLBs was found between Charcoal and Coconut water. At 40 DAI, the numbers of PLBs in Coconut water were lowest (12.68 /explant). No significant variation of the number of PLBs was found between the organic extracts Sabri banana pulp and Charcoa. At 60 DAI, Coconut water showed the highest (26.26/explant) number of PLBs (Table.1). Chen and Chen (1998) reported that the best plantlets were grown on banana extract. It appears from the present study that Coconut water showed superiority over others.

**Table 1: Effects of different organic extracts on plantlet regeneration from PLB and its subsequent growth in *Dendrobium***

Treatment	Fresh weight of PLBs (g) at different days after inoculation			Number of PLBs at different days after inoculation			Number of shoots at different days after inoculation			Length of shoots (cm) at different days after inoculation			Number of leaves at different days after inoculation			Length of leaves (cm) at different days after inoculation		
	20	40	60	20	40	60	20	40	60	20	40	60	20	40	60	20	40	60
Sb	0.31 <sup>a</sup>	0.47 <sup>a</sup>	0.71 <sup>a</sup>	11.85 <sup>a</sup>	17.13 <sup>a</sup>	24.28 <sup>ab</sup>	8.27 <sup>a</sup>	15.01 <sup>ab</sup>	25.32 <sup>a</sup>	0.61 <sup>a</sup>	0.93 <sup>a</sup>	1.30 <sup>a</sup>	1.04 <sup>a</sup>	1.81 <sup>a</sup>	2.26 <sup>a</sup>	0.36	0.38 <sup>a</sup>	0.57 <sup>a</sup>
C	0.16 <sup>c</sup>	0.28 <sup>b</sup>	0.41 <sup>b</sup>	7.95 <sup>b</sup>	16.04 <sup>b</sup>	23.74 <sup>b</sup>	2.13 <sup>b</sup>	5.74 <sup>b</sup>	14.04 <sup>b</sup>	0.36 <sup>b</sup>	0.69 <sup>b</sup>	0.99 <sup>b</sup>	0.46 <sup>b</sup>	1.21 <sup>b</sup>	1.97 <sup>b</sup>	0.21	0.25 <sup>b</sup>	0.46 <sup>a</sup>
Cw	0.24 <sup>b</sup>	0.34 <sup>b</sup>	0.47 <sup>b</sup>	8.43 <sup>b</sup>	12.68 <sup>b</sup>	26.26 <sup>a</sup>	2.15 <sup>b</sup>	6.85 <sup>b</sup>	13.01 <sup>b</sup>	0.28 <sup>b</sup>	0.66 <sup>b</sup>	0.97 <sup>b</sup>	0.42 <sup>b</sup>	1.18 <sup>b</sup>	1.91 <sup>b</sup>	0.12	0.20 <sup>b</sup>	0.33 <sup>b</sup>
LSD at 5%	0.037	0.096	0.088	1.361	1.884	2.064	0.813	1.600	1.915	0.099	0.188	0.125	0.092	0.306	0.157	NS	0.652	0.116

In a column, figures followed by same letter(s) don't differ significantly at P<0.05 as per DMRT

Note: Sb = Sabri banana pulp, C = Charcoal, Cw = Coconut water, LSD = Least significant difference

NS = Non significant

### Number of shoots

Different media showed a significant effect on number of shoots at 20, 40 and 60 DAI. At 20 DAI, the highest number of shoots (7.27 /explant) was found in ½MS media and lowest (1.94 /explant)

was in NP media. No significant difference of the number of shoots was observed between KC and VW media. At 40 DAI, the number of shoots in  $\frac{1}{2}$ MS medium was significantly higher than those of other media. No significant difference of the number of shoots was observed between KC and NP media (Fig. 3). From the present experiment, it appears that the number of shoots gradually increased with time and  $\frac{1}{2}$ MS medium had superiority over other media. This result was partially support result observed by Talukder *et al.* (2003), where they obtained the highest number of proliferated shoots (11.13/explant) on MS medium containing BAP.

The number of shoot of in Sabri banana pulp was significantly higher than those of other organic extracts at all DAIs. No significant variation of the number of shoots per explants was found between the organic extracts Charcoal and Coconut water (Table. 1). Sabri banana pulp appeared the best at all DAIs due to higher percentage (27%) of sucrose concentrations, Singh (1995).

### **Length of shoots**

Length of shoots on  $\frac{1}{2}$ MS medium was significantly higher (0.64 cm) at 20 DAI than those on other media. However, at 40 and 60 DAI, length of single shoots on KC medium was significantly higher than those on other media. At 40 DAI, no significant difference of the length of single shoots was observed among KC, VW and  $\frac{1}{2}$ MS media. At 60 DAI, the higher (1.29 cm) and lower (0.84 cm) length of single shoots was found from KC and NP media, respectively (Fig. 4). Martini (2001) observed large number of plantlets on MS medium in *Gongora*.

The length of shoot on Sabri banana pulp was significantly higher than those on others at all DAIs. No significant variation of the length of single shoot was found between Charcoal and Coconut water. (Table.1). Higher percentage (27%) of sucrose in of Sabri banana pulp might be responsible for the promotive effect on the length of shoot at all DAIs.

### **Number of leaves**

The number of leaves on  $\frac{1}{2}$ MS medium was significantly higher (2.83 / plantlet) than those on other media at all DAIs (Fig. 5). The number of leaves on NP medium was significantly lower (1.47/ plantlet) than those on other media. No significant difference of the number of leaves was found between the KC and VW media. The number of leaves gradually increased with time and  $\frac{1}{2}$ MS medium had superiority to others media at all DAIs. This result is in agreed with that of Davidson (1994) who reported that the number of leaves of *Dendrobium* species was higher in MS medium than in KC medium. Hye (2003) also observed highest number of leaves (5.28/ plantlet) in hybrid orchid on MS medium at 90 DAI.

The highest number (2.26 / plantlet) of leaves was obtained from Sabri banana pulp (Table. 1) at all DAIS. It might be due to the higher concentrations of sucrose and other nutrient contents in sabri banana pulp, Singh (1995).

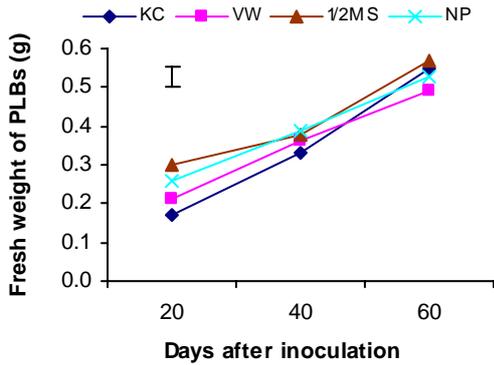
### **Length of leaves**

At 40 DAI, the length of leaves of NP medium was significantly lower (0.22cm) than those on other media. No significant difference of the length of leaves was observed among the KC, VW and half strength MS media (Fig. 6). At 60 DAI, the highest length (0.48 cm) of leaves was found in both KC and half strength MS media.

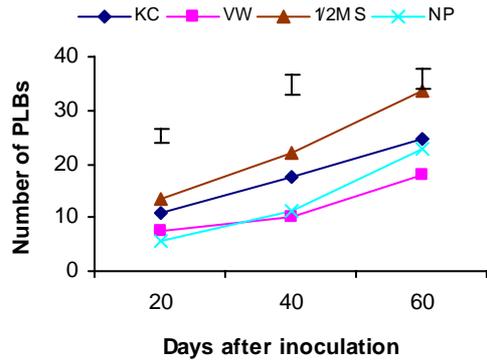
Different organic additives showed a significant variation in leaf length at 40 and 60 DAIs. The length of leaves on Sabri banana pulp was significantly higher than those on others (Table. 1). Higher percentage (27%) of sucrose in Sabri banana pulp extract might have a promotive effect on the length of leaves.

It revealed from the present study that  $\frac{1}{2}$ MS medium supplemented with Sabri banana pulp is the most suitable for organogenesis of *Dendrobium* orchid.

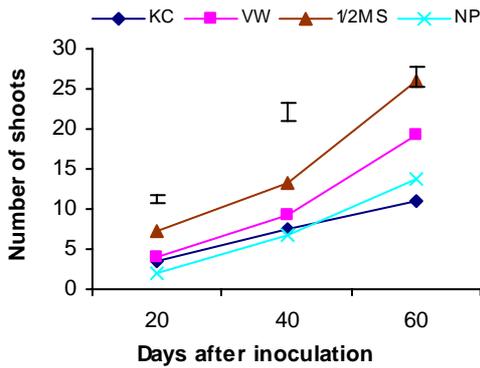
Organogenesis of *dendrobium* orchid using traditional media and organic extracts



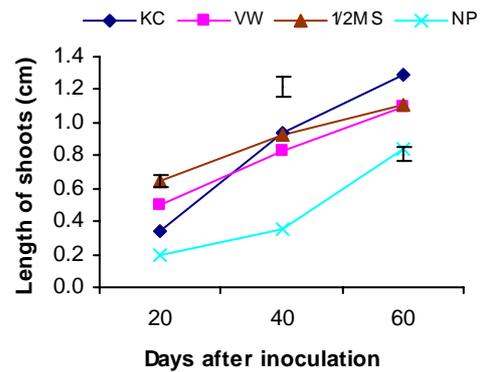
**Fig. 1.** Effect of different media on fresh weight of PLBs at different DAIs. Vertical bar represent LSD at 5% level of probability



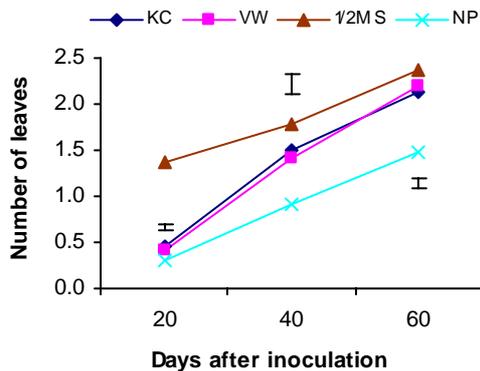
**Fig. 2.** Effect of different media on number of PLBs at different DAIs. Vertical bars represent LSD at 5% level of probability



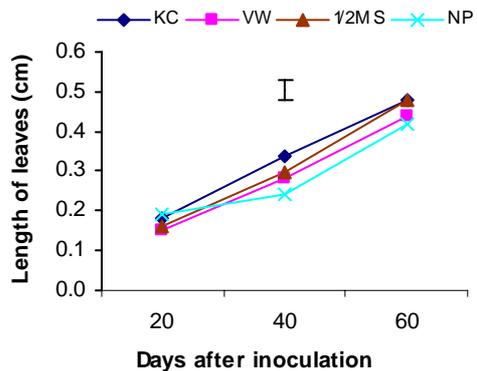
**Fig. 3.** Effect of different media on number of shoots at different DAIs. Vertical bars represent LSD at 5% level of probability



**Fig. 4.** Effect of different media on length of shoots at different DAIs. Vertical bars represent LSD at 5% level of probability



**Fig. 5.** Effect of different media on number of leaves at different DAI. Vertical bars represent LSD at 5% level of probability



**Fig. 6.** Effect of different media on length of leaves at different DAIs. Vertical bars represent LSD at 5% level of probability

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