

## Effects of Different Media and Organic Additives Interaction on *In Vitro* Regeneration of *Dendrobium* Orchid

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### ABSTRACT

Interaction of different media and organic additives showed significant effects on growth and development of protocorm like bodies (PLBs) and subsequent plantlets regeneration of *Dendrobium* orchid in laboratory conditions. Except the length of shoots and leaves, the highest values of all parameters were obtained from the interaction of ½MS medium with Sabri banana pulp. However, the longest shoots were found in KC medium with Sabri banana pulp and the longest leaves were found in both KC and ½MS media with Sabri banana pulp.

**Key words:** Interaction, regeneration, *Dendrobium*, media and organic additives.

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### INTRODUCTION

*Dendrobium* orchids grow in nature through seeds but in absence of appropriate hosts the seeds don't germinate in adequate percentage. These obstacles may be overcome by adopting tissue culture technique. *In vitro* plant regeneration is an important and essential component of plant biotechnology. The frequency of callus inductions and plant regeneration are influenced by many factors, such as genotypes, type of explants and composition of media (Jain, 1997). Nutrient composition is considered to be major sources of variation in plant tissue culture (Khanna and Raina, 1998). Different culture media have been used for efficient plant regeneration in orchid tissue culture. Among them, MS medium (Murasigh and Skoog, 1962) was found to be efficient for PLBs formation and plantlet regeneration of *Dendrobium* orchid when supplemented with 2,4-D (Nasiruddin *et al.*, 2003). Lim-Ho *et al.* (1982) showed that VW medium (Vacin and Went, 1949) with organic substrate yielded the highest increase in weight and most numbers of leaves and roots formed in *Aranda*. NP medium (New *Phalaenopsis*) gave better results when supplemented with high concentrations of BAP (Chowdhury *et al.*, 2003). 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 were reported satisfactory while some were unsatisfactory and even inhibitory (Arditti, 1967). For this, suitable media and organic additives are needed to be identified for large-scale utilization in orchid tissue culture. Therefore, the present study was undertaken to observe the interactive effects of different media and organic additives on formation of PLBs and their subsequent plantlet regeneration capability of *Dendrobium*.

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## MATERIALS AND METHODS

The experiment was carried out at the USDA Biotechnology Laboratory, Bangladesh Agricultural University, Mymensingh during the period of February to April 2006 to investigate the effects of different media and organic additives interaction on growth and development of PLBs and subsequent plantlets regeneration of *Dendrobium* orchid. *In vitro* multiple PLBs were cultured on the VW (Vacin and Went, 1949), KC (Knudson C, 1946), half strength MS (Murasighe and Skoog, 1962) and NP (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,  $P^H$  was adjusted to 5.8. Agar powder (10 gL<sup>-1</sup>) 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 labelled and set up under twelve treatments with three replications. Four *in vitro* multiple PLBs were placed in each culture vial (Plate 1). 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. 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, number of PLBs explant<sup>-1</sup>, number of shoots explant<sup>-1</sup>, length of shoot, number of leaves plantlet<sup>-1</sup> and length of leaves. The treatment means were compared based on Duncan's Multiple Range Test (DMRT) at 1% level of probability.



Fig. 1. PLBs after inoculation in media

## RESULTS AND DISCUSSION

The effects of different media and organic additives interaction on growth and development of PLBs and subsequent plantlets regeneration of *Dendrobium* orchid are presented in Table 1 (a,b) and the performance of *in vitro* regeneration of *Dendrobium* orchid is showing in Fig. 2.

**Table 1a. Effect of different media and organic additives interaction on growth and development of mini plantlet of *Dendrobium* orchid at different days after inoculation (DAI)**

Media	Organic additives	Fresh weight of PLBs (g)			Number of PLB explant <sup>-1</sup>			Number of shoots explant <sup>-1</sup>		
		20 DAI	40 DAI	60 DAI	20 DAI	40 DAI	60 DAI	20 DAI	40 DAI	60 DAI
KC	Sb	0.25	0.50 <sup>abc</sup>	0.70 <sup>b</sup>	8.50 <sup>cd</sup>	12.50 <sup>de</sup>	15.3 <sup>e</sup>	4.20 <sup>c</sup>	10.20 <sup>c</sup>	15.30 <sup>de</sup>
	C	0.12	0.20 <sup>d</sup>	0.47 <sup>cd</sup>	18.30 <sup>b</sup>	30.20 <sup>b</sup>	40.36 <sup>b</sup>	3.20 <sup>cd</sup>	6.52 <sup>de</sup>	9.93 <sup>fg</sup>
	Cw	0.13	0.28 <sup>d</sup>	0.48 <sup>cd</sup>	5.50 <sup>e</sup>	10.20 <sup>ef</sup>	18.50 <sup>de</sup>	3.4 <sup>cd</sup>	5.50 <sup>def</sup>	7.50 <sup>g</sup>
VW	Sb	0.30	0.50 <sup>abc</sup>	0.70 <sup>b</sup>	10.20 <sup>c</sup>	12.3 <sup>def</sup>	17.50 <sup>de</sup>	10.20 <sup>b</sup>	20.40 <sup>b</sup>	35.50 <sup>b</sup>
	C	0.15	0.25 <sup>d</sup>	0.31 <sup>d</sup>	2.50 <sup>f</sup>	3.15 <sup>g</sup>	6.25 <sup>f</sup>	1.10 <sup>ef</sup>	2.50 <sup>f</sup>	12.50 <sup>ef</sup>
	Cw	0.17	0.34 <sup>bcd</sup>	0.49 <sup>cd</sup>	10.10 <sup>c</sup>	15.20 <sup>d</sup>	30.00 <sup>c</sup>	0.50 <sup>f</sup>	5.50 <sup>def</sup>	9.50 <sup>f</sup>
½MS	Sb	0.50	0.60 <sup>a</sup>	0.91 <sup>a</sup>	22.50 <sup>a</sup>	35.50 <sup>a</sup>	50.00 <sup>a</sup>	17.50 <sup>a</sup>	25.20 <sup>a</sup>	40.30 <sup>a</sup>
	C	0.21	0.31 <sup>bcd</sup>	0.44 <sup>cd</sup>	10.10 <sup>c</sup>	20.20 <sup>c</sup>	30.20 <sup>c</sup>	2.10 <sup>def</sup>	6.70 <sup>cde</sup>	15.20 <sup>de</sup>
	Cw	0.20	0.24 <sup>d</sup>	0.34 <sup>d</sup>	8.02 <sup>cde</sup>	10.10 <sup>ef</sup>	20.30 <sup>d</sup>	2.20 <sup>def</sup>	8.20 <sup>cd</sup>	22.30 <sup>c</sup>
NP	Sb	0.17	0.30 <sup>cd</sup>	0.55 <sup>bc</sup>	6.20 <sup>de</sup>	8.20 <sup>ef</sup>	14.12 <sup>c</sup>	1.20 <sup>ef</sup>	4.25 <sup>ef</sup>	10.20 <sup>g</sup>
	C	0.14	0.34 <sup>bcd</sup>	0.44 <sup>cd</sup>	0.90 <sup>cf</sup>	10.60 <sup>ef</sup>	18.13 <sup>de</sup>	2.13 <sup>def</sup>	7.51 <sup>cde</sup>	18.52 <sup>cd</sup>
	Cw	0.46	0.52 <sup>ab</sup>	0.59 <sup>bc</sup>	10.10 <sup>c</sup>	15.20 <sup>d</sup>	36.25 <sup>b</sup>	2.50 <sup>cde</sup>	8.21 <sup>cd</sup>	12.75 <sup>ef</sup>
LSD at 1%	NS	0.1921	0.1767	2.721	3.768	4.128	1.627	3.201	3.831	

In column, figures followed the same letter(s) don't significant at P<0.01

Where,

KC = Knudson C, VW = Vacin and Went, ½MS = ½Murasighe and Skoog, NP = New *Phalaenopsis*, C = Charcoal, Sb = Sabri banana, Cw = Coconut water, DAI = Different days after inoculation.

NS= Non-significant, LSD= Least significant difference.

**Table 1b. Interaction effect of different media and organic additives on growth and development of mini plantlet of *Dendrobium* orchid at different days after inoculation (DAI)**

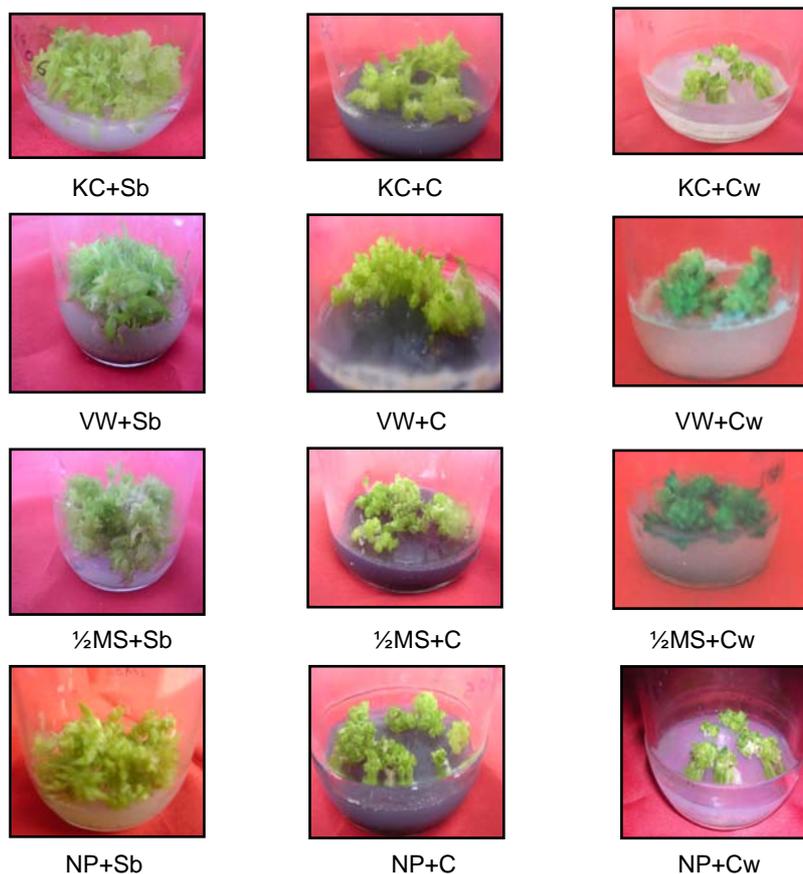
Media	Organic additives	Length of shoot (cm)			Number of leaves plantlet <sup>-1</sup>			Length of leaf (cm)		
		20 DAI	40 DAI	60 DAI	20 DAI	40 DAI	60 DAI	20 DAI	40 DAI	60 DAI
KC	Sb	0.41 <sup>bcd</sup>	1.10	1.50 <sup>a</sup>	1.01 <sup>b</sup>	2.11	2.50 <sup>a</sup>	0.31	0.52	0.62
	C	0.32 <sup>bcd</sup>	0.91	1.25 <sup>ab</sup>	0.20 <sup>e</sup>	1.20	2.10 <sup>b</sup>	0.11	0.31	0.51
	CW	0.28 <sup>cde</sup>	0.82	1.11 <sup>bc</sup>	0.15 <sup>e</sup>	1.20	1.80 <sup>bc</sup>	0.11	0.20	0.31
VW	Sb	0.80 <sup>a</sup>	1.00	1.30 <sup>ab</sup>	0.81 <sup>c</sup>	1.91	2.51 <sup>a</sup>	0.21	0.42	0.53
	C	0.50 <sup>bc</sup>	0.80	1.10 <sup>bc</sup>	0.21 <sup>e</sup>	1.20	2.15 <sup>b</sup>	0.12	0.23	0.50
	CW	0.20 <sup>de</sup>	0.70	0.90 <sup>cd</sup>	0.21 <sup>e</sup>	1.11	1.90 <sup>b</sup>	0.11	0.20	0.30
½MS	Sb	0.90 <sup>a</sup>	1.11	1.20 <sup>b</sup>	1.81 <sup>a</sup>	2.21	2.52 <sup>a</sup>	0.21	0.40	0.62
	C	0.51 <sup>b</sup>	0.83	1.10 <sup>bc</sup>	1.20 <sup>e</sup>	1.61	2.51 <sup>a</sup>	0.15	0.30	0.52
	CW	0.52 <sup>b</sup>	0.81	1.04 <sup>bc</sup>	1.10 <sup>b</sup>	1.51	2.12 <sup>b</sup>	0.12	0.20	0.31
NP	Sb	0.32 <sup>bcdde</sup>	0.52	1.20 <sup>b</sup>	0.51 <sup>d</sup>	1.00	1.50 <sup>c</sup>	0.70	0.20	0.50
	C	0.12 <sup>e</sup>	0.21	0.51 <sup>e</sup>	0.21 <sup>e</sup>	0.82	1.10 <sup>d</sup>	0.45	0.18	0.30
	CW	0.13 <sup>e</sup>	0.32	0.81 <sup>d</sup>	0.22 <sup>e</sup>	0.91	1.81 <sup>bc</sup>	0.12	0.19	0.40
LSD at 1%		0.1994	NS	0.2500	0.1846	NS	0.3153	NS	NS	NS

In column, figures followed the same letter(s) do not significant at P<0.01

Where,

KC = Knudson C, VW = Vacin and Went, ½MS = ½Murasighe and Skoog, NP = New *Phalaenopsis*, C = Charcoal, Sb = Sabri banana, Cw = Coconut water, DAI = Different days after inoculation.

NS= Non-significant, LSD= Least significant difference.



**Fig. 2. Showing *in vitro* regeneration of *Dendrobium* orchid at 60 days after inoculation**

### Fresh weight of PLBs

Interaction of different media and organic additives showed significant difference in the fresh weight of PLBs at different days after inoculation. At 20 DAI, the highest (0.50 g) and lowest (0.12 g) fresh weights of PLBs were obtained from interaction of ½MS and Sb and KC and C, respectively. At 40 DAI, the highest (0.60 g) and lowest (0.20 g) fresh weights of PLBs were obtained from interaction of ½MS and Sb and KC and C, respectively. Accordingly at 60 DAI, the highest (0.91 g) and lowest (0.31 g) of the fresh weight of PLBs were obtained from the interaction of ½MS and Sb and VW and C, respectively (Table. 1). It appears from the present study, ½MS and Sb interaction showed superiority effect on fresh weight of PLBs over others at all DAIs. It might be due to presence of higher percentage (27%) of sucrose concentrations in Sb extract and higher nitrate, sulphate and relatively lower phosphate content of ½MS medium than others. Haque (1996) found similar effect in garlic (*Allium sativum*). Pathania *et al.* (1998) observed KC medium to be the best for PLBs formation of *Dendrobium* orchid supplemented with BAP and NAA. Baker *et al.* (1987) reported that *Phalaenopsis* developed protocorms on VW medium. Goh and Wang (1990) observed that PLB proliferation of *Aranda* orchid was better on liquid VW medium combined with Cw and sucrose.

### Numbers of PLBs

It was found that the number of PLBs explant<sup>-1</sup> varied significantly with interaction of different media and organic additives at different days after inoculation. At 20 DAI, the highest number (22.50/explant) and lowest (0.9/explant) of PLBs were obtained from interaction of ½MS and Sb and NP and C, respectively. Similar trend was obtained at 40 and 60 DAI (Table. 1). Higher nitrate, sulphate and relatively lower phosphate content of ½MS medium had a promotive effect on the number of PLBs (Haque, 1996). Hye (2003) observed combination of KC+Sb+Cw+C produced 34.22/explant PLBs. Pathania *et al.* (1998) obtained 13.6 PLBs on VW medium with Cw after 45 days. Wang *et al.* (1996) found a few protocorm was induced on KC medium. Rovindra *et al.* (2004) observed the highest percentage of protocorm like bodies (95%) of *Vanda coeruleae* survived in VW medium supplemented with 11.35 µM thidiazuron. Kalpona *et al.* (2000) observed VW medium supplemented with a combination of 3% banana pulp and 10% coconut water was more effective and enhanced the production of PLBs of *Dendrobium* orchid. Lee and Lee (2003) found 13 PLBs on MS medium supplemented with 4.44 µM N6 benzyl adenine. These partially support the present study.

### Number of shoots

Interaction of different media and organic additives showed significant effect on the number of shoots explant<sup>-1</sup> at different days after inoculation. At 20 DAI, the highest (17.50/explant) and lowest (0.5/explant) was obtained from interaction of ½MS and Sb and VW and C, respectively. At both 40 and 60 DAI, the highest and lowest of the number of shoots explant<sup>-1</sup> were obtained from interaction of same media and organic extracts. (Table.1). ½MS and Sb appeared to be the best interaction over others at all DAIs due to presence of higher percentage (27%) of sucrose concentrations in Sb extract and higher nitrate, sulphate and relatively lower phosphate content of ½MS medium than others. Lee and Lee, (2003) found that number shoots of *Sarcanthus scolopndrifotius* were increased on KC with NAA. Yesmin (2005) showed that VW medium supplemented Sb and C was the best for shoot (3.33/explant) production of *Dendrobium* hybrid orchid. Rovindra *et al.* (2004) found proliferating shoots of *Vanda coeruleae* when PLBs were cultured on VW media with 11.35 µM thidiazuron. Shoot regeneration from the protocorm of *Vanda teres* was found by Sinha and Roy (2003) in ½MS medium with coconut water. Bhadra and Hossain (2003) observed that the elongated seedlings of *Goedrum* grew on ½MS medium. Sudeep *et al.*, (1997) observed that coconut water (5, 10 or 15%) increased the number of shoots of *Dendrobium nobile* with ½MS medium. Tasy (2000) observed that axillary shoots of *A. formosanus* elongated and rooted when subcultured on agar-gelled ½MS medium with 2.0 mg/BA+0.5 mg/NAA activated charcoal.

### Length of shoot

Interaction of different media and organic additives showed a significant difference on the number of shoots explant<sup>-1</sup> at 20 and 60 DAI. No significant variation of the length of shoots was found

among the interaction of different media and organic additives at 40 DAI. At 20 DAI, the longest (0.90 cm) and shortest (0.12 cm) shoots were found from the interaction of ½MS and Sb and NP and Cw, respectively. Accordingly, at 60 DAI the longest (1.5 cm) and shortest (0.51 cm) shoots were found from the interaction of KC and Sb and NP and C, respectively (Table. 1). It might be due to decrease the vitamin contents of ½MS medium with timing. Yesmin (2005) found 1.90, 3.00 and 4.10 cm length shoot of *Dendrobium* orchid from KC+Sb+ C at 20, 40 and 60 DAI. Hye (2005) found the maximum length of shoot (6.88 cm) on KC+Sb+CW+C at 90 DAI. Sudeep *et al.*, (1997) observed that VW media supplemented with 10% banana pulp increased shoot length of *Vanda* orchid. They also observed that the shoot of *Dendrobium nobile* was the highest on ½MS medium with 2.0 mg/l NAA.

### Number of leaves

Interaction of different media and organic extracts showed a significant difference on the number of leaves plantlet<sup>-1</sup> at 20 and 60 DAI. At 20 DAI, the highest (1.81/plantlet) and lowest (0.15/plantlet) number of leaves explant<sup>-1</sup> was found on the interaction of ½MS and Sb and KC and Cw, respectively. At 40 DAI, no significant difference of the number of leaves explant<sup>-1</sup> was found among the interaction of different media and organic extracts. At 40 and 60 DAI, the highest number of leaves was found from the ½MS and Sb interaction (Table 1). Partially support result showed by Suddep *et al.* (1997), where they observed banana pulp with VW medium significantly increased the leaf number of *Dendrobium nobile*.

### Length of leaves

No significant differences of the length of leaves were found among the interaction of different media and organic additives at all DAIs. At 40 and 60 DAI, the highest length of leaves (0.52 and 0.62 cm) was found from the KC and Sb interaction (Table 1). It might be due to the presence of higher percentage (27%) of sucrose concentrations in Sb extract. It partially agrees with Minea *et al.* (2004) where they observed that VW medium with 10 gL<sup>-1</sup> blended banana had positive effect on leaf size of *Spathoglottis kimbali*.

### CONCLUSION

From the above findings, it may be concluded that ½MS medium and Sabri banana pulp interaction performed best for *in vitro* regeneration of *Dendrobium* orchid comparison than that of other media and organic additives. The protocol developed in this study may be useful for improving the orchid species of Bangladesh using different biotechnological approaches. It is also important factor for shortening of growth period and rapidly propagates of orchid.

### LITERATURE CITED

- Arditti, J. 1967. "Orchid Biology: Reviews and Perspective". Cornell University Press, Ithaca, New York. pp. 114-1255
- Baker, K. M., Mathes, M. L. and Wallace, B. J. 1987. Germination of *Pantheva* and *Cattleya* seeds and development of *Phalaenopsis* protocorms. *Lindleyana* **2**(2), 7783.
- Bhadra, S. K. and Hossain, M. M. 2003. *In vitro* germination and micropropagation of *Geodorum densiflorum* (Lam.) Schltr., an endangered orchid species. *Plant Tissue Cult* **13**(2), 165-171.
- Chowdhury, I., Rahman. A. R. M., Islam, M. O and Matsui., S. 2003. Effects of plant growth regulators on callus proliferation, plantlet regeneration and growth of plantlets of *Doritaenopsis* orchid. *J Biotec* **3**(2), 214-221.
- Goh, C. J. and Wang, P. F. 1990. Micropropagation of the monopodial orchid hybrid *Aranda deborah* using inflorescence explants. *Scientia Hort* **44**(3-4), 315-321.
- Haque, M. S. 1996. Studies on the micropropagation of garlic (*Allium sativum*). Unpublished [MS Thesis], Lab. of Plant Genetics and Breeding, School of Agricultural Science, Nagoya University, Japan.

- Hye, M. A. 2003. Organogenesis of hybrid orchid with different media supplementation. Unpublished [MS Thesis], Department of Horticulure, Bangladesh Agricultural University, Mymensingh.
- Jain, R. K. 1997. Effects of some factor on plant regeneration from *indica* rice cells and protoplasts: A review. *Indian J Biol* **35**, 323-331.
- Kalpona, S., Sathyanarayana, B. N. and Sachdev, K. 2000. Effect of coconut water and banana pulp on *in vitro* culture of *Dendrobium*. *J Plant Biol* **29**(2), 209- 210.
- Khanna, H. K. and Raina, S. K. 1998. Genotype X culture media interaction effects on regeneration response of three *indica* rice cultivars. *Plant Cell Tissue Organ Cult* **52**(3), 145-153.
- Knudson, C. 1946. A new nutrient solution for germination of orchid seed. *Amer Orchid Soc Bull* **15**, 214-217.
- Lee, Y. L. and Lee, N. 2003. Plant regeneration from protocorm derived callus of *Cypripedium formosanum*. *In vitro Cell Dev Biol* **39**(5), 475-479.
- Lim-Ho, E. L., Lee, G. C. and Phua, L. K. 1982. Clonal propagation of orchids from flower buds. In "Proceedings of 50<sup>th</sup> Asian Orchid Congress", Singapore, pp. 90-110.
- Minea, M., Piluek, C., Menakanit, A. and Tantiwiwat, S. 2004. A study on seed germination and seedling development of *Spathoglottis* orchids, *Kasetsart J Nati Sci* **38**, 141-156.
- Murashige, I. and Skoog, F. 1962. A revised medium for rapid growth and bioassys with tobacco tissue culture. *Plant Physiol* **15**, 473-497.
- Nasiruddin, K. M., Begum, R. and Yesmin, S. 2003. Protocorm like bodies and plantlet regeneration from *Dendrobium formosum* leaf callus. *J Plant Sci* **2**(13), 955-957.
- Pathania, N. S., Sehgal, O. P., Debojit, P., Dilta, B. S. and Paul, D. 1998. Studies on micropropagation in *Dendrobium* cv. Sonia. *J Orchid Soc* **12**(1-2), 35-38.
- Rovindra, B., Mulagund, G. S. and Natarija, K. 2004. Efficient regeneration of *Vanda coerulea* an endangered orchid using thidiazuron. *Plant Tissue Cult* **14**(1), 55-61.
- Sinha, P. and Roy, S. K. 2003. Regeneration of an indigenous orchid, *Vanda teres* (roxb.) *Plant Tissue Cult* **13**(2), 165-171.
- Suddep, R., Rajeevan, P. K., Valsalakumari, P. K. and Geetha, C. K. 1997. Influence of organic supplements on shoot proliferation in *Dendrobium*. *J Hort* **3**(1-2), 38-44.
- Tasy, H. S. 2000. Use of tissue culture for the mass propagation of pathogen free plants. *Plant Cell Reports* **17**, 670-674.
- Vacin, E. and Went, F. 1949. Some pH changes in nutrient solution. *Bot Gardens Conserv News* **110**, 605-613.
- Wang, Y., Yang, Z., Wang, Y. F. and Yang, Z. P. 1996. A study on *in vitro* propagation of *Phalaenopsis* hybrid and *Cattleya auranliace*. *Acta Agric* **12**(4), 59-62.
- Yesmin, S. 2005. Organogenesis of *Dendrobium* orchid with different media and organic supplementation. Unpublished [MS Thesis], Department of Biotechnology, BAU, Mymensingh.