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ON

Micropropagation of *Dendrobium* Orchid as Influenced by Different Culture Media

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Course Instructors	Major Professor
Dr. Md. Mizanur Rahman Professor **Dr. A. K. M. Aminul Islam** Professor **Dr. Md. Rafiqul Islam** Professor **Dr. Dinesh Chandra Shaha** Assistant professor **BSMRAU**	**Dr. M. Mofazzal Hossain** **Professor** **Department of Horticulture** **BSMRAU, Gazipur**

SUBMITTED BY

Rafia Akhtar Rimu
Reg. no.:13-05-2964
Department of Horticulture

Bangabandhu sheikh Mujibur Rahman Agricultural Univarsity

Salna, Gazipur-1706

Micropropagation of *Dendrobium* Orchid as Influenced by Different Culture Media

Abstract

Orchids are normally propagated through micropropagation technique. For successful micropropagation , culture media is the most important components. *Dendrobium* orchid is normally is normally cultivated in Bangladesh. For regeneration of *Dendrobium* orchid , among different combination of culture media with organic additives, MS medium with sabri banana performs better compare to other combination. Number of shoots, number of PLBs and fresh weight, in all cases this combination give better results. In case of Plant growth regulators, among different concentration of 2,4-D, 10 mgL^{-l} 2,4-D gives better performance in plantlet regeneration in *Dendrobium* orchid. In *Dendrobium chrysanthum,* Gamborg et al.(B_5) media give high germination (96.7) percentage. But MS media performs better in growth and developments of *Dendrobium chrysanthum.* Best result found in 1.0 BA + 1.5 IBA (mg/l), in case of *Dendrobium bensoniae.* MS medium performs better in case of *Dendrobium transparens.* The maximum seed germination (78%) was observed in MS medium, which was followed by Hyponex medium (73%). Survival percentage also higher in MS medium in case of *Dendrobium transparens.*

Key word : Micropropagation, Culture media, Plant Growth Regulators.

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CHAPTER I
INTRODUCTION

Orchids are flowering plants, commercially grown worldwide as cut flower and potted plants in floriculture trade. Orchidaceae is the largest, including most multifariousness family of flowering plants, consisting of more than 25,000 species belonging to 700 - 800 genera (Begum, 2000). It included both terrestrial and epiphytic orchids. Due to their ornamental and medicinal importance they demand a very high price in the international market. Different kinds of orchids are indigenous to Bangladesh; those are *Rhyncostylis* sp., *Pierardi* sp., *Arides* sp., *Dendrobium* sp., *Cymbidium* sp., *Arnada* sp., *Arathera* sp., *Bokthara* sp., *Eridis* sp., *Miltonia* sp., *Hoya* sp., *Vanda* sp. These orchids are found naturally growing or anchoring on the mango tree, wood apple tree, tamarind tree, rain tree, sissoo etc. (Kabir, 2012). In the world, the genus Dendrobium having more than 1100 species. They are widely distributed and cultivated in the world ranging from southern Asia to New Guinea and Australia (Puchooa, 2004). It is the most popular orchids all over the world, also in Bangladesh. Different characteristics of *dendrobium* such as rapid growth, easiness of plantlet regeneration, beauty of the flower, and year round production in control flowering and long lasting of the flower stalk are very advantageous of *this* genus. (Talukder *et al.,* 2003). Different *Dendrobium* species including *D. aphyllum, D. transparens, D. densiflorum, D. fimbriatum* and *D. nobile* are the indegenous species found in India. Orchids are normally grown in commercially, the tropical regions of different countries of Asia like Nepal, Bhutan, India, Thailand, Bangladesh etc. Orchids can grow in nature through seeds but due to lack of suitable hosts they don't germinate in adequate numbers, so still now it considered as a rare species. This problem may be solved by adopting tissue culture technique. For appropriate germination of orchid seeds, micropropagation is appropriate for multiplication rather than *in vivo* (Arditti, 1979). A large number of orchid varieties with beautiful flowers are available in Bangladesh. In Bangladesh, the environmental conditions essential for the survival and culture of orchid are adequately suitable throughout the year. Different species of orchids also *Dendrobium* sp. are abundantly distributed in the country (Chowdhury, 1975. There is a great scope for large scale production of orchid as *Dendrobium* orchid in Bangladesh to meet the demand of international market and to earn foreign currency through export (Mondal, 2011). For cultivating commercially, appropriate combination and concentrations of hormones, organic

additives and the composition of macro and micro elements in the culture medium were of key importance for micropropagation of *Dendrobium* orchid.

Objectives

1. To assess the effects of organic additives with culture media for regeneration of *Dendrobium* orchid.
2. To identify the effects of Plants Growth Regulators on growth and developments of *Dendrobium* orchid.
3. To review the suitable culture media for germination, growth and development of *Dendrobium chrysanthum*.
4. To investigate the combined effect of BA+IBA for regeneration of *Dendrobium bensoniae.*
5. To know the effects of culture media on *Dendrobium transparens.*

CHAPTER II

MATERIALS AND METHODS

This seminar paper is exclusively a review paper. All data and information are adopted as a secondary data. It has been prepared by reviewing the various articles published in different Books, Proceedings, Abstracts, Review papers, Journals, MS thesis, Ph.D. Dissertation etc. available in the library of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur . For collecting recent information, I visited different websites through internet. The necessary thoughts, ideas, facts and findings has been collected through internet searching and incorporated with the body of the seminar. I prepared this paper in consultation with my learned major professor, and other concerned experts. After collecting necessary information, it has been compiled and arranged chronologically for better understanding and clarification.

CHAPTER III
REVIEW of FINDINGS

Orchid propagation

Orchids can easily grow naturally through seeds but in absence of hosts they can not germinate properly. Always it is considered that orchids are difficult to grow or cultivate. It can also propagated through budding and grafting but this technique has limitation both qualitatively and quantitatively (Nilufar , 2000).These propagation technique are beneficial because its produce exact clones unlike sexual reproduction. However, that technique is not use for the fastest method of propagation. Seed germination is another method of propagation but through this method, genetically identical plantlets cannot be obtained. Also, as the orchids seeds are very small with small reserves and having no endosperm, they need to be germinated in a nutrient medium for best results. Moreover, tissue culture techniques or micropropagation have been widely used for the mass multiplication of several commercially important orchids like Dendrobium (Kanjilal *et al*., 1999; Malabadi *et al*., 2004). If a plant is attacked by a disease, it is possible in tissue culture technique, to take a very small piece of the apical meristem from a shoot, as meristem is virus free and culture it to create a disease free plant. So for large scale rapid propagation it is essential to go through micropropagation. Orchids can easily propagated through in virto propagation or tissue culture technique by using different plant parts as explants such as seeds, shoot tip , flower bud segment, lateral bud, young inflorescence, inflorescence node and pseudobulb (Martin K. P., Madassery, 2006). Other part, as leaf and root also propagated (Nilufar, 2000). Through micropropagation technique we can produce large number of disease free plantlets at a very low cost. As orchid seeds are minute, numerous numbers produced in each capsule, these seeds possess small amount or no stored food material. These minute embryo can germinate easily and seedlings can grow, when the seeds are placed in suitable culture media. So for appropriate germination and survivability of seedlings, it is very essential to select proper culture media. Culture media is varied from variety to variety and species to species. For different *dendrobium* species, different culture media is suitable. Culture media also varied in a same species, as different plant part use as explant for same variety.

Effects of organic additives with different culture media for regeneration of *Dendrobium* Orchid

In vitro regeneration of plant is an essential element of plant biotechnology. The frequency of callus initiation and plantlets regeneration are influenced by many factors, such as genotypes, type of explants and composition of media (Jain, 1997). Nutrient composition and its concentration are considered to be major sources of variation in plant tissue culture (Khanna and Raina, 1998). Different culture media with different organic additives have been used for efficient plant regeneration in orchid tissue culture. MS medium was found to be most effective for PLBs formation and plantlet regeneration of *Dendrobium* orchid when added with 2, 4- D, compare to other culture media (Nasiruddin *et al*., 2003). Lim-Ho *et al*. (1982) reported that VW medium with organic additives gives the maximum increase in fresh weight and highest numbers of leaves and roots regenerated in NP medium (New *Phalaenopsis*) gave better results when mixed with high concentrations of BAP (Chowdhury *et al*., 2003). A large number of organic additives as coconut water, banana pulp, tomato juice, slap honey and beef extract can be very efficiently used with undefined mixture of organic nutrients and growth factors. Some complex organic additives were reported satisfactory while some were unsatisfactory and may also inhibitory, in case of plantlets regeneration (Arditti, 1967). Due to this reasons, suitable media and organic additives are needed for large-scale multiplication of orchid.

In vitro multiple PLBs of dendrobium orchid were cultured on the VW (Vacin and Went, 1949), KC (Knudson C, 1946), half strength MS (Murasighe and Skoog, 1962) media , that was supplemented with charcoal (0. 1% w/v), Sabri banana (Sb) pulp (10% w/v) and coconut water (10% v/v). And the media pH was adjusted to 5.8. To solidify the culture media, agar powder (10 gL^{-1}) was added. The culture media were autoclaved with 1210C for 20 minutes. Those culture vials were placed in a growth chamber and kept to grow at 25°C under 16 hour photoperiod with fluorescent tube of 2000-3000 lux. The data were collected and recorded at 40 days interval up to 60 days on fresh weight of PLBs, number of PLBs per explant, number of shoots per explant (S. Aktar *et al*., 2008).

Fresh weight of PLBs per explant

Interaction of different media, in combination with organic additives showed significant difference in the fresh weight of PLBs at different days after inoculation. At 40 DAI, the maximum (0.60 g) and minimum (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 maximum (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 means that, ½MS and Sb interaction showed superiority effect on fresh weight of PLBs over others at 40 and 60 DAIs. It occurred due to presence of higher percentage (27%) of sucrose concentrations in Sb extract and higher amount of nitrate, sulphate and relatively lower phosphate content of ½MS medium than others. Haque (1996) found similar results in garlic micropropagation. Pathania *et al*. (1998) reported KC medium to be the best for PLBs formation of *Dendrobium* orchid supplemented with BAP and NAA. Goh and Wang (1990) stated that PLB regeneration of *Aranda* orchid was better on liquid VW medium combined with Cw and sucrose (S. Aktar *et al*., 2008).

Numbers of PLBs per explant

It reported that the number of PLBs per explant significantly different with interaction of culture media and organic additives at different days after inoculation. At 40 and 60 DAI, the maximum and minimum number of PLBs was obtained from interaction of ½MS and Sb and VW and C, respectively. Higher nitrate, sulphate and relatively lower phosphate content of the medium had a promotive effect on the number of PLBs (Haque, 1996) that is occurred in ½MS and Sb media. Hye (2003) reported that combination of KC+Sb+Cw+C produced 34.22/explant PLBs. Pathania *et al*. (1998) reported that 13.6 PLBs on VW medium with Cw after 45 days. Wang *et al*. (1996) found that only a few protocorn was induced on KC medium. Kalpona *et al*. (2000) reported that, VW medium mixed with a combination of 3% banana pulp and 10% coconut water was more useful and enhanced the production of PLBs of *orchid*. Lee and Lee (2003) observed that 13 PLBs on MS medium supplemented with N6 benzyl adenine. That findings also partially support the AKTAR *et al*. , (2008) findings.

Table 1: Effect of different combination of culture media and organic additives on growth and development of plantlet at 40 and 60 days after inoculation (DAI)

Media	Organic additives	Fresh weight of PLBs (g)		Number of PLBs per explant		Number of shoots per explant	
		40 DAI	60 DAI	40 DAI	60 DAI	40 DAI	60 DAI
KC (Knudson C)	Sb (Sabri banana)	0.50	0.7	12.50	15.3	10.20	15.30
	C(Charcoal)	0.20	0.47	13.20	40.36	6.52	9.93
	Cw (Coconut water)	0.28	0.48	10.20	18.50	5.50	7.50
VW (Vacin and Went)	Sb (Sabri banana)	0.5	0.70	12.3	17.50	20.40	35.50
	C (Charcoal)	0.25	0.31	3.15	6.25	2.50	12.50
	Cw (Coconut water)	0.34	0.49	15.20	30	5.50	9.50
½MS (½Murasighe and Skoog)	Sb (Sabri banana)	0.6	0.91	35.50	50.00	25.20	40.30
	C (Charcoal)	0.31	0.44	20.20	30.20	6.70	12.20
	Cw(Coconut water,)	0.24	0.34	10.10	20.30	8.20	22.30

(Source: Aktar *et al.,* 2008)

Number of shoots per explant

Interaction of different culture media and organic additives showed significant difference on the number of shoots per explant at different days after inoculation. At 40 DAI, the highest (25.20/explant) and lowest (2.5/explant) was obtained from interaction of ½MS and Sb and VW and C, respectively. At 60 DAI, the highest and lowest of the number of shoots per explant were obtained from interaction of same media and organic extracts. (Table.1). ½MS and Sb appeared

to be the best interaction over others interaction 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 and Sb medium than others. Yesmin (2005) found that VW medium added with Sb and C was reported to the best for shoot (3.33/explant) production of *Dendrobium* hybrid orchid. Sudeep *et al.*, (1997) found that coconut water (5, 10 or 15%) increased the number of shoots of *Dendrobiun nobile*, supplemented with ½MS medium.

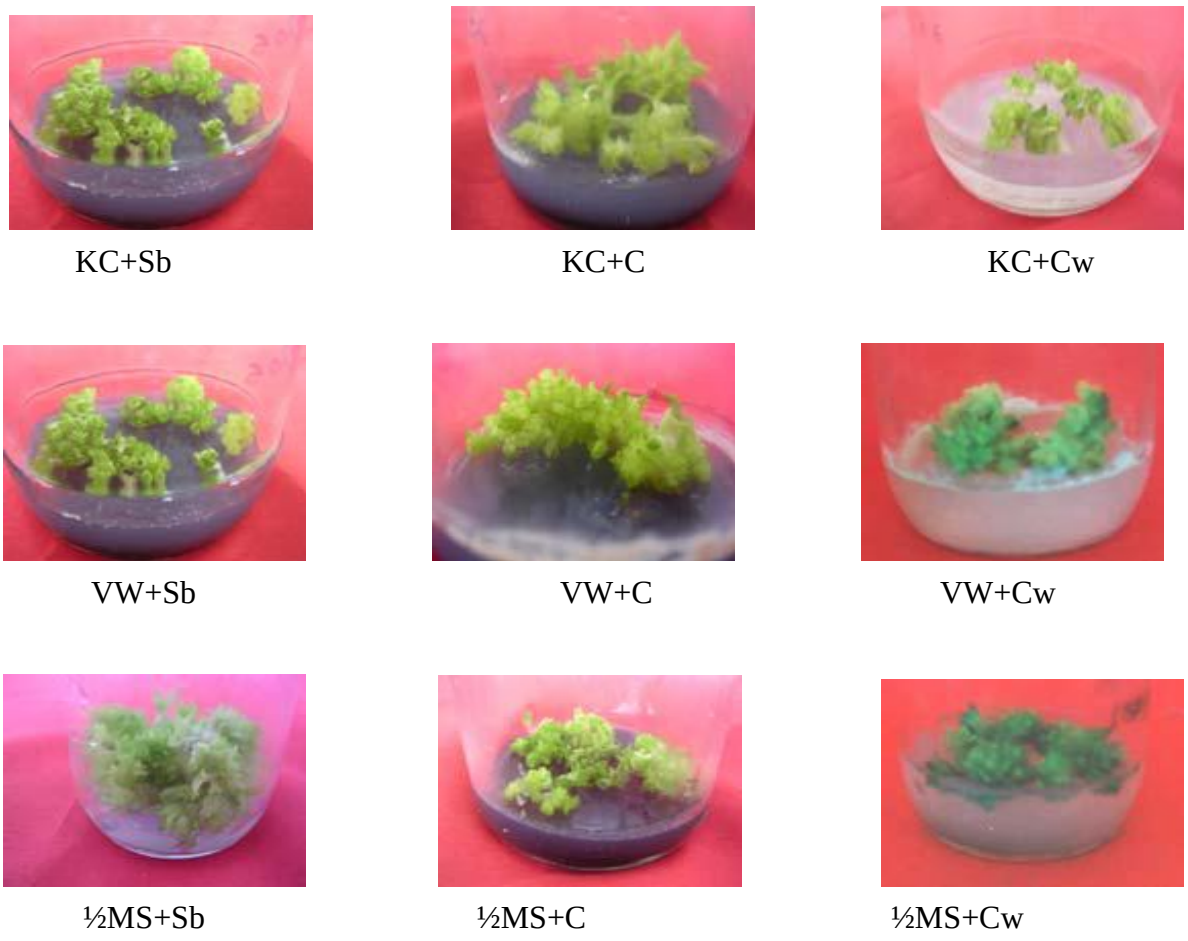


Figure 1 : *In vitro* regeneration of *Dendrobium* orchid at 60 days after inoculation.

(Source: S. Aktar et al., 2008)

Effects of Plant Growth Regulators on growth and development of *Dendrobium* orchid

In this experiment leaf tips of *Dendrobium* sp. of orchid were used as explants. Explants were placed on the MS (Murashige and Skoog, 1962) medium, added with plant growth regulators. The pH of the medium was setted to 5.2 with 1N KOH or HCl before autoclaving for 15 min at 121^0C. Full strength MS (Murashige and Skoog) medium was used for the development of PLBs (Protocorm Like Bodies) from leaf tips and shoots regeneration from PLBs. Without this, half strength MS medium was also used for the subculture of PLBs and development of roots. Five different concentrations of 2, 4-D (0, 0.5, 2.5, 5, 10 mgL^{-1}) were added to MS medium for the development of PLBs from leaf tips. And for the development of shoot, BAP (0, 0.5, 2.5, 5 mgL^{-1}) and NAA (0, 0.5, 2.5, 5 mgL^{-1}) was used in different concentration. After 60 days of culture, data were recorded in different parameters as number and length of PLBs, root, shoot (K. Goswami *et al*., 2015).

Effect of 2, 4-D (2,4-D dichlorophenoxy acetic acid) on growth and development of PLBs (Protocorm like bodies), from leaf tips

Number of PLBs (Protocorm like bodies) per vial:

Concentration of 2, 4–D having significant effect on the growth and development of plant (Samad, 2011). 2, 4-D is a synthetic auxin, and regarded as plant hormones. It is assimilated through the leaves and is translocated to the meristems of the plant. The maximum number of PLBs (16.0) was recorded in 10 mgL^{-1} 2, 4-D (Fig.2) and minimum number of PLBs (2.0) was observed at 0 mgL^{-1} 2, 4-D after 60 days of culture using leaf tips (Fig. 2)

High concentration of 2, 4-D has the capabilities to proliferate plant within very short time (Fig.2). This result partially supports the findings of Jaime and Teixeira (2014) who observed that the fresh weight of plantlets and PLBs production increased in the presence of high levels (8mg-l) of 2, 4-D. (K. Goswami et al., 2015).

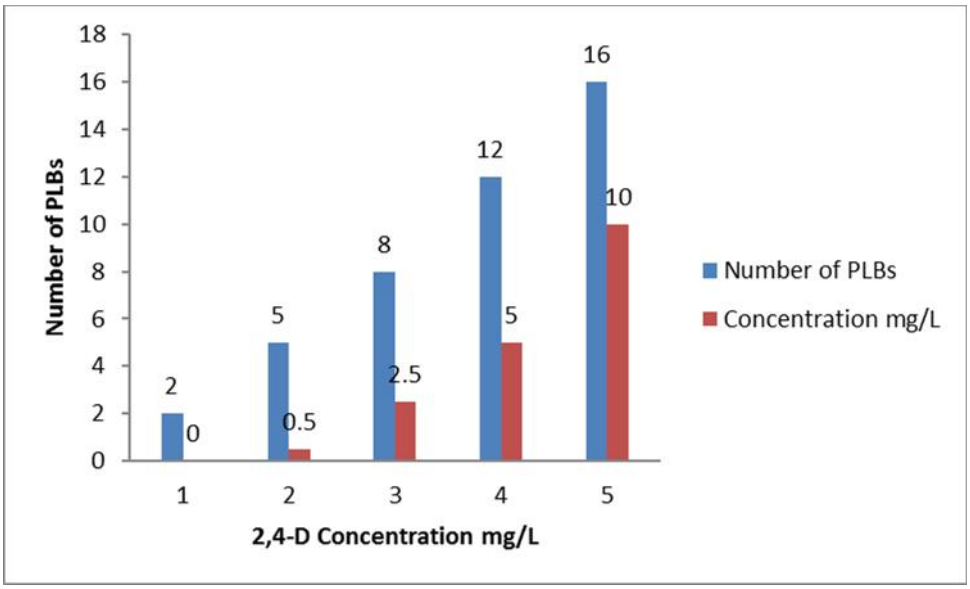


Figure 2: Effects of 2, 4-D on the number of PLBs (Protocorm like bodies) formation from leaf tips. (Source: K. Goswami et al., 2015)

Subculture of PLBs

Proliferated PLBs were subcultured onto half strength MS medium. Large number of PLBs was found by the subculture within few days (Fig.3.III). I. Initiation of PLBs from single leaf tip of *Dendrobium* sp. On MS medium supplemented with 10 mgL^{-1} 2, 4–D after 22 days of culture. II. Proliferation of PLBs in MS medium supplemented with 10 mgL^{-1} 2, 4-D after 60 days of culture III. Subculture of PLBs into half strength MS media.

I II III

Figure 3: Initiation of PLBs from single leaf tip of *Dendrobium* sp. on MS medium.

(Source: K. Goswami *et al*., 2015).

PLBs formation (%)

Plant regeneration is influenced by high concentration of 2, 4-D (Gaj, 2004). The highest number of PLBs formation (90%) was observed in 10 mgL^{-1} 2, 4-D and minimum response (30%) was found at 0 mgL^{-1} 2, 4-D after 60 days of culture (Fig.4). This founding partially supports the observation of Lee (1999) who treated *Cymbidium* orchid with 2, 4-D and PLBs formation response was accelerated with the 2, 4-D concentration. (K. Goswami *et al*., 2015).

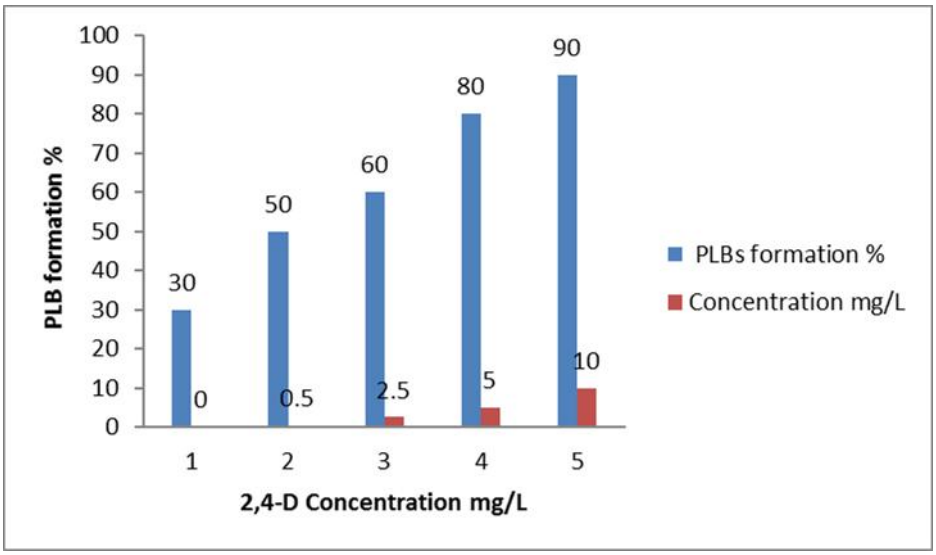


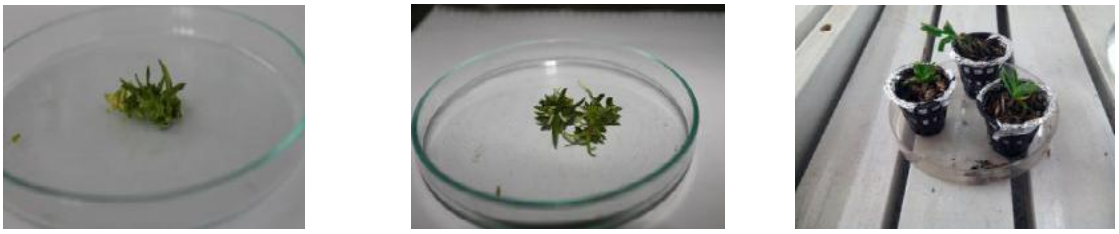
Figure 4: Effects of different concentrations of 2, 4-D on PLBs formation (%) after 60 days of culture. (Source: K. Goswami *et al*., 2015)

Combined effect of NAA (Napthaleneacetic acid) and BAP (Benzyl amino purine) on plantlet regeneration

Effects on shoot regeneration

MS medium prepared with different concentrations and combination of NAA and BAP significantly influenced the number, weight and height of shoots. The highest number of shoots (11.00) was observed in 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP (Fig.3.a). Again lowest number of shoots (0.66) per vial was found at 0 mgL^{-1} NAA + 0 mgL^{-1} BAP followed by 5 mgL^{-1} BAP + 0 mgL^{-1} NAA after 60 days of culture (Table-1). BAP is a first-generation synthetic cytokinin that affects plant growth and development responses, setting flower and stimulating fruit richness by stimulating cell division. NAA acts as auxin. Although high concentration of auxin and

cytokinin sometimes induced toxicity. The shoots length were influenced significantly due to supplement of NAA and BAP into the medium. After 60 days of culture. The highest shoot length (3.61cm) was observed in 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP and lowest shoot length (1.33 cm) was found at 0 mgL^{-1} NAA + 0 mgL^{-1} BAP (Table-2). That findings also partially supported by Khatun (2005) who recorded that 0.5 mg each of BAP and NAA performed better growth and development of orchid. **.** I. Initiation of shoot in MS medium supplemented with 0.5mgL^{-l} NAA+ 0.5 mgL^{-l} BAP, II. Proliferation of roots of *Dendrobium* sp. orchid on half strength MS medium after 60 days of culture and III. Hardening of rooted plantlets. (K. Goswami *et al*., 2015).



I II III

Figure 5: Shoot initiation in MS medium supplemented with NAA and BAP.

(Source: K. Goswami *et al*., 2015).

Effects on fresh weight of shoot

Fresh weight of shoot was also significantly affected by combination of NAA and BAP. The highest fresh weight (0.62g) of was observed in 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP and lowest fresh weight (0.06g) of shoots was found (Prasad *et al.* 2001) at 0 mgL^{-1} NAA + 0 mgL^{-1} BAP (Table-2).

Effects on number of root

The highest number of roots (4.00) was observed in 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP and lowest number of roots (0.33) per vial was found at 5 mgL^{-1} NAA + 5 mgL^{-1} BAP (Table2). These findings were supported by Nayak *et al.* (1998) who found that a NAA and BAP combination induced rooting in regenerated shoots thereby producing complete plantlets. Significant effect was observed on the effect of NAA and BAP which influenced the roots length

after 60 days of culture. The highest root length (1.62cm) was observed in 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP and minimum root length (0.41cm) was found at 5 mgL^{-1} NAA + 5 mgL^{-1} BAP. That findings partially supported by Khatun (2005) who showed that 0.5 mgL^{-1} each of BAP and NAA performed better growth and development of orchid. However, high concentrations of auxin inhibit root elongation instead of initiating adventitious root formation. Similarly, cytokinin also could not act properly in high concentration. (K. Goswami *et al*., 2015).

Plantlets regeneration (%)

NAA and BAP had significant effects on the percentage of plantlet regeneration. Although individually BAP and NAA having capabilities to regenerate plant but BAP along with NAA was very effective for plant regeneration. The maximum percentage of plantlets regeneration (93.33%) was observed in 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP and the minimum plantlets regeneration (26.67%) was found at 0 mgL^{-1} NAA + 0 mgL^{-1} BAP (Table-2). (K. Goswami *et al*., 2015).

Table 2. Combined effect of different concentrations of NAA and BAP on plantlet regeneration at 60 days after culture

BAP (mg L^{-1})	NAA (mg L^{-1})	Number of shoots	Weight of shoots (g)	Length of shoot (cm)	Number of roots	Planlet regeneration (%)
0	0	0.667	0.66	1.33	1.00	26.67
0.5	0.5	11	0.623	3.61	4.00	93.33
2.5	2.5	4.00	0.423	2.82	2.66	66.67
5	5	1.00	0.2	2.00	0.333	46.67

(Source: K. Goswami *et al.,* 2015)

Effects of culture media on germination, growth and development of *Dendrobium chrysanthum*

Figure 6: Picture of *Dendrobium chrysanthum*

(Source: www.google.com)

Asymbiotic seed germination in in-vitro conditions is one of the best methods of conservation and propagation of orchids seed (Rasmussen, 1995). The highest seed germination percentage was found in B5 medium (96.7%) followed by Nitsch and Nitsch (NN) (94.4%), MS (94.1%) and the least was recorded in KC (29.7%) (Table 3). A balanced supply of both organic and inorganic nutrients or additive is needed for the germination of orchid seeds (Arditti, 1982). Plant growth regulators, i.e., cytokinins are recorded to play vital role in orchid seed germination (Manning, 1987). But in this experiment the seeds of *D. chrysanthum* germinated in medium that is devoid of growth regulators. This could be possible due to the availability of sufficient endogenous growth regulators needed for the initial stages of germination of orchid seeds. The presence of nitrogen in the form of potassium nitrate in B5 medium could have possible reason for the high percentage of germination of orchid seeds. Also, the presence of the vitamin, thiamine in higher amount in B5 might have influenced the germination of these seeds (Sharma, 1991). The largest protocorm volume was recorded in MS media (29.95x 10^{-4} mm3). This happened due to MS medium is rich in both micro nutrients and macro nutrients. The smallest protocorm volume was found in KC media (1. 8 x 10^{-4} mm3). The poor response in terms of seed germination and growth of the protocorms was recorded in KC (Knudson C) medium.(Subarna, 2010).

Table 3: Effect of different media on germination of seed and protocorms development of *Dendrobium chrysanthum*

Media	Germination %	Volume (mm^3)
Murashige and Skoog	94.1b	**29.95 x 10^{-4}a**
Nitsch and Nitsch	94.4b	8.78 x 10^{-4}c
Gamborg et al.(B_5)	**96.7a**	13.61 x 10^{-4}b
Knudson C	29.7c	1.80 x 10^{-4}d

(Source: Modified from Subarna Hajong et al., 2010)

The efficient assimilation and utilization of nitrogen is needed for the further development of protocorms into seedlings, in the form of ammonium nitrate that present in the MS medium. The growth of the seedlings viz., shoot number (1.90), shoot length (1.17), number of leaves (2.80), root number (3.05) and root length (0.80) was also found to be highest in MS medium (Table 4). In Nitsch and Nitsch (NN) and Gamborg et al. (B5) media, the protocorms formed shoots and roots but growth stopped after 90 days. This might be attributed to the negative impact of nitrogen in the form of ammonium sulphate in B_5 medium or mixtures of vitamins present in both B_5 and NN media on seedling growth (Sharma, 1991).

Table 4: Effect of different media on growth and development of *Dendrobium chrysanthum* seedlings at 120 days

Media	Shoot no.	Shoot length (cm)	No. of leaves	Root no.	Root length (cm)
Murashige and Skoog	**1.90a**	**1.17a**	**2.80a**	**3.05a**	**0.80a**
Nitsch and Nitsch	1.26b	0.63b	2.55b	2.41b	0.50b
Gamborg et al.	1.43b	0.67b	2.41b	2.26b	0.38c

(Source: Modified from Subarna Hajong *et al*., 2010)

That might have been occurred due to the lower amount of nutrients and vitamins present in KC medium that were not sufficient for complete development of the seedlings. There are also similar finding of inhibition of germination of seed in epiphytic orchids in KC medium (Chaturvedi, 1987). The differential effects of orchid seeds to different nutrient media is due to

specific requirement of the specific species. Seedlings were hardened in a compost mixture comprising brick, charcoal, decaying litter in a ratio of 1:1:1 and a layer of moss on upper part. During the time of hardening it was observed that the transferred seedlings initially shed their leaves then produced new leaves. According to Preece and Sutter (1991) when seedlings transferred, must produce new leaves to adjust to new conditions due to enable effective photosynthesis and growth of the in vitro - raised plants (Preece and Sutter ,1991). This method that can be used for in vitro mass scale propagation of *D. chrysanthum* through asymbiotic seed germination wherein a maximum number of plants can be produced from seeds in basal MS medium.

Combined effect of BA (benzyl adenine) + IBA (indole-3-butyric acid) for regeneration of *Dendrobium bensoniae*

Figure 7: Picture of *Dendrobium bensoniae* .

(Source: www.google.com)

Explants of *D. bensoniae* were inoculated onto media composed of basal MS (Murashige and Skoog, 1962). This medium supplemented with the plant growth regulators. Different hormones were added separately to media according to the requirements. The trimmed shoot nodes were used as explant in this experiment. The explants were cultured on MS nutrient medium added with different concentrations of BA and IBA. The findings of the combined effect of different concentrations of BA+IBA had been presented below (Sahida , 2016).

Percentage of explant showing shoots induction

Significant difference were observed on percentage of explant showing shoot induction in presence of different concentrations of cytokinin and auxin supplementations. The maximum percentage (90%) of shoot induction was found in the treatment with 1.0 BA+2.0 IBA (mg/l) and the minimum percentage (25%) was induced in hormone free media in case of *D. bensoniae* (Table 5). Addition of IBA along with BA reduced induction and regeneration (Arditti , 1967), whereas, others reported that an appropriate combination of BA and IBA stimulated shoot formation (Roy and Banerjee, 2003).

Days to shoot initiation

The initiation of regeneration frequency was late in the control treatment, where no IBA and BA were added. The maximum number of days to shoot induction was recorded in the control treatment of *D. bensoniae* (26 days). Best result found in 1.0 BA + 1.5 IBA (mg/l), where required lowest days for *D. bensoniae* (15 days) (Table 5).

Average number of shoots, found per explant

Among the various combinations tested, BA (1.0 mg/1) and IBA (1.5 mg/l) was found to be most effective for the shoot multiplication. That findings showed that combination of BA and IBA is also suitable for shoot multiplication. Here 1.0 BA + 1.5 IBA (mg/l) gave the maximum number of shoots (3.67), whereas the minimum number of shoots (0.95) was found with hormone free MS media in *D. bensoniae* (Table 5). Vij and Kaur (1998) also reported similar findings where BA-enriched medium in combination with IBA favoured multiple shoot bud formation in *Dendrobium bensoniae*.

Average Days to leaf initiation

The maximum number of days to leaf initiation was recorded in control of *D. bensoniae* (37.21 days) and minimum days for *D. bensoniae* (17 days) recorded from 1.0 BA+1.5 IBA (mg/l) (Table 5).

Average number of leaves per explant

The number of leaves also increased with days increased after inoculation. The highest number of leaves was obtained at 60 DAI from these treatments compared to control. The maximum number of leaves per explant (9.33) was noticed from 1.0 BA+2.0 IBA (mg/l), whereas the minimum were (1.23 ± 1.0) in control treatment of *D. bensoniae* (Sahida , 2016).

Table 5: Efficacy of BA+IBA combinations in induction of shoots and leaves in *D. bensoniae*

Hormonal (BA+IBA) concentration (mg/l)	Number of shoots per explant	% of explants showing shoot induction	Initiation of regeneration (Days)	Days to leaf induction	Average number of leaves per explants (60 DAI)
MS (Control)	**0.95**	**25**	**26**	**37**	**1.23**
0.5 + 0.5	3	55	20	18	6.35
0.5 + 1.0	3.34	60	19	27	8
1.0 + 1.5	**3.67**	75	**15**	**17**	8.25
1.0+ 2.0	2.65	**90**	21	19	**9.33**

(Source: Sahida, 2016)

Average length of leaves per explant

The length of leaves was varied due to the various concentrations of BA+IBA supplementations. The maximum length of leaves per explant (1.00 cm) was founded from 0.5 BA+1.0 IBA (mg/l), whereas the minimum was (0.75 cm) in control of *D. bensoniae* (Sahida, 2016).

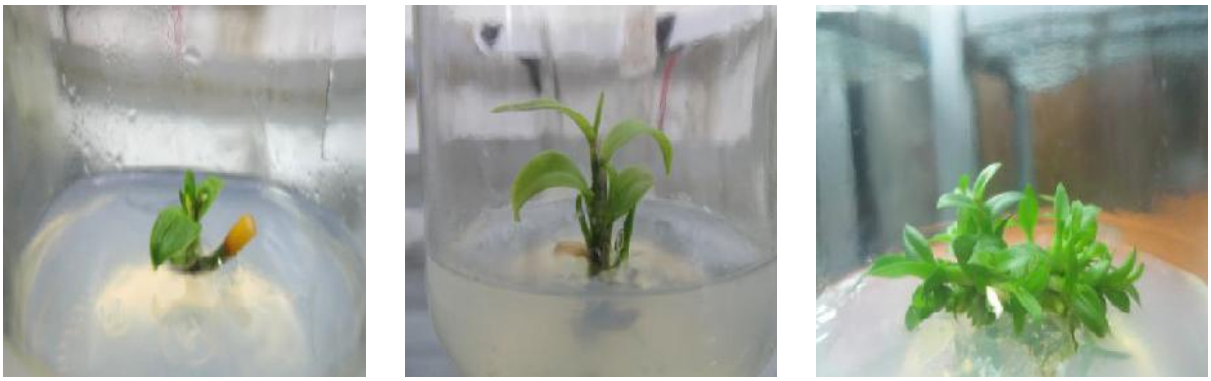


Figure 8: Shoot initiation, regeneration and multiplication of *D. bensoniae.*

(Source: Sahida, 2016)

Effects of culture media on *Dendrobium transparens*

Figure 9: Picture of *Dendrobium transparens.* (Source: www.google.com)

Four different media namely- Hyponex, Murashige and Skoog (MS), OKF1 and Knudson C (KC), were tested for multiplication of native orchid, *Dendrobium transparens* through seeds. MS medium was reported to be best for characters recorded in *Dendrobium transparens*, followed by Hyponex medium but OKF1 medium gave the worst performance. Days required to seed germination was the least (50 days) in MS medium while the highest days (59 days) was required by OKF1 medium. And other parameters, as days required for protocorm formation and plantlet regeneration, number of leaves, plantlet height and lastly plant survivability percentage, in all cases MS medium gave significantly better performance for in vitro seed propagation of *Dendrobium transparens* (Nilufar, 2000).

In vitro seed germination percentage

There was a highly significant variation was recorded in percentage of seed germination among different media.The maximum seed germination (78%) was observed in MS medium, which was followed by Hyponex medium (73%), on the other hand OKF1 gave the poor performance (58%). This findings is similar with that of Ismat (1982) findings who conducted an experiment with Dendrobium *pierardii* on MS and Hyponex media and observed percentage of seed germination was 79 and 70 respectively. Partially similar results were found by Hoque (1993) in case of *Dendrobium formusum* that percentage of seed germination was 81 and 74 in MS and Hyponex media respectively (Nilufar, 2000).

Required days to seed germination

Highly significant difference among different media had been recorded in required days to seed germination (Table 6). Maximum days (59 days) required to seed germination was recorded by OKF_1 medium while the minimum days (50 days) was required by MS medium. Ismat (1982) and Hoque (1993) reported that required days for seed germination of *Dendrobium* sp. in MS medium is 51 and 55 days respectively. That findings having similarity with this results.

Days required to protocorm formation and development of plantlet

The required days to protocorm formation and plantlet development were significantly different from different media (Table 6). In case of protocorm formation, highest duration (48 days) was required by OKF_1 medium which was significantly similar to Knudson C medium (43 days). But in case of MS medium it required the least period (36 days). Similar findings were also observed in planlet development (Table 6). Hoque (1993) reported that protocorms transformed into plantlets within 72-78 days in different culture media which is partially similar to the present findings.

Table 6: Effects of different media on percentage of seed germination percentage and days required to seed germination, protocorm formation and development of plantlet of *Dendrobium transparens*

Treatments	%Seed germination	Days required to		
		Seed germination	Protocorm formation from seed germination	Plantlet development from protocorm formation
Hyponex	73a	55ab	39bc	83bc
MS	**78a**	50b	36c	79c
OKF1	58b	**59a**	**48a**	**92a**
Knudson C	66a	56ab	43ab	86ab

(Source: Modified from Nilufar, 2000)

Plant survivility percentage: Plant survivility percentage also showed significant differences among treatments at 12 WAPD (Figure 10). The maximum survival plant (76%) was observed in MS medium which was statistically similar to that of Hyponex medium (71%) whereas OKF_1 medium gave the lowest survival percentage (30%).

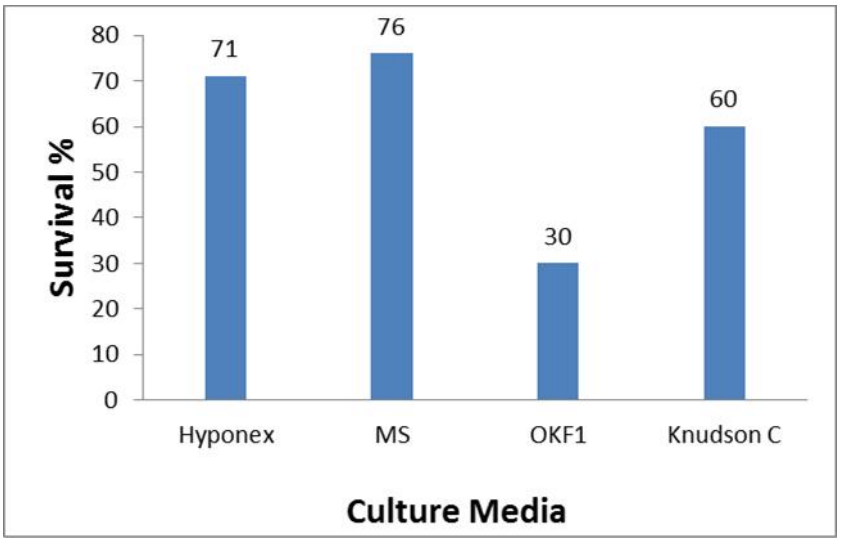


Figure 10: Plant survivility percentage in different culture media. (Source: Nilufar, 2000)

CHAPTER IV

CONCLUSION

Different culture media with different organic additives have been used for efficient plant regeneration of *Dendrobium* orchid. Among different combination of culture media with organic additives, MS medium with Sabri banana combination give the best results compare to other combination.

Different concentration of 2, 4 -D was supplemented with MS media. But the best concentration of 2, 4-D is 10 mgL^{-1}. That gives better results than other concentration. And in combined effect of NAA and BAP on plantlet regeneration give better results in 0.5 mgL^{-1} NAA + 0.5 mgL^{-1} BAP for plant growth and development.

In case of *Dendrobium chrysanthum* , highest germination percentage is found in Gamborg et al.(B_5) media. But in all cases of growth and development MS media performs better than other media.

In *Dendrobium bensoniae* , among different combination of growth regulators, 1.0 BA + 1.5 IBA (mg/l) give better performance in many cases as days required to shoot initiation, average number of shoots per explants. But the maximum percentage (90%) of shoot induction was found in the treatment with 1.0 BA+2.0 IBA (mg/l).

Among different culture media, *Dendrobium transparens* performs better in MS media in all cases. But in case of survivability percentage, with MS media, Hyponex media also perform better.

CHAPTER V

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