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Research Paper

IN VITRO SEED GERMINATION AND FLOWERING OF *Dendrobium palpebrae* LINDL. ORCHID

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Abstract

Dendrobium palpebrae Lindl. is a natural orchid species from Bangladesh. The immature seeds were germinated aseptically on lactose, sucrose, glucose supplemented KC, MS, MVW and PM basal medium and produced protocorm like bodies (PLBs). PLBs were sub-cultured on PGRs supplemented eighteen types of agar solidified MS and PM media. Fifteen cultured vessel were induced to flower after 6-7 months. The most significant result is that, cultured protocorms can shorten the flowering time from 3-5 years to 6-7 months. Orchid is one of the popular flowers that take a long time to flowering after seed germination and *in vitro* regeneration. The culture media composition and concentration have a marked effect on explants growth or *in vitro* flower formation. Therefore, the aim of this study is to show the *in vitro* conditions for shortening the juvenile period and induction of orchid flowering. Plant growth regulators, polyamines, additives, growth retardant, phosphorus and nitrogen contents, temperature, day length affect, age of explants and origin of the explants might have triggered the *in vitro* flowering process. *In vitro* flowering can shorten the relatively long juvenile phase of most orchids.

Key words: *In vitro* flowering, *Dendrobium palpebrae*, epiphytic orchid, PLBs, PGRs.

INTRODUCTION

Orchids belong to the largest and most diverse group among the angiosperms. The genus *Dendrobium* is the third largest in the family orchidaceae comprising of about 1184 species worldwide and they are primarily distributed from Southeast Asia to New Guinea and Australia [1]. In Bangladesh, 27 *Dendrobium* species distributed throughout the country especially Chittagong, Chittagong Hill Tracts, Cox's Bazar, greater Sylhet, Gazipur and Sundarbans forest [2]. *Dendrobium palpebrae* Lindl. is an epiphytic perennial orchid, occasionally distributed in Chitagong and Chittagong Hill Tracts of Bangladesh. The precise mechanism of flowering is still unclear despite it being of significance both physiologically and as a main regulator of fruit or seed yield in horticulture and agriculture. Artificial pollination and breeding could increase *D. palpebrae* propagation. *In vitro* flowering could shorten the juvenile phase, making rapid breeding of this rare orchid possible. Induction of precocious flowering *in vitro* has been shown in several *Dendrobium* orchids [3-5]. With viable seed production, *in*

vitro flowering in culture could play an important role in orchid breeding. However, production of functional orchid seeds in culture following *in vitro* flowering and pollinating has only been reported in a *Dendrobium* hybrid [3].

The minute orchid seeds are produced profusely but are non-endospermous and contain almost no nutrients. In nature, germination and early development are therefore reliant upon a highly specialized fungal association [6]. Asymbiotic seed germination by *in vitro* culture is essential. Since then, *in vitro* seed germination protocols have been established for many orchid species and a number of media and salts have been used for germination and propagation [7]. The natural habitat of *D. palpebrae* has been largely destroyed and populations are declining due to over exploitation for attractive flowers and low rates of natural propagation. The entire breeding cycle of *D. palpebrae* lasts three to five years, due to the long juvenile phase of this orchid. Hence tissue culture is a solution which can help in large scale multiplication. *Dendrobium palpebrae* Lindl. *in vitro* seed germination studies and flowering in sub-cultured plantlets is selected here for the present investigation which deals with the selection of a suitable medium for the asymbiotic culture.

In this study, we established an entire regeneration system for *D. palpebrae*, describing the processes of seed germination, protocorm like bodies (PLBs) formation, plantlet development and flowering. These findings are important for providing the potential to advance the breeding programs and commercial purposes.

MATERIALS AND METHODS

Dendrobium palpebrae fruit capsules approximately 90 days old were collected from National Botanical Garden, Mirpur, Dhaka, Bangladesh. Two protocols were used for surface sterilization of capsules. Healthy mature fresh capsules were first cleaned with detergent and rubbed with savlon soaked cotton and finally washed in running tap for 30 minutes till all the detergent was washed off clearly. Then the capsule is washed in liquid detergent to remove the surface dirt, rinsed in double distilled water to remove the traces of detergent. After that, green capsules were surface sterilized sequentially with 70% ethyl alcohol for 1 minute, 0.1% $HgCl_2$ solution for 10 minutes and finally and rinsed with autoclaved double distilled water for three times. Chemical surface sterilization process was carried out on laminar airflow cabinet. The capsule was cut open and the seeds were dusted on the surface of the nutrient medium and tissue culture bottles and test tubes were kept under controlled conditions of growth room.

Inoculations of disinfected explants and sub-culturing were carried out under aseptic environment, in a Laminar Air Flow Cabinet. Explants were placed on the nutrient medium in culture bottles/tubes with a sterilized forceps. Various basal media like KC, MS, PM and MVW were used for germination of orchid seeds. 0.8% agar solidified MS and PM media supplemented with various combinations of auxins and cytokinins (PGRs) were used for PLBs culture. pH of the medium was maintained at 5.4 -5.8 and the cultures were incubated at 25±2 ^{0}C temperature. Humidity level with air condition was between 50-60% and 14/10 h photoperiod with 4000-5000 lux illumination from cool white fluorescent tubes. Cultures were regularly sub-cultured based on the type of cultures, designed in an experiment. Each experiment was repeated twice and consisted of 3 replicates of 10 explants for each treatment. Observations were made regularly once in a week.

The germinated PLBs underwent elongation when sub-cultured on PGRs supplemented elongation media. 0.8% agar solidified MS and PM media supplemented with eighteen combinations of auxins and cytokinins (PGRs) were used for PLBs culture. After 6-7 months later, *in vitro* plantlet inside the tissue culture bottles were seen with the inflorescences production.

A protocol was developed for *in vitro* rooting of *Dendrobium* palpebrae Lindl. Half strength MS0 and nine different types of 0.8% (w/v) agar solidified MS medium supplemented with 3% (w/v) sucrose and three kinds of auxins *viz*. IAA, IBA, NAA were used for induction of strong and stout root system. The efficiency of the media in terms of enhancing the development of root system was assessed based on the increase in number and length of roots that developed within 30d of culture in rooting media.

90 days old plantlets with good *in vitro* rooting and with 3-4 leaf conditions were selected for
hardening. Tissue cultured bottles with plantlets were shifted from growth room conditions and were exposed to natural light conditions inside the laboratory area for 4 days. Further plantlets were carefully removed from the nutrient medium and were subjected to gentle washing of the root system with double distilled water to remove the adhering agar. Plants were treated with auxins to induce *ex vitro* rooting. Roots were treated with fungicide and transferred to pots containing moistened coir, saw dust and coal. Plants were covered with perforated plastic bags with optimum humidity conditions. Plants were shifted to green house after 10 days.

RESULTS AND DISCUSSION

The seeds of *Dendrobium palpebrae* aseptically grown on 0.8% (w/v) agar solidified MS [8]; PM [9]; MVW [10] and 1.2% (w/v) agar solidified KC [11] media with three different sources of carbohydrates *viz.* sucrose, glucose and lactose. The results obtained are briefly summarized in Tables-1. The overall results indicate that PM was better than the other three media in respect of the percentage of germination and required time for germination of this orchid species (Fig.1a). Similar result was also noted by [12-13] the references. MS medium was found best for germination of *Aerides odorata orchid seeds* was reported by [14] the reference*.* Sugar is an important component of any kind of nutrient medium used in tissue culture research. Normally sucrose is used in the medium but in some cases other carbohydrates such as lactose, glucose, maltose, fructose, dextrose, galactose, mannitol, cellulose, inulin, mannose have also been used [15-16]. Carbon source play a great role for *in vitro* orchid seed germination. The seeds failed to germinate on lactose containing KC medium. Among three carbon sources, the percentage of seed germination was higher in sucrose containing medium than in two glucose and lactose containing media.

Generally seed germination media contain only minerals, sugars, and vitamins and rarely enriched with PGRs and complex additives. It is indicated that as a rule of flower induction in orchid seedlings, it is not mediated by any of the known PGRs, vitamins and additives. The available evidences also indicate that temperature, illumination, photoperiods and interaction among these factors may not play important roles [4-5]. Therefore it is not yet clear that which factor is controlled *in vitro* flowering. In germination media, the germinated protocorms turned into mini seedlings and for continued further growth, germinated seedlings were transferred to elongation media

(Table-2) prepared with different combinations and concentrations of PGRs. Eighteen types of elongation media were prepared using MS and PM basal media with different concentrations and combinations of PGRs (BAP, NAA, IAA and Picloram) for enhancing elongation of seedlings. MS based medium was better than PM based medium for enhancing elongation of shoot system of the seedlings (Fig. 1b). After 6-7 months in elongation media elongated seedlings of *D. palpebrae* having 4-6 leaves started flowering on the some of the media concentrations. Among eighteen types of media *D. palpebrae* flowering took place on only 0.8% (w/v) agar solidified MS medium supplemented with 3% (w/v) sucrose + 0.5 mg/l Pic + 1.0 mg/l BAP (Fig. 1c) and PM medium fortified with 2% (w/v) sucrose + 0.5 mg/l NAA + 1.0 mg/l BAP. Cytokinin requirement for the growth and development of flower buds which are believed to be important signals for flowering and reported in several plants [17-18]. Cytokinin is also a common requirement for *in vitro* flowering [19]. BAP was found to be effective for early floral induction in orchids [4, 20-21].

In vitro flowering of *Dendrobium* has been a research focus based on the application of cross breeding and investigation on molecular mechanism of flowering. Conventional orchid breeding is time consuming because the entire breeding cycle could take at least 3-5 years. *In vitro* flowering protocols could shorten the time to 5 or 6 months [3, 5]. Most successful *in vitro* flowering in *Dendrobium* was obtained with green pods was noted by [4] the reference. *In vitro* flowering of *Dendrobium* is influenced by various hormones. BAP plays an essential role in inducing inflorescence for *Dendrobium* [3-5]. Plantlets grown in BAP free medium did not produce an inflorescence [3, 5]. In some cases, BAP alone was effective for floral induction [4, 22]. Furthermore, BAP played a more powerful function in flowering and in combination with other substances [4].

Sugars have been considered as important determinant in induction of *in vitro* flowering in some plants [23]. In case of orchid culture media as MS and PM used here always contain sugar, which is sucrose at 3% (w/v) and 2% (w/v) respectively. Thus, it is clear that if sugar could induce flowerings and it would be a common rather than a rare occurrence in the culture of orchid seedlings. EDTA, which can induce flowering in some plants [23] is often present in the tissue culture media as chelating agent. The available information does not support any apparent connection between the presence of EDTA in the media and orchid flowering *in vitro* [24]. It may be mentioned here that both MS and PM media used here contained EDTA as a component. Both NH_4^+ and NO_3^- ratio and nutrient concentrations were considered to have an effect on flowering [25]. Specific photoperiod was reported to induce flowering under *in vitro* condition in several plants [23, 26]. Our results do not support this view, as all the cultures were maintained in the same photoperiod *i.e.*, in a regular cycle of 14h light and 10h dark.

Table-1: Results of *in vitro* germination of seeds *Dendrobium palpebrae*.

Medium	Carbohydrate	Number of culture vessels used	Number of culture vessels in which seeds germinated		Time (d) required for germination	Remarks
			No.	%		
KC	2% sucrose	10	05	50	42 - 45	Deep green PLBs
	2% glucose	10	02	20	48 - 50	Green PLBs
	2% lactose	10	—	—	—	No response
MS	3% sucrose	10	07	70	35 - 38	Yellowish green PLBs
	3% glucose	10	03	30	38 - 42	Yellowish green PLBs
	3% lactose	10	02	20	40 - 44	Light green PLBs
	2% sucrose	**10**	**08**	**80**	**42 - 45**	**Green PLBs**
PM	2% glucose	10	07	70	45 - 48	Green PLBs
	2% lactose	10	04	40	38 - 42	Green PLBs
MVW	2% sucrose	10	06	60	40 - 42	Yellowish green PLBs
	2% glucose	10	04	40	36 - 40	Yellowish green PLBs
	2% lactose	10	02	20	38 - 42	Light green PLBs

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Table-2: Flowering in *in vitro* grown seedlings of *D. palpebrae* when cultured on 0.8% (w/v) agar solidified PGRs supplemented MS and PM elongation media.

Culture medium	No. of seedlings cultured on the medium	No. of seedlings flowered on the medium	Time required (week) for induction of flower after germination of seeds	Frequency (%) of seedlings that flowered
MS+1.0 mg/l IAA+0.5 mg/l BAP	-	-	-	-
MS+0.5 mg/l IAA+1.0 mg/l BAP	-	-	-	-
MS+1.0 mg/l IAA+1.0 mg/l BAP	-	-	-	-
MS+1.0 mg/l NAA+0.5 mg/l BAP	-	-	-	-
MS+0.5 mg/l NAA+1.0 mg/l BAP	-	-	-	-
MS+1.0 mg/l NAA+1.0 mg/l BAP	-	-	-	-
MS+1.0 mg/l Pic+0.5 mg/l BAP	-	-	-	-
MS+ 0.5 mg/l Pic+1.0 mg/l BAP	**25**	**15**	**24 - 28**	**60.00**
MS+1.0 mg/l Pic+1.0 mg/l BAP	-	-	-	-
PM+1.0 mg/l IAA+0.5 mg/l BAP	-	-	-	-
PM+0.5 mg/l IAA+1.0 mg/l BAP	-	-	-	-
PM+1.0 mg/l IAA+1.0 mg/l BAP	-	-	-	-
PM+1.0 mg/l NAA+0.5 mg/l BAP	-	-	-	-

PM+0.5 mg/l NAA+1.0 mg/l BAP	**20**	**12**	**22 - 25**	**60.00**
PM+1.0 mg/l NAA+1.0 mg/l BAP	-	-	-	-
PM+1.0 mg/l Pic+0.5 mg/l BAP	-	-	-	-
PM+ 0.5 mg/l Pic+1.0 mg/l BAP	-	-	-	-
PM+1.0 mg/l Pic+1.0 mg/l BAP	-	-	-	-

'—' indicates no response

Table-3: Mean increased length (cm) and number of roots per seed originated seedling of 30 days of culture on agar solidified ½ MS0 and Auxin supplemented MS rooting media.

Rooting medium			Average increased length and number of roots per seed derived seedling	
			Mean length (cm) ± S.E.	Mean no. of roots/ seedling ± S.E.
½ MS0			4.03 ± 0.20	2.31 ± 0.11
Auxin (mg/l)	**IAA**	**0.5**	**4.05 ± 0.18**	**2.42 ± 0.09**
		1.0	3.48 ± 0.27	2.05 ± 0.11
		1.5	3.95 ± 0.21	2.34 ± 0.16
	IBA	**0.5**	**4.63 ± 0.22**	**2.53 ± 0.14**
		1.0	3.14 ± 0.21	1.96 ± 0.11
		1.5	3.78 ± 0.23	2.28 ± 0.15
	NAA	0.5	2.67 ± 0.25	1.83 ± 0.13
		1.0	3.14 ± 0.20	2.03 ± 0.17
		1.5	2.65 ± 0.15	1.58 ± 0.13

***Based on observations from 50 seedlings taking five at random from each of ten culture vessels.**

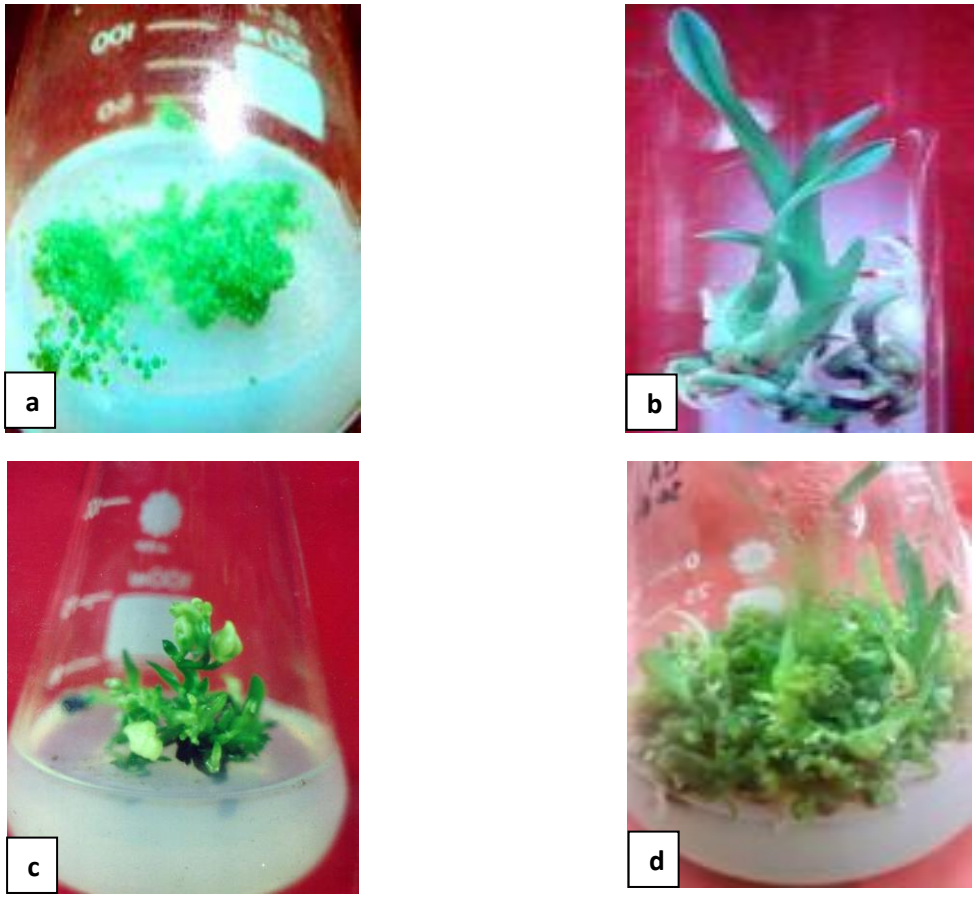

Fig.1. Different stages of *in vitro* seed germination, seedling development and *in vitro* flowering of *D. palpebrae*: a. *In vitro* PLBs; b. Elongation of germinated seedlings; c. Development of SPS; d. *in vitro* flowering on agar solidified medium.

In vitro flowering plants were above 2-10 cm height excluding the inflorescence, where a normal flowering green house grown plant measured about 11-13 cm height. Orchids were grown mainly for their exquisite flowers [4]. In this paper, we have demonstrated the possibility of *in vitro* flowering of *Dendrobium palpebrae* was earlier than time required for conventional breeding method. Not only flowers could be induced early in culture when compared to conventional orchid growing methods. There is a possibility of flowering *in vitro* throughout the year than seasonal flower between November to January. The auxin and cytokine used have also enhanced the *in vitro* flowering. Most important of all, there will be tremendous saving of time effort space, man power and cost in orchid breeding by adapting in orchid breeding programmes. These methods will undoubtedly contribute and benefit the orchid industry as whole.

When the seed originated seedlings were cultured in elongation media those produced masses of shoot primordia like structures (SPSs) at the base of the shoots. These SPSs were used for mass scale production of seedlings. MS medium was more effective than PM medium for SPSs induction. Most of the SPSs were greenish (Fig. 1d), few were yellowish in color. Similar finding was noted by [27-30] the reference.
The elongated seed originated seedlings produced roots in elongation media but those were weak and few in number. Half strength MS0 and nine different types of PGR (IAA, IBA, NAA) supplemented MS media were used for induction of strong and stout root

system (Table-3). The efficiency of the rooting media was evaluated based on the increase in length and number of roots developed per seedling within 30d of culture in rooting media. Induction of strong and stout root system of *D. palpebrae* was best when cultured on MS medium supplemented with 3% (w/v) sucrose and 0.5 mg/l IBA followed by MS + 3% (w/v) sucrose and 1.0 mg/l IAA. The reference [31] shown that in *Acampe praemorsa*; [32] in *Cymbidium iridioides* observed that IBA was effective for rooting. But the reference [33] found that NAA was most appropriate in inducing roots in *Cymbidium*. The references [34] also reported that IAA and auxin supplemented medium was more efficient for induction of strong and stout root system. It is noted that low concentration of auxin is more suitable than high concentration for induction of well developed root system.

Hardening of *in vitro* raised seedlings is an important aspect of plant tissue culture. Usually *in vitro* grown seed derived seedlings cannot adjust directly to outside natural environment. For that reason it is considered important to formulate efficient protocols for quick hardening technique. With this idea a number of mature *in vitro* developed seedlings were adjusted to outside natural environment through successive phases of acclimatization.

CONCLUSION

PM was found superior then KC, MS & MVW media for promoting germination of orchid seeds. The effect of different carbohydrates namely, sucrose, glucose and lactose was also studied in terms of promoting seed germination and the overall results indicate that sucrose supplemented media was better for germination of orchid seeds but failed in lactose supplemented KC medium. The overall results indicated that increased in root length and number of roots is higher in shoot bud derived seedlings than that of seed originated seedlings. Low concentration of auxin (o.5mg/l IBA) was more effective for enhancing rooting. Tissue originated plants has a great value in commercial firm and *ex situ* conservation. This clonal propagation technique created intense interest among the orchid growers and had a tremendous impact on the development of orchid industries. *In vitro* flowering of *Dendrobium palpebrae* orchids holds great potential in commercial application. This 'mini' orchid with flowers can be sold as souvenirs and novelty gifts. Orchid breeders can also use this *in vitro* flowering technique for quick production of hybrid orchids.

REFERENCES

[1] Puchooa, D. Comparison of different culture media for the *In vitro* culture of *Dendrobium* (Orchidaceae). *Intternational Journal of Agriculture and Biollogy*, v. 6, pp. 884-888, 2004.

[2] Huda, M. K. An updated enumeration of the family orchidaceae from Bangladesh. *The Journal of the Orchid Society of India*, v. 21, n. (1-2), pp. 35-49, 2007.

[3] Hee, K. H.; Loh, C. S.; Yeoh, H. H. Early *In vitro* flowering and seed production in culture in *Dendrobium* Chao Praya Smile (Orchidaceae). *Plant Cell Report*, v. 26, pp.2055-2062, 2007.

[4] Sim, G. E.; Loh, C. S.; Goh, C. J. High frequency early *in vitro* flowering of *Dendrobium Madame* Thong-In (Orchidaceae). *Plant Cell Reports*, 26: 383-393, 2007.

[5] Wang, Z. H.; Wang, L.; Ye, Q. S. High frequency early flowering from *in vitro* seedlings of *Dendrobium nobile*. *Science Horticulture*, v. 122, pp. 328-331, 2009.

[6] Hazarika, R. B.; Sarma, C. M. *In vitro* germination and regeneration of *Dendrobium transparens* Lindl, *Journal of the Orchid Society of India,* v. 9, pp. 51–54, 2009.

[7] Arditti, J.; Ernst, R. Micropropagation of orchids. New York: John Wiley and Sons Inc, 1993.
[8] Murashige, T.; Skoog, F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, v. 15, pp. 473-497, 1962.
[9] Arditti, J. Clonal propagation of orchids by means of tissue culture: A manual *In*: J. Arditti (ed.). *Orchid Biology: Reviews and Perspectives,* I. University Press, Ithaca, New York, 1977.
[10] Knudson, L. A new nutrient solution for germination of orchid seed. *American Orchid Society Bulletin*, v. 15, pp. 214–217, 1946.
[11] Vacin, E.; Went, F. Some pH change in nutrient solution. Botanic Gardens, Conservation News, v. 110, pp. 605–613, 1949.
[12] Bhadra, S. K.; Bhowmik, T. K. Axenic germination of seeds and rhizome-based micropropagation of an orchid *Arundina graminifolia* (D.Don) Hochr. *Bangladesh Journal of Botany*, v. 34 n. 2, pp. 59-64, 2005.
[13] Hoque, M. I.; Roy, A. R.; Sarker, R. H., Haque, M. M. Micropropagation of *Cymbidium* bicolor through *in vitro* culture. *Plant Tissue Culture*, v. 4, n. 1, pp. 45-51, 1994.
[14] Pant, B.; Gurrung, R. *In vitro* seed germination and seedling development in *Aerides odorata* Lour. *The Journal of the Orchid Society of India*, v. 19 n. 1-2, pp. 51-55, 2005.
[15] Harvais, G. Growth requirements and development of *Cypripedium reginae* in axenic culture. *Canadian Journal of Botany*, v. 51, pp. 327-332, 1973.
[16] Tsutsui, Tomita. "Suitability of several carbohydrates as the carbon sources for symbiotic seedling growth of two orchid species". Lindleyana, 5(2)., pp. 134-139, 1990.
[17] Huang, X. L.; Yang, B.; Hu, C. C.; Yao, J. L. *In vitro* induction of inflorescence in *Dioscorea zingiberensis*. *Plant Cell, Tissue and Organ Culture,* v. 99, pp. 209-215, 2009.
[18] Zhang, T. *In vitro* flowering of *Perilla frutescens*. *In Vitro Cell Development of Plants*, v. 43, pp. 91-94, 2007.
[19] Bernier, G.; Havelange, A.; Housa, C.; Petitjean, A.; Lejeune, P. Physiological signals that induce flowering. *Plant Cell Reports*, V. 5, pp. 1147-1155, 1993.
[20] Chang, C.; Chang, W. C. Cytokinins promotion of flowering in *Cymbidium ensifolium* var *misericors In vitro Plant Growth Regulators*, v. 39, pp. 217-221, 2003.
[21] Kim, H. H.; Chiang, S. L.; Hock, H. Y. Early *in vitro* flowering and seed production in culture in *Dendrobium* Chao Praya Smile (Orchidaceae). *Plant Cell Reports*. V. 26, pp. 2055–2062, 2007.
[22] Tee, C. S.; Maziah, M.; Tan, C. S. Induction of *in vitro* flowering in the orchid *Dendrobium* Sonia 17. *Biology Plantarum*. v. 52, pp.723-726, 2008.
[23] Scorza, R. *In vitro* flowering. *Horticulture Reviews,* v. 4, pp. 106-127, 1982.
[24] Chia, T. F.; Arditti, J.; Segeren, M. I.; Hew., C. S. Review: *In vitro* flowering of orchids. *Lindleyana,* v. 14, n. 2, pp. 60-76, 1999.
[25] Duan, J. X.; Yazawa, S. 6-Benzyladenine and low night temperature treatments induce early flowering in young *Phalaenopsis* seedlings. *Japan Journal of Tropical Agriculture,* v. 38, pp. 258-260, 1994.
[26] Deniel, Mc. C. N., King, R. W.; Evans, L. T. Floral determination and *in vitro* floral differentiation in isolated shoot apices of *Lolium temulentum* L. *Plantarum,* v. 185, pp. 9-16, 1991.

[27] Bhadra, S. K.; Hossain, M. M. *In vitro* germination and micropropagation of *Geodorum densiflorum* (Lam.) Scheltr., an endangered orchid species". *Plant Tissue Culture*, v. 13, n. 2, pp. 165-171, 2003.

[28] Malabadi, R. B.; Mulgund, G. S.; Nataraja, K. Efficient regeneration of *Vanda coerulea*, an endangered orchid using thidiazuron. *Plant Cell, Tissue and Organ Culture*, v. 76, pp. 289-93, 2004.

[29] Sinha, P.; Roy, S. K. Regeneration of an indigenous orchid, *Vanda teres* (Roxb.) Lindl. through *in vitro* culture. *Plant Tissue Culture*, v. 14, n. 1, pp. 55-62, 2004.

[30] Martin, K. P. Cost effective *in vitro* conservation of the threatened *Ipsea Malabarica* Orchid using protocorm like bodies. *Plant Cell Biotechnology and Molecular Biology*, v. 6, n. 1-2, pp. 57-60, 2005.

[31] Nayak, N. R. S.; Pathak, P.; Rath, S. P. Direct shoot regeneration from foliar explants of an epiphytic orchids *Acampae praemorsa* (Roxb.) Blatter and Mccann. *Plant Cell Reports*, v. 16, pp. 583-586, 1997.

[32] Pant, B.; Swar, S. Micropropagation of *Cymbidium iridioides* D. Don. *The Journal of the Orchid Society of India*, v. 25, n. 1-2, pp. 9-12, 2011.

[33] Banerjee, B.; Mandal, A. B. *In vitro* germination of immature *Cymbidium* seeds for rapid propagation of plantlets in lands. *Cell and Chromosome Research*, v. 21, pp. 1-5, 1999.

[34] Gangaprasad, A. N.; Decruse, W. S.; Seeni, S.; Menon, S. Micropropagation and restoration of the endangered Malabar daffodil orchid *Ipsea malabarica*. *Lindleyana*, v. 14, n. 1, pp. 38-48, 1999.