International Journal of Biological and Pharmaceutical Sciences Archive

ISSN: 0799-6616 (Online)

Journal homepage: https://ijbpsa.com/

(RESEARCH ARTICLE)

Check for updates

In vitro seed propagation of endangered *Dendrobium* - *Dendrobium lituiflorum* Lindl. and *D. aduncum* Lindl.

Kuntala Neog Barua *, Bebija L. Singha, Bonanya Bora and Satyam Bordoloi

Rain Forest Research Institute (Indian Council of Forestry Research and Education), Sotai, Jorhat-785010, Assam, India.

International Journal of Biological and Pharmaceutical Sciences Archive, 2022, 03(02), 108–115

Publication history: Received on 03 April 2022; revised on 13 May 2022; accepted on 16 May 2022

Article DOI: https://doi.org/10.53771/ijbpsa.2022.3.2.0056

Abstract

Genus *Dendrobium* of Orchidaceae prized for their floricultural excellence, that have fascinated the commercial growers all over the world and attention has paid to exploit this group. Destruction of specific habitats encourage the species vanished and need attention to conservation and restoration of rare and endangered taxa. *In vitro* seed propagation is the most popular multiplication technique and indispensable components of plant genetic resource management. Present observation deals with the effect of different media on *In vitro* seed proliferation and growth of plantlets of two endangered *Dendrobium* – *D. lituiflorum* Lindl. and *D. aduncum* Lindl. for large scale propagation. In *D. lituiflorum*, the highest germination percentage (95.48%) was recorded in MS medium supplemented with yeast extract, otherwise, in *D. aduncum*, 90.43 % germination was recorded in medium supplemented with potato extract. Quick proliferation of *D. lituiflorum* by the use of banana extract exhibited 12.5±1.77 number of shoot and 12.5±1.77 length. However, maximum number of shoots as 14.5±1.77 and length 4.84±0.58 cm. recorded in *D. aduncum*. After 12 weeks of culture, the medium containing 1.5 mgL-1 IBA supplemented with 0.2% banana extract showed maximum root formation (5.9±0.95) in *D. lituiflorum*. Otherwise, in *D. aduncum*, the highest number of roots7.9±1.33 per shoot was recorded the medium supplemented with 1.5mgL-1 IBA and 0.2% potato extract after 9 week of culture. In green house condition the seedlings of *D. lituiflorum* showed better performance in the medium containing brick chips: charcoal: coconut husk (1:1:2) and brick chips: charcoal: sphagnum moss (1:1:2) is better for *D. aduncum*. Seed derived propagation is very easy approach for multiplication of these species and could be successfully applied for mass multiplication intended for future conservation, and commercial aspects.

Keywords: *In vitro* seed propagation; MS medium; *Dendrobium lituiflorum*; *Dendrobium aduncum*; mass multiplication; conservation

1. Introduction

Dendrobium is the second largest genus of Orchidaceae family having 1184 species belongs to the subfamily Epidendroideae, and subtribe Dentrobiinae [1, 2]. India with its perfect climate is the harbor of nearly 103 species and 77 species from north-eastern region of the country [3]. The name *Dendrobium* was derived from the Greek words dendron meaning "tree" and bios meaning "life", resemblance of the epiphytic habit of nearly everyone species. It exhibited an incredible range of diversity in size, colour and curious shapes. They are prized for their floricultural excellence, beautiful foliage, fragrant and long-lasting flower that have fascinated the commercial growers all over the world and attention has paid to exploit this group for commerce. It is also the high valued orchids in the market for their traditional medicine [4, 5]. Indian sub-continent has been a desired orchid hunting ground for the past centuries [6]. It augmented destruction of specific habitats that enhanced and some species vanished from the Indian regions.

*Corresponding author: Barua Kuntala N
Rain Forest Research Institute, Sotai, Jorhat-785010, Assam, India.

In vitro seed propagation is the most popular multiplication method of orchids and indispensable components of plant genetic resource management, conservation and restoration of rare and endangered plant species [7]. Propagation of *Dendrobium* has been done by various authors time to time [8, 9, 10, 11] but very limited work was reported through *In vitro* seed propagation [12, 13]. Present study deals with *In vitro* seed propagation of two endangered *Dendrobium* namely *D. lituiflorum* Lindl. and *D. aduncum* Lindl. for successful conservation.

D. lituiflorum, commonly known the bent raceme *Dendrobium* is native to Southern China, Arunachal Pradesh, Assam, Bangladesh Thailand, Laos, and Vietnam [14] at an altitude up to 1,700 m. It is a sympodial epiphyte with long pendulous pseudobulbs. Flowers are attractive borne on fascicles of 3 or more from the nodes, inner surface of lip pale purple with a deep purple spot surrounded by a white circle. *D. aduncum,* the inward bent *Dendrobium* is native to Southern China, the Eastern Himalaya and Northern Indonesia at an elevation of 300 to 1300 m above sea level. It comes into flower in axillary raceme with pale pink spreading flower and deep purple anther cap. Both the species are blooming in March-April.

2. Material and methods

2.1. Seed capsule collection and surface sterilization

The fully mature capsules of *D. lituiflorum* and *D. aduncum* collected from the forest of North Eastern coal field under Digboi Forest division of Assam in the month of January 2019. The capsules were washed properly in tap water followed by washing in Tween-20 solution for 10 minutes (2-3 drops in 100 ml sterile distilled water). These capsules were surface sterilized using 0.5% $HgCl_2$ solution for 10 minutes after dripped in 70% ethanol for 60 seconds. The capsules were washed by sterile distilled water for 4-5 times to completely remove $HgCl_2$ followed by flaming of the capsules. The surface sterilised capsules were split open longitudinally by using sterile surgical blade to scooped out minute seeds and spread out on the culture media. The whole procedure was performed in aseptic condition under laminar flow to prevent any kind of contaminations. The mother plants are also conserved in Orchidarium of Rain Forest Research Institute, Jorhat.

2.2. *In vitro* seed culture medium

To study the best medium for seed germination, the mature seeds of each species of orchid were inoculated on different gelled medium viz., full-strength of MS medium [15], half-strength of MS and IY [16] and commercial Dendrobium seed germination medium. These mediums contain potassium nitrate and ammonium nitrate which serve as a source of nitrogen which is ideal for efficient germination and seedling development. The media were solidified by using 0.7% agar. The pH of the medium was adjusted to 5.8 with 1N NaOH or 1N HCl and then autoclaved at 121°C for 15 minutes. The cultures were maintained at 25°C±2°C with proper light illumination under 16/24 h photoperiod. The data of seed germination were recorded for each medium to study the percentage of seed germination.

2.3. *In vitro* shoot formation and its regeneration

To study the shoot formation and regeneration of seedlings, the protocorms were scooped and culture in MS medium supplemented with various plant growth regulators like 6-Benzyl Amino Purine (0.5-2 mgL^{-1}) and Indole-3-butyric Acid (0.5-2 mgL^{-1}), either singly or in combination. Yeast extract, banana extract, potato extract and activated charcoal powder were used as natural additives in this study. Sub culturing was done at regular interval and data were recorded.

2.4. *In vitro* root formation

To study the root initiation and development, the individual shoot bearing 5-6 cm long were selected and transferred to MS medium supplemented with different concentration of auxins, Indole-3-butyric Acid (0.5-2 mgL^{-1}) and 1-Naphthalene Acetic Acid (0.5-2 mgL^{-1}) along with 0.2% banana extract for *D. lituiflorum* and 0.2% potato extract for *D. aduncum.*

2.5. Acclimatization of seedlings

Completely rooted seedlings were taken out from culture vessels for hardening by using sterile forceps, thoroughly washed with distilled water to remove the gel adhered and treated with 0.1% (w/v) bavistin solution for 10-15 minutes to control fungal contaminants if any. These Seedlings were transferred in three different potting media viz., i) brick chips: charcoal: sphagnum moss (1:1:2); ii) brick chips: charcoal: coconut husk (1:1:2) and iii) brick chips: charcoal: Leaf mould (1:1:1). During hardening, the seedlings were covered with transparent plastic sheets for at least 2 weeks to maintained humidity and temperature. The sheets were gradually removed to reduce humidity. Spraying of 1% bavistin twice a week was also done to keep fungus away from the seedlings. Survival percentage was calculated.

2.6. Data analysis

The experiment was done with 10 replications per treatment and repeated twice. The results were calculated as mean ± standard deviation by application of Microsoft Excels software.

3. Results and discussion

3.1. *In vitro* seed culture medium

In *D. lituiflorum,* the highest germination percentage (95.48%) was recorded in full strength MS medium supplemented with yeast extract followed by 85.45% in half strength MS medium. In case of *D. aduncum,* the highest germination percentage 90.43% was recorded in full strength MS medium supplemented with potato extract followed by 84.35% in half strength MS medium (Table-1). Successful germination was observed after 3-7 weeks of culture in different medium, evidence by the enlargement of the embryos and ultimately produces irregular shaped parenchymatous cell mass spherules. The spherules were transformed into round, oval, elongated, branched or spindle shaped after 5-9 weeks of culture. Former *In vitro* seed culture of *D. chrysotoxum* was found encouraging in Mitra medium supplemented by auxins and cytokinins [13] and suggested their protocol for mass propagation of *Dendrobium* orchids. However, in the present study MS medium were found suitable for seed germination of *D. lituiflorum* and *D. aduncum*.

Table 1 Seed germination and development of protocorm in different culture medium

Name of Species	Medium	Natural additives (2%)	Germination %	Spherule formation (In weeks)	Protocorms formation (In weeks)	
D. lituiflorum	MS (Full strength)		-	85.30	3-5	6-8
			BE	83.24	3-5	6-8
			YE	95.48	3-4	5-7
	MS (Half strength)		-	81.30	4-5	6-8
			BE	84.50	3-6	7-9
			YE	85.45	4-5	6-8
	IY		-	75.34	4-7	8-10
			BE	76.40	4-6	7-9
			YE	78.20	3-5	6-9
	Dendrobium seed germination		-	80.00	4-6	7-9
			BE	81.20	4-6	7-9
			YE	82.50	3-5	6-8
D. aduncum	MS (Full strength)		-	85.95	3-5	5-8
			AC	87.24	3-4	5-7
			PE	90.43	3-4	5-6
	MS (Half strength)		-	82.30	4-5	6-9
			AC	83.65	3-5	6-8
			PE	84.35	4-5	6-8
	Dendrobium seed germination		-	80.06	4-6	7-8
			AC	81.29	4-6	7-8
			PE	83.25	4-5	6-8
	IY		-	75.29	4-7	9-10
			AC	78.40	4-7	7-9
			PE	80.20	4-5	6-9

Result based on average of 10 replicate per treatment denotes mean and ± standard deviation; AC: Activated charcoal and PE: Potato extract

3.2. *In vitro* shoot formation and its regeneration

The optimal media for tissue culture of *D. lituiflorum* was reported earlier [8].During the present study protocorms were proliferated to multiple shoots in MS medium supplemented with various concentrations and combinations of BAP and IBA with 2% banana and yeast extract as natural additives. Mass multiplication of *D. primulinum,* a critically endangered orchid was done through shoot tip culture with MS medium supplemented by NAA & BAP (for shoot proliferation), as well as IAA & IBA effective for rooting [11].

Table 2 Effect of plant growth regulators and natural additives on shoot development

Name of Species	IBA	BAP	Additives (0.2%)	Mean number of shoots	Mean length of shoot (cm.)	Plantlet length(cm.)
D. lituiflorum	Control		BE	3.2 ± 0.82	1.65±0.70	3.1±0.99
	-	0.5	BE	3.5 ± 1.26	1.84±0.58	3.18±0.16
	-	1	BE	5.5 ± 2.06	2.48±0.70	2.48±0.25
	-	1.5	BE	12.5±1.77	5.78±0.46	4.84±0.58
	-	2	BE	7.3 ± 1.49	4.15±0.58	3.48±0.36
	0.5	0.5	BE	6.3 ± 1.49	2.53±0.66	4.01±0.66
	0.5	1	BE	7.9 ± 1.66	3.61±0.33	3.03±0.73
	0.5	1.5	BE	15.2±1.75	4.75±0.58	2.53±0.66
	0.5	2	BE	7.5 ± 1.56	3.43±0.85	3.61±0.33
	Control		YE	3.8 ± 1.56	2.48±0.25	3.43±0.85
	-	0.5	YE	4.9 ± 1.26	1.64±0.58	3.18±0.16
	-	1	YE	4.5 ± 0.89	3.48±0.36	2.48±0.25
	-	1.5	YE	10.5 ±3.62	4.01±0.66	1.84±0.58
	-	2	YE	8.4 ± 1.03	3.43±0.73	3.48±0.36
	0.5	0.5	YE	6.6 ± 1.41	2.53±0.66	4.01±0.66
	0.5	1	YE	6.1 ± 1.10	3.61±0.33	2.3±0.46
	0.5	1.5	YE	9.5±1.50	3.68±0.46	2.4±0.57
	0.5	2	YE	7.8 ± 1.07	3.43±0.85	3.3±1.33
D. aduncum	Control		PE	3.3± 0.82	1.65±0.70	2.9±0.79
	-	0.5	PE	3.8 ± 1.26	3.18±0.16	3.47±1.17
	-	1	PE	5.3± 2.06	2.48±0.25	4.18±1.16
	-	1.5	PE	14.5±1.77	4.84±0.58	4.48±0.25
	-	2	PE	6.3 ± 1.49	3.48±0.36	4.14±0.58
	0.5	0.5	PE	4.3 ± 1.49	4.01±0.66	3.28±0.36
	0.5	1	PE	8.9 ± 1.66	3.03±0.73	4.11±0.67
	0.5	1.5	PE	13.2±1.75	2.53±0.66	3.13±0.83
	0.5	2	PE	7.5 ± 1.56	3.61±0.33	2.33±0.65
	Control		AC	4.8 ± 1.56	2.48±0.25	3.43±0.85
	-	0.5	AC	3.9 ± 1.26	1.84±0.58	3.18±0.16
	-	1	AC	4.5 ± 0.89	3.48±0.36	2.48±0.25
	-	1.5	AC	11.5 ±3.62	4.01±0.66	1.84±0.58
	-	2	AC	8.4 ± 1.03	3.03±0.73	3.48±0.36
	0.5	0.5	AC	6.6 ± 1.41	2.53±0.66	4.01±0.66
	0.5	1	AC	6.1 ± 1.1	3.61±0.33	2.3±0.46
	0.5	1.5	AC	12.5±1.50	3.78±0.46	2.4±0.57
	0.5	2	AC	7.8 ± 1.07	3.43±0.85	3.3±1.33

Result based on average of 10 replicate per treatment denotes mean and ± standard deviation; BE: Banana extracts; YE: Yeast extract; AC: Activated charcoal and PE: Potato extract

In *D. lituiflorum* maximum number of shoots formation was observed after 12 weeks of culture and the highest numbers (12.5±1.77) of shoots in MS with 1.5 mg/L BAP and 0.2% banana extract with an average (5.78±0.46) length [Table-2, Figure -1(F)]. Previous report supported the application of banana extract for quick proliferation of *D. lituiflorum* [10]. Banana extract showed better response in comparison with yeast extract. The shoot multiplication was initiated after 4 weeks of primary culture. The combined effect of 0.5 IBA and 1.5 BAP (15.2±1.75) with banana extract showed highest multiple shoot development (Table-2).

In case of *D. aduncum,* the medium supplemented with activated charcoal and potato extract as natural additives, shows better result in both shoot and root development. Maximum number of shoots was observed after 13 weeks of culture in the medium supplemented with BAP (1.5 mgL^{-1}) and 0.2% of potato extract (Table-2). The highest number of shoots (14.5±1.77) was recorded with an average (4.84±0.58) cm length [Table-2; Figure-2-(F)]. Medium containing activated charcoal shows average number (12.5±1.50) of shoot with (3.78±0.46) cm long plantlet (Table-2). The shoot multiplication was initiated after 4 weeks of primary culture in combination of auxins and cytokinins. As the concentration of BAP from 0.5 to 2.0mg/l considerably increased the multiple shoot production. However, increase in concentration of BAP to 2 mg/l decreased the multiplication rate.

3.3. *In vitro* root formation

After 12 weeks of culture, the medium containing 1.5 mgL^{-1} IBA supplemented with 0.2% banana extract showed maximum number of root formation (5.9±0.95) with 6.5±0.45 cm length in *D. lituiflorum* [Table-3, Figure-1(H)]. However, in *D. aduncum,* the highest number of root (7.9±1.33) per shoot was recorded in MS medium supplemented with 1.5 mgL^{-1} IBA and 0.2% potato extract with an average length of (4.6±1.71) after 9 week of culture [Table-3, Figure -2(I)].

Table 3 Effect of different plant growth regulator in multiple root development

Name of Species	PGR		Natural Additives (2%)	Mean	
	NAA	IBA		No. of roots per shoots	Root length (cm)
D. lituiflorum	0.5	-	BE	3.7±1.33	2.75±0.87
	1.0	-	BE	3.4±1.17	1.05±0.33
	1.5	-	BE	3.6±1.4	2.75±0.87
	2.0	-	BE	3.7±1.33	3.5±0.60
	-	0.5	BE	4.0±0.81	2.75±0.87
	-	1.0	BE	3.6±1.4	3.9±0.53
	-	1.5	BE	5.9±0.95	6.5±0.45
		2.0	BE	4.5±1.4	4.61±1.36
D. aduncum	0.5	-	PE	4.1±0.82	3.76±0.51
	1.0	-	PE	3.7±0.77	3.9±1.27
	1.5	-	PE	5.3±0.42	4.9±1.44
	2.0	-	PE	4.9±0.64	7.47±0.60
	-	0.5	PE	3.8±0.84	2.9±0.55
	-	1.0	PE	4.5±0.73	3.44±1.4
	-	1.5	PE	7.9±1.33	4.6±1.71
	-	2.0	PE	5.9±1.19	3.76±0.72

Result based on average of 10 replicate per treatment denotes mean and ± standard deviation; BE: Banana extract and PE: Potato extract

3.4. Acclimatization of seedlings

Plantlets with 3-5 cm long shoot and 5-7 cm long root were transferred to greenhouse condition in three potting media after the process of acclimatization. The seedlings of *D. lituiflorum* showed better performance in the medium containing Brick chips: Charcoal: Coconut husk (1:1:2) [Table-4, Figure 1(J)]. Otherwise, brick chips: charcoal: sphagnum moss (1:1:2) is better for *D. aduncum* [Table-4, Figure -2(K)].

Table 4 Effect of potting media on growth and development of plantlets

Name of Species	Composition of potting media	Survival rate (%)	Average leaf number	Average leaf length (cm)	Average Leaf width (cm)	Response *
D. lituiflorum	Brick chips:Charcoal: Coconut husk (1:1:2)	90.34	7.2±1.48	5.02±0.63	1.15±0.20	+++
	Brick chips:Charcoal:moss (1:1:2)	81.90	5.9±0.83	6.34±0.44	1.01±0.49	++++
	Brick chips: Charcoal: Leaf mould (1:1:2)	70.35	5.4±1.14	5.86±0.40	1.25±0.25	++
D. aducum	Brick chips: Charcoal: Coconut husk (1:1:2)	89.03	5.87±1.37	5.02±1.03	1.10±0.50	+++
	Brick chips:Charcoal: moss (1:1:2)	91.90	6.98±1.14	6.54±1.24	1.11±0.54	++++
	Brick chips: Charcoal: Leaf mould (1:1:2)	74.25	5.36±1.24	3.96±0.87	1.02±0.55	++

*++ Moderate +++ Satisfactory ++++ Highly Satisfactory; Result based on average of 10 replicate per treatment denotes mean and ± standard deviation

Figure 1 Different stages of *In vitro* propagation of *D. lituiflorum* – (A) Capsule of *D. lituiflorum;* (B) Seed swelling; (C&D) Protocorm formation; (E) Leaf initiation;(F) Multiple shoot development; (G) Root initiation; (H) Seedlings with well-developed root & shoot; (I) Hardening of seedlings; (J) Hardened seedling and (K-L) Blooming of *D. lituiflorum*

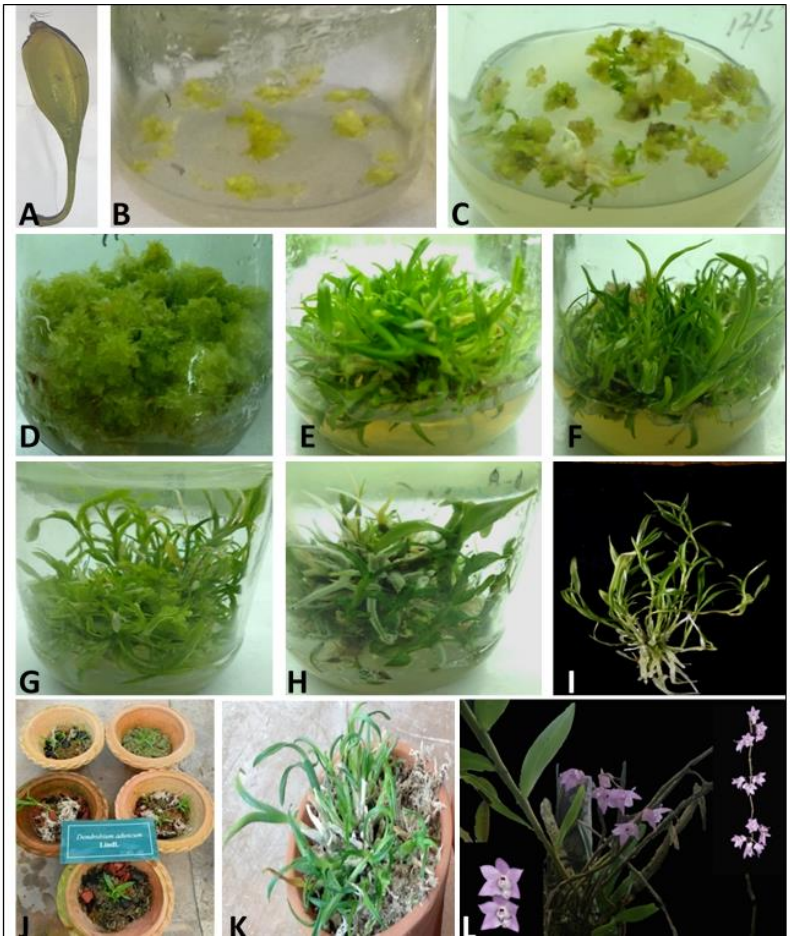

Figure 2 Different stages of *In vitro* propagation of *D. aduncum*– (A) Capsule *D. aduncum;* (B&C) Seed swelling; (D) Protocorm formation; (E) Leaf initiation; (F) Multiple shoot formation; (G&H) Root initiation; (I) Well developed root & shoot; (J) Hardening with three potting media; (K) Best potting media-brick chips: charcoal: sphagnum moss; (L) Blooming of *D. aduncum*

4. Conclusion

Dendrobium lituiflorum Lindl. and *D. aduncum* Lindl. are the most beautiful orchids having immense commercial value~~?~~. Seed derived propagation is very prompt and easy approach for multiplication of these species. It could be successfully applied for mass multiplication of these orchids intended for large scale propagation, future conservation, reintroduction in their natural ecological niche and commercial aspects.

Compliance with ethical standards

Acknowledgments

Authors are very grateful to Director, Rain Forest Research Institute, Jorhat for his supervision and facilities. Authors expressed deep sense of thankfulness to Central Mine and Planning Design Institute, Ranchi for providing financial support.

Disclosure of conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and the financial support for this work received from Central Mine and Planning Design Institute, Ranchi has not influenced its outcome.

References

[1] Begum R, Alam SS, Menzel G, Schmidt T.Comparative molecular cytogenetics of major repetitive sequence families of three *Dendrobium* species (Orchidaceae) from Bangladesh. Annals of Botany. 2009; 104: 863–872.

[2] Leitch IJ, Kahandawala I, Suda J, Hanson L, Ingrouille MJ, Chase MW, Fay MF. Genome size diversity in orchids: consequences and evolution. Annals of Botany. 2009; 104: 469–481.

[3] Singh KP, Phukan S, Bujarbarua P. Floristic Diversity and Conservation Strategies in India. Botanical Survey of India. 2001; 4: 1736-1764.

[4] Chen QX, Ji ZH. Encyclopedia of China orchid Beijing, China: China Forestry Publishing House. 1998.

[5] Singh A, Duggal S. Medicinal orchids: an overview. Ethnobot. Leaflets. 2009; 13: 351-363.

[6] Vij SP. Orchidology in India current status in orchid conservation, culture, Forming and trade. In: Hedge SN, eds. Himalayan Publishers, Logi shopping Complex, Itanagar, Arunachal Pradesh. 2001; 79(11): 11.

[7] Fay M. Conservation of rare and endangered plants using *In vitro* methods. *In vitro* Cellular Developmental Biology. 1992; 28: 1–4.

[8] Chung J, Ding X, Bao SL, Ley DY, He J, Tang F, Ding BZ. Study on tissue culture of *Dendrobium lituiflorum* China Journal of Chinese Materia Medica. 2004; 29(4): 7-313.

[9] Ray J, Naha S, Majumdar M, Banerjee N. Direct and callus - mediated protocorm like body induction from shoot tips of *Dendrobium chrysotoxum* Lindl. (Orchidaceae). Plant Cell Tissue and Organ Culture. 2007; 90(1):31-39.

[10] Vyas S, Guha S, Bhattacharya M, Rao U. Rapid regeneration of plant of *Dendrobium lituiflorum*Lindl. (Orchidaceae) by using banana extract. Scientia Horticulturae. 2009; 121: 32-37. DOI: 10.1016/j.scienta 2009.01.012

[11] Pant B, Thapa D. *In vitro* mass propagation of an epiphytic orchid, *Dendrobium primulinum* Lindl. Through shoot tip culture. African Journal of Biotechnology. 2012; 11(42): 9970-9974.

[12] Soetopo L, Pumamaningsih SL. *In vitro* propagation of *Dendrobium* and *Phalaenopsis* through tissue culture for conservation. Agrivita. 2012; 34(2): 115-126.

[13] Nongdam P, Tikandra L. Establishment of an efficient *In vitro* regeneration protocol for rapid and mass propagation of *Dendrobium chrysotoxum* Lindl. using seed culture. The Scientific World Journal. 2014; Article ID 740150, 8 pages. https://doi.org/10.1155/2014/740150

[14] Averyanov LV, Averyanova AL. Updated Checklist of the Orchids of Vietnam. 1st ed. Hanoi: Vietnam National University Publishing House; c2003.

[15] Murashige T, Skoog F. A revised medium for rapid growth and bioassays with Toba cultures. Physiologia Plantarum. 1962; 15: 473-497.

[16] Ichihashi S, Yamashita M. Studies on the media for orchid seed germination. The effect of balances inside each cation group for the germination and seedling development of *Bletilla striata* seeds. Journal of Japan Soc Hortic Sci. 1977; 45: 407–413.