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In vitro seed propagation of *Dendrobium moschatum* as influenced by different plant growth regulators

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Abstract

This study aimed to develop an effective *in vitro* protocol for germinating and multiplying *Dendrobium moschatum* seeds by identifying optimal concentrations of plant growth regulators for shoot proliferation and root formation. Five different MS media formulations were utilized, including a control (M_1) and those supplemented with varying concentrations of kinetin and NAA (M_2, M_3, M_4) and BAP (M_5). Results showed significant variation in seed germination time across media, with the longest duration observed in M_4 (76 days) and the shortest in M_1 (55 days). Protocorm formation and plantlet development were quickest in M_1, while M_3 and M_5 exhibited the longest duration. The initiation of leaves or shoots occurred earliest in M_3 (103 days) and latest in M_5 (149 days). Notably, leaf and plantlet growth differed between three- and five-month intervals, with M_3 demonstrating optimal growth over the longer term. These findings underscore the importance of carefully balanced plant growth regulator concentrations in achieving successful seed germination and subsequent growth in *D. moschatum*.

Key words: *Dendrobium moschatum*, growth regulators, *in vitro*, propagation, tropical orchid

Introduction

Orchids are one of the largest groups of ornamental plants highly-priced in the international market due to their spectacularly designed flowers which have attractive shades of colour, myriad sizes and shapes along with delightful appearance and forms and the plants have curious growth habits, belonging to the largest plant family Orchidaceae among the monocotyledons (De *et al.,* 2014). It has 29,199 accepted species (Govaerts *et al.*, 2017) and 31000 species estimated to exist (Hinsley *et al.,* 2018). *Dendrobium* belongs to one of the three most important and prominent families among the genera in Orchidaceae (Leitch *et al.,* 2010). *Dendrobium* comprises approximately 1600 species sympodial epiphytic species (Tikendra *et al.*, 2019), which has been considered the second largest genus in the family Orchidaceae. Nearly 1/4th of the species are used for ornamental perspective and 300 species are available in India (Chen and Ji, 1998). *Dendrobium* is among the most demanding cut flowers in domestic and international markets. It can also be used as a potted plant. In the Philippines, Indonesia and New Guinea, the stems of *Dendrobium* are used for making baskets and pseudo bulbs of *Dendrobium tokai* are used for oral contraceptives (Bose and Bhattacharjee, 1999). *Dendrobium* is widely distributed worldwide, specifically in southern Asia to New Guinea and Australia (Puchooa, 2004).

D. moschatum, also called musky-smelling *Dendrobium*, is native to the Himalayan region. Orchids are listed in CITES Appendix II, and most of them are categorized as critically endangered and are therefore legally protected (Subedi, 2011; Pant, 2013). *D. moschatum* is an epiphytic endangered medicinal species of orchid that grows naturally on tree trunks in the forests of the North Eastern Himalayas, including India, China, Vietnam, Burma, Laos and Thailand. Different literature revealed phytocomponents with several biological activities, including antioxidant, antibacterial, anticancer, anti-inflammatory, and anti-HIV (Kovács *et al.*, 2008; Rajput and Saikia, 2020). The stems of *D. moschatum* grow up to 1-2 m long and the spike contains fragrant flowers 5-8cm in diameter and 7-15 numbers (Baker and Baker, 2009). The reproduction of *Dendrobium* occurs in two ways: asexually through keikis and sexually through seeds. But the primary constraint in sexual propagation is that the seeds of *Dendrobium* do not have endosperm; thus, natural germination is restricted due to the lack of a suitable host or medium.

In the natural world, just 1% of seeds germinate because they require a certain mycorrhizal fungus relationship (Kumaria and Tandon, 2010; Shah *et al.*, 2019; Zhang *et al.*, 2012). Due to habitat degradation and indiscriminate, widespread harvesting, the species is now endangered (Secretariat of the Convention on Biological Diversity, 2011; Luo *et al.,* 2013). The entire family of *Dendrobium* orchids is classified in CITES Appendix II, which lists closely endangered plant and animal species. *Dendrobium* orchids, listed under CITES Appendix II and in the IUCN Red Data Book, face conservation challenges. In vitro techniques like tissue culture are crucial for germinating seeds and ensuring the survival of these endangered species (Teixeira da Silva *et al.*, 2014).

Therefore, this study aimed to develop an appropriate protocol for *In vitro* germination and propagation of *D. moschatum* seeds while also determining the optimal concentrations of plant growth regulators for enhancing shoot proliferation and root development.

Materials and methods

The present study was carried out at the Plant Tissue Culture Labouratory of the Department of Floriculture, Medicinal and Aromatic Plants of Uttar Banga Krishi Viswavidyalaya, Cooch Behar, West Bengal. Seeds of *D. moschatum* were used as explant. The matured fresh seed pods of *D. moschatum* were collected from ICAR-NRCO, Pakyong, Sikkim. The seedpods were cleaned thoroughly under tap water and immersed in 70% ethanol for 30 seconds for disinfestations (Rao and Barman 2014) and surface sterilization was done under running tap water by adding 3 drops of Tween-20 detergent for 20 min. (Thokchom and Maitra, 2017) followed by 20% sodium hypochlorite treatment (Ma *et al.,* 2020) and washing in sterilized distilled water and drying. Seedpods were cut longitudinally with a sharp sterilized knife in laminar air flow chamber, and seeds were placed in the MS media (Murashige and Skoog, 1962) with the addition of different plant growth regulators. The addition of 5 mL vitamin solution prepared M.S. basal mediums, 30 g sucrose and 8 g potato dextrose agar per litre. pH was adjusted at 5.8 before autoclaving (Rao and Barman, 2014).

Media were sterilized in an autoclave at 15 psi at 121^0C for 15 min.. Five different M.S. medium were used as culture media for seed germination and *In vitro* growth. MS media with no hormone (M_1) as control, MS media supplemented with 1mg kinetin/L (M_2), MS media supplemented with 2 mg kinetin/L (M_3), MS media supplemented with 1 mg NAA (2-naphthalene acetic acid)/L (M_4), MS media supplemented with 2mg BAP (6-benzyl amino purine)/L (M_5). All the culture media were placed under cool fluorescent light and at 24± 2ºC and continuous light duration for 14 hours.

The present experiment was designed in a completely randomized block design with six replications in each treatment. The parameters were recorded simultaneously, such as the days required for seed germination, protocorm formation, plantlet development, and first initiation of leaf or shoot. The observations on the number of leaves, length of leaves and length of plantlets were recorded after three months and five months of seed germination, respectively.

Results were analysed by one-way analysis of variance. Duncan's multiple range tests at probability level 0.5 were used to determine the significance between treatment means.

Results and discussion

Days required for seed germination: It is evident from the observations presented in the Table 1, that days required for seed germination of *D. moschatum* varied significantly among the media having different plant growth regulators under the study. The maximum days required for seed germination was observed in MS media supplemented with 1mg NAA (2-naphthalene acetic acid)/L (M_4), which was 76 days, whereas the least days required for seed germination was observed in MS media with no hormone (M_1) which was 55 days.

Days required for protocorm formation and plantlet development: The seeds showed yellow colour, but at the time of germination and protocorm development, it turned into light green to white and turned green at the time of plantlet development. It is evident from the data presented in Table 1 that the minimum days required for protocorm formation and plantlet development were observed in MS media with no hormone (M_1). In contrast, the maximum days required for protocorm formation and plantlet development (98 days) was observed in MS media supplemented with 2 mg kinetin/L (M_3), which was at par with M_5 (97 days) and M_2 (96 days).

Days required for first initiation of leaf or shoot: The days required for first initiation of shoot or leaves varied significantly among the media, which had different plant growth regulators under study (Table 1). Maximum days required for the first initiation of leaf or shoot (149 days) was observed in MS media supplemented with 2mg BAP (6-benzyl amino purine)/L (M_5). In contrast, the least days required for the first initiation of leaf or shoot (103 days) was observed in MS media supplemented with 2mg kinetin/L (M_3). Kinetin or 6-furfurolaminopurine, associated with the cytokinin group, promotes cell division and activates cellular growth and segregation processes. The same trend was also observed by Nguyen *et al.* (2011).

The number of leaves: Maximum numbers of leaves (7.83) were observed in MS media supplemented with 1 mg NAA (2-naphthalene acetic acid)/L (M_4) followed by M_3(6.16) and M_2(6.13) which were at par each other and the minimum numbers of leaves (2.83) were observed in MS media with no hormone (M_1) (Table 1). But a perusal of data presented in Table 2 showed that the maximum number of leaves five months after germinations (7.16) was observed in MS media supplemented with 2 mg kinetin/L (M_3) which was at par (6.50) with MS

Table 1. Effect of different plant growth regulators on days required for seed germination, protocorm development, first initiation of shoot, number of leaves, length of leaves and length of plantlets 3 months after germination of *Dendrobium moschatum* seeds

Media	Days required for seed germination	Days required for protocorm formation and plantlet development	Days required for first initiation of leaf or shoot	Number of leaves 3 months after germination	Length of leaves (cm) 3 months after germination	Length of plantlet (cm) 3 months after germination
M_1	55.83±3.6^a	75.66±3.5^a	109.50±4.5^b	2.83±0.7^a	0.46±0.1^a	0.85±0.1^a
M_2	66.50±1.8^c	96.50±1.8^c	143.66±3.4^d	6.13±1.1^b	0.50±0.08^a	1.28±0.1^b
M_3	73.16±1.7^d	98.16±0.7^c	137.16±1.1^c	6.16±1.1^b	0.70±0.08^b	1.46±0.1^c
M_4	76.33±2.1^e	88.50±1.3^b	103.83±1.6^a	7.83±1.1^c	0.88±0.09^c	1.90±0.1^d
M_5	63.16±1.4^b	97.83±0.7^c	149.16±1.9^e	4.00±0.8^a	0.38±0.07^a	1.15±0.1^b
C.D.at 5%	2.761	2.359	3.401	1.259	0.115	0.153
S.Em±	0.936	0.800	1.153	0.427	0.039	0.052

The results are based on six replications per treatment in three repeated experiments. ± indicates the Standard Deviation values. Means followed by the same letter are not significantly different at *P*=0.05. CD indicates critical difference and SEm indicates standard error of means.

media supplemented with 1mg kinetin/L (M_2). In contrast, the minimum number of leaves (3.83) was observed in MS media with no hormone (M_1).

Length of leaves: Maximum length of leaves (0.88cm) was observed in MS media supplemented with 1mg NAA (2-naphthalene acetic acid)/L (M_4) whereas minimum length of leaves (0.38cm) was observed in MS media supplemented with 2 mg BAP (6-benzyl amino purine)/L (M_5) which was statistically at par with MS media with no hormone (M_1) and MS media supplemented with 1mg kinetin/L (M_2) (Table 1). Five months after germination, the maximum length of leaves (2.18cm) was observed in MS media supplemented with 2mg kinetin/L (M_3), which was at par with (2.05cm) MS media with no hormone (M_1) followed by (1.95 cm) MS media supplemented with 1mg NAA (2-naphthalene acetic acid)/L (M_4). In contrast, the minimum length of leaves (1.28cm) was observed in MS media supplemented with 2mg BAP (6-benzyl amino purine)/L (M_5) (Table 2).

Length of plantlets after germination: Length of plantlets 3 months after germination showed a statistically significant difference among the treatments under study. The maximum length of plantlets (1.90cm) was observed in MS media supplemented with 1 mg NAA (2-naphthalene acetic acid)/L (M_4) followed by (1.46 cm) MS media supplemented with 2 mg kinetin/L (M_3). M_2 and M_5 showed statistically at par result in the length of plantlets, whereas the minimum length (0.85cm) was observed in MS media with no hormone (M_1). However, perusal of data presented in the Table 2 revealed that length of plantlets 5 months after germination showed statistically significant values. The maximum length of plantlets (3.23cm) was observed in MS media supplemented with 2mg kinetin/L (M_3) which was at par with M_1 (3.05cm) followed by M_4 (2.98cm). In contrast, the minimum length (2.30cm) was observed in MS media supplemented with 1mg kinetin/L (M_2).

Table 2. Effect of different plant growth regulators on number of leaves, length of leaves and length of plantlets 5 months after germination of *Dendrobium moschatum* seeds

Media	No. of leaves at 5 months after germination	Length of leaves (cm) at five months after germination	Length of plantlet (cm) at 5 months after germination
M_1	3.83±0.7[a]	2.05±0.1[cd]	3.05±0.3[cd]
M_2	6.50±0.5[c]	1.60±0.2[b]	2.30±0.1[b]
M_3	7.16±0.7[c]	2.18±0.1[d]	3.23±0.1[d]
M_4	5.33±0.5[b]	1.95±0.1[c]	2.98±0.1[c]
M_5	4.83±0.7[b]	1.28±0.09[a]	2.06±0.1[a]
C.D.at 5%	0.811	0.174	0.233
S.Em±	0.275	0.059	0.079

The results are based on six replications per treatment in three repeated experiments. ± indicates the Standard Deviation values. Means followed by the same letter are not significantly different at P=0.05. CD indicates critical difference and S.Em indicates standard error of means.

Discussion

In this study, MS media with five different plant growth hormones were utilised among these days taken for seed germination and protocorm formation was found least in MS media with no hormone. This occurred as a result of the growth regulators' inclusion at varying concentrations to the media, which led to an excess of the additional concentration needed for *Dendrobium moschatum* seeds. Both kinetin and NAA at excessively high and low concentrations will impede plant growth and cell division,

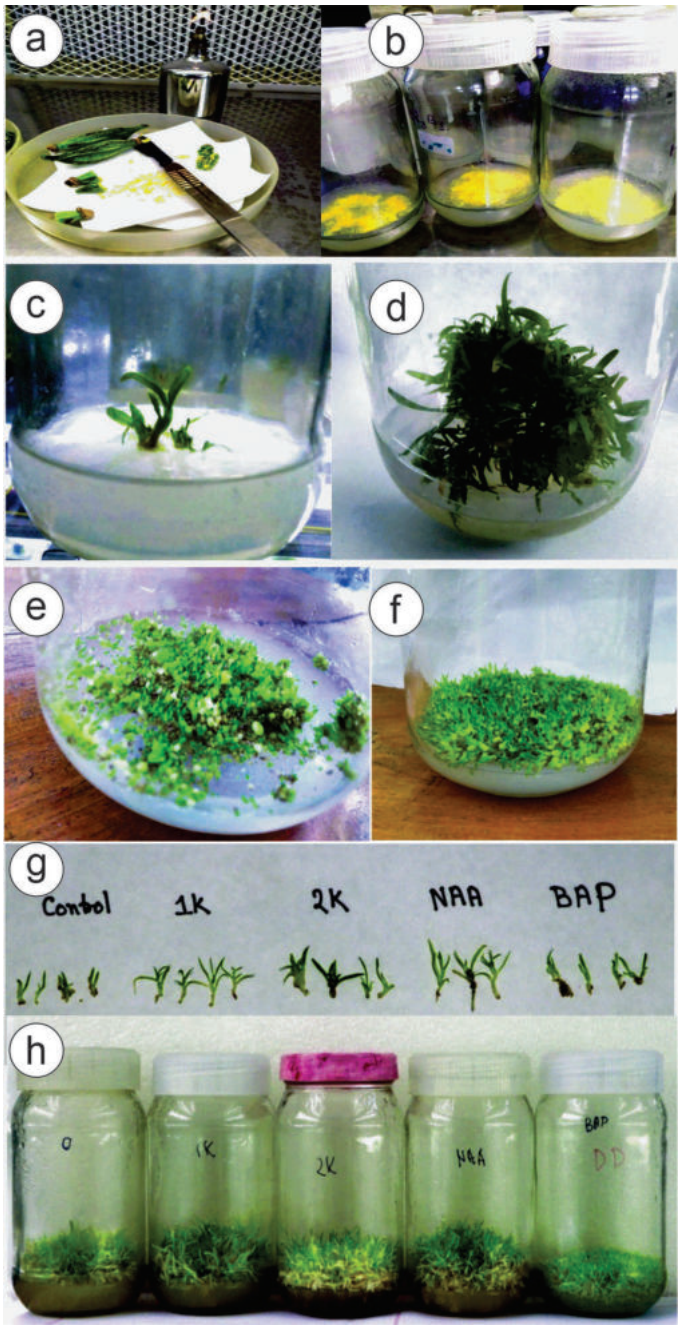

Fig. 1. (a): Seedpods of *D. moschatum.* (b): Seeds of *D. moschatum.* (c): After subculture of plantlets. (d) After proper emergence of leaves. (e): Development of protocorm. (f): Development of plantlet from protocorm. (g-h): Plantlets of *D. moschatum* developed plantlets under different media.

negatively affecting plants. This is because excess nutrients will negatively affect plants. After all, each plant has an adequate amount of endogenous cytokinins while maintaining an active meristem network. Puri *et al.* (2022) noted a similar pattern, greater kinetin doses impede cell proliferation. According to Chin *et al.* (2019), some cultures are believed to have enough endogenous cytokinins to suit their needs, negating the necessity for PGR. According to Dohling *et al.* (2008), adding GA_3 to the medium inhibits seed germination and plantlet development in *Dendrobium* species. Alam *et al.* (2002) found that the *Dendrobium transparens* seeds needed 50 days for germination in MS media without adding any plant growth promoter. Hoque (1993) and Ismat (1982) found that seeds of *Dendrobium* sp.

took 55 days and 51 days for germination in M.S. medium, respectively. The present findings show a resemblance with their results.Parmar and Pant (2016) reported that *Coelogyne stricta* seeds took a minimum of five weeks to germinate, both in the control group and in Murashige and Skoog (M.S.) medium supplemented with 1 mg/L of BAP and 1 mg/L of NAA. But the minimum days required for the first initiation of leaf was observed in MS media supplemented with 2mg kinetin/L. Kinetin or 6-furfurolaminopurrine, associated with cytokinin group, which promotes cell division and activates the processes of cellular growth and segregation. The same trend was also observed by Nguyen *et al.* (2011).

Initially maximum number of leaves was found best in NAA-supplemented media but after 5 months kinetin showed better results. Islam *et al.* (2014) found similar results that the maximum weight of PLBs and the highest number of plantlets were observed on M.S. medium containing 1.0 mg/L NAA while maximum PLBs multiplication and highest number of PLBs were found on M.S. medium supplemented with 2.0 mg/L IAA+0.5 mg/L of kinetin. In a study Pradhan *et al.* (2013) concluded that MS media supplemented with NAA (0.5 mg/L) and BAP (2 mg/L) was ideal for proficient regeneration of *D. densiflorum*. Shoot induction and proliferation may vary because of different plant growth regulator concentrations, especially cytokinin (Anuchai and Sasiangdee, 2020). Cytokinins can promote cell division and have also been shown to influence both root and shoot development (Moubayidin et al., 2009; Heriansyah et al., 2020).Tikendra *et al.* (2018) reported that MS media supplemented with 2 mg kinetin/lit showed the best result in shoot number per plantlet (3.16) after 30 weeks of the culture of *Dendrobium thysiflorum* Rchb.f. which is an endangered medicinal orchid. Rahman (2001) found the maximum number of regenerated plantlets (4.66/vial) with the supplementation of 0.5 mg/L NAA in *Doritaenopsis* orchid. According to Parvin *et al.* (2009), longer shoots were produced by a higher concentration of NAA and NAA would work better for shoot elongation.

The length of plantlets showed similar trends as the number of leaves. Auxin's role in encouraging *In vitro* plant shoots has been well investigated by Aloni *et al.* (2006). Similar findings of increased shoot formation in response to auxins were previously documented in *Dendrobium pendulum, D. primulinum, D. heterocarpum, D. aqueum, D. microbulbon* and *D. chrysanthum* (Li *et al.*, 2013; Parthibhan *et al.,* 2015; Sharma *et al.*, 2007; Rao and Barman 2014). Kaviani *et al.* (2013) reported that MS media supplemented with 2mg kinetin/L showed best shoot length of *Matthiola incana* (an ornamental plant) where seeds were used as explants. Tikendra *et al.* (2018) reported that MS media supplemented with 2mg kinetin/L showed the best result in shoot length (3.36cm) after 30 weeks of culture of *Dendrobium thysiflorum* Rchb. f. Kinetin stimulated rapid cell division to produce several shoots in *Dendrobium huoshanense*, which affected *In vitro* shoot proliferation (Luo *et al.,* 2009). According to Abeed *et al.* (2021), the hormone kinetin is involved in cell division in plant tissue culture. Similarly, combining kinetin to the medium improved the development of shoots in *D. thyrsiflorum*, demonstrating the role that exogenous cytokinin in encouraging multiple shootings (Rizal *et al.*, 2017; Sulichantini, 2016). This may be because the available nutrients were fully utilized for the development of multiple shoots. Growing on kinetin-enriched medium, *Aerides ringens* formed the longest roots and the most significant number of shoots (Srivastava *et al.*, 2015). Asghar *et al.* (2011) also reported that longest shoot development in *Dendrobium nobile* on medium supplemented with 1.5 mg/L kinetin. Puri *et al.* (2022) also found 1.5 mg/L kinetin concentration optimum for the multiplication of *Dendrobium* Sonia. Maharjan *et al.* (2020) reported that the highest number of shoots of *D. chryseum* developed on ½-M.S. medium fortified with 2.0 mg/L of kinetin.

The present study revealed the possibility of developing effective and reproducible *In vitro* regeneration protocols for rapidly propagating *D. moschatum*. From the above experimental results, it may be concluded that different plant growth promoters have different activity on plant cells. However, in seed germination, the effect was slight, but in the case of plantlet growth and development, plant growth promoters showed significantly different effective results. The plant growth regulator, kinetin @ 2mg/L, was the most effective for shoot proliferation of *Dendrobium* moschatum in in-vitro conditions.

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References

Abeed, A.H., M.A. Eissa and D.A. Abdel-Wahab, 2021. Effect of exogenously applied jasmonic acid and kinetin on drought tolerance of wheat cultivars based on morpho-physiological evaluation. *J. Soil Sci. Plant Nutr.*, 21: 131-144.

Alam, M. K., M.A. Rashid, M.S. Hossain, M.A. Salam and M.A. Rouf, 2002. *In vitro* Seed Porpagation of *Dendrobium* (*Dendrobium transparens*) Orchid as Influenced by Different Media. *Biotechnol.,* 1: 111-115.

Aloni, R., E. Aloni, M. Langhans and C.I. Ullrich, 2006. Role of auxin in regulating *Arabidopsis* flower development. *Planta*, 223: 315-328.

Anuchai, J. and A. Sasiangdee, 2020. Effect of growth regulator on shoot induction from protocorm of *Dendrobium* Anna. *Int. J. Agric. Technol.*, 16(6): 1319-1330.

Asghar, S., T. Ahmad, I.A. Hafiz and M. Yaseen, 2011. *In vitro* propagation of orchid (*Dendrobium nobile*) var. Emma White. *Afr. J. Biotech.,*10: 30973103.

Baker, M.L. and C.O. Baker, 2009. Orchid Species Culture: *Dendrobium*, Timber Press (OR).

Bose, T.K. and S.K. Bhattacharjee, 1999. Orchid of India (Edited). Naya Prakash Calcutta, 70006; 4-5.

Chen, Q.X. and Z.H. Ji, 1998. Encyclopedia of China orchid. Beijing, China: China Forestry Publishing House.

Chin, C.K., Z.H. Lee, S.A. Mubbarakh, J.J.J. Antony, B.L. Chew and S. Subramaniam, 2019. Effects of plant growth regulators and activated charcoal on somaclonal variations of protocorm-like bodies (PLBs) of *Dendrobium Sabin* Blue orchid. *Biocat. Agric. Biotechnol.*, 22: 101426.

De, L.C., A.N. Rao, P.K. Rajeevan and P. Pathak, 2014. Orchid Improvement- An overview. *J. Orchid Soc. India,* 28: 35-45.

Dohling S, Kumaria S, Tandon P (2008) Optimization of nutrient requirement for asymbiotic seed germination of Dendrobium longicornu Lindl. and D. formosum Roxb. *Proc. Indian Natl. Sci. Acad.,* 74(4):167–171.

Govaerts, R., P.Bernet, K.Kratochvil, G. Gerlach, G. Carr, P. Alrich, A.M. Pridgeon, J. Pfahl, M.A. Campacci, B.D. Holland, H. Tigges, J. Shaw, P. Cribb, A. George, K. Kreuz and J.J. Wood, 2017. World checklist of Orchidaceae. The Royal Botanic Gardens. http: //apps. kew.org/wcsp.

Heriansyah, P., H.B. Jumin and M. Maizar, 2020. *In vitro* rooting induction on the embryo somatic of *Dendrobium* species from Riau Province Indonesia. *Paspalum: J. Ilmiah Pertanian*, 8(2): 93-98.

Hinsley, A., H.J. De Boer, M.F. Fay, S.W. Gale, L.M. Gardiner, R.S. Gunasekara, P. Kumar, S. Masters, D. Metusala, D.L. Roberts and S. Veldman, 2018. A review of the trade in orchids and its implications for conservation. *Bot. J. Linnean. Soc.*, 186(4): 435-455.

Hoque, N.N. 1993. Micro-propagation of native and exotic orchids. M.Sc. Thesis, IPSA, Salna, Gazipur, Bangladesh: 19-31.

Islam, M.N., K.M. Nasiruddin and M. Al-Amin, 2014. *In vitro* growth and multiplication of a hybrid orchid (*Dendrobium alba*× *Ascanda dongtarm*) with different concentrations of plant growth regulators. *J. Biosci. Agr. Res.*, 1: 27-33.

Ismat, N. 1982. An attempt towards micro-propagation of some orchids of Bangladesh. M.Sc. Thesis, Department of Botony, University of Dhaka, Bangladesh: 75-76.

Kaviani, B., A. A. Hesar, A. Tarang, S. B. Zanjani, D. Hashemabadi and M.H. Ansari, 2013.Effect of kinetin (Kn) and naphthalene acetic acid (NAA) on the micropropagation of *Matthiolaincana* using shoot tips, and callus induction and root formation on the leaf explants. *Afr. J. Agric. Res.,* 8(30): 4134-4139.

Kong, Q., S.Y. Yuan and G. Y. Végvári, 2007 Micropropagation of an orchid *Dendrobium strongylanthum* Rchb. f. *Int. J. Hortic. Sci.,*13(1): 61-64.

Kovács, A., A. Vasas and J. Hohmann, 2008. Natural phenanthrenes and their biological activity. *Phytochem.*, 69(5): 1084-1110.

Kumaria, S. and P. Tandon, 2010. Biotechnological approaches to the conservation of orchids, the wondrous and mystic plants of North-East India. Man and Society. *J. NorthEast Stud.*, 4: 61-68.

Leitch, I.J., J. M. Beaulieu, M.W. Chase, A.R. Leitch and M.F. Fay, 2010. Genome size dynamics and evolution in monocots. Review Article. Hindawi Publishing Corporation. *J. Bot.,* 2010: 1-18.

Li, Y., D.H. Zhu, H.T. Pan and Q.X. Zhang, 2013. *In vitro* propagation of three *Dendrobium* species from stems. *J. Northeast Forest. Univ.,* 41(8): 77–81.

Luo, X.Q., M.K. Wu, G. Shen and X.B. Zhang, 2013. Guizhou Karst areas *Dendrobium officinale* re-introduce conservation and sustainable utilization. *Chin. Wild. Plant. Resour.*, 32(6): 47-50.

Luo, J.P., C. Wawrosch and B. Kopp, 2009. Enhanced micropropagation of *Dendrobium huoshanense* C.Z. Tang et S.J. Cheng through protocormlike bodies: The effects of cytokinins, carbohydrate sources and cold pretreatment. *Sci. Hortic.,* 123: 258262.

Ma, N.L., S.C. Khoo, J.X. Lee and C.F. Soon, 2020. Efficient micropropagation of *Dendrobium aurantiacum* from shoot explant. *Plant. Sci. Today,* 7(3): 476-482.

Maharjan, S., L.S. Thakuri, B.B. Thapa, S. Pradhan, K.K. Pant, G.P. Joshi and B. Pant, 2020. *In vitro* propagation of the endangered orchid *Dendrobium chryseum* Rolfe from protocorms culture. *Nepal. J. Sci. Technol.*, 19(1). DOI: https://doi.org/10.3126/njst.v19i1.29737

Moubayidin, L., D.R. Mambro and S. Sabatini, 2009. Cytokinin-auxin cross talk. *Trends. Plant. Sci.*, 14: 557-562.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologiae Plantarum*, 15: 473-497.

Nguyen, V.S., H.V. Phan and T.B.P. Truong, 2011. *In vitro* Micropropagation of *Dendrobium chrysotoxum*. *Hue. Univ. J. Sci.,*10: 24-30

Pant, B. 2013. Medicinal orchids and their uses: Tissue culture, a potential alternative for conservation. *Afr. J. Plant. Sci.*, 7(10): 448-467.

Parmar, G. and B. Pant, 2016. *In vitro* seed germination and seedling development of the orchid *Coelogyne stricta* (D. Don) Schltr. *Afr. J. Biotechnol.*, 15(5): 105-109.

Parthibhan, S., T.K. Senthil and M.V. Rao, 2015. Phenology and reintroduction strategies for *Dendrobium aqueum* Lindley – An endemic, near-threatened orchid. *J. Nat. Conserv.*, 24: 68-71.

Parvin, M.S., M.E. Haque, F. Akhter, M. Moniruzzaman and A.B. Khaldun, 2009. Effect of different levels of NAA on *in vitro* growth and development of shoots of *Dendrobium* orchid. *Bangladesh J. Agric. Res.*, 34(3): 411-6.

Pradhan, S., Y.P. Paudel and B. Pant, 2013. Efficient regeneration of plants from shoot tip explants of *Dendrobium densiflorum* Lindl., a medicinal orchid. *Afr. J. Biotechnol.*, 12: 1378-1383.

Puchooa, D. 2004. Comparison of different culture media for the *in vitro* culture of *Dendrobium* (Orchidaceae). *Int. J. Agril. Biol.*, 6(5): 884-888.

Puri, S., P. Heriansyah and T. Nopsagiarti, 2022. Potassium dihydrogen phosphate (KH_2PO_4) and kinetin enhance the growth of *Dendrobium* Sonia somatic embryos (Kalium Dihidrogen Fosfat (KH_2PO_4) dan Kinetin Meningkatkan Untuk Pertumbuhan Embrio Somatik *Dendrobium* Sonia). *J. Bio. Indonesia.*, 18(1): 41-50.

Rahman, A.R.M.M. 2001. Studies on the improvement of *in vitro* plantlet regeneration in *Dorieaenopsis* and Cattleya orchids. M.S. Thesis, Dept. of Crop Bot., Bangladesh Agricultural University, Mymensingh.

Rajput, D. and L.R. Saikia, 2020. GCMS analysis of phyto components of the musky smelling *Dendrobium –Dendrobium moschatum*. *Eco. Env. Cons.*, 26: 215-220.

Rao, S. and B. Barman, 2014. *In vitro* Micropropagation of *Dendrobium chrysanthum* Wall. Ex Lindl. –A Threatened Orchid. *Sch. Acad. J. Biosci.*, 2(1): 39-42.

Rizal, S., W.E. Murdiono and E. Nihayati, 2017. Pengaruh pemberian beberapa konsentrasi kinetin terhadap induksi tunas aksilar tanaman kakao (*Theobroma cacao* L.) secara *in vitro*. *J. Produksi Tanaman*, 5(9): 1512-1517.

Secretariat of the Convention on Biological Diversity, 2011. List of Parties. http: //www.cbd.int/convention/ parties/list/. Accessed 17 May 2011

Shah, S., B.B. Thapa, K. Chand, S. Pradhan, A. Singh, A. Varma, L.T. Sen, P. Joshi and B. Pant, 2019. *Piriformospora indica* promotes the growth of the *in vitro* raised *Cymbidium aloifolium* plantlet and their acclimatization. *Plant Sig. Beh.*, 14(6): 1596716. doi: 10.1080/15592324.2019.1596716

Sharma, U., R. Rama, J. Mohan and A. Reddy,2007. *In vitro* propagation of *Dendrobium microbulbon* A. Rich – A rare ethno medicinal herb. *Indian J. Biotechnol.*, 6: 381384.

Srivastava, D., M.C. Gayatriand S.K. Sarangi, 2015. *In vitro* seed germination and plant regeneration of an epiphytic orchid *Aerides ringens* (Lindl.) Fischer. *Indian J. Biotech.*, 14: 574580.

Subedi, A. 2011. New species, pollinator interactions and pharmaceutical potential of Himalayan orchids Ph.D. Thesis, Leiden University, The Netherlands.

Sulichantini, E.D. 2016. Pengaruh Konsentrasi Zat Pengatur Tumbuh terhadap Regenerasibawang Putih (*Allium Sativum* L) secara Kultur Jaringan. *Agrifor: Jurnal Ilmu Pertanian dan Kehutanan*, 15(1): 29-36.

Teixeira da Silva, J.A., S. Zeng, J.C. Cardoso, J. Dobránszki and G.B. Kerbauy, 2014. *In vitro* flowering of *Dendrobium*. *Plant Cell, Tissue. Organ Cult.* (*PCTOC*), 119: 447-456.

Thokchom, R. and S. Maitra, 2017. Standardization of surface sterilization technique for aseptic *in vitro* establishment and survival of leaf explants in *Gerbera jamesonii* cv. Rosalin. *Int. J. Bas. App. Bio.*, 4(2): 112-114.

Tikendra, L., T. Amom and P. Nongdam, 2019. Molecular genetic homogeneity assessment of micropropagated *Dendrobium moschatum* Sw.-A rare medicinal orchid, using RAPD and ISSR markers. *Plant Gene.*, 19: 100196.

Tikendra, L., T. Amom and P. Nongdam, 2018. Effect of Phytohormones on Rapid *In vitro* Propagation of *Dendrobium thyrsiflorum* Rchb.f.: An Endangered Medicinal Orchid. *Pharmacognosy Magazine.,* Published by Wolters Kluwer –Medknow, 14(58), ISSN: 0973-1296.

Yeung, E.C. 2017. A perspective on orchid seed and protocorm development. *Yeung Bot. Stud.*, 58(1): 1-14.

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