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In vitro Micropropagation of an Endangered Medicinal Orchid, *Dendrobium transparens* (Wall. ex. Lindl.) from Bangladesh

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Key words: Endangered, Medicinal, Protocorm like bodies (PLBs), Multiple shoot Buds (MSBs), Plant growth regulators (PGRs)

Abstract

Dendrobium transparens is an endangered medicinal and ornamental orchid species of Bangladesh. The nodal segments of *in vitro* grown plantlets were used as explant for this study. MS medium provides the highest percentage (85.0 ± 0.37) of primary (PLBs) formation. While the highest percentage of secondary PLBs (88.0 ± 0.58) and its maximum weight (1.15 ± 0.05 g) was recorded when the cultures medium were fortified with 2.0 mg/l Kn + 1.0 mg/l NAA. The maximum percentage of shoots (80.0 ± 0.45) were recorded when 0.5 mg/l BAP was used. The highest length (4.34 ± 0.10 cm) of plantlets was found when the medium was enriched with 2.0 mg/l BAP and 1.0 mg/l NAA. The maximum number of multiple shoot buds (MSBs) (9.60 ± 0.15) was recorded on a medium containing 1.5 mg/l BAP + 0.75 mg/l NAA. Following acclimatization well rooted plantlets were shifted to the orchid shed house of the Institute of Biological Sciences (IBSc), University of Rajshahi. The protocol established during this study might be very useful for mass multiplication and further improvement of orchids in Bangladesh.

Introduction

Orchids belong to the plant family Orchidaceae, which appears to be one of the most popular well-known commercially grown plants worldwide with a significant share in floriculture trades with high potential value (De 2020, Bhattacharjee et al. 2022, Ayesha and Islam 2024). It is popular as a cut flower and potted plant because of its captivating long-lasting blooms with diverse colors, shapes and form (Kndlmann et al. 2023). Orchids are not only commercially but also medicinally important. Many orchid genera are eminent for pharmacological and medicinal attributes (Bhattacharjee and Islam 2015, Gantait et al. 2021). *Dendrobium* is one of the largest orchid genera, which composed of

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1600 species (Hartati et al. 2021). It is distributed throughout the sub-tropical and tropical regions of Asia and Oceania with enormous morphological diversity which made it difficult to classify them (Nguyen 2020). Most of the species of this genus is epiphytic with high rate of self-incompatibility, which might be a reason for their high level of species diversity (Niu et al. 2018). Around 40 *Dendrobium* species have been applied in traditional Chinese medicine (Niu et al. 2018, Ma et al. 2020). It is common traditional herbal medicinal plants in China due to their pharmacological significance of health benefits for human. *Dendrobium* stems are commonly used in traditional Chinese medicine (TCM) to improve digestion, promote fluid production, nourish, clear heat, and serve as a tonic (Teixeira da Silva and Ng 2017, Joshi et al. 2023). Large quantities of polysaccharides, phenanthrenes and polyphenols that have strong cytotoxic and antioxidant properties and certain of them have shown immunoregulatory, anticancer, anti-angiogenic, anti-platelet aggregation and antimicrobial activity, are commonly found in *Dendrobium* species (Bhattacharjee et al. 2015, Pant et al. 2022, Joshi et al. 2023, Biswas et al. 2024).

There have been 72 genera of orchids reported to exist in Bangladesh. Among them, *Dendrobium* is the most diverse genus with 27 species (Rahman et al. 2017). *Dendrobium transparens* is a medicinal as well as ornamental orchid in Bangladesh (Bhowmik and Rahman 2020a). It used to treat geriatric illnesses and problems in Indian traditional medicine (Joshi et al. 2023). Paste of its pseudobulb is also used to treat fractured and dislocated bones (Yonzone 2018). Extracts of *D. transparens* displayed high cytotoxicity against cancer cell lines like HeLa and U251 (Shukla et al. 2022). This species is at risk of extinction due to habitat destruction (Mutum et al. 2022). Rashid et al. (2017) reported that it is critically endangered in Bangladesh and even listed as a possibly lost orchid. Vegetative propagation of *D. transparens* is very slow and its seed germination rate is highly poor. It is normally propagated by dividing pseudobulbs, generating about 2-4 plants per year. For this reason, tissue culture micropropagation is extremely useful to sustain their germplasm and for healthcare needs (Joshi et al. 2023). *In vitro* derived PLBs are one of the most commonly used explants in micropropagation of *Dendrobium* plants (Fritsche et al. 2022). The main objective of this study was to establish an effective *in vitro* micropropagation protocol of *D. transparens* using different explants for its conservation and further investigations.

Materials and Methods

Disease free *D. transparens* were collected from Sylhet, Bangladesh and kept in the natural condition at the orchid shed house, Institute of Biological Sciences (IBSc), University of Rajshahi. Immature capsules were collected from these plants as a seed source. Plantlets were successfully developed under *in vitro* condition through seed germination. Under this study, nodal segments of these plantlets were used as explants and primary PLBs generated from these nodal segments were used as explants for micropropagation of *Dendrobium transparens.*

Six-month-old shoots of *in vitro* grown plantlets of *D. transparens* were cut into small segments by a sterile surgical blade under aseptic condition. Each segment was 1- 1.5 cm long with single node. These nodal segments were cultured in glass vessels that contained four types of culture medium. MS, B5 (Gamborg et al. 1968), PM (Phytamax™, Sigma, USA) and MVW (Vacin and Went 1949) were used in this study. As basal media, MS and B5 were amended with 3% (w/v) sucrose and the PM and MVW were amended with 2% (w/v) sucrose. The pH for all mediums was adjusted to 5.6-5.8. Inoculated vessels were maintained in the culture room for a period of 16 hrs light and dark, for 8 hrs at 25 ± 2°C. After two weeks of inoculation, some of the nodal segments developed primary PLBs. Once the primary protocorm like bodies (PLBs) were developed, then data about percentage of primary PLBs formation were recorded by following formula:

$$\text{Percentage of primary PLBs formation} = \frac{\text{Number of explant responded} \times 100}{\text{Total number of cultured explants.}}$$

Primary PLBs from nodal segments of *D. transparens* were collected and approximately 0.25 g of them were cultured as explants on MS medium supplemented with various concentrations of plant growth regulators either alone or in combination for secondary PLBs/shoots regeneration and its subsequent development. Various PGRs e.g. BAP (0.5, 1.0, 1.5 and 2.0 mg/l), Kn (0.5, 1.0, 1.5 and 2.0 mg/l) and NAA (0.5, 1.0 and 2.0 mg/l) were used in this study. In this case MS basal medium was considered as control. Data were recorded on the basis of the percentage of secondary PLBs or shoots production from primary PLBs, increase weight of secondary PLBs and their viability to developed plantlets. Percentage of secondary PLBs/ shoots formation were recorded by following formula:

$$\text{Percentage of secondary PLBs/shoots formation} = \frac{\text{Total number of explant responded} \times 100}{\text{Total No. of cultured explants.}}$$

About 0.25 g of primary PLBs were cultured as explant into the culture vessels containing the same types of media that were able to regenerate secondary PLBs. Data on increased weight of secondary PLBs were recorded after 30 days of inoculation by following formula:

Increased weight of secondary PLBs = Final weight of PLBs - Initial weight of PLBs

$$\text{Increased weight of secondary PLBs} = \frac{\text{Total increased weight of secondary PLBs}}{\text{Total no. of cultured explants.}}$$

Young plantlets regenerated from PLBs were transferred to MS medium enrich with PGRs in order to induce rapid growth. MS medium supplemented with different concentration of PGRs either single or in combination were used to evaluate their efficacy on height elongation of plantlets. For multiple shoot bud induction, the shoot segments

were transferred into fresh MS medium fortified with the above mentioned PGRs of same concentration either single or in combination. MS basal medium was used as a control.

The experiment was designed by a completely randomized design (CRD). Data were recorded for different parameters and were statistically analyzed by analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The calculations were done at a 5% level of significance ($p < 0.05$). All data were represented as the mean with a standard error (mean ± SE). Five culture vessel each with eight explants were taken per medium for production of primary PLBs from nodal segments. Ten culture vessels each with five explants were taken per treatment for secondary PLBs/Shoots regeneration. For elongation of plantlets and multiple shoot buds (MSBs) formation, ten explants were taken for each treatment. All the experiments were carried out three times. The data analysis was performed using the IBM SPSS software.

Results and Discussion

Nodal segments of *D. transparens* were inoculated on four types of PGRs free agar solidified basal media to examine their effectiveness on primary PLBs production. Primary PLBs production was influenced by the types of medium and they responded in different ways and at different times. Two weeks after inoculation small green round shape primary PLBs were started to form and became visible around the nodal region of the nodal segments (Fig. 1a-c). Data were recorded every week after PLBs formation. In few cases, direct shoot regeneration along PLBs production also occurs on MS medium (Fig. 1b).

Table 1. Comparative performance of four culture media on primary PLBs formation of *D. transparens*.

Medium	Total no. of cultured explant	% of primary PLBs formation (Mean ± SE)	Required time week
MS	40	85.0 ± 0.37[a]	2-3
PM	40	70.0 ± 0.40[b]	3-4
Modified VW	40	57.5 ± 0.51[c]	4-5
B5	40	-	-

MS = Murashige and Skoog (1962), PM = Phytamax™, P-1056, Sigma, USA; B5 = Gamborg et al. (1968), MVW (modified after Vacin and Went medium -1949). Means in a column with the differentletter (superscript) are significantly different according to least significant difference at ($p < 0.05$).

The highest percentage (85.0 ± 0.37%) of primary PLBs production was achieved on MS basal medium with lowest the required time (Fig. 1b-c) followed by PM (70.0 ± 0.40%) (Fig. 1a) and MVW (57.5 ± 0.51%). B_5 medium failed to produce any PLBs on nodal segments (Table 1).

Primary PLBs were sub cultured on MS medium supplemented with various forms of auxin and cytokinin to evaluate their efficacy in promoting the development of secondary PLBs/shoots and multiplication of secondary PLBs. Different types of PGRs with different concentration were used either alone or in combination (Table 2). MS basal media were used as control. Secondary PLBs/shoots were started producing from primary PLBs after 2 weeks of inoculation. The highest percentage of shoots (80 ± 0.45) occurs on MS medium enriched with 0.5 mg/l BAP while 2.0 mg/l Kn + 1.0 mg/l NAA supplemented medium provides the highest percentage (88.0 ± 0.58) of secondary PLBs and its maximum increase in weight (1.15 ± 0.05g) were also obtained from the same condition followed by 2.0 mg/l Kn + 1.0 mg/l NAA (Table 2). The combined effect of Kn and NAA was more effective than other PGRs on development of secondary PLBs of *D. transparens* (Fig. 1e), while single effect of BAP in low concentration was proved to be best for shoot formation (Fig. 1d). Secondary PLBs later converted successfully into healthy plantlets within 4 weeks (Fig. 1f). A significant percentage (60 ± 0.45) of shoot formation was obtained from control medium but it failed to produce secondary PLBs (Table 2). Plantlets derived from secondary PLBs were used to study for other parameters in the present study (Fig. 1g).

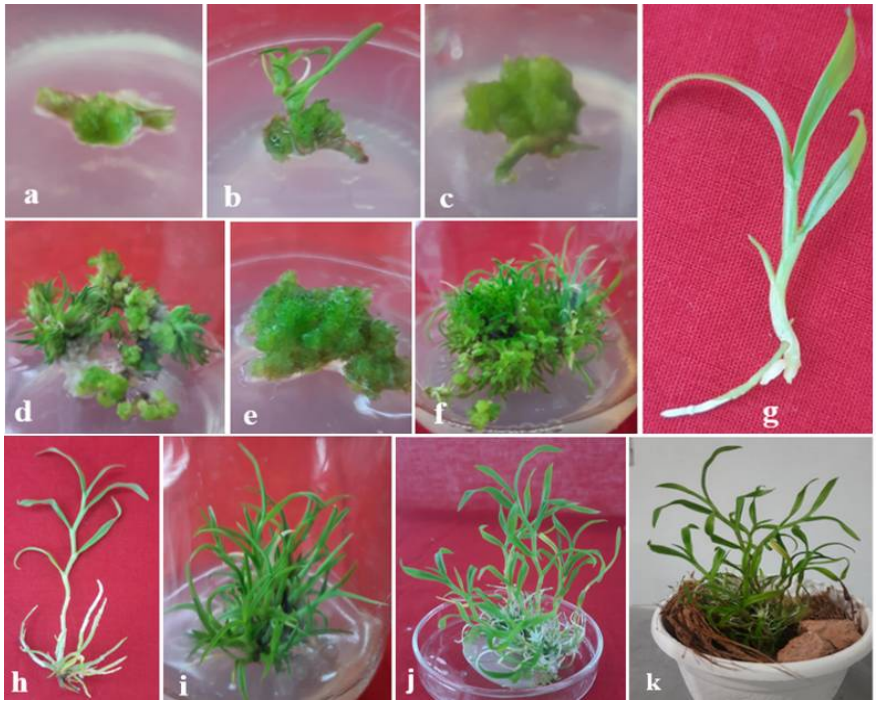

Fig. 1 (a-k). Primary and secondary PLBs development of *D. transparens:* (a) Primary PLBs on PM medium, (b-c) Primary PLBs on MS medium, (d) Shoot regeneration on primary PLBs, (e) Secondary PLBs developed from primary PLBs, (f) Young plantlets developed from secondary PLBs, (g) A single young plantlet, (h) An elongated plantlet with well-developed roots, (i) Multiple branching from a single shoot, (j) Mature plantlets with roots ready to hardening and (k) After acclimatization transferred of plants in pot.

Table 2. Effects of PGRs on development of PLBs/shoots and increase weight of secondary PLBs in *D. transparens*.

PGRs (mg/l)			% of secondary PLBs formation (Mean ± SE)	% of shoot formation (Mean ± SE)	Increase weight of secondary PLBs(g) (Mean ± SE)	Required time for shoot/secondary PLBs formation (Week)
BAP	Kn	NAA				
0.5	-	-	-	80 ± 0.45^{a}	-	
1.0	-	-	28 ± 0.37^{c}	62 ± 0.49^{b}	0.29 ± 0.05^{c}	
1.5	-	-	36 ± 0.40^{b}	48 ± 0.37^{c}	0.40 ± 0.06^{b}	4-5
2.0	-	-	42 ± 0.37^{a}	34 ± 0.51^{d}	0.45 ± 0.09^{a}	
-	0.5	-	20 ± 0.32^{c}	70 ± 0.55^{a}	0.36 ± 0.04^{c}	
-	1.0	-	38 ± 0.37^{b}	54 ± 0.24^{b}	0.38 ± 0.07^{bc}	5-6
-	1.5	-	40 ± 0.32^{ab}	40 ± 0.45^{c}	0.44 ± 0.08^{b}	
-	2.0	-	54 ± 0.51^{a}	22 ± 0.37^{d}	0.62 ± 0.09^{a}	
0.5	-	0.5	30 ± 0.32^{f}	64 ± 0.51^{a}	0.42 ± 0.04^{e}	
1.0	-	0.5	50 ± 0.45^{e}	44 ± 0.24^{b}	0.61 ± 0.06^{d}	
0.5	-	1.0	56 ± 0.51^{d}	32 ± 0.58^{c}	0.68 ± 0.05^{c}	3-4
1.0	-	1.0	68 ± 0.58^{c}	24 ± 0.40^{d}	0.74 ± 0.04^{b}	
2.0	-	1.0	78 ± 0.37^{b}	-	0.85 ± 0.05^{ab}	
1.0	-	2.0	80 ± 0.32^{a}	-	0.90 ± 0.07^{a}	
2.0	-	2.0	62 ± 0.37^{bc}	-	0.66 ± 0.05^{cd}	
-	0.5	0.5	56 ± 0.40^{d}	42 ± 0.37^{a}	0.51 ± 0.04^{e}	
-	1.0	0.5	82 ± 0.37^{ab}	20 ± 0.32^{c}	1.03 ± 0.02^{ab}	
-	0.5	1.0	66 ± 0.40^{c}	30 ± 0.45^{b}	0.65 ± 0.04^{d}	2-3
-	1.0	1.0	74 ± 0.51^{bc}	-	0.71 ± 0.05^{c}	
-	2.0	1.0	88 ± 0.58^{a}	-	1.15 ± 0.05^{a}	
-	1.0	2.0	78 ± 0.37^{b}	-	0.94 ± 0.05^{b}	
-	2.0	2.0	70 ± 0.45^{c}	-	0.75 ± 0.06^{bc}	
Control (MS0)			-	60 ± 0.45	-	5-6

PGRs = Plant growth regulators, values represent mean ± SE. Each treatment was repeated three times. Means in a column with different letters (superscript) are significantly different according to the least significant difference at $p < 0.05$ levels.

In vitro grown plantlets of *D. transparens* were cultured on MS medium fortified with different types of PGRs for elongation of plants and induction of MSBs. Different concentrations of BAP, Kn and NAA were used either alone or in combination to assess their efficacy on the above mentioned parameters. Data was recorded after 30 days of inoculation. The maximum elongation (4.34 ± 0.10 cm) of plants was observed on MS medium enriched with 2.0 mg/l BAP and 1.0 mg/l NAA. The effects combined of BAP + NAA were superior on elongation of plantlets followed by Kn + NAA (Fig. 1h). Among PGRs fortified medium the minimum elongation (1.18 ± 0.08 cm) was obtained from

medium with 0.5 mg/l BAP + 1.0 mg/l Kn while control media showed the lowest increased height of plantlets (Table 3).

Table 3. Effects of MS medium fortified with different PGRs on elongation of plants of *D. transparens* after 30 days of culture initiation.

PGRs (mg/l)			Elongation of plant height (cm)			No. of MSBs Mean ± SE
BAP	Kn	NAA	Final length (Mean)	Initial length (Mean)	Increased length (Mean ± SE)	
0.5	-	-	4.50	3.00	1.50 ± 0.08^{d}	4.03 ± 0.15^{d}
1.0	-	-	4.91	3.06	1.85 ± 0.10^{c}	5.30 ± 0.12^{b}
1.5	-	-	5.61	2.98	2.63 ± 0.11^{a}	6.33 ± 0.19^{a}
2.0	-	-	5.15	3.10	2.05 ± 0.07^{b}	4.90 ± 0.21^{c}
-	0.5	-	4.39	2.95	1.44 ± 0.07^{d}	3.70 ± 0.12^{d}
-	1.0	-	4.77	3.10	1.67 ± 0.06^{c}	4.80 ± 0.20^{b}
-	1.5	-	5.37	3.12	2.25 ± 0.09^{a}	5.50 ± 0.17^{a}
-	2.0	-	4.94	3.03	1.91 ± 0.10^{b}	4.10 ± 0.15^{c}
0.5	-	0.25	5.32	3.00	2.32 ± 0.07^{d}	6.00 ± 0.21^{d}
1.0	-	0.5	5.81	2.93	2.88 ± 0.11^{c}	8.53 ± 0.19^{b}
1.5	-	0.75	6.88	3.02	3.86 ± 0.12^{b}	9.60 ± 0.15^{a}
2.0	-	1.0	7.33	2.99	4.34 ± 0.10^{a}	7.90 ± 0.12^{c}
-	0.5	0.25	5.30	3.13	2.17 ± 0.10^{c}	5.70 ± 0.17^{d}
-	1.0	0.5	5.91	2.91	3.00 ± 0.09^{b}	6.50 ± 0.15^{c}
-	1.5	0.75	6.86	3.11	3.75 ± 0.13^{a}	8.30 ± 0.10^{a}
-	2.0	1.0	5.64	3.08	2.56 ± 0.07^{bc}	7.10 ± 0.12^{b}
0.5	0.5	-	4.91	3.10	1.81 ± 0.10^{b}	3.80 ± 0.15^{bc}
1.0	0.5	-	5.30	3.04	2.26 ± 0.11^{a}	4.63 ± 0.19^{a}
0.5	1.0	-	4.30	3.12	1.18 ± 0.08^{c}	4.07 ± 0.18^{b}
1.0	1.0	-	4.43	3.06	1.37 ± 0.08^{bc}	3.30 ± 0.12^{c}
Control (MS0)			3.87	3.03	0.84 ± 0.09	2.97 ± 0.18

PGRs = Plant growth regulators, values represent mean ± SE. Each treatment was repeated three times. Means in a column with different letters (superscript) are significantly different according to the least significant difference at p-value (<0.05) levels.

To investigate the effect of PGRs on MSBs induction of *D. transparens* single shoots were transferred on fresh culture medium with the same PGRs in the same condition used to investigate height elongation of plants. The maximum number of MSBs (9.60 ± 0.15) was found on MS medium supplemented with 1.5 mg/l BAP + 0.75 mg/l NAA (Fig. 1i). The minimum number of MSBs (3.30 ± 0.12) per explant was obtained on MS medium fortified with 1.0 mg/l BAP + 1.0 mg/l Kn. We obtained lowest numbers of MSBs on MS0 medium which were used as control (Table 3). Data were recorded after 30 days of inoculation.

This is the first complete protocol that has been developed for mass propagation of *D. transparens* by PLBs collected from *in vitro* grown nodal segments. Considering the

rate of amount and time the present findings suggest that, among the four investigated basal media MS medium was the most suitable one for primary PLBs (85.0 ± 0.37%) generations of *D. transparens* with a significant difference from other media (Table 1). These results are in contrast with those reported earlier in*, Dendrobium chrysotoxum*, *Vanda cristata*, and *Vanilla planifolia* where the PGRs fortified medium favoured regeneration of maximum PLBs (Kaur 2017, Pathak et al. 2022, Malhotra et al. 2023). Limited work on the percentage of secondary PLBs and shoot formation from primary PLBs has been reported by the researchers. Maximum researchers reported proliferation of number/weight of secondary PLBs from primary PLBs. This study found that the highest percentage of secondary PLBs (88.0) and their heaviest weight (1.15 g) from primary PLBs occurred in MS medium with 2.0 mg/l Kn and 1.0 mg/l NAA. On the contrary, BAP + NAA and TDZ + NAA supplemented medium provides highest number of secondary PLBs regeneration respectively in *Dendrobium* sp. and *Aerides crispum* (Hossen et al. 2021, Pyati 2022). ½MS semi-solid medium fortified with BAP and Kn displayed the highest rate of PLBs proliferation in Oncidium golden anniversary orchid (Zakaria et al. 2021). In the present study, the highest percentage of shoot formation (80.0 ± 0.45) from primary PLBs was observed on medium enrich with 0.5 mg/l BAP. Multiple shoot buds induction and increase of plant height are two key points of *in vitro* micropropagation because of its importance for rapid multiplication and batter hardening. Different types and combination of PGRs in various concentrations and combinations enriched medium were used to study their efficacy on height increasement of *in vitro* grown plantlets of *D. transparens*. We observed that 2 mg/l BAP + 1.0 mg/l NAA was best for the height elongation of plantlets. In this medium the average length of the plantlets was about 4.34 cm (Table 2). The present findings are supported by those, who reported positive influence of BAP + NAA on the height elongation of plantlets across many orchid species (Islam et al. 2015a, Bhowmik and Rahman 2020b). Instead of BAP+NAA, MS medium supplemented with Kn + NAA provide highest length of shoots in *Dendrobium anosmum* (Nguyen et al. 2022). On the other hand, some researchers reported the boosting effect of sole PGRs on shoot height increase (Parthibhan et al. 2015).

In case of MSBs induction, we obtained the highest number (in average of 9.60 shoots per explants) of adventitious shoots of *D. transparens* in the media fortified with 1.5 mg/l BAP + 0.75 mg/l NAA. Our findings are in total agreement with the result reported on *Cymbidium finlaysonianum* (Islam et al. 2015b). The beneficial effect of BAP + NAA on MSBs induction was also reported as best on shoot multiplication in some orchid species, (Islam et al. 2015a, Bhowmik and Rahman 2020b). Some researchers noted a stimulated effect of Kn combined with auxin on shoot multiplication in *Dendrobium fimbriatum* (Tikendra et al. 2021). In contrast, the positive effect of BAP fortified KC medium and BAP+ half strength MS medium on shoot multiplication was reported respectively in *Dendrobium chryseum* and *Cymbidium aloifolium* (Maharjan et al. 2020, Kumar et al. 2022). It was observed that, like many orchid species in the addition of BAP and NAA to the MS

medium led to optimum height elongation of plants and MSBs induction of *D. transparens*. The well-rooted plantlets were taken out and successfully hardened in a potting mixture which included coconut husk, charcoal and brick pieces in a 2 : 1 : 1 ratio (Fig. 1j-k). Maximum plantlets survived the acclimatization process and grew to a usual flowering plant in the natural conditions of field environments.

The results of this study suggest that, PLBs that developed from nodal segments are a reliable and appropriate source for rapid mass clonal propagation of *D. transparens*. The highest percentage of primary PLBs (85.0 ± 0.37) obtained from MS medium. Single PGR provides the highest percentage of shoot regeneration (80.0 ± 0.45) while, combined effect of PGRs were more effective for secondary PLBs development (88.0 ± 0.45), plant height elongation (4.34 ± 0.10) and multiple shoot induction (9.60 ± 0.15) of *D. transparens*. Under this study an efficient regeneration protocol for *in vitro* micropropagation in *D. transparens* through PLBs from nodal segment has been established for the first time.

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References

Ayesha MN and **Islam SMS** (2024) *In vitro* germination and mass multiplication of an endangered medicinal orchid *Bulbophyllum crassipes* Hook. f. from Bangladesh. Plant Tiss. Cult. Biotech. **34**(2): 93-104.

Bhattacharjee B and **Islam SMS** (2015) Assessment of antibacterial and antifungal activities of the extracts of *Rhynchostylis retusa* Blume- A Medicinal Orchid. World J. Phar. Pharma. Sci. **4**(2): 74-87.

Bhattacharjee B, Hossain ME, Ayesha MN and **Islam SMS** (2022) Morphological diversity analysis of some existing and endangered *Dendrobium* orchid species in Bangladesh. J. Bio-Sci. **30**(2): 47-57.

Bhattacharjee B, Islam SMS and **Subramaniam S** (2015) Rapid development of PLBs derived from immature seeds and mass multiplication of *Vanda tessellata* (Roxb.) Hook. ex. G. Don a *threatened* orchid in Bangladesh. Plant Tiss. Cult. Biotech. **25**(2): 181-191.

Bhowmik TK and **Rahman MM** (2020a) *In vitro* mass scale propagation and seedlings development of medicinal orchid *Dendrobium transparens* Wall ex Lindl. J. Medicin. Plant Stud. **8**(3): 141-146.

Bhowmik TK and **Rahman MM** (2020b) *In vitro* seed germination and micropropagation of *Dendrobium chrysotoxum* Lindl (Golden Bow): A highly fragrant orchid species of Bangladesh. J. Orchid Soci. India. **34**: 69-77.

Biswas SS, Kalaivanan NS, Pal R, Alam BK, Ngangom NM and **Natta S** (2024) Traditional knowledge of medicinal Orchids at different parts of India. Indian J. Tradit. Know. **23**(5): 452-463.

De LC (2020) Morphological diversity in orchids. Int. J. Bot. Stud. **5**(5): 229-238.

Fritsche Y, Ornellas TS, Stefenon VM and **Guerra MP** (2022) Ploidy mosaics: does endopolyploidy in explants affect the cytogenetic stability of orchids regenerated from PLBs? Plant Cell Tiss. Org. Cult. **149**(3): 697-713.

Gamborg OL, Miller R and **Ojima K** (1968) Nutrient requirements of suspension cultures of soybean root cells, Experimental Cell Res. **50**(1): 151-158.

Gantait S, Das A, Mitra M and **Chen JT** (2021) Secondary metabolites in orchids: Biosynthesis, medicinal uses, and biotechnology. South African J. Bot. **139**: 338-351.

Hartati S, Cahyono O and **Hariyadi AN** (2021) Morphological characterization of natural orchids *Dendrobium* spp. In IOP Conference Series: Earth Environ. Sci. **905**(1): 012139.

Hossen MM, Saha S, Khatun F and **Yasmin S** (2021) Effects of plant growth regulators on in vitro growth and development of orchid (*Dendrobium* sp.) from protocorm like bodies (PLBs). J. Bangladesh Agricul. Univ. **19**(3): 294-301

Islam SMS, Islam T, Bhattacharjee B, Mondal TK and **Subramaniam S** (2015a) *In vitro* pseudobulb based micropropagation for mass development of *Cymbidium finlaysonianum* Lindl. Emirates J. Food Agric. **27**(6): 469-474

Islam T, Bhattacharjee B, Islam SMS, Uddain J and **Subramaniam S** (2015b) Axenic seed culture and *in vitro* mass propagation of Malaysian wild orchid *Cymbidium finlaysonianum* Lindl. Pakistan J. Bot. **47**(6): 2361-2367.

Joshi PR, Pandey S, Maharjan L and **Pant B** (2023) Micropropagation and assessment of genetic stability of *Dendrobium transparens* wall. Ex Lindl. Using RAPD and ISSR markers. Front. Conserv. Sci. **3**: 1083933.

Kaur S (2017) *In vitro* regeneration of shoots from nodal explants of *Dendrobium chrysotoxum* Lindl. J. Hort. Res. **25**(1): 27-34.

Kumar A, Chauhan S, Rattan S, Warghat AR, Kumar D and **Bhargava B** (2022) *In vitro* propagation and phytochemical assessment of *Cymbidium aloifolium* (L.) Sw.: an orchid of pharma-horticultural importance. South African J. Bot. **144**: 261-269.

Kndlmann P, Kull T and **McCormick M** (2023) The distribution and diversity of orchids. Diversity **15**(7): 810.

Murashige T and **Skoog F** (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant **15**: 473-497.

Ma NL, Khoo SC, Lee JX and **Soon CF** (2020) Efficient micropropagation of *Dendrobium aurantiacum* from shoot explant. Plant Sci. Today. **7**(3): 476-482.

Maharjan S, Thakuri LS, Thapa BB, Pradhan S, Pant KK, Joshi GP and **Pant B** (2020) *In vitro* propagation of the endangered orchid *Dendrobium chryseum* Rolfe from protocorms culture. Nepal J. Sci. Technol. **19**(1): 39-47.

Malhotra EV, Bansal S, Pathania P, Mali SC and **Agrawal A** (2023) Regeneration of plantlets through protocorm-like bodies: An alternative method for vanilla micro propagation. Indian J. Biotech. **20**: 388-395.

Mutum RD, Chanu NM, Khanganba TN and **Thongam B** (2022) Propagation and conservation of selected orchids of Manipur. J. Orchid Soc. India. **36**: 95-101.

Nguyen HT, Dinh ST, Ninh TT, Nong HT, Dang TT, Khuat QV and **Kalashnikova EA** (2022) *In vitro* propagation of the *Dendrobium anosmum* Lindl. collected in Vietnam. Agronomy **12**(2): 324.

Nguyen NH, Le TL, Vu HT, Tran KD, Tran H, Duong HX and **Tran HD** (2020) Analysis and categorization of several *Dendrobium* species based on morphological traits. Annual Res. Review Biol. **35**(3): 97-114.

Niu SC, Huang J, Xu Q, Li PX, Yang HJ, Zhang YQ and **Liu ZJ** (2018) Morphological type identification of self-incompatibility in *Dendrobium* and its phylogenetic evolution pattern. Int. J. Mol. Sci. **19**(9): 2595.

Pant B, Chand K, Paudel MR, Joshi PR, Thapa BB, Park SY and **Rajbhandari P** (2022) Micropropagation, antioxidant and anticancer activity of pineapple orchid: *Dendrobium densiflorum* Lindl. J. Plant Biochem. Biotech. **31**: 399-409.

Parthibhan S, Rao MV and **Kumar TS** (2015) *In vitro* regeneration from protocorms in *Dendrobium aqueum* Lindley- An imperiled orchid. J. Gene. Eng. Biotech. **13**(2): 227-233.

Pathak P, Kumari A and **Thakur B** (2022) Regeneration competence of an endangered orchid, *Vanda cristata* Wall. ex Lindl. using leaf explants: A study *in vitro*. South African J. Bot. **151**: 1018-1024.

Pyati AN (2022) Multiple protocorm like body (MPLB) formation and plant regeneration from the PLB culture of a rare orchid *Aerides crispum* Lindl. Indian J. Appl. Pure Biol. **37**(2): 555-563.

Rahman MA, Huda MK and **Rashid ME** (2017) Orchid species diversity in Bangladesh and their revised nomenclatural updates. Biodiver. Bull. Bangladesh **10**: 1-70.

Rashid ME, Rahman MA and **Huda MK** (2017) Possibly lost orchid treasure of Bangladesh and their enumeration with conservation status. J. Biodiver. Conser. Biores. Manag. **3**(1): 65-84.

Shukla MK, Thakur A, Verma R, Lalhlenmawia H, Bhattacharyya S, Bisht D and **Kumar D** (2022). Unravelling the therapeutic potential of orchid plant against cancer. South African J. Bot., **150**: 69-79.

Teixeira da Silva JA and **Ng TB** (2017) The medicinal and pharmaceutical importance of *Dendrobium species*. Appl. Microbiol. Biotech. **101**: 2227-2239.

Tikendra L, Potshangbam AM, Dey A, Devi TR, Sahoo MR and **Nongdam P** (2021) RAPD, ISSR, and SCoT markers based genetic stability assessment of micropropagated *Dendrobium fimbriatum* Lindl. var. *oculatum* Hk. f.- An important endangered orchid. Physiol. Molecul. Biol. Plants **27**: 341-357.

Vacin EF and **Went FW** (1949) Use of tomato juice in the asymbiotic germination of orchid seeds, Botanical Gazette. **111**(2): 175-183.

Yonzone R (2018). Medicinal evaluation of six additional *Dendrobium* species (Orchidaceae) with taxonomic enumeration, field status and threats in Darjeeling Himalaya of West Bengal, India. Agricul. Int. **5**(1): 49-64.

Zakaria S, Antony JJJ, Chin CK, Zakaria R and **Subramaniam S** (2021) Development of *in vitro* culture system for proliferation of protocorm-like bodies of *Oncidium* golden anniversary orchid. Songklanakarin J. Sci. Technol. **43**(6): 1542-1549.

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