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Development of an efficient micropropagation protocol for *Geodorum densiflorum* (Lam.) Schltr: A terrestrial medicinal orchid of Bangladesh

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Abstract
Geodorum densiflorum (Lam.) Schltr. is an endangered terrestrial orchid of Bangladesh, highly valued for its ornamental appeal and therapeutic applications, including the treatment of diabetes, menstrual irregularities and wound healing. Due to habitat destruction and the inherent difficulties of natural seed germination without symbiotic fungi, this species faces severe conservation threats. This study established a highly reproducible *in vitro* propagation protocol via asymbiotic seed germination and micropropagation utilizing rhizome and leaf explants. Four basal media (MS, PM, MVW and KC) were evaluated. The highest seed germination rate and earliest seedling development were achieved on Murashige and Skoog (MS) medium followed by PM basal medium. For mass micropropagation, rhizome segments exhibited maximum multiple shoot buds (MSBs) induction (58.67%; 5.76 ± 0.16 shoots/explant) on MS medium fortified with 2.0 mg/l BAP and 1.0 mg/l NAA. Conversely, leaf segments successfully produced Protocorm-Like Bodies (PLBs) with the highest frequency (58.67%) on MS medium supplemented with 2.0 mg/l BAP and 1.0 mg/l Picloram. Shoot elongation was optimal on solid MS medium containing 1.0 mg/l Kn and 0.6 mg/l IAA, yielding a maximum length increase of 4.00 cm. Strong and stout root systems were successfully induced on MS medium supplemented with 1.0 mg/l IBA and 1.0 mg/l NAA, generating an average of 4.17 roots per seedling. Regenerated seedlings were successfully acclimatized with a 85% survival rate in a potting mixture of sterile sand, sawdust, small bricks and charcoal. This optimized protocol provides a robust pathway for the rapid mass propagation and ex situ conservation of this critically endangered medicinal orchid.

Keywords: *Geodorum densiflorum*, micropropagation, multiple shoot buds (MSBs), protocorm-like bodies (PLBs), ex situ conservation

Introduction

Orchids represent natures most extravagant group of flowering plants, distributed globally and renowned for their incredible diversity in shape, size and color. Beyond their aesthetic brilliance, many orchids play a significant role in traditional systems of medicine due to their rich phytochemical reservoirs of alkaloids, flavonoids, glycosides and other bioactive compounds (Rahman and Husen 2003, Rosa *et al.* 2009) [18, 19]. *Geodorum densiflorum* (Lam.) Schltr., commonly known as the nodding swamp orchid, is an endangered, herbaceous terrestrial orchid native to the moist grasslands and semi-deciduous forests of Bangladesh, India, Nepal and Australia (Datta *et al.* 1999) [8].

In traditional medicine, the underground rhizome and roots of *G. densiflorum* are ethno-medicinally utilized to treat various ailments. Root pastes are applied as insecticides and wound-healing agents, while rhizome extracts are used to regularize menstrual cycles, control diabetes and cure carbuncles (Patil and Patil 2005, Dash *et al.* 2008, Nath *et al.* 2011) [7, 14, 15]. Unfortunately, increasing commercial demand for its ornamental and medicinal properties has led to continuous over exploitation from its natural habitats.

Like other members of the Orchidaceae family, *G. densiflorum* produces millions of extremely minute, non-endospermic seeds. In nature, successful germination is strictly dependent on a symbiotic association with specific mycorrhizal fungi, resulting in less than 1% of seeds successfully developing into mature plants (Arditti 1967) [1]. Furthermore, conventional vegetative propagation via rhizome division is a slow and expensive process, rendering it insufficient for large scale commercial cultivation or conservation efforts.

Recent advancements in plant biotechnology emphasize the critical need for robust *in vitro* propagation techniques to counteract the rapid depletion of medicinal orchids (Rahman and Huda 2021) [17]. Contemporary studies on endangered orchids like *Robiquetia spathulata* and *Pholidota articulata* have demonstrated that optimized media can significantly enhance seed germination, PLBs mass proliferation and rapid seedlings development (Bhowmik and Rahman 2021; San Win 2021; Hussien *et al.* 2024) [5, 10, 17, 22]. *In vitro* tissue culture, particularly asymbiotic seed germination and clonal micropropagation, offers a powerful alternative for overcoming these natural dormancy barriers and producing mass planting materials (Sheelavantmath *et al.* 2000; Bhadra and Hossain 2003) [3, 23].

While some protocols exist for commercial epiphytic orchids, highly efficient and reproducible micropropagation protocols for indigenous terrestrial medicinal orchids of Bangladesh remain under researched. The present study was designed to establish an optimized, Multiple Shoot Buds (MSBs) development, PLBs induction and root development.

Materials and Methods

1. **Plant Material and Sterilization:** Mature, un-dehisced green capsules of *Geodorum densiflorum* (Lam.) Schltr. were collected from the wild populations in Bangchari, Kaptai, Rangamati, Bangladesh. The capsules were

washed under running tap water to remove superficial dust and treated with a standard detergent solution. Surface sterilization was conducted in a laminar airflow cabinet by treating the capsules with 0.2% (w/v) mercuric chloride ($HgCl_2$) for 5 minutes, followed by a one-minute rinse in 70% ethanol. The capsules were finally washed 3-4 times with sterile double distilled water to eliminate any residual sterility.

2. **Media Preparation and Culture Conditions:** Four distinct basal media were evaluated for seed germination: MS (Murashige and Skoog 1962) [12], PM: Phytamax (Arditti 1977) [2], MVW (Vacin and Went 1949) [24] and KC (Knudson 1946) [11]. Sucrose was added at 3% (w/v) for MS medium and 2% (w/v) for PM, MVW and KC media. The media were solidified with 0.8% (w/v) agar. The pH of the media was adjusted to 5.8 (MS), 5.4 (PM, MVW) and 5.0 (KC) using 1N NaOH or 1N HCl prior to autoclaving at 121°C and 1.9 kg/cm^2 pressure for 20 minutes. All cultures were incubated in a controlled culture room maintained at 25 ± 2°C under a 14/10-hour (light/dark) photoperiod provided by cool white fluorescent tubes.

3. **Asymbiotic Seed Germination:** Surface sterilized capsules were longitudinally dissected using a sterile surgical blade. The powdery seeds were aseptically scooped out and uniformly spread over the surface of the four basal media (MS, PM, MVW and KC. Regular observations were made to record the time taken for the initiation of germination, protocorm formation and complete seedling development.

4. **Micropropagation via Rhizome and Leaf Explants:** To evaluate mass multiplication capabilities, *in vitro* generated seedlings (5-6 cm in height) were utilized as source material. Rhizome segments (1.0-1.5 cm) and leaf segments (0.5-1.0 cm) were excised aseptically and inoculated onto full strength MS media. The media were supplemented with 17 different concentration combinations of cytokinins (BAP, Kinetin) and auxins (NAA, IAA, Picloram) to evaluate their potential for MSBs and PLBs induction.

5. **Shoot Elongation and Root Induction:** Vigorous MSBs generated from rhizome explants were subcultured for rapid elongation. Both solid (0.8% agar) and liquid MS basal media supplemented with varying concentrations of Kinetin (Kn), BAP, IAA and NAA were assessed for 30 days. For root induction, elongated shoots (2-3 cm) were individually transferred to MS media fortified with different combinations of auxins (IAA, IBA and NAA) ranging from 0.5 to 1.0 mg/l.

6. **Hardening and Acclimatization:** Well-developed seedlings with stout root systems were subjected to sequential acclimatization. Culture vessels were kept open in the culture room for 24 hours, subsequently moved to an external environment for 6 hours and then for 12 hours the following day. Seedlings were gently removed and roots were washed thoroughly under running tap water to remove adhering agar. The seedlings were transplanted into small pots containing a sterilized mixture of sand, sawdust, small brick pieces and charcoal and watered regularly.

7. **Statistical Analysis:** All experiments were conducted using a Completely Randomized Design (CRD). Data on multiple shoot counts, PLBs development, root number and length were recorded. The values are presented as Mean ± Standard Error (SE) of each experiment, consisting of appropriate replicates (5 for multiplication and elongation, 6 for rooting).

Results

1. ***In vitro* Seed Germination and Differentiation:** The seeds of *G. densiflorum* were cultured asymbiotically, demonstrating variable developmental responses across the four distinct basal media. Germinating seeds on the MS medium (Fig. 1) not only exhibited the highest germination rate but also rapidly formed robust followed by PM medium (Fig. 2), intensely green protocorms compared to the pale, slower growing counterparts observed on the control media. Germination was poorest on the KC medium. The transition from protocorms to fully developed seedlings was fastest on the same MS medium.

2. **Micropropagation via Rhizome and Leaf Explants:** Rhizome segments exhibited direct organogenesis leading to Multiple Shoot Buds (MSBs), while leaf segments underwent embryogenesis to form PLBs (Table 1). The highest percentage of induced MSBs (58.67 ± 1.44%) and the maximum number of MSBs per rhizome segment (5.6 ± 0.51) were recorded on MS medium supplemented with 2.0 mg/l BAP and 1.0 mg/l NAA within 5.76 ± 0.16 weeks (Fig. 3). The generated MSBs were exceptionally healthy and lacked hyper-hydricity, ensuring high quality shoot multiplication. A closely comparable response was noted with 2.0 mg/l BAP + 1.0 mg/l IAA (5.4 ± 0.60 shoots/segment).
   For leaf segments, the peak embryogenic response was observed on MS medium containing 2.0 mg/l BAP combined with 1.0 mg/l Picloram (Fig. 4). This synergistic formulation yielded a 58.67 ± 1.44% induction rate of PLBs within the shortest timeframe of 7.44 ± 0.11 weeks. The induced PLBs were distinguished by their yellowish-green coloration and friable texture, indicating high embryogenic competence and regenerative potential. Explants cultured on hormone free MS control media exhibited minimal to zero proliferative responses.

3. **Shoot Elongation:** To counter the naturally slow growth rate of the newly formed MSBs, they were transferred to solid and liquid elongation media (Table 2). Solid MS media generally proved superior to liquid media for shoot elongation. The maximum shoot length increase (4.00 ± 0.05 cm) was achieved over 30 days on solid MS medium fortified with 1.0 mg/l Kn and 0.6 mg/l IAA (Fig. 5). In contrast, the maximum elongation observed in the corresponding liquid medium was slightly lower (3.93 ± 0.04 cm, Fig. 6). Furthermore, seedlings cultured on solid MS medium exhibited broader leaves, sturdier stems and a healthier overall morphology compared to those in liquid media, where early signs of water-logging were occasionally

observed. Media lacking PGRs (MS0) displayed the lowest elongation rates in both solid (2.86 cm) and liquid (2.59 cm) forms.

4. **Root Induction and Plantlet Development:** Elongated shoots (2-3 cm) transferred to rooting media demonstrated robust responses to exogenous auxins (Table 3). Among the 13 formulations tested, MS medium supplemented with 1.0 mg/l IBA and 1.0 mg/l NAA yielded the most exceptional results. This combination induced the highest average number of roots per seedling (4.17 ± 0.40) and the maximum root length increase (3.85 ± 0.06 cm) within 30 days (Fig. 7). The roots induced by the IBA and NAA combination were distinctly thick, fleshy and covered with dense root hairs, architectural traits that are vital for subsequent ex *vitro* acclimatization. Formulations utilizing IAA and NAA also performed well (4.00 roots/seedling), but combinations heavily reliant on a single auxin or hormone free control media (1.67 roots/seedling) produced significantly weaker and thinner root networks.

5. **Hardening and Ex *Vitro* Establishment:** Rooted seedlings displaying 3-5 leaves and stout root systems were successfully transferred to ex *vitro* conditions (Fig. 8). A potting mix comprising sterilized sand, sawdust, small bricks and charcoal provided excellent aeration and moisture retention. Following the acclimatization period, the seedlings demonstrated a survival rate of 85% after two months. The surviving plants effectively adapted to the external environment, exhibiting the development of new functional roots and continuous foliage growth.

Table 1: Development of MSBs/PLBs from *in vitro* raised rhizome and leaf explants of *Geodorum densiflorum* on agar solidified full strength MS medium with different kinds of PGRs

Sl. No.	PGR Concentration (mg/l)					Rhizome Segment			Leaf Segment		
	BAP	Kn	NAA	IAA	Pic	% of induced MSBs/ segment (Mean ± SE)	Time required (week) for development of MSBs (Mean ± SE)	No. of MSBs produced/ segment (Mean ± SE)	% of induced PLBs/ segment (Mean ± SE)	Time required (week) for development of PLBs (Mean ± SE)	Nature of PLBs (Colour)
1.	1.0	-	-	-	-	20.00±1.72	7.42±0.10	2.2±0.37	0.00±0.00	0.00±0.00	-
2.	2.0	-	-	-	-	33.33±1.72	6.64±0.15	3.8±0.58	13.33±1.72	9.14±0.15	G
3.	-	1.0	-	-	-	6.67±1.22	7.78±0.18	1.4±0.24	0.00±0.00	0.00±0.00	-
4.	-	2.0	-	-	-	13.33±1.22	7.66±0.17	1.6±0.24	0.00±0.00	0.00±0.00	G
5.	1.0	-	0.5	-	-	46.67±1.72	6.16±0.14	4.6±0.51	26.67±1.22	8.44±0.11	G
6.	2.0	-	1.0	-	-	58.67±1.44	5.76±0.16	5.6±0.51	40.00±1.22	8.22±0.14	YG
7.	1.0	-	-	0.5	-	46.67±1.72	6.46±0.09	4.4±0.24	33.33±1.72	8.12±0.12	WG
8.	2.0	-	-	1.0	-	57.67±1.44	6.14±0.16	5.4±0.60	52.00±1.44	7.68±0.14	YG
9.	1.0	-	-	-	0.5	33.33±1.72	6.76±0.16	3.6±0.40	45.33±1.44	8.12±0.12	G
10.	2.0	-	-	-	1.0	52.00±1.44	6.26±0.15	5.2±0.37	58.67±1.44	7.44±0.11	YG
11.	-	1.0	0.5	-	-	26.67±1.72	7.22±0.15	2.8±0.37	0.00±0.00	0.00±0.00	WG
12.	-	2.0	1.0	-	-	52.00±1.44	6.12±0.17	5.0±0.71	20.00±1.72	8.68±0.14	G
13.	-	1.0	-	0.5	-	20.00±1.22	7.12±0.14	2.4±0.24	6.67±1.22	9.20±0.14	-
14.	-	2.0	-	1.0	-	26.67±1.72	7.14±0.14	3.0±0.71	13.33±1.22	8.72±0.12	YG
15.	-	1.0	-	-	0.5	14.67±1.44	7.54±0.15	2.0±0.45	20.00±1.72	8.64±0.15	-
16.	-	2.0	-	-	1.0	20.00±1.72	7.12±0.12	2.6±0.40	26.67±1.72	8.56±0.05	YG
17.	MS0 (Control)					5.33±1.44	8.22±0.17	1.2±0.20	0.00±0.00	0.00±0.00	-

Multiple Shoot Buds (MBSs); Protocorm Like Bodies (PLBs); '-' Indicate no response; Greenish PLBs (G), Yellowish Green PLBs (YG), Whitish Green PLBs (WG); Values represent mean ± SE of each experiment consist of five replicates.

Table 2: Elongation of MSBs developed from rhizome explant of *Geodorum densiflorum* on 0.8% (w/v) agar solidified and liquid full-strength MS medium with different kinds of PGRs

Sl. No.	PGR Conc. (mg/l)				Solid media			Liquid media		
	BAP	Kn	NAA	IAA	Initial length (cm) of seedlings after germination (Mean± SE)	Length (cm) of seedlings after 30d of culture on elongation medium (Mean± SE)	Increased in length (cm) of seedlings within 30d of culture on elongation medium (Mean± SE)	Initial length (cm) of seedlings after germination (Mean± SE)	Length (cm) of seedlings after 30d of culture on elongation medium (Mean± SE)	Increased in length (cm) of seedlings within 30d of culture on elongation medium (Mean± SE)
1.	1.0	-	-	-	1.47±0.02	4.66±0.04	3.19±0.05	1.48±0.02	4.44±0.07	2.96±0.06
2.	1.5	-	-	-	1.43±0.02	4.56±0.07	3.14±0.05	1.42±0.02	4.31±0.06	2.89±0.04
3.	2.0	-	-	-	1.42±0.02	4.44±0.04	3.03±0.05	1.43±0.02	4.19±0.04	2.76±0.04
4.	-	1.0	-	-	1.44±0.02	5.21±0.05	3.76±0.05	1.46±0.03	4.89±0.03	3.43±0.05
5.	-	1.5	-	-	1.42±0.02	4.93±0.04	3.51±0.04	1.44±0.03	4.75±0.03	3.31±0.04
6.	-	2.0	-	-	1.45±0.03	4.90±0.04	3.45±0.05	1.47±0.02	4.55±0.07	3.08±0.05
7.	1.0	-	0.6	-	1.43±0.02	5.36±0.06	3.93±0.05	1.45±0.03	5.16±0.03	3.71±0.05
8.	1.5	-	0.9	-	1.48±0.02	5.36±0.05	3.88±0.05	1.48±0.02	5.07±0.06	3.59±0.05
9.	2.0	-	1.2	-	1.40±0.02	4.74±0.04	3.34±0.04	1.47±0.02	4.60±0.05	3.13±0.04
10.	1.0	-	-	0.6	1.47±0.02	5.32±0.03	3.85±0.04	1.45±0.02[a]	5.09±0.05	3.64±0.04
11.	1.5	-	-	0.9	1.46±0.02	5.27±0.03	3.81±0.04	1.47±0.02	5.03±0.05	3.56±0.05
12.	2.0	-	-	1.2	1.40±0.02	4.65±0.05	3.25±0.04	1.43±0.02	4.45±0.04	3.02±0.05

13.	-	1.0	0.6	-	1.43±0.03	5.38±0.07	3.96±0.04	1.42±0.02	5.22±0.04	3.80±0.04
14.	-	1.5	0.9	-	1.44±0.03	4.49±0.06	3.05±0.04	1.48±0.02	5.23±0.05	3.75±0.05
15.	-	2.0	1.2	-	1.47±0.02	4.86±0.06	3.39±0.05	1.45±0.02	4.69±0.07	3.24±0.05
16.	-	1.0	-	0.6	1.42±0.03	5.41±0.03	4.00±0.05	1.46±0.02	5.39±0.06	3.93±0.04
17.	-	1.5	-	0.9	1.46±0.03	4.66±0.02	3.20±0.04	1.43±0.02	5.11±0.06	3.68±0.04
18.	-	2.0	-	1.2	1.41±0.02	4.71±0.07	3.30±0.05	1.45±0.02	4.65±0.04	3.20±0.04
19.	MS0 (Control)				1.48±0.02	4.34±0.04	2.86±0.04	1.48±0.02	4.07±0.07	2.59±0.05

Values represent mean ± SE of each experiment consist of five replicates.

Table 3: Mean increase in length (cm) and number of roots per seed derived seedlings of *Geodorum densiflorum* in auxin supplemented MS and MS0 rooting media

Sl. No.	PGR Concentration (mg/l)			Initial length (cm) per seed derived seedling (Mean ± SE)	Initial number of roots per seed derived seedling (Mean ± SE)	Increased length (cm) per seed derived seedling (Mean ± SE)	Increased number of roots per seed derived seedling (Mean ± SE)
	IAA	IBA	NAA				
1.	0.5	-	-	1.51±0.03	0.83±0.31	2.67±0.06	2.67±0.21
2.	1.0	-	-	1.45±0.03	0.50±0.22	2.22±0.06	3.33±0.42
3.	-	0.5	-	1.44±0.03	0.33±0.21	2.32±0.06	2.33±0.21
4.	-	1.0	-	1.53±0.03	0.50±0.22	1.98±0.06	3.00±0.45
5.	-	-	0.5	1.53±0.03	0.33±0.21	2.87±0.06	3.00±0.37
6.	-	-	1.0	1.39±0.02	0.83±0.40	3.30±0.05	3.50±0.43
7.	0.5	0.5	-	1.40±0.03	0.50±0.22	3.10±0.06	3.17±0.48
8.	1.0	1.0	-	1.46±0.03	0.83±0.40	3.41±0.04	3.50±0.43
9.	-	0.5	0.5	1.45±0.04	0.83±0.31	2.71±0.05	3.83±0.48
10.	-	1.0	1.0	1.42±0.03	0.33±0.21	3.85±0.06	4.17±0.40
11.	0.5	-	0.5	1.48±0.03	0.50±0.22	2.52±0.06	3.67±0.49
12.	1.0	-	1.0	1.49±0.02	0.83±0.31	3.63±0.05	4.00±0.37
13.	MS0 (Control)			1.43±0.03	0.67±0.33	1.81±0.07	1.67±0.21

Values represent mean ± SE of each experiment consist of six replicates.

Fig 1: Germinated PLB's turned into small shoots on MS medium

Fig 3: Development of MSBs sprouted from *in vitro* raised rhizome segment on MS + 2.0 mg/l BAP + 1.0 mg/l NAA

Fig 2: Development of seedlings on PM medium

Fig 4: Development of PLBs from *in vitro* developed leaf segment on MS + 2.0 mg/l BAP + 1.0 mg/l Pic

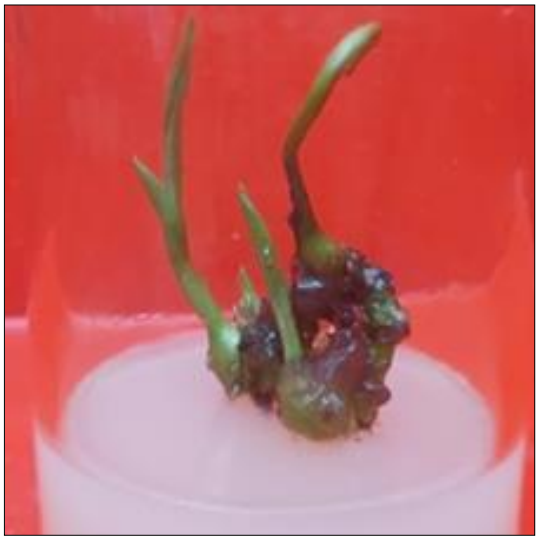

Fig 5: Germinated seedlings undergoing elongation on agar solidified MS + 1.0 mg/l Kn + 0.6 mg/l IAA

Fig 6: Germinated seedlings undergoing elongation in liquid MS + 1.0 mg/l Kn + 0.9 mg/l IAA

Fig 7: Induction of strong and stout root system in seed originated seedling on MS + 1.0 mg/l IBA + 1.0 mg/l NAA

Fig 8: *In vitro* developed *Geodorum densiflorum* seedlings growing in pot outside of the culture room

Discussion

The development of a highly efficient in vitro propagation protocol is pivotal for the conservation of threatened medicinal orchids like G. densiflorum. The results of this study confirm that the nutritional complexity of the basal medium profoundly impacts asymbiotic seed germination.

The overarching superiority of the MS medium over PM, MVW and KC media can be attributed to its high concentrations of nitrogen in the form of ammonium and nitrate salts. Nitrogen is fundamentally essential during the early heterotrophic stages of orchid seed germination for the rapid synthesis of amino acids, proteins and nucleic acids (Roy and Banerjee 2001, Bhadra and Hossain 2003 [3], Prasad *et al.* 2021) [16, 20].

In the micropropagation assays, a distinct morphogenic divergence was observed based on explant type. Rhizome segments exhibited direct organogenesis, while leaf tissues favored somatic embryogenesis resulting in PLBs. The "Auxin-Cytokinin Synergism" was paramount here. The combination of 2.0 mg/l BAP with 1.0 mg/l NAA maximized the multiplication of MSBs from rhizomes. Cytokinins like BAP are critical for breaking apical dominance and stimulating axillary meristems, while NAA aids in stabilizing cell elongation and vascular differentiation (Roy and Banerjee 2002, Borah *et al.* 2015) [6, 21]. For leaf segment embryogenesis, the combination of BAP with Picloram yielded the highest PLBs induction. This synergistic interaction between auxins and cytokinins is crucial for massive PLBs proliferation, aligning with recent findings by Hussien *et al.* (2024) [10], who emphasized the necessity of dual-hormone treatments for avoiding single-hormone-induced organogenesis retardation in orchids. Picloram, a synthetic systemic auxin, acts as a potent stressor that drives somatic cell dedifferentiation, highly effective for PLBs initiation in terrestrial orchids (Naing *et al.* 2011) [13].

During the shoot elongation phase, solid MS media marginally outperformed liquid media. While liquid systems can increase nutrient uptake due to continuous tissue contact, they often lead to hyper-hydricity (vitrification), which causes fragile, water-logged tissues with poor survival prospects ex *vitro*. The agar solidified medium supplied adequate physical support while allowing optimal transpiration and nutrient diffusion, corroborating contemporary findings in the micropropagation of *Dendrobium palpebrae* (Bhowmik and Rahman 2020) [4].

For robust field establishment, high quality root architecture is essential. The synergistic application of IBA and NAA produced thick, stout roots. IBA is a highly stable, potent rooting hormone that initiates adventitious root primordia, while NAA sustains subsequent root elongation (Devi and Neelashree 2018) [9].

Finally, the acclimatization phase, critical for the survival of *in vitro* raised clones, achieved a 85% success rate. Epiphytic and terrestrial orchids are notoriously susceptible to desiccation shock when transitioned from the humid, heterotrophic environment of a culture vessel to autotrophic ex *vitro* conditions. The porous substrate mixture utilized in this study (sand, sawdust, charcoal) effectively mimicked the natural, well-aerated micro-environment of *G. densiflorum*, mitigating transplantation shock.

Conclusion

This study successfully established a highly reproducible, efficient and rapid *in vitro* mass propagation protocol for the endangered medicinal orchid *Geodorum densiflorum* (Lam.) Schltr. The optimal strategy requires asymbiotic seed germination on MS medium followed by high frequency MSBs induction from rhizome explants utilizing 2.0 mg/l BAP and 1.0 mg/l NAA. Robust root induction was perfected using a combined treatment of IBA and NAA. This standardized micropropagation framework offers a commercially viable solution for producing mass planting materials, alleviating the pressure on wild populations and providing a sustainable platform for the ex situ conservation and pharmaceutical exploitation of this valuable orchid species.

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