

Development of an efficient *in vitro* propagation protocol for *Aerides odoratum* Lour: Seed germination, micropropagation, rooting and acclimatization of an indigenous medicinal orchid of Bangladesh

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Abstract

Aerides odoratum Lour. is an indigenous, epiphytic orchid of Bangladesh holding immense ornamental and medicinal value. Due to excessive wild harvesting and significant natural propagation barriers such as the lack of endosperm in seeds and strict mycorrhizal dependence the species is currently facing the threat of localized extinction. The present study was undertaken to establish an efficient, highly reproducible *in vitro* seed germination and direct micropropagation protocol for *A. odoratum* to facilitate its conservation and mass propagation. Seeds from wild collected capsules were cultured on four basal media: Knudson C (KC), Murashige and Skoog (MS), Phytamax (PM) and Modified Vacin and Went (MVW), both with and without plant growth regulators (PGRs). While MS medium supplemented with PGRs initiated germination the earliest (9.12 weeks), MS medium supplemented with 0.5 mg/l 6-benzylaminopurine (BAP) and 0.5 mg/l alpha-naphthaleneacetic acid (NAA) proved fundamentally superior for overall development, yielding the highest germination frequency (83.34%) and the most rapid complete plantlet development (27.01 weeks). For micropropagation *via* direct organogenesis, *in vitro* raised nodal segments exhibited the maximum multiple shoot buds (MSBs) induction (5.7 ± 0.41 shoots/explant, $73.13 \pm 1.58\%$ response) on MS medium fortified with 2.0 mg/l BAP and 1.0 mg/l NAA within 7.84 ± 0.19 weeks. Conversely, leaf segments produced the optimal protocorm-like bodies (PLBs) ($49.00 \pm 1.48\%$) on MS medium containing 2.0 mg/l BAP and 1.0 mg/l Indole-3-acetic acid (IAA). Subsequent shoot elongation was optimized on MS medium supplemented with 2.0 mg/l Kinetin (Kn) and 1.2 mg/l NAA, while robust rhizogenesis was achieved using a synergistic combination of 1.0 mg/l IAA and 1.0 mg/l Indole-3-butyric acid (IBA). Hardened seedlings demonstrated successful *ex vitro* acclimatization with a 75% survival rate. This optimized protocol offers a viable pathway for the commercial cultivation of *A. odoratum*, effectively mitigating wild harvesting pressures and supporting long term biodiversity conservation.

Keywords: *Aerides odoratum*, conservation, direct organogenesis, micropropagation, rhizogenesis

Introduction

The Orchidaceae family, encompassing over 25,000 species across 700 to 800 genera, represents one of the most evolutionarily advanced, diverse and largest groups of angiosperms globally (Chugh *et al.* 2009, Dressler 1993) [8, 11]. Orchids account for nearly 8% of all angiosperm species diversity and have successfully colonized almost every terrestrial habitat, exhibiting complex adaptations such as perennial epiphytic, terrestrial and saprophytic growth forms (Chase *et al.* 2015, Arditti 1992, Fay 2018) [7, 2, 13]. Despite their ecological success, orchids are exceptionally vulnerable to anthropogenic disturbances. The dual pressures of illegal wildlife trafficking driven by their immense ornamental appeal and traditional therapeutic applications and localized habitat destruction have pushed numerous orchid taxa to the verge of extinction (Swarts and Dixon 2009; Hossain *et al.* 2010, Wraith and Pickering 2018) [31, 15, 35].

In Bangladesh, the evergreen and semi-evergreen forests of the Chittagong Hill Tracts, Sylhet and Cox's Bazar serve as

critical biodiversity hotspots for indigenous orchids (Huda 2007, Bhowmik and Rahman 2020) [18, 5]. Among these, *Aerides odoratum* Lour. (syn. *Aerides odorata*) is a prominent, commercially valuable monopodial epiphytic species (Huda and Kashem 2021) [17]. Taxonomically distinguished by its robust, scandent stems, dense foliage and pendulous racemes of fragrant yellow-green flowers, *A. odoratum* is highly prized in the global horticultural industry (Paraste *et al.* 2023, Thiyam *et al.* 2025) [27, 33]. Furthermore, the species holds profound ethnomedicinal significance. Various indigenous communities utilize extracts from its leaves and roots for treating ailments ranging from joint pain, rheumatism and tuberculosis to external cuts and bone fractures (Yonzon *et al.* 2013, Medhi and Chakrabarti 2009, Hossain 2011) [36, 21, 16].

Despite its significance, the natural populations of *A. odoratum* in Bangladesh are declining precipitously. In the wild, orchid reproduction is severely constrained. Orchid seeds are minute, lack endosperm (nutritional reserves) and depend entirely on specific mycorrhizal fungal associations

for successful germination and early protocorm development (Bhowmik *et al.* 2024, Pant 2013, Kauth *et al.* 2008, Rasmussen 1995) [4, 26, 19, 29]. Consequently, the natural seed germination rate is remarkably low, taking 2 to 8 years for a plant to reach maturity. Seed morphology variations further complicate natural propagation across different terrains (Zeng *et al.* 2012, Arditti and Ghani 2000) [37, 1]. To circumvent these biological bottlenecks, asymbiotic *in vitro* seed germination and micropropagation have emerged as vital biotechnological tools. Plant tissue culture techniques provide a reliable alternative for mass scale propagation, ensuring the production of genetically uniform, disease free clones while preserving wild populations (Thiyam *et al.* 2025, Thapa *et al.* 2026, Engelmann 2004, Murthy *et al.* 2018) [33, 32, 12, 23].

Currently, there is a critical research gap regarding highly optimized, reproducible *in vitro* regeneration pathways for *A. odoratum* utilizing varied basal nutritional matrices and precise phytohormonal combinations (Devadas *et al.* 2016) [10]. Therefore, the primary objective of this study was to standardize an efficient *in vitro* seed germination and micropropagation protocol for *A. odoratum*. Specifically, this research aimed to compare the efficacy of four standard basal media Murashige and Skoog (MS), Phytamax (PM), Modified Vacin and Went (MVW) and Knudson C (KC) on seed germination kinetics, optimize the plant growth regulators (PGRs) matrices for multiple shoot bud (MSB) induction and protocorm-like bodies (PLBs) differentiation and establish a robust timeline for subsequent shoot elongation, rhizogenesis and successful *ex vitro* acclimatization (Murashige and Skoog 1962, Arditti 1977, Vacin and Went 1949, Knudson 1946) [22, 3, 34, 20].

Materials and Methods

1. Source of explants and seeds

Undehisced capsules (fruits) of *Aerides odoratum* Lour. were collected from naturally growing populations in Bengchari, Kaptai, Rangamati, Bangladesh. The capsules were extensively washed under running tap water to remove superficial dust and debris, followed by 3-4 rinses with sterile distilled water. Surface sterilization was conducted in a laminar airflow cabinet. The capsules were treated with a 0.2% (w/v) mercuric chloride (HgCl₂) solution for 5 minutes, followed by 2-3 washes with double distilled sterile water. A final surface disinfection was performed using 70% ethanol for 1 minute, concluding with sequential washing using sterile distilled water.

2. Basal media preparation

For asymbiotic seed germination, four basal nutritional media were evaluated: Murashige and Skoog (MS), Phytamax: PM (Arditti 1977), Modified Vacin and Went (MVW) and Knudson C (KC). MS medium was supplemented with 3% (w/v) sucrose, whereas PM, MVW

and KC media were supplemented with 2% (w/v) sucrose. For PM medium, 2.0 g/l peptone was added as an organic supplement. The pH of the respective media was digitally adjusted prior to the addition of the gelling agent: MS was adjusted to 5.8, PM and MVW to 5.4 and KC to 5.0 using 1N NaOH or 1N HCl. All media were solidified with 0.8% (w/v) bacteriological agar. Media were dispensed into culture vessels (30 ml in 2.5 cm × 15 cm test tubes or 50 ml in 100 ml conical flasks), sealed and autoclaved at 1.9 kg/cm² pressure at 121°C for 20 minutes.

3. Growth regulators setup

Plant Growth Regulators (PGRs) were supplemented primarily into the MS basal medium to facilitate direct micropropagation, shoot elongation and rhizogenesis. Stock solutions of cytokinins (BAP and Kinetin) and auxins (NAA, IAA, IBA) were prepared by dissolving 10 mg of the respective powder in 1 ml of 1N NaOH, making the final volume up to 100 ml with distilled water. Picloram was dissolved in ethanol. PGRs were added to the media individually or in synergistic combinations prior to pH adjustment and sterilization.

4. Culture conditions

Surface sterilized capsules were longitudinally dissected using a sterile surgical blade on an aluminum slab. Powdery seeds were scooped and inoculated onto the surface of the agar solidified germination media. All culture vessels, encompassing both germination and subsequent micropropagation phases (using *in vitro* derived nodal and leaf segments), were incubated in a controlled culture room. The environmental parameters were strictly maintained at 25 ± 2°C with a 14h light and 10h dark photoperiod provided by cool-white fluorescent tubes. Sub-culturing was routinely performed at 20-25 day intervals.

5. Acclimatization setup

Well-rooted seedlings were subjected to gradual *ex vitro* acclimatization. The *in vitro* developed seedlings were kept outside the culture room with gradually increasing time up to three days. Seedlings were then gently extracted, their roots washed under running tap water to remove residual agar and transferred into small plastic pots containing saw dust, coal, dried wood and moistened coir. They were kept at room temperature for 3-5 days. Surviving seedlings were then monitored for strong root development.

6. Experimental design and statistical analysis

All experiments were structured in a completely randomized design. Morphogenic responses (germination percentage, MSBs induction, elongation and root count) were recorded meticulously. Data represent the Mean ± Standard Error (SE) calculated from experimental replicates.

Results

1. *In vitro* seed germination and initial proliferation

Seeds of *A. odoratum* were germinated on agar solidified KC, MS, PM and MVW media with or without PGRs. The results are summarized in Table 1. The percentage of germination, germination time, protocorms and plantlet development varied significantly across different types of media.

The highest rate of germination was recorded on MS medium supplemented with PGRs (0.5 mg/l BAP + 0.5 mg/l NAA), achieving an 83.34% germination rate (Fig. 1), followed by PGRs fortified PM medium (75.00%, Fig. 2). Seeds took the minimum time to initiate germination on PGRs contained MS medium (9.12 ± 0.35 weeks), followed closely by PGRs fortified PM medium (9.47 ± 0.27 weeks). Maximum time was required for seed germination on PGRs free MVW medium (16.25 ± 0.28 weeks).

However, for all subsequent developmental stages, the PGRs supplemented MS medium proved unequivocally superior. Protocorm development was observed within the minimum time on PGRs supplemented MS medium (11.60 ± 0.37 weeks, Fig. 3), followed by PGRs fortified PM medium (13.58 ± 0.46 weeks). Furthermore, the minimum time for differentiation of the first leaf primordia (18.13 ± 0.59 weeks), first root primordia (21.03 ± 0.51 weeks) and complete plantlet development (27.01 ± 0.43 weeks, Fig. 4) were all recorded on the PGRs fortified MS medium. The maximum time for complete plantlet development was recorded on PGRs free KC medium (34.62 ± 0.52 weeks).

2. Direct MSBs proliferation and PLBs induction

This experiment was held for the mass scale propagation of seedlings. *In vitro* raised nodal and leaf segments were cultured on agar solidified MS media supplemented with different concentrations of BAP, Kn, NAA, IAA and Picloram. The results are depicted in Table 2.

Multiple shoot buds (MSBs) were produced from the nodal segments through direct organogenesis. The highest percentage of induced MSBs ($73.13 \pm 1.58\%$) and the highest average number of shoot buds (5.7 ± 0.41 MSBs/segment) sprouted on MS medium supplemented with 2.0 mg/l BAP + 1.0 mg/l NAA within 7.84 ± 0.19 weeks (Fig. 5). This was followed by MS medium containing 2.0 mg/l BAP + 1.0 mg/l Picloram, which yielded $66.67 \pm 1.92\%$ induction and 4.8 ± 0.72 MSBs/segment in 8.16 ± 0.09 weeks. The media supplemented with 1.0 mg/l Kn alone yielded the minimum number of MSBs (2.0 ± 0.35 /segment).

Conversely, leaf segments underwent proliferation to produce PLBs through embryogenesis. The highest PLBs induction response ($49.00 \pm 1.48\%$) was achieved on MS media fortified with 2.0 mg/l BAP + 1.0 mg/l IAA (Fig. 6).

This media composition took a minimum of 8.72 ± 0.14 weeks to develop yellowish green (YG) PLBs. Leaf segments failed to produce any PLBs or callus on media with 1.0 mg/l BAP alone, 1.0 mg/l Kn alone, 1.0 mg/l Kn + 0.5 mg/l IAA, or 1.0 mg/l Kn + 0.5 mg/l Picloram.

3. Elongation of MSBs derived shoot buds

Comparatively, Kn is better than BAP for the elongation of MSBs. Multiple shoot buds produced from nodal explants were transferred to elongation media. The detailed results on the initial length, length after 30 days of culture and finally the increased length of plantlets are summarized in Table 3. The highest increase in length (3.18 ± 0.03 cm) was recorded on MS medium supplemented with 2.0 mg/l Kn + 1.2 mg/l NAA (Fig. 7). The medium containing 1.5 mg/l Kn + 0.9 mg/l IAA also showed highly favorable performance, yielding a length increase of 3.04 ± 0.03 cm (Fig. 8). In contrast, the lowest overall increase among the treated media (1.47 ± 0.04 cm) was recorded on MS medium supplemented with 1.0 mg/l BAP alone.

4. Results on rooting

The roots developed from MSBs on elongation media were very weak and poor in numbers. For the induction of a well-developed root system, the plantlets (2-3 cm in height) were transferred to rooting media. Auxin (IAA, IBA and NAA) supplemented MS and MS basal media (control) were used. The results are shown in Table 4.

The highest increased length (3.39 ± 0.05 cm) and the maximum number of roots (5.33 ± 0.38) per seedling were induced on agar solidified MS medium supplemented with 1.0 mg/l IAA + 1.0 mg/l IBA (Fig. 9). This was followed by the MS medium containing 0.5 mg/l IAA + 0.5 mg/l IBA, which yielded an increased length of 3.19 ± 0.07 cm and 5.17 ± 0.31 roots per seedling. It was unequivocally found that MS medium with synergistic combinations of IAA and IBA gave better responses than other rooting media using solitary auxins or NAA.

5. Hardening and transplantation of *in vitro* developed seedlings

The *in vitro* developed seedlings of *A. odoratum* were kept outside the culture room with gradually increasing the time up to three days for acclimatization. The mature, well-rooted seedlings were shifted successfully from the growth room to external pots (Fig. 10). This process was done in step-by-step phases of acclimatization. The seedlings were transferred to small plastic pots containing saw dust, coal, dried wood and moistened coir and kept at room temperature for 3-5 days. A number of seedlings died, but those which survived (65%) continued to grow and develop strong roots.

Table 1: Effect of different media with or without PGRs on *in vitro* seed germination, differentiation and plantlet development of *Aerides odoratum* Lour.

Medium	Culture condition	Time taken in weeks					% of culture vessel germinated	Remarks
		Initiation of germination (Mean $\pm$ SE)	Development of protocorm (Mean $\pm$ SE)	Differentiation of 1st leaf primordial (Mean $\pm$ SE)	Differentiation of 1st root primordial (Mean $\pm$ SE)	Development of plantlet (Mean $\pm$ SE)		
KC	*	14.37 $\pm$ 0.33	17.04 $\pm$ 0.36	25.91 $\pm$ 0.47	27.85 $\pm$ 0.42	34.62 $\pm$ 0.52	33.34	+
	**	12.80 $\pm$ 0.35	15.26 $\pm$ 0.59	23.10 $\pm$ 0.53	23.81 $\pm$ 0.50	29.42 $\pm$ 0.43	50.00	++
MS	*	10.51 $\pm$ 0.29	13.21 $\pm$ 0.48	20.99 $\pm$ 0.56	23.86 $\pm$ 0.47	28.34 $\pm$ 0.55	58.34	++
	**	9.12 $\pm$ 0.35	11.60 $\pm$ 0.37	18.13 $\pm$ 0.59	21.03 $\pm$ 0.51	27.01 $\pm$ 0.43	83.34	+++
PM	*	10.61 $\pm$ 0.36	14.11 $\pm$ 0.48	21.85 $\pm$ 0.47	23.91 $\pm$ 0.37	29.27 $\pm$ 0.51	50.00	++
	**	9.47 $\pm$ 0.27	13.58 $\pm$ 0.46	19.92 $\pm$ 0.35	21.59 $\pm$ 0.58	27.29 $\pm$ 0.55	75.00	+++
MVW	*	16.25 $\pm$ 0.28	17.05 $\pm$ 0.42	26.35 $\pm$ 0.52	27.52 $\pm$ 0.49	33.61 $\pm$ 0.46	41.67	+
	**	13.11 $\pm$ 0.26	15.57 $\pm$ 0.50	20.96 $\pm$ 0.55	24.68 $\pm$ 0.41	29.15 $\pm$ 0.49	58.34	++

*Full strength without PGRs, **Full strength with PGRs (0.5mg/l BAP + 0.5mg/l NAA); + = Minimum germination (0% $\leq$ + $\leq$ 49%), ++ = Medium germination (50% $\leq$ ++ $\leq$ 74%), +++ = Maximum germination (75% $\leq$ +++ $\leq$ 100%). Values represent Mean $\pm$ SE of each experiment consist of 12 replicates.

Table 2: Development of MSBs/PLBs from *in vitro* raised nodal and leaf segments of *A. odoratum* on MS medium with different kinds of PGRs.

Sl. No.	PGR Concentration (mg/l)					Nodal Segment			Leaf Segment		
	BAP	Kn	NAA	IAA	Pic	% of induced MSBs/Segment (Mean $\pm$ SE)	Time required (week) for development of MSBs (Mean $\pm$ SE)	No. of MSBs produced/segment (Mean $\pm$ SE)	% of induced PLBs/segment (Mean $\pm$ SE)	Time required (week) for development of PLBs (Mean $\pm$ SE)	Nature of PLBs (Colour)
1.	1.0	-	-	-	-	30.00 $\pm$ 1.40	9.46 $\pm$ 0.12	2.4 $\pm$ 0.54	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	-
2.	2.0	-	-	-	-	43.13 $\pm$ 1.46	9.23 $\pm$ 0.14	2.7 $\pm$ 0.68	6.33 $\pm$ 1.27	10.26 $\pm$ 0.09	G
3.	-	1.0	-	-	-	15.77 $\pm$ 1.48	10.36 $\pm$ 0.18	2.0 $\pm$ 0.35	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	-
4.	-	2.0	-	-	-	22.33 $\pm$ 2.28	9.68 $\pm$ 0.16	1.9 $\pm$ 0.48	6.33 $\pm$ 1.26	9.78 $\pm$ 0.13	G
5.	1.0	-	0.5	-	-	61.67 $\pm$ 1.91	8.36 $\pm$ 0.14	4.6 $\pm$ 0.42	31.37 $\pm$ 1.67	9.93 $\pm$ 0.14	G
6.	2.0	-	1.0	-	-	73.13 $\pm$ 1.58	7.84 $\pm$ 0.19	5.7 $\pm$ 0.41	45.00 $\pm$ 2.29	9.13 $\pm$ 0.09	YG
7.	1.0	-	-	0.5	-	54.33 $\pm$ 1.90	8.22 $\pm$ 0.07	3.5 $\pm$ 0.54	42.67 $\pm$ 1.42	8.76 $\pm$ 0.08	WG
8.	2.0	-	-	1.0	-	60.37 $\pm$ 1.92	8.18 $\pm$ 0.11	4.6 $\pm$ 0.52	49.00 $\pm$ 1.48	8.72 $\pm$ 0.14	YG
9.	1.0	-	-	-	0.5	54.33 $\pm$ 1.94	8.34 $\pm$ 0.15	4.3 $\pm$ 0.47	30.33 $\pm$ 1.74	9.54 $\pm$ 0.15	G
10.	2.0	-	-	-	1.0	66.67 $\pm$ 1.92	8.16 $\pm$ 0.09	4.8 $\pm$ 0.72	37.67 $\pm$ 1.67	10.12 $\pm$ 0.10	YG
11.	-	1.0	0.5	-	-	36.67 $\pm$ 1.40	9.58 $\pm$ 0.13	2.4 $\pm$ 0.44	11.33 $\pm$ 1.26	10.65 $\pm$ 0.14	WG
12.	-	2.0	1.0	-	-	42.37 $\pm$ 1.48	8.84 $\pm$ 0.16	2.7 $\pm$ 0.53	18.00 $\pm$ 1.68	9.78 $\pm$ 0.16	G
13.	-	1.0	-	0.5	-	29.67 $\pm$ 1.47	9.74 $\pm$ 0.012	1.9 $\pm$ 0.56	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	-
14.	-	2.0	-	1.0	-	42.33 $\pm$ 1.85	8.87 $\pm$ 0.11	3.1 $\pm$ 0.70	25.33 $\pm$ 1.24	10.23 $\pm$ 0.08	YG
15.	-	1.0	-	-	0.5	36.33 $\pm$ 1.48	9.26 $\pm$ 0.14	2.6 $\pm$ 0.55	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	-
16.	-	2.0	-	-	1.0	48.67 $\pm$ 1.92	8.68 $\pm$ 0.12	3.8 $\pm$ 0.48	18.00 $\pm$ 2.15	9.66 $\pm$ 0.09	YG
17.	MS0 (Control)					10.00 $\pm$ 1.40	9.46 $\pm$ 0.09	1.2 $\pm$ 0.23	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	-

Multiple Shoot Buds (MSBs); Protocorm Like Bodies (PLBs); ‘-’ Indicate no response; Greenish PLBs (G), Yellowish Green PLBs (YG), Whitish Green PLBs (WG); Values represent Mean $\pm$ SE of each experiment consist of five replicates.

Table 3: Elongation of MSBs developed from nodal segments of *A. odoratum*.

Sl. No.	PGR Conc. (mg/l)				Solid media		
	BAP	Kn	NAA	IAA	Initial length (cm) of plantlets (Mean±SE)	Length (cm) of plantlets after 30d of culture (Mean±SE)	Increased length (cm) of plantlets after 30d of culture (Mean±SE)
1.	1.0	-	-	-	1.37±0.02	2.84±0.05	1.47±0.04
2.	1.5	-	-	-	1.33±0.03	3.06±0.04	1.73±0.05
3.	2.0	-	-	-	1.31±0.02	3.18±0.06	1.87±0.05
4.	-	1.0	-	-	1.34±0.03	3.27±0.08	1.93±0.02
5.	-	1.5	-	-	1.35±0.02	3.39±0.06	2.04±0.03
6.	-	2.0	-	-	1.32±0.03	3.64±0.05	2.32±0.03
7.	1.0	-	0.6	-	1.31±0.02	3.13±0.03	1.82±0.02
8.	1.5	-	0.9	-	1.33±0.03	3.63±0.03	2.30±0.04
9.	2.0	-	1.2	-	1.35±0.03	4.11±0.02	2.76±0.05
10.	1.0	-	-	0.6	1.30±0.02	3.52±0.05	2.22±0.02
11.	1.5	-	-	0.9	1.34±0.03	3.94±0.07	2.60±0.04
12.	2.0	-	-	1.2	1.33±0.02	3.05±0.05	1.72±0.04
13.	-	1.0	0.6	-	1.31±0.03	3.68±0.07	2.37±0.05
14.	-	1.5	0.9	-	1.34±0.03	3.02±0.03	1.68±0.02
15.	-	2.0	1.2	-	1.36±0.02	4.54±0.04	3.18±0.03
16.	-	1.0	-	0.6	1.30±0.03	4.12±0.02	2.82±0.02
17.	-	1.5	-	0.9	1.35±0.03	4.38±0.06	3.04±0.03
18.	-	2.0	-	1.2	1.33±0.03	3.22±0.08	1.89±0.05
19.	MS0 (Control)				1.31±0.02	2.82±0.05	1.51±0.04

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

Table 4: Length (cm) and number of roots per plantlet of *A. odoratum* in auxin supplemented MS and MS0 rooting media.

Sl. No.	PGR Concentration (mg/l)			Initial length of roots per plantlet (Mean±SE)	Initial number of roots per plantlet (Mean±SE)	Increased length (cm) of roots after 30d of culture (Mean±SE)	Increased number of roots after 30d of culture (Mean±SE)
	IAA	IBA	NAA				
1.	0.5	-	-	1.35±0.02	0.67±0.21	2.17±0.05	4.33±0.54
2.	1.0	-	-	1.33±0.01	0.50±0.25	2.50±0.06	4.67±0.49
3.	-	0.5	-	1.32±0.03	1.00±0.37	2.25±0.03	4.00±0.58
4.	-	1.0	-	1.33±0.02	0.67±0.33	2.26±0.04	4.50±0.58
5.	-	-	0.5	1.32±0.01	1.00±0.39	2.57±0.05	4.67±0.31
6.	-	-	1.0	1.35±0.03	0.67±0.36	2.79±0.06	5.00±0.32
7.	0.5	0.5	-	1.31±0.03	1.00±0.35	3.19±0.07	5.17±0.31
8.	1.0	1.0	-	1.28±0.03	0.50±0.36	3.39±0.05	5.33±0.38
9.	-	0.5	0.5	1.33±0.02	0.50±0.24	2.69±0.04	4.67±0.38
10.	-	1.0	1.0	1.34±0.02	1.00±0.39	3.03±0.03	4.83±0.37
11.	0.5	-	0.5	1.31±0.03	0.83±0.38	2.92±0.06	3.33±0.29
12.	1.0	-	1.0	1.32±0.02	1.00±0.35	3.18±0.02	3.16±0.30
13.	MS0 (Control)			1.30±0.02	1.00±0.00	1.27±0.05	2.99±0.28

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



Fig 1: Germinated seeds of *A. odoratum* on 0.5 mg/l BAP + 0.5 mg/l NAA fortified MS medium

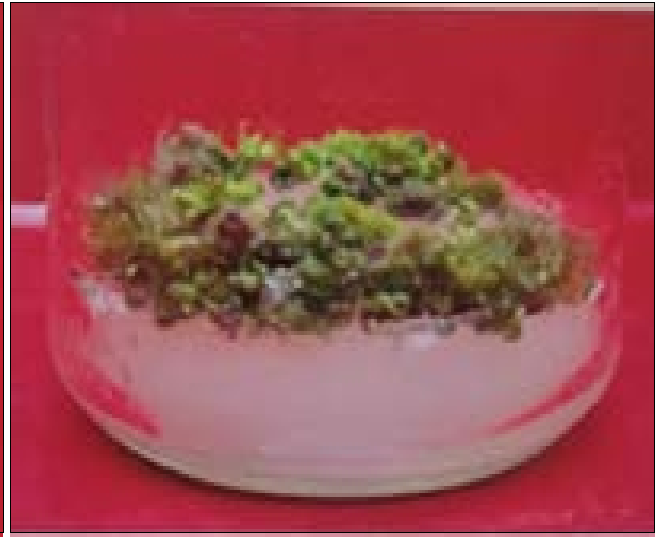


Fig 2: Germinated seeds of *A. odoratum* on 0.5 mg/l BAP + 0.5 mg/l NAA fortified PM medium



Fig 3: Development of protocorms on 0.5 mg/l BAP + 0.5 mg/l NAA fortified MS medium



Fig 4: Development of plantlets on 0.5 mg/l BAP + 0.5 mg/l NAA fortified MS medium



Fig 5: MSBs developed in *A. odoratum* from in vitro raised nodal segment on 2.0 mg/l BAP + 1.0 mg/l NAA supplemented MS medium



Fig 6: Development of PLBs in *A. odoratum* from in vitro derived leaf segment on 2.0 mg/l BAP + 1.0 mg/l IAA fortified MS medium



Fig 7: Elongation of mini shoot bud of *A. odoratum* on 2.0 mg/l Kn + 1.2 mg/l NAA supplemented MS



Fig 8: Elongation of mini shoot bud of *A. odoratum* on 1.5 mg/l Kn + 0.9 mg/l IAA fortified MS medium



Fig 9: Induction of strong and stout root system of *A. odoratum* on MS medium with 1.0 mg/l IAA + 1.0 mg/l IBA



Fig 10: Induction of strong and stout root system of *A. odoratum* on MS medium with 1.0 mg/l IAA + 1.0 mg/l IBA

Discussion

The establishment of an efficient *in vitro* micropropagation protocol for endangered epiphytic orchids like *A. odoratum* is governed heavily by the complex biochemical interactions between basal nutrient formulations and exogenous phytohormones (Rao and Ravishankar 2002) [28].

MS medium supplemented with BAP and NAA significantly outperformed PM, MVW and KC media in terms of overall germination percentage and subsequent developmental transitions (protocorm, leaf and root formation). This superior overall response on MS medium aligns with the findings of Novak *et al.* (2014) [24] and Roy *et al.* (2011) [30], who observed that the robust macronutrient profile of MS provides the necessary metabolic fuel for complete plantlet differentiation in monopodial orchid species, once the initial germination barrier is overcome. While the initial rapid swelling on PM may be attributed to

its specific organic additives like peptone and balanced osmotic pressure (Thiyam *et al.* 2025, Paraste *et al.* 2023) [33, 27], MS medium ultimately offered a more sustained nutritional scaffold for full organogenesis.

In the micropropagation phase, the induction of multiple shoot buds (MSBs) from nodal segments was strictly dependent on the synergistic action of a potent cytokinin (BAP) and an auxin (NAA). The highest proliferation (5.7 shoots per explant) on MS medium with 2.0 mg/l BAP and 1.0 mg/l NAA highlights the fundamental physiological role of BAP in breaking apical dominance and stimulating axillary meristematic activity. Concurrently, NAA acts as a critical signaling molecule, facilitating initial cellular differentiation and establishing the root-shoot axis. This cytokinin-auxin synergy is well-documented in Orchidaceae tissue culture, specifically for genera like *Aerides* and *Vanda*, where balanced ratios are mandatory to avert callus

hyperhydricity and direct the morphogenic pathway strictly toward organogenesis (Thapa *et al.* 2026, Chugh *et al.* 2009, Decruse *et al.* 2003, Bhowmik and Rahman 2025) ^[32, 8, 9, 6].

Following induction, the necessity for a distinct elongation phase is a well-recognized bottleneck in orchid biotechnology (Pant and Thapa 2012) ^[25]. The current study revealed that Kinetin (Kn) in combination with NAA was vastly superior to BAP for longitudinal shoot development. While BAP is highly effective for inducing cell division and multiplication, its prolonged presence often leads to stunted, rosette-like shoots. Kinetin, however, promotes sustained cell elongation and internodal expansion without the risk of phenotypic abnormalities, ensuring the development of healthy, elongated plantlets ready for rhizogenesis.

Rhizogenesis architecture was optimally developed using a combination of IAA and IBA. The physiological mechanism dictating this success revolves around the metabolic stability of the auxins (Novak *et al.* 2014) ^[24]. IBA is widely classified as a highly stable, slow releasing auxin. Unlike IAA, which is rapidly degraded by peroxidases and light within the plant tissue, IBA is gradually metabolized, providing a continuous, sustained release of auxin signaling over an extended period. This continuous stimulation is crucial for epiphytic orchids, ensuring the initiation of robust, multi-branched root architectures capable of absorbing ambient moisture and anchoring to phorophytes *ex vitro*. The addition of IAA provides the initial rapid burst of rooting stimulus, complementing the sustained action of IBA perfectly.

Ultimately, the successful *ex vitro* acclimatization of these plantlets in porous, well aerated substrates (saw dust, coal, dried wood and moistened coir) simulates their natural epiphytic microhabitats. The 75% survival rate indicates a strong transition to autotrophic growth, mirroring survival trends observed in other acclimatized epiphytic orchid taxa (Gonzalez-Arno *et al.* 2008) ^[14].

Conclusion

This study successfully standardizes a rapid, efficient and reproducible *in vitro* propagation protocol for the endangered and highly valuable medicinal orchid, *Aerides odoratum*. While PM medium accelerates initial seed germination, full-strength MS medium fortified with 0.5 mg/l BAP and 0.5 mg/l NAA ensures the highest overall germination success (83.34%) and fastest complete seedling development. Concurrently, the strategic manipulation of PGRs matrices specifically 2.0 mg/l BAP and 1.0 mg/l NAA for maximal MSBs induction (5.7 shoots/explant), followed by elongation on Kinetin and NAA and robust rhizogenesis *via* 1.0 mg/l IAA and 1.0 mg/l IBA provides a seamless developmental continuum. This optimized biotechnological approach offers a foundational framework for the long term ecological conservation of *A. odoratum* in Bangladesh and establishes a reliable supply chain for the horticultural and pharmaceutical industries.

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