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Research Article

In vitro Micropropogation of Endangered Orchid *Aerides odotrata* Lour. from Seed Culture

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ABSTRACT

Aerides odorata Lour. is an endangered orchid, famous for its appealing, fragrant blooms and its epiphytic growth. This study examined the effects of culture medium composition, plant growth regulators, and organic additives on the *in vitro* growth of *Aerides odorata* Lour. Full-strength MS medium enriched with 10% coconut water produced the highest seed germination rate (85%), showing earlier protocorm and shoot development in comparison to other strength of MS medium and medium with plant growth regulators. Shoot multiplication was found a notable increase with 2.0 mg/l of BAP, yielded the highest shoot count (13.4 per explant), whereas shoot elongation was most effective in MS medium with 1.0 mg/l of BAP and 0.1 mg/l of NAA. The effective of root number induction was showed the highest in MS medium with NAA and IBA at 3 mg/l, while root length induction was in MS medium with IBA at 3 mg/l, highlighting its prolonged auxin effectiveness. The results emphasize the combined effect of coconut water, BAP and IBA in enhancing effective micropropagation of *A. odorata*, offering a dependable method for the conservation and extensive propagation of this orchid species.

Keywords: *Aerides*; Auxin; Coconut water; Cytokinin; Micropropogation

Introduction

Orchids (Orchidaceae) are among the most varied families of flowering plants globally, with an estimated 31,480 species spread across 758 genera (Elliott et al., 2025). They have inhabited almost every environment from sea level to mountain peaks, exhibiting impressive morphological and ecological modifications, which makes them essential elements of worldwide plant diversity (Kindlmann & Rocamora, 2023). In Nepal, the orchids exhibits considerable diversity because of the country's diverse topography and climate. Nepal's orchid diversity comprises around 501 species within 108 genera, found in both tropical and alpine regions (Shrestha et al., 2022).

Aerides odorata Lour. is a critically endangered orchid species, recognized for its beautiful, aromatic blooms and epiphytic nature (Huda et al., 2021). It is extensively found throughout subtropical and tropical region in Nepal, China, India, Bhutan, Bangladesh, Myanmar, Thailand, Laos, Cambodia, Vietnam, Malaysia,

Indonesia and Philippines. In Nepal, *A. odorata* thrives in mixed forests at low to mid elevations (200–1100 m) and often develops as an epiphyte on the trunks and branches of trees (Rajbhandari & Rai, 2017; Huda et al., 2021). It has thick and branching stems with elongated leaves. This blooms from late spring to summer, yielding clusters of fragrant flowers that are frequently utilized decoratively. *odorata* has been utilized for addressing skin diseases, wounds, fevers, ear infections, digestive issues and inflammatory ailments (Singh et al., 2001). Phytochemical investigations have revealed biologically active secondary metabolites, including flavonoids, alkaloids, phenolic compounds, terpenoids, and glycosides, which play a role in its known antioxidant, antimicrobial and cytotoxic characteristics (Hossain, 2011; Pant & Raskoti, 2013; Saifur et al., 2025). In Nepal, wild orchids have been collected for medicinal, horticultural and commercial purposes for a long time, with more than 60 species traditionally used to address issues like wounds, fever, bone fractures and skin ailments (Subedi et al., 2013). Despite the scarcity of specific ethnomedicinal documentation for *A. odorata* in Nepalese customs, regional research from South Asia shows that this plant is utilized typically for healing wounds and as a tonic, incorporating different parts of the plant in traditional therapies (Panda & Mandal, 2013; Teoh, 2016). Initial phytochemical assessments of *A. odorata* leaf extracts have identified the existence of alkaloids, coumarins, flavonoids, glycosides, phenols, and terpenoids, substances frequently linked to medicinal benefits (Katta et al., 2019). Later research also indicated antioxidant and liver-protective properties, implying that the species contains bioactive substances with medicinal potential.

Though varied and valuable, orchids encounter major conservation issues around the world. Many species are at risk due to threats such as habitat loss, climate change, illegal collection and overexploitation putting orchids in danger (Gale et al., 2018; Hinsley et al., 2018). *Aerides odorata* is classified as endangered category by the IUCN because of habitat loss and decreasing wild populations. In Nepal, the unlawful collection and sale of orchids for traditional medicine and commerce have intensified population pressures, underscoring the necessity for conservation strategies and sustainable management (Subedi et al., 2013)

Materials and Methods

Explant source and surface sterilization

Mature unopened capsules of *Aerides odorata* Lour. were collected from naturally growing plants in the Makwanpur district of Hetauda, Nepal. To remove surface contaminants and debris, the capsules were first rinsed under running tap water for 15–20 min after being agitated in 0.01% (v/v) Tween 20 solution for 30 min on a rotary shaker. Subsequent surface sterilization was performed under aseptic conditions in a laminar airflow cabinet. The capsules were briefly immersed in 70% (v/v) ethanol for 30 s and flamed to eliminate superficial microorganisms, followed by treatment with 1% (w/v) sodium hypochlorite solution for 8 min for further disinfection. After sterilization, the capsules were thoroughly rinsed 4–5 times with sterile distilled water to remove any traces of sterilizing agents. Finally, the disinfected capsules were longitudinally opened using a sterile scalpel, and the immature seeds were carefully inoculated onto the prepared culture media under aseptic conditions in culture jar.

Culture media and growth regulators

The basal culture medium comprised Murashige and Skoog (MS) medium (Murashige & Skoog, 1962) supplemented with 3% (w/v) sucrose as a carbon source and solidified with 0.8% (w/v) agar. The pH was carefully adjusted to 5.8 ± 0.02 prior to sterilization. The medium was autoclaved at 121 °C under 1.05 kg/cm² pressure for 15–20 min. For seed germination and protocorm development, the MS medium was supplemented with various concentrations and combinations of plant growth regulators (PGRs) and coconut water. To induce shoot formation and shoot proliferation, cytokinins including 6-benzylaminopurine (BAP; 0.5-2.0 mg/l) and kinetin (Kn; 0.5-2.0 mg/l) were incorporated at different levels. For the root induction medium was supplemented with auxins such as α-naphthaleneacetic acid (NAA; 0.5-4.0 mg/l), indole-3-butyric acid (IBA; 0.5-4.0 mg/l), and indole-3-acetic acid (IAA; 0.5-4.0 mg/l), either individually or in selected combinations. All chemicals and media constituents were procured from standard commercial suppliers and used without further modification.

Culture conditions

All cultures were maintained in a controlled growth room at 25 ± 2 °C under a 16 h light/8 h dark photoperiod. Illumination was supplied by cool white fluorescent lamps. The relative humidity of the culture room was regulated at around 60-70% to ensure stable growth conditions.

Data analysis

Observations of seed germination, protocorm development, shoot induction and multiplication, as well as root formation, were systematically recorded at

regular intervals typically every 4 weeks. For each treatment, 10-15 explants were cultured, and all treatments were performed in triplicate to ensure reproducibility. Quantitative data were expressed as mean ± standard deviation (SD). Statistical comparisons among treatments were carried out using Duncan's multiple range test (DMRT) at a significance level of $p \leq 0.05$.

Results and Discussion

Effect of media composition on seed germination

The response of mature seeds of *Aerides odorata* Lour. varied markedly with the composition of the culture medium. Nine different media formulations, including full strength MS (FMS), half strength (HMS), quarter strength MS (QMS), and media supplemented with or without plant growth regulators and organic additives, were evaluated for seed germination, protocorm development, and shoot initiation.

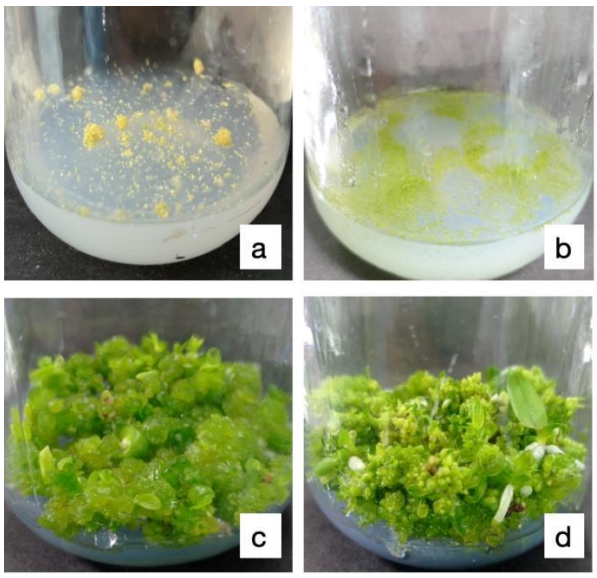

Figure 1: Seed germination stages in MS medium. a) Initiation of seed germination in MS medium with 10 % CW; (b) Protocorm like bodies; (c) Protocorm formation in MS medium with 10 % CW; (d) Shoot initiation.

Among all treatments, FMS supplemented with 10% (v/v) coconut water (CW) exhibited the most rapid and efficient response which is 85 % of total germination (Figure 1a &b, Figure 2). This results is consistent with the previous studies reporting enhanced seed germination in the presence of 10 % coconut (Thapa et al., 2020; Pant et al., 2020). Coconut water contains natural cytokinins which stimulate cell division and differentiation of the embryo. Initial seed germination was observed within 4 weeks of culture, followed by FMS where initiated of seed germination took place after 5 weeks of culture. In quarter-strength MS medium supplemented with 0.5 mg/l BAP and 0.1 mg/l NAA, seed germination was initiated only after 8 weeks of culture (Figure 3) and seed germination rate is only 39.33 % (Figure 2). As the concentration of MS salts decreased from FMS to quarter-strength MS (QMS), a consistent delay in developmental milestones was recorded. For instance, in basal media without additives, germination initiation shifted from 5.0 weeks (FMS) to 6.6 weeks (QMS). Furthermore, the integration of synthetic plant growth regulators (0.5 mg/l BAP + 0.1 mg/l NAA) did not enhance the growth rate compared to CW (Figure 2). Similar inhibitory effects of full concentrations MS salts have been reported on seed germination of some orchid species (Joshi et al., 2023; Azad et al., 2025).

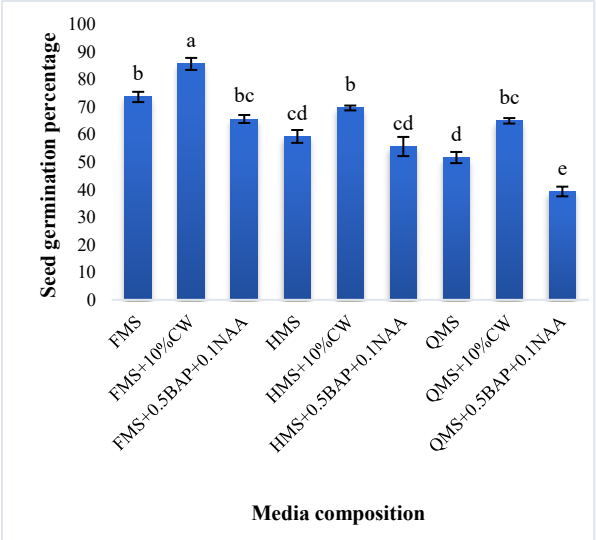

Figure 2: Seed germination percentage of *A. odorata* in different media composition.

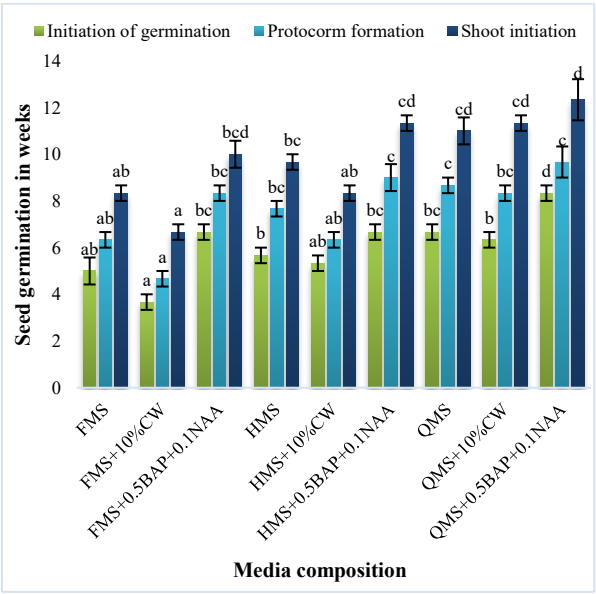

Figure 3: Seed germination of *A. odorata* in different media composition.

Effect of media composition on protocorm formation and shoot initiation

The most rapid and efficient response of protocorm formation (Figure 1c) and shoot initiation (Figure 1d) was also observed with FMS supplemented with 10% (v/v) coconut water (CW). The effect of coconut water aligns with its known content of natural cytokinins, vitamins, and organic compounds that enhance cell division and morphogenesis (Nambiar et al., 2012; da Silva et al., 2017). Initiation of protocorm formation was started in 4.67 days while shoot initiation was started in 6.67 days (Figure 3). Media supplemented with plant growth regulators showed a reduced and delayed response compared to organic additive-based media. In quarter-strength MS medium supplemented with 0.5 mg/l BAP and 0.1 mg/l NAA, seed germination was initiated only after 8 weeks of culture (Figure 3). Protocorm formation was observed at approximately 9 weeks, while shoot initiation required up to 12 weeks (Figure 3). Moreover, this treatment exhibited the lowest overall response.

Overall, the results clearly indicate that medium composition with coconut water significantly influences *in vitro* seed germination and subsequent developmental stages in *A. odorata.* This results is consistent with the previous studies where in the presence of coconut water enhance the seed germiantion and protocorm formation (Maharjan et al., 2020; Pant et al., 2022).

Effect of media composition on shoot induction

Shoot number of Aerides odorata was significantly affected by the type and concentration of plant growth regulators. Among all treatments, MS medium supplemented with 2.0 mg/l BAP produced the highest number of shoots (13.4 ± 0.30 shoots per explant), followed by MS + 1.5 mg/l BAP (12.1 shoots) and MS + 1.0 mg/l BAP (11.2 shoots) (Figure 4 & Figure 6a) after 12 weeks of culture. Coconut water supplementation also enhanced shoot proliferation (9.0 shoots) compared with the control MS medium (6.8 shoots). In contrast, kinetin containing media resulted in comparatively lower shoot numbers (3.4–7.2 shoots), with the minimum response observed at 0.5 mg/l Kn. The addition of low NAA (0.1 mg/l) to BAP containing media moderately influenced shoot number but did not surpass the proliferation achieved with 2.0 mg/l BAP alone.

The superior performance of BAP over kinetin observed in this study is consistent with numerous recent reports indicating that BAP is more effective in stimulating axillary bud break and shoot multiplication in orchids due to its higher cytokinin activity and stability *in vitro* (Pant & Gurung, 2005; da Silva et al., 2025;). Similar dose-dependent effectively increases in shoot proliferation with increasing BAP concentrations have been reported in *Dendrobium*, *Cymbidium* and *Vanda* species (Tao et al., 2011; Lukatkin et al., 2019). The enhanced shoot number at higher BAP levels may be attributed to increased meristematic activity and suppression of apical dominance, which promotes lateral bud proliferation (Su et al., 2011). Overall, the results demonstrate that BAP at 2.0 mg/l is optimal for maximizing shoot multiplication in *A. odorata*, while kinetin is comparatively less effective for shoot proliferation in this species.

Shoot length varied significantly among the different media compositions tested (Figure 5), indicating that the nature and concentration of plant growth regulators substantially influenced shoot elongation in Aerides odorata. The longest shoots (3.8 ± 0.14 cm) were obtained on MS medium supplemented with 1.0 mg/l BAP and 0.1 mg/l NAA (Figure 6b), significantly producing greater elongation than all other treatments ($p \leq 0.05$). Moderate shoot growth was observed on MS supplemented with 10% coconut water (~2.3 cm), MS + 2.0 mg/l BAP (~2.1 cm), and MS + 1.5 mg/l BAP (~2.0 cm), while media containing kinetin exhibited comparatively limited elongation. The lowest shoot lengths (0.4-0.6 cm) occurred in MS + 0.5-2.0 mg/l Kn treatments (Figure 5). These results are consistent with multiple recent studies reporting that shoot proliferation and elongation in orchids are highly responsive to the type and balance of cytokinins and auxins. Similarly, Bhattacharjee & Islam (2014) and Talukder et al. (2003) reported BAP and NAA enhanced shoot elongation in *Vanda* and *Dendrobium*, consistent with the present study.

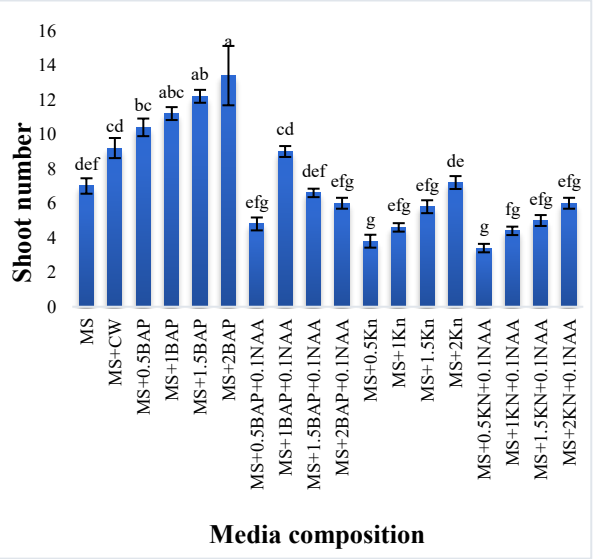

Figure 4: Number of shoot proliferation in different media composition.

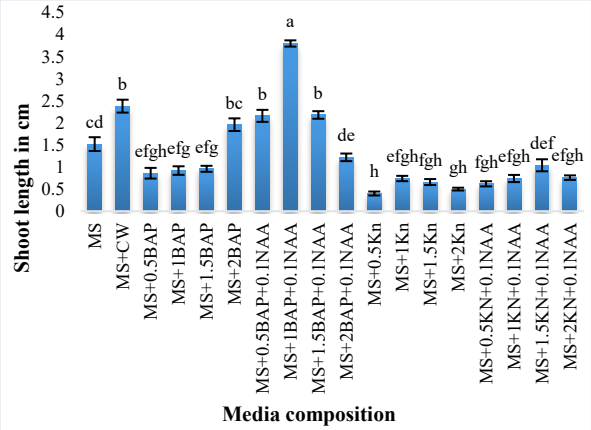

Figure 5: Shoot length in different media composition.

In vitro root formation using plant growth regulators

The present study demonstrated that root induction in *Aerides odorata* was maximized at 3 mg/l NAA and 3 mg/l IBA, with both treatments producing significantly higher root numbers compared to IAA (Table 1). These findings are consistent with previous reports in orchids, where NAA has been shown to stimulate root primordia formation (Thiyam et al., 2025; Mirani et al., 2017), while IBA provides sustained auxin activity leading to vigorous root development (Frick & Strader, 2018; Kaushik & Shukla, 2020). The comparatively weaker response to IAA corroborates earlier observations that natural auxins are less stable *in vitro* (Nissen & Sutter 1990). Thus, synthetic auxins, particularly NAA and IBA, remain the most effective regulators for *in vitro* rooting of *A. odorata*.

Table 1: Effect of different media composition on root number and root length induction of *A. odorata*.

Media Combination	Root Number (Mean ± SE)	Root Length (cm) (Mean ± SE)
MS + 0.5 NAA	2.0 ± 0.0566^{bc}	0.64 ± 0.0072^{f}
MS + 1.0 NAA	2.2 ± 0.0358^{bc}	1.14 ± 0.0313^{de}
MS + 2.0 NAA	2.6 ± 0.0438^{b}	1.68 ± 0.0256^{cd}
MS + 3.0 NAA	3.0 ± 0.0800^{ab}	2.20 ± 0.0247^{c}
MS + 4.0 NAA	2.4 ± 0.0438^{bc}	1.20 ± 0.0179^{de}
MS + 0.5 IAA	1.8 ± 0.0358^{c}	0.44 ± 0.0145^{f}
MS + 1.0 IAA	2.0 ± 0.0566^{bc}	1.06 ± 0.0145^{e}
MS + 2.0 IAA	2.4 ± 0.0716^{bc}	2.04 ± 0.0440^{cd}
MS + 3.0 IAA	2.6 ± 0.2683^{b}	1.52 ± 0.0119^{d}
MS + 4.0 IAA	2.2 ± 0.0669^{bc}	1.24 ± 0.0308^{de}
MS + 0.5 IBA	2.2 ± 0.0358^{bc}	1.70 ± 0.0247^{cd}
MS + 1.0 IBA	2.8 ± 0.0358^{ab}	2.42 ± 0.0409^{bc}
MS + 2.0 IBA	3.0 ± 0.0566^{ab}	2.94 ± 0.0308^{b}
MS + 3.0 IBA	3.2 ± 0.1315^{a}	3.86 ± 0.0762^{a}
MS + 4.0 IBA	2.4 ± 0.0716^{bc}	3.68 ± 0.0296^{a}

Root length in *Aerides odorata* was significantly influenced by both the type and concentration of auxin incorporated into the culture medium. Among the treatments evaluated, IBA at 3 mg/l consistently produced the longest and most well-developed roots (Figure 6c), whereas NAA resulted in intermediate root elongation and IAA produced comparatively shorter roots. The superior performance of IBA observed in the present study aligns with earlier findings in orchids and other micropropagated species, where IBA has been reported to be more effective than other auxins in stimulating *in vitro* rooting and enhancing root elongation (Rafique et al., 2012).

Figure 6: Shoot and root induction. (a) shoot formation; (b) shoot elongation in terms of length; (c) root formation and root length.

The enhanced rooting response under IBA may be attributed to its physiological behavior within plant tissues. IBA is known to function as a relatively stable auxin precursor that can be converted into the biologically active form, IAA, thereby providing a more sustained auxin supply during root initiation and elongation (Frick & Strader, 2018). Exogenous supplied IAA is more susceptible to rapid degradation through conjugation and oxidative inactivation pathways, which can limit its persistence and reduce its effectiveness in supporting prolonged root growth under *in vitro* conditions (Hayashi et al., 2021). From a practical perspective, the use of IBA at 3 mg/l in rooting media is therefore recommended to obtain robust and elongated roots prior to acclimatization, which is critical for improving survival and establishment under *ex vitro* conditions.

Conclusion

The present study establishes an efficient and reproducible *in vitro* regeneration protocol for *Aerides odorata* Lour., highlighting the critical influence of medium composition and growth regulator balance on morphogenetic responses. Full-strength MS supplemented with 10% (v/v) coconut water significantly enhanced seed germination, accelerated protocorm formation, and promoted earlier shoot initiation compared to basal and PGR supplemented media, confirming the stimulatory role of organic additives during early orchid development. For shoot

multiplication, MS medium containing 2.0 mg/l BAP produced the highest shoot number, demonstrating the superior cytokinin efficiency of BAP over kinetin. However, optimal shoot elongation was achieved with 1.0 mg/l BAP in combination with 0.1 mg/l NAA, indicating that a balanced cytokinin auxin interaction improves shoot quality. Root induction was maximized with 3 mg/l NAA and IBA, and IBA (3 mg/l) produced the greatest root length, supporting its sustained effectiveness in promoting root elongation. Collectively, the optimized combinations identified in this study provide a robust micropropagation system for *A. odorata*, facilitating large scale propagation, conservation, and commercial utilization of this ornamental orchid.

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