In vitro propagation of Hoang Nhan orchid (*Aerides odorata x houlletiana)*

Cao Thi Viet Nga[*], Nguyen Thi Nhu Quynh, Nguyen Thi Nhu Quynh, Vu Quang Nam
Vietnam National University of Forestry

Nhân giống lan Hoàng Nhạn (*Aerides odorata* x *houlletiana*) bằng kỹ thuật nuôi cấy *in vitro*

Cao Thị Việt Nga[*], Nguyễn Thị Như Quỳnh, Nguyễn Thị Như Quỳnh, Vũ Quang Nam
Trường Đại học Lâm nghiệp
**Corresponding author*: *ngactv@vnuf.edu.vn*

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ABSTRACT

The Hoang Nhan orchid (Aerides odorata × houlletiana) is a hybrid species within the genus Aerides, valued for its beautiful flowers, characteristic sweet fragrance, and high economic potential. This plant species is currently Endangered and requires protection under the CITES Convention (2017). In Vietnam, this orchid is predominantly distributed in the South Central Coast, particularly along the Truong Son mountain range, bordering Cambodia and Vietnam. This study aimed to develop an effective in vitro propagation protocol for the Hoang Nhan orchid. The optimal sterilization method for the orchid pod involved triple flaming with 96% ethanol, yielding 92.2% contamination-free culture rate and a 9.04% seed germination rate. For shoot multiplication, the most effective medium was MS (Murashige and Skoog, 1962) supplemented with 1.25 mg/L BAP, 0.75 mg/L kinetin, and 0.4 mg/L NAA. This formulation resulted in a 90.0% shoot regeneration rate, a multiplication coefficient of 5.23, and an average shoot height of 3.4 cm. The regenerated shoots exhibited good development, characterized by thick stems and dark green leaves. Successful rooting was achieved using MS medium supplemented with 0.75 mg/L NAA and 0.5 g/L activated charcoal, yielding an 88.9% rooting rate, an average of 6.2 roots per plantlet, and a mean root length of 3.1 cm. The roots developed healthily and uniformly. These findings demonstrate the potential to apply in vitro culture techniques for the large-scale propagation of the Hoang Nhan orchid, contributing significantly to the conservation and sustainable development of this valuable genetic resource.

Keywords:
Aerides odorata x A. houlletiana, Aerides, conservation, in vitro propagation, seed propagation.

TÓM TẮT

*Lan Hoàng Nhạn (*Aerides odorata × houlletiana*) là một loài lan lai thuộc chi* Aerides*, nổi bật với hoa đẹp, mùi thơm đặc trưng dịu ngọt và giá trị kinh tế cao. Loài thực vật này đang bị đe dọa tuyệt chủng và cần được bảo vệ theo Công ước CITES 2017. Tại Việt Nam, loài lan này phân bố chủ yếu ở miền Nam Trung Bộ, đặc biệt là khu vực dọc theo dãy Trường Sơn, giáp ranh với Campuchia và Việt Nam. Kết quả nghiên cứu cho thấy, phương pháp khử trùng hiệu quả đối với quả lan Hoàng Nhạn là nhúng và đốt 3 lần bằng cồn 96°, đạt tỷ lệ mẫu sạch 92,2% và tỷ lệ hạt nảy mầm 94%. Môi trường nhân nhanh chồi tối ưu là môi trường MS (Murashige và Skoog, 1962) có bổ sung 1,25 mg/L BAP, 0,75 mg/L kinetin và 0,4 mg/L NAA, cho tỷ lệ tái sinh chồi đạt 90%, hệ số nhân chồi 5,23 lần và chiều cao chồi trung bình 3,4 cm; chồi phát triển tốt, thân mập, lá xanh đậm. Môi trường cảm ứng ra rễ hiệu quả nhất là MS bổ sung 0,75 mg/L NAA và 0,5 g/L than hoạt tính, cho tỷ lệ ra rễ đạt 88,9%, trung bình 6,2 rễ/cây và chiều dài rễ 3,1 cm; rễ phát triển khỏe và đồng đều. Kết quả nghiên cứu này khẳng định tiềm năng ứng dụng kỹ thuật nuôi cấy in vitro để nhân giống lan Hoàng Nhạn, góp phần bảo tồn và phát triển nguồn gen quý.*

Từ khóa:
Aerides odorata x A. houlletiana, Aerides, bảo tồn, nhân giống in vitro, nhân giống từ hạt.

1. INTRODUCTION

Vietnam is home to a rich and diverse tropical forest ecosystem that supports numerous rare and economically valuable plant and animal species. Among them, the Hoang Nhan orchid (*Aerides odorata × houlletiana*), a natural hybrid within the genus Aerides (Orchidaceae), stands out due to its striking floral morphology, pleasant fragrance, and considerable commercial potential.

In addition to its ornamental value, this orchid hybrid has shown promise in the cosmetics industry through essential oil extraction. It is native to Southeast Asia, with significant populations found in the forests of Gia Lai province (Vietnam) and parts of Cambodia. However, the species faces increasing threats from rapid socio-economic development, particularly due to deforestation and habitat fragmentation. Overexploitation for the ornamental plant trade has further contributed to its decline. As a result, Aerides odorata × houlletiana was included in the list of protected species under the 2017 CITES Convention.

Under natural conditions, the Hoang Nhan orchid propagates slowly, primarily through offshoot division or keiki production. These traditional methods are inefficient, highly dependent on environmental conditions, and vulnerable to pest and disease outbreaks, making them unsuitable for large-scale propagation or conservation purposes.

In recent decades, plant tissue culture has emerged as an effective approach for the propagation and conservation of rare and endangered orchids. This technique allows for rapid clonal multiplication under controlled conditions, minimizing the risk of genetic erosion and supporting ex situ conservation efforts. Various studies, both domestic and international, have successfully developed in vitro protocols for orchids such as *Aerides odorata* (Phung Thi Ha et al., 2023), *Dendrobium anosmum* (Phan Van Hoai Lan et al., 2024), and *Aerides multiflora* (Paramanik et al., 2024), among others [1-7].

Given the endangered status and horticultural value of *Aerides odorata × houlletiana*, establishing a reliable *in vitro* propagation protocol is essential. Such a method would contribute to the conservation of this genetic resource and support sustainable cultivation to meet increasing market demand.

2. RESEARCH METHODS

1. Plant materials

Physiologically orchid capsules of the *Hoang Nhan* orchid (*Aerides odorata × houlletiana*), approximately six months old with fully developed, firm, and glossy surfaces, were utilized as the plant material in this study. The capsules were collected from Xuan Mai Orchid Garden, located in Xuan Mai town, Hanoi, Vietnam.

2. Methods

2.1. Establishment of aseptic cultures

The collected orchid capsules were initially washed with detergent and thoroughly rinsed under running tap water. Surface sterilization was subsequently performed within a laminar airflow cabinet by immersing the capsules in 96% ethanol followed by flaming to completely burn off residual ethanol from the capsule surface. This sterilization procedure was repeated between one and five times according to the treatment groups. Following sterilization, capsules were aseptically dissected, and seeds were inoculated onto initiation medium consisting of Murashige and Skoog (MS) basal salts (Murashige and Skoog, 1962) supplemented with 20 g/L sucrose and solidified with 7 g/L agar. The cultures were maintained for 10 weeks, and data on survival rate, contamination rate, and the timing of protocol emergence were recorded.

2.2. Shoot multiplication

Shoots exceeding 1 cm in length were excised from aseptically grown seedlings and transferred to MS medium supplemented with various concentrations of 6-benzylaminopurine (BAP; 0.0–2.5 mg/L), kinetin (0.25–1.25 mg/L), and naphthaleneacetic acid (NAA; 0.1–0.5 mg/L). 0 After an 8-week incubation period, the shoot regeneration rate, average number of shoots per explant, and shoot height (cm) were assessed.

2.3. Plantlet development and rooting

Shoots measuring 2–3 cm in height and bearing 1–2 adventitious roots (~1 cm in length) were transferred to MS medium supplemented with 30 g/L sucrose, 0.5 g/L activated charcoal, and varying concentrations of NAA (0, 0.5, 0.75, and 1.0 mg/L) to induce rooting and promote complete plantlet formation. After 8 weeks of culture, root number per plantlet, average root length (cm), and root quality were evaluated.

All culture media were adjusted to pH 5.8 ±

0.1 using 1N NaOH before sterilization by autoclaving. All experiments were conducted at 25 ± 2°C with a 12-hour photoperiod and a light intensity of approximately 2,000 lux. The light was provided by white LED lamps. Each treatment was replicated three times, with 30 explants per replicate.

3. Data Analysis

Data were analyzed using Microsoft Excel and SPSS version 20. Before performing one-way ANOVA, we checked the assumptions of normal distribution and equal variances using the Shapiro–Wilk and Levene's tests, respectively. Both assumptions were met ($p > 0.05$), allowing us to proceed with the analysis. Post-hoc comparisons were made using Bonferroni and Duncan tests, with significance set at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Establishment of aseptic *in vitro* cultures

In plant tissue culture, obtaining aseptic explants at the initial stage is a crucial determinant of successful micro-propagation. One of the key steps in this process is the selection of an appropriate sterilization method that can effectively eliminate microbial contaminants while maintaining the viability and regenerative potential of the explants.

In the present study, 96% ethanol was used as a surface sterilizing agent for *Aerides odorata × houlletiana* fruits, combined with varying numbers of flame exposures to assess their effectiveness in achieving asepsis. The efficiency of each sterilization treatment was evaluated, and the results are summarized in Table 1.

Table 1. Influence of different sterilization treatments on the in vitro establishment of aseptic explants in *Aerides odorata × houlletiana*

Experimental procedure	Number of times burning the fruit peel with 96° alcohol	Evaluation criteria	
		Percentage of clean samples	Percentage of clean samples germinated
KT1	1	66.7	58.3
KT2	2	77.8	85.7
KT3	3	**92.2**	**94.0**
KT4	**4**	94.4	75.3
KT5	**5**	96.7	69.0
Sig		*0.0001*	*0.0001*

As shown in Table 1, the number of flaming repetitions using 96° alcohol significantly affected both microbial decontamination efficiency and seed regeneration capacity in *Hoang Nhan* orchid capsules. All five sterilization treatments resulted in clean sample rates of at least 66%, indicating that 96° alcohol is an effective disinfectant for this type of plant material. However, differences in performance across treatments highlight the critical role of optimizing the number of flaming steps to achieve effective sterilization without compromising tissue viability.

The KT5 treatment, involving five flaming repetitions, produced the highest proportion of clean samples (96.7%). This suggests that increasing the frequency of flaming enhances the removal of surface contaminants. Nevertheless, this treatment also yielded the highest percentage of clean but non-regenerating samples (31%), indicating that overexposure to heat and alcohol may cause cellular damage, particularly to the embryo, thereby impairing seed germination. This result emphasizes the importance of maintaining a balance between sterilization strength and tissue preservation in *in vitro* culture.

In contrast, the KT3 treatment (three flaming repetitions) demonstrated a more balanced outcome, with a slightly lower clean sample rate but the highest proportion of clean and regenerating samples (94%). This finding suggests that moderate sterilization intensity may be optimal, as it sufficiently reduces microbial contamination while minimizing damage to embryonic tissues. Reduced exposure to high temperatures and ethanol likely contributes to better preservation of cellular integrity and physiological function, promoting successful germination and growth post-treatment.

Overall, the results suggest that evaluating sterilization effectiveness based solely on clean sample rates may be misleading. A more comprehensive assessment incorporating both decontamination efficiency and regenerative performance is necessary to determine the most suitable protocol. Given its favorable balance between these two outcomes, the KT3 treatment -three flaming steps using 96° alcohol is recommended as the optimal sterilization method for *Aerides odorata × houlletiana* orchid capsules in tissue culture applications.

Figure 1. Shoot initiation from Hoang Nhan orchid seeds cultured on MS medium following sterilization using the KT3 treatment after 10 weeks of incubation

3.2. Effects of plant growth regulators on shoot multiplication capacity

The application of plant growth regulators (PGRs) significantly influenced the shoot proliferation of *Aerides odorata × houlletiana* (Table 2). Among the treatments, MS medium supplemented with 1.5 mg/L BAP combined with 0.5 mg/L NAA resulted in the highest shoot multiplication rate (4.27 ± 0.15 shoots/explant) and optimal shoot length (2.83 ± 0.12 cm). This combination also produced healthy and well-formed shoots, making it the most efficient protocol for shoot induction in this hybrid.

By contrast, media containing kinetin (Kn) alone or at higher concentrations induced fewer shoots and often resulted in callus formation or abnormal growth. This suggests that BAP, particularly in combination with a low level of NAA, plays a critical role in shoot initiation and development.

These findings are consistent with previous studies on other orchid hybrids, where cytokinins—especially BAP—were shown to promote shoot proliferation more effectively than kinetin (Zhao et al., 2020; Luan et al., 2019). The favorable response under the BAP + NAA treatment highlights the importance of optimizing cytokinin-auxin balance for in vitro propagation protocols.

3.2.1. Effects of BAP on the shoot multiplication capacity of the orchid

The effects of BAP on the shoot multiplication capacity of Hoang Nhan orchid after 8 weeks are detailed in Table 2.

Table 2. Shoot cluster formation of *Hoang Nhan* orchid at varying concentrations of BAP after 8 weeks of culture

Experimental procedure	BAP (mg/l)	Regeneration Rate (%)	Shoot Multiplication Rate	Shoot Height (cm)
CT1.2	0	39.1	0.97^{a}	0.97^{a}
CT2.2	0.5	52.2	2.50^{b}	1.5^{b}
CT3.2	0.75	58.9	3.10^{c}	1.8^{bc}
CT4.2	1.0	67.8	3.30^{c}	2^{cd}
CT5.2	**1.25**	**77.8**	$\mathbf{3.90^{d}}$	$\mathbf{2.1^{d}}$
CT6.2	1.5	62.2	3.1^{c}	2.2^{d}
	Sig		*0.0001*	

Note: *Within the same column, mean values followed by the same letter are not significantly different at P = 0.05 (Duncan's multiple range test).*

The data presented in Table 2 highlight the significant influence of BAP on shoot regeneration in the Hoang Nhan orchid. All treatments containing BAP resulted in substantially higher shoot regeneration rates and multiplication coefficients compared to the control without BAP, indicating its primitive role in shoot initiation and development.

Among the tested concentrations, the medium supplemented with 1.25 mg/L BAP (treatment CT5.2) achieved the highest regeneration rate (77.8%) and a shoot multiplication coefficient of 3.9. Shoots regenerated under this treatment were generally thick, green, and morphologically uniform, although the average height (2.1 cm) remained moderate. These observations suggest that this BAP concentration not only enhances shoot number but also supports satisfactory shoot morphology.

In comparison, both lower and higher BAP concentrations yielded less favorable outcomes. For instance, treatments CT4.2 (1.0 mg/L BAP) and CT6.2 (1.5 mg/L BAP) produced slightly lower multiplication coefficients (3.3) and regeneration rates of 67.8% and 66.7%, respectively. Notably, at 1.5 mg/L BAP, some decline in growth parameters was observed, which may reflect a threshold beyond which BAP becomes inhibitory. On the other hand, treatments with low BAP concentrations (e.g., CT2.2 and CT3.2) resulted in weak shoot formation, with thin, pale-green shoots, indicating reduced vigor and growth quality.

Based on these results, 1.25 mg/L BAP (CT5.2) appears to offer the most effective balance between regeneration rate and shoot quality. This treatment was therefore selected for subsequent experiments in the propagation protocol.

Figure 2. Shoot development of Hoang Nhan orchid cultured on MS medium supplemented with varying concentrations of BAP after 8 weeks.

3.2.2. Effects of combined BAP and Kinetin on the shoot multiplication capacity of the orchid.

Following the identification of the optimal BAP concentration for shoot multiplication in Hoang Nhan orchid, a subsequent experiment was conducted to assess the combined effects of two plant growth regulators—BAP and Kinetin—on shoot proliferation. The objective was to determine the most effective concentration combination to enhance shoot multiplication efficiency. The results obtained after 8 weeks of culture are presented in Table 3.

The analysis demonstrated that the combined application of the two plant growth regulators, BAP and Kinetin, in the culture medium significantly outperformed the use of BAP alone. This was evidenced by notable increases in both the regeneration rate and the shoot multiplication coefficient across all five experimental treatments. Among these, treatment CT3.3 (1.25 mg/L BAP + 0.75 mg/L Kinetin) exhibited the most favorable results, achieving the highest regeneration rate of 85.6%, a shoot multiplication coefficient of 4.63, and an average shoot height of 2.73 cm—surpassing all other tested combinations. This suggests that an appropriate balance of BAP and Kinetin not only strongly stimulates shoot differentiation and development but also maintains superior shoot morphology.

Table 3. Effects of BAP and Kinetin combinations on the shoot multiplication capacity of Hoang Nhan orchid

Experimental procedure	BAP (mg/l)	Kinetin (mg/l)	Percentage of samples with multiple shoots (%)	Shoot multiplication rate (times)	Average shoot length (cm)	Quality of shoot buds
CT1.3	1.25	0.25	65.6	3.30^{a}	2.00^{ab}	Short, thick, dark-green shoots
CT2.3	1.25	0.50	73.3	4.00^{b}	2.26^{abc}	Short, thick, dark-green shoots
CT3.3	**1.25**	**0.75**	**85.6**	$\mathbf{4.63^{c}}$	$\mathbf{2.73^{d}}$	**Tall, thick, dark-green shoots**
CT4.3	1.25	1.0	70.0	4.16^{b}	2.40^{bc}	Short, thick, dark-green shoots
CT5.3	1.25	1.25	61.1	3.50^{a}	1.83^{a}	Short, weak, green shoots
Sig				*0.004*		

Note: *Within the same column, mean values followed by the same letter are not significantly different at P = 0.05 (Duncan's multiple range test).*

In contrast, increasing the Kinetin concentration to 1.0 mg/L and 1.25 mg/L in treatments CT4.3 and CT5.3 resulted in declines in all growth parameters, indicating that excessive cytokinin levels may induce physiological imbalances that inhibit shoot development. Therefore, treatment CT3.3 was identified as the optimal combination in this experiment, effectively promoting shoot cluster formation in both quantity and quality. This combination will serve as the foundation for subsequent experiments incorporating auxin supplementation (e.g., NAA) to further enhance regeneration capacity and optimize the in vitro propagation protocol for Hoang Nhan orchid.

Figure 3. Development of Hoang Nhan orchid shoots on MS medium supplemented with different combinations of BAP and Kinetin after 8 weeks of culture

3.2.3. Effects of combined BAP, Kinetin, and NAA on the shoot multiplication capacity of *Aerides odorata × houlletiana.*

Building on the previous experiments, the research team further investigated the effect of adding NAA (α-Naphthaleneacetic acid) to the optimal combinations of BAP and Kinetin. The purpose of including NAA was to assess the synergistic interaction between auxin and cytokinin in promoting shoot formation and development of *Hoang Nhan* orchid, with the goal of optimizing the culture medium for enhanced shoot multiplication. The results after 8 weeks of culture are summarized in Table 4.

Table 4. Effects of Combined BAP, Kinetin, and NAA on shoot multiplication capacity of Hoang Nhan orchid

Experimental procedure	BAP (mg/l)	Kinetin (mg/l)	NAA (mg/l)	Percentage of samples with multiple shoots (%)	Shoot multiplication rate (times)	Average shoot length (cm)	Quality of shoot buds
CT1.4	1.25	0.75	0.1	70.0	3.90[a]	1.9[a]	Short, thick, dark-green shoots
CT2.4	1.25	0.75	0.2	76.7	4.20[ab]	2.46[b]	Short, thick, dark-green shoots
CT3.4	1.25	0.75	0.3	83.3	4.40[b]	2.60[b]	Tall, thick, dark-green shoots
CT4.4	**1.25**	**0.75**	**0.4**	**90.0**	**5.23[c]**	**3.40[c]**	Short, thick, dark-green shoots
CT5.4	1.25	0.75	0.5	80.0	4.43[b]	2.73[b]	Short, slender, green shoots
Sig					*0.014*		

Note: *Within the same column, mean values followed by the same letter are not significantly different at P = 0.05 (Duncan's multiple range test).*

The results of Experiment 4 demonstrated that the simultaneous supplementation of three plant growth regulators—BAP, Kinetin, and NAA—into the culture medium exerted a pronounced synergistic effect. This combination significantly enhanced the regeneration rate, shoot multiplication coefficient, and shoot morphological quality, compared to treatments with individual hormones or binary combinations. All formulations tested in this experiment achieved regeneration rates exceeding 70% and shoot multiplication coefficients above 4.0, indicating a marked improvement in in vitro propagation efficiency.

In comparison with the previous experiments, the best-performing formula in Experiment 1 (CT5.2 – 1.25 mg/L BAP) achieved a regeneration rate of 77.8%, a shoot multiplication coefficient of 3.9, and an average shoot height of 2.1 cm. In Experiment 2 (BAP + Kinetin), formula CT3.3 (1.25 mg/L BAP + 0.75 mg/L Kinetin) yielded superior results, with 85.6% regeneration, a multiplication coefficient of 4.63, and a shoot height of 2.73 cm. However, formula CT4.4 (1.25 mg/L BAP + 0.75 mg/L Kinetin + 0.4 mg/L NAA) in Experiment 4 outperformed all previous treatments, achieving a regeneration rate of 90%, a multiplication coefficient of 5.23, and an average shoot height of 3.4 cm.

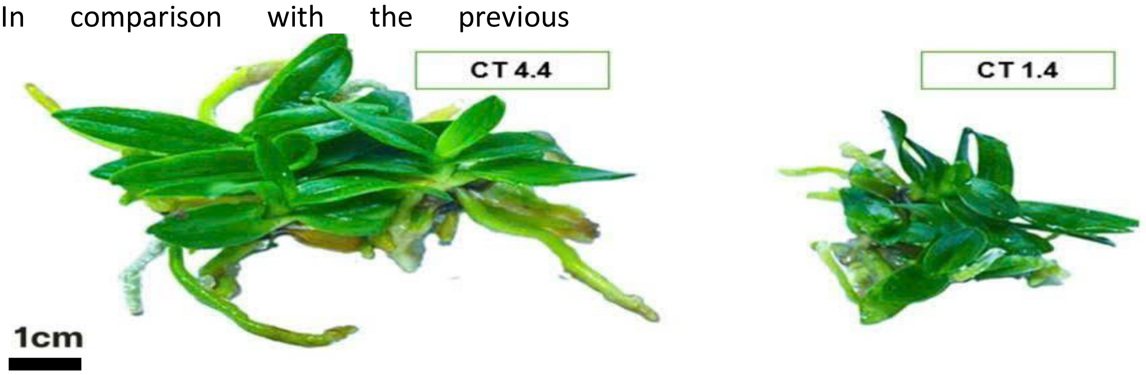

Figure 4. Effects of combined BAP, Kinetin, and NAA supplementation on shoot development of Aerides odorata × houlletiana cultured on MS medium for 8 weeks

The regenerated shoots in treatment CT4.4 were thick, uniformly dark green, and showed consistent morphological development, suggesting a favorable response to the combined application of BAP, Kinetin, and NAA. These differences in shoot quality and multiplication coefficient were statistically significant ($P < 0.05$), indicating that this specific growth regulator combination was more effective than other tested treatments. Based on these results, CT4.4 was selected as the most suitable medium formulation for the in vitro shoot multiplication stage of *Aerides odorata × houlletiana*, and was subsequently applied in further steps of the propagation protocol to support the production of morphologically stable and healthy plantlets.

3.3. Effects of NAA concentration on root Induction in shoots of Hoang Nhan orchid

The in vitro plantlet formation stage constitutes the final phase of the tissue culture process for *Aerides odorata × houlletiana* under controlled laboratory conditions. The survival and subsequent growth of plantlets in ex vitro environments are highly dependent on both the physiological quality of the shoots and the establishment of a functional root system during in vitro culture.

Due to the biological characteristics of this orchid hybrid, root primordia may initiate during the shoot multiplication stage, even when cultured on shoot induction media. However, these roots are typically underdeveloped—characterized by a simple anatomical structure, small size, and absence of root hairs—which limits their capacity for effective water and nutrient uptake.

Therefore, the formulation of an appropriate rooting medium is critical to stimulate the complete development and functionality of roots, thereby enhancing the survival rate and growth performance of plantlets during acclimatization and nursery stages.

The results of the experiment evaluating the effect of varying NAA concentrations on root development after six weeks of culture are presented in Table 5.

Table 5. Effect of different NAA concentrations on root development in *Aerides odorata × houlletiana*

Experimental procedure	NAA (mg/l)	AC	Roots/Plant	Root length (cm)
CT0.5	0	0.5g	2.1^{a}	1.06^{a}
CT1.5	0.50		4.13^{b}	2.56^{b}
CT2.5	**0.75**		$\mathbf{7.5^{d}}$	$\mathbf{5.1^{d}}$
CT3.5	1.00		5.46^{c}	3.13^{c}
Sig				*0.0001*

Note: *Within the same column, mean values followed by the same letter are not significantly different at P = 0.05 (Duncan's multiple range test).*

The data presented in Table 5 show that the addition of NAA (α-naphthaleneacetic acid) to the culture medium had a notable effect on root induction in *Aerides odorata × houlletiana*. While most shoots initiated root development under in vitro conditions, root growth quality varied depending on the NAA concentration. In general, the roots were thin and limited in elongation unless an optimal hormonal balance was achieved.

At the lowest concentration tested (0.5 mg/L NAA, CT1.5), rooting efficiency was limited, with a mean of 4.10 roots per plantlet and an average root length of 1.56 cm. Increasing the NAA concentration to 0.75 mg/L in CT2.5 improved rooting outcomes, producing 6.50 roots per plantlet and a mean root length of 3.10 cm—the highest values observed among all treatments. This suggests that 0.75 mg/L NAA is suitable for enhancing both root number and length.

However, further increasing the concentration to 1.0 mg/L (CT3.5) led to reduced rooting performance, with parameters comparable to those of the lowest concentration. This decline implies that excessive NAA may exert inhibitory effects on root development, possibly through hormonal imbalance or altered physiological responses.

In addition, the inclusion of 0.5 g/L activated charcoal in all treatments may have contributed positively by stabilizing the pH and adsorbing phenolic compounds or other growth-inhibiting substances, thereby improving the rooting environment.

Taken together, formulation CT2.5 (0.75 mg/L NAA with 0.5 g/L activated charcoal) was identified as the most effective treatment for in vitro root induction in this hybrid orchid and was selected for use in the subsequent acclimatization stage.

Figure 5. Effect of NAA on shoot development of *Aerides odorata × houlletiana* (Hoang Nhan) cultured on MS medium after 6 weeks

4. CONCLUSION

The in vitro propagation protocol for *Aerides odorata × houlletiana* was successfully established through a series of optimized procedures. The most effective seed sterilization method involved triple immersion and flaming in 96% ethanol, which resulted in a contamination-free rate of 92.2% and a seed germination rate of 94%.

For shoot multiplication, the optimal medium was MS supplemented with 1.25 mg/L BAP, 0.75 mg/L kinetin, and 0.4 mg/L NAA. This formulation produced a shoot regeneration rate of 90%, a multiplication coefficient of 5.23, and an average shoot height of 3.4 cm. Shoots developed with compact morphology and uniformly green coloration.

Root induction was most effective on MS medium supplemented with 0.75 mg/L NAA and 0.5 g/L activated charcoal, which supported an 88.9% rooting rate, with an average of 6.2 roots per plantlet and a mean root length of 3.1 cm.

During acclimatization, a substrate consisting of 50% coconut coir and 50% pine bark facilitated the highest survival rate (88.9%) and promoted shoot elongation, with an average plant height of 6.16 cm. These findings provide a reliable micropropagation system for the large-scale production of this ornamental orchid hybrid.

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