International Journal of Agricultural Technology 2026 Vol. 22(4):1845-1858
DOI: https://doi.org/10.63369/ijat.2026.22.4.1845-1858
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Effects of plant growth regulators on nodal regeneration of *Rhynchostylis* orchid by plant tissue culture

Poeaim, S., Poeaim, A. and Boonmee, W.*

School of Biology, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang (KMITL), Ladkrabang, Bangkok 10520, Thailand.

Poeaim, S., Poeaim, A. and Boonmee, W. (2026). Effects of plant growth regulators on nodal regeneration of *Rhynchostylis* orchid by plant tissue culture. International Journal of Agricultural Technology 22(4):1845-1858.

Abstract The effects of plant growth regulators (PGRs) in the tissue culture of *Rhynchostylis* orchids was investigated. The result found that the best sterilizing method involved using distilled water combined with 0.15 percent mercuric chloride, resulting in the highest average number (86.67 percent) of uncontaminated samples. Furthermore, the best condition for plant regeneration from nodal segments was the MS medium supplemented with 2 mg/L BAP, which yielded the highest average measurements for shoot length (1.81 cm), number of shoots (4.20), number of leaves (8.20), and number of roots (5.00) over 8 weeks. This condition also produced the highest number of protocorms. However, the results showed that the MS medium was superior to the KC medium for the culture of these explants. The plantlets successfully acclimatized after being transplanted into pots and covered with plastic bags for five weeks.

Keywords: Plant growth regulators, Protocorms, *Rhynchostylis*, Sterilization

Introduction

Orchids (Orchidaceae) are flowering plants highly popular domestically and internationally. Their appeal largely stems from their blossoms' vibrant and aesthetically pleasing nature. In Thailand, orchids represent a crucial economic crop, with exports increasing annually. Notably, elephant orchids are distributed globally generating an annual export value of at least 4,000 baht. It is native to Southeast Asia, such as Thailand, Burma, Malaysia, and the Philippines, as well as countries in Indochina, India, and Sri Lanka.

Rhynchostylis sp. is characterized as a monopodial, epiphytic species exhibiting medium to large stems and thick leaves. In Thailand, this genus comprises three recognized species: *R. gigantea*, *R. retusa*, and *R. coelestis*. Their floral morphology and coloration vary displaying combinations of white adorned with red-purple spots, as well as hues of red-purple and orange. This genus is frequently used in the creation of interspecific hybrids and is also

* **Corresponding Author:** Boonmee, W.; **Email:** wimonmat.bo@kmitl.ac.th

popular for cultivation in pots (Thammasiri, 2016). However, *Rhynchostylis* sp. has been classified as an endangered species in Thailand (Suphuntee *et al*., 2017; Thitiprasert *et al*., 2007). According to Chugh *et al*. (2009), this status is attributed to factors such as deforestation and unregulated harvesting. Thus, the species face a significant risk of extinction in the near future, necessitating the implementation of effective, conservation measures.

Plant tissue culture serves as prominent method not only for large-scale micropropagation but also for the conservation of threatened orchid species. The formulation of the culture medium is critical for the successful in vitro cultivation of orchids. The most commonly used media include Knudson C (KC) medium and Murashige and Skoog (MS) medium (Dutra *et al*., 2009; Godo *et al*., 2010; Suzuki *et al*., 2012). Moreover, plant growth regulators are vital for the growth and development of orchids. Among these, cytokinins are significant plant growth regulators that facilitate cell differentiation during the early stages of growth and development in vitro. Cytokinins influence various physiological processes, such as promoting cell division and expansion, differentiating plastids, delaying leaf senescence, activating metabolite uptake, and inducing shoot formation from calluses (Lomin *et al*., 2012). This research focused on the in vitro culture of *Rhynchostylis* sp. derived from nodes using two different culture media supplemented with cytokinins to promote mass propagation and mitigate the risk of extinction in the foreseeable future.

Materials and methods

Plant materials

The nodes of *Rhynchostylis* were rinsed in water for 10 minutes. Next, the nodes were sterilized using a solution of 0.1% mercuric (II) chloride ($HgCl_2$) combined with 2 drops of tween 20 in 80 mL of water, while being shaken at 250 rpm for 3 minutes. Following this, the nodes were rinsed in sterilized water, with three separate one-minute shaking intervals. The nodes were then cultured in MS medium (Murashige and Skoog, 1962) and KC (Knudson, 1946) both supplemented with various concentrations of plant growth regulators including 6-benzylaminopurine (BAP), N-phenyl-N-1,2,3-thidizuron-5-yl urea (TDZ), meta-topolin (mT), and 6-(4-hydroxy-3-methyl-trans-2-butenylamino) purine (zeatin) at levels of 0, 0.5, 1, 2, 3, 4, and 5 mg/L. The media also contained 30 g/l sucrose, and 2.6 g/l phytagel, with some treatments conducted without the addition of plant growth regulators. The growth responses were monitored by counting the number of protocorms that developed. After 8 weeks of culture, measurements were taken using a vernier caliper to determine the average shoot

length, average number of shoots, average number of leaves, and average number of roots. Subsequently, the plantlets were transferred to pots containing a mixture of soil and coconut dust and covered in plastic bags to facilitate acclimatization to their new environment. After five weeks, the surviving plants were assessed.

Statistical analysis

The study was conducted with 10 replicates for each treatment to facilitate the induction of shoots and roots in vitro. An analysis of variance (ANOVA) was carried out, confirming the normal distribution of the data before analysis. The significance of differences in means was evaluated using Duncan's multiple comparison test ($p < 0.05$).

Results

The study focused on culturing *Rhynchostylis* orchids from joint fragments to promote the development of new plantlets achieved by utilizing two synthetic media (MS and KC) supplemented with plant growth regulators such as BAP, TDZ, mT, and zeatin at varying concentrations of 0.5, 1, 2, 3, 4, and 5 mg/L. This research followed an examination of the methods for bleaching and disinfecting *Rhynchostylis* orchids. As a result, it was revealed that the most suitable disinfection formula involved the use of sterile distilled water in conjunction with a 0.15 percent mercuric chloride solution, which demonstrated several uncontaminated samples. The average survival rate achieved was as high as 86.67 percent, accompanied by a satisfactory regeneration rate.

Following an 8-week culture of *Rhynchostylis* nodes on synthetic MS medium (Table 1; Figures 1-4, and 9), the best growth of new plantlets was observed in the medium supplemented with the growth regulator BAP at a concentration of 2 mg/L. This particular formulation demonstrated significantly enhanced growth characteristics, yielding the highest number of protocorms, averaging 4 protocorms per explant. Additionally, it recorded the highest average shoot length of 1.81 centimeters and the highest average number of shoot counts of 4.20. The shoots also exhibited a maximum average leaf count of 8.60 leaves and an average root count of 5.00. However, an increase in BAP concentration resulted in a decline in the number of shoots produced.

Table 1. The development of protocorms, average shoot length, average number of shoots, average number of leaves, and average number of roots, as observed following an 8-week cultivation period on MS media supplemented with the growth regulators BAP, TDZ, mT, and zeatin for *Rhynchostylis*

Plant growth regulators (mg/L)		Sample number (node)	Number of protocorms	Average shoot length (cm)	Average number of shoots (shoot)	Average number of leaves (leaf)	Average number of roots (root)
Control	0	10	0	0.66 ± 0.32^{bc}	1.00^{d}	1.60^{ef}	1.40^{def}
BAP	0.5	10	0	0.69 ± 0.23^{bc}	1.40^{cd}	1.60^{ef}	1.60^{cdef}
	1	10	1	1.07 ± 0.32^{abc}	1.80^{bcd}	3.20^{bcdef}	2.60^{bcd}
	2	10	4	1.81 ± 0.65^{a}	4.20^{a}	8.60^{a}	5.00^{a}
	3	10	3	0.93 ± 0.33^{bc}	1.60^{cd}	2.60^{cdef}	2.00^{bcdef}
	4	10	3	0.80 ± 0.30^{bc}	1.60^{cd}	3.00^{bcdef}	2.20^{bcdef}
	5	10	0	0.60 ± 0.38^{bc}	1.00^{d}	2.00^{cdef}	1.60^{cdef}
TDZ	0.5	10	0	0.42 ± 0.06^{c}	1.00^{d}	1.40^{f}	0.00^{f}
	1	10	0	0.64 ± 0.15^{bc}	1.40^{cd}	1.80^{def}	0.00^{f}
	2	10	1	0.63 ± 0.09^{bc}	1.60^{cd}	2.80^{bcdef}	0.20^{ef}
	3	10	3	0.80 ± 0.35^{bc}	1.80^{cd}	3.20^{bcdef}	1.60^{cdef}
	4	10	2	1.03 ± 0.30^{abc}	2.00^{bcd}	4.20^{bcdef}	2.00^{bcdef}
	5	10	0	0.50 ± 0.20^{c}	1.40^{cd}	2.60^{cdef}	1.40^{def}
***m*T**	0.5	10	0	0.49 ± 0.14^{b}	1.20^{d}	2.40^{cdef}	1.60^{cdef}
	1	10	0	0.64 ± 0.11^{bc}	1.40^{cd}	2.60^{cdef}	1.80^{bcdef}
	2	10	0	0.75 ± 0.11^{bc}	1.40^{cd}	2.80^{bcdef}	2.20^{bcdef}
	3	10	0	1.09 ± 0.18^{abc}	2.40^{abcd}	4.40^{bcdef}	3.40^{abcd}
	4	10	1	1.32 ± 0.59^{ab}	3.20^{abc}	5.00^{bc}	3.60^{abc}
	5	10	0	0.88 ± 0.21^{bc}	1.20^{d}	2.20^{cdef}	1.60^{cdef}
zeatin	0.5	10	0	0.57 ± 0.25^{bc}	1.40^{cd}	2.60^{cdef}	0.00^{f}
	1	10	0	0.94 ± 0.24^{bc}	2.60^{abcd}	4.80^{bcd}	2.20^{bcde}
	2	10	0	1.36 ± 0.58^{ab}	3.60^{ab}	5.80^{ab}	3.80^{ab}
	3	10	1	1.11 ± 0.48^{abc}	2.20^{bcd}	4.60^{bcde}	3.00^{abcd}
	4	10	0	0.70 ± 0.25^{bc}	2.00^{bcd}	3.60^{bcdef}	2.40^{bcd}
	5	10	0	0.63 ± 0.19^{bc}	1.60^{cd}	3.20^{bcdef}	2.00^{bcdef}

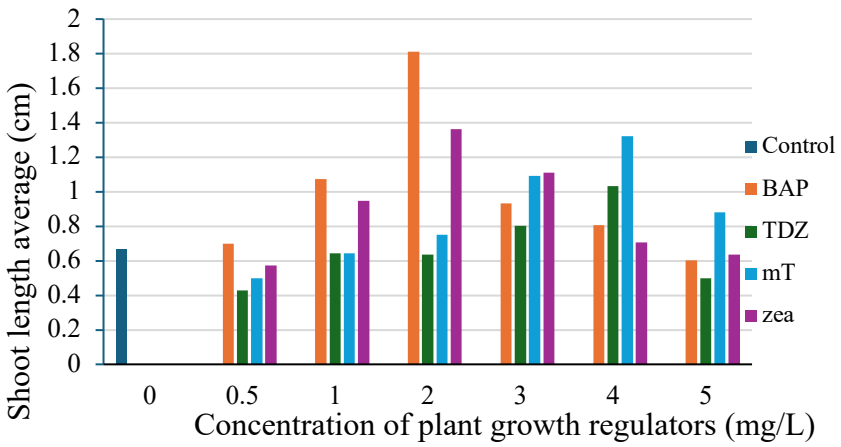

Figure 1. The average shoot length of *Rhynchostylis* following 8-week cultivation on MS medium supplemented with growth regulators BAP, TDZ, mT, and zeatin at concentrations of 0, 0.5, 1, 2, 3, 4, and 5 mg/L

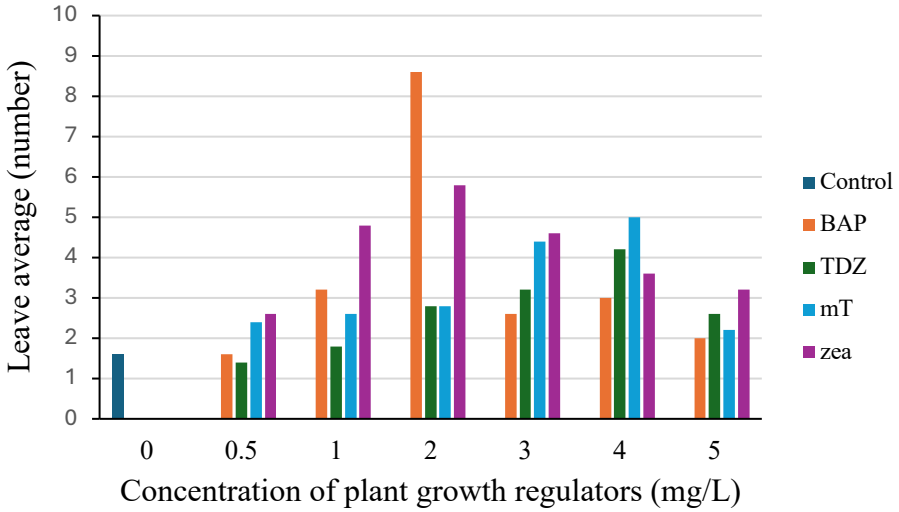

Figure 2. The average number of shoots of *Rhynchostylis* following 8-week cultivation on MS medium supplemented with growth regulators BAP, TDZ, mT, and zeatin at concentrations of 0, 0.5, 1, 2, 3, 4, and 5 mg/L

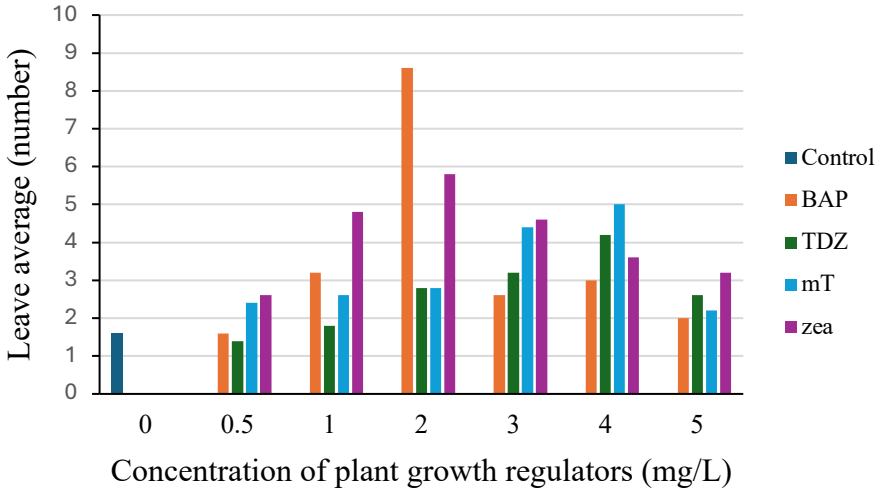

Figure 3. The average number of leaves of *Rhynchostylis* following 8-week cultivation on MS medium supplemented with growth regulators BAP, TDZ, mT, and zeatin at concentrations of 0, 0.5, 1, 2, 3, 4, and 5 mg/L

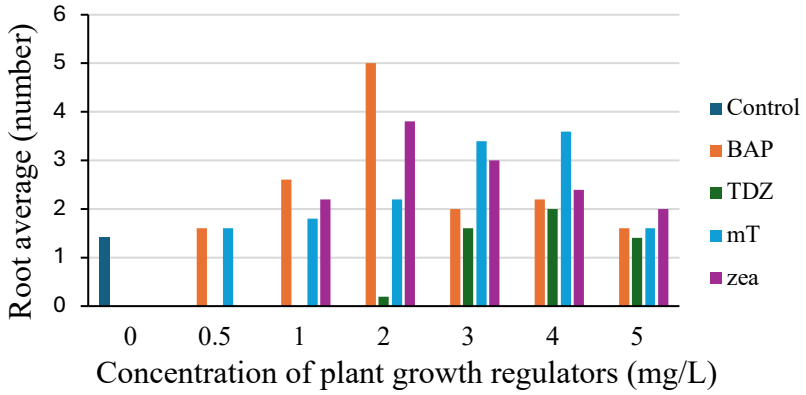

Figure 4. The average number of roots of *Rhynchostylis* following 8-week cultivation on MS medium supplemented with growth regulators BAP, TDZ, mT, and zeatin at concentrations of 0, 0.5, 1, 2, 3, 4, and 5 mg/L

Following an 8-week culture of *Rhynchostylis* nodes on synthetic KC medium, the highest growth of new plantlets was observed in the medium supplemented with the growth regulator BAP at a concentration of 2 mg/L, yielding an average shoot length of 0.88 centimeters. A comparable outcome was noted in the medium supplemented with 4 mg/L of mT. The average number of shoots per explant was consistent at 2.40, while the highest average number of

leaves reached 4.20 leaves. Additionally, the growth condition supplemented with the plant growth regulator zeatin at 2 mg/L resulted in the highest average number of roots, which was 3 roots per explant (Table 2; Figures 5-8, and 10).

Table 2. The formation of protocorms, average shoot length, average number of shoots, average number of leaves, and average number of roots, as observed following an 8-week cultivation on KC media supplemented with growth regulators BAP, TDZ, mT, and zeatin for *Rhynchostylis*

Plant growth regulators (mg/L)		Sample number (node)	Number of protocorms	Average shoot length (cm)	Average number of shoots (shoot)	Average number of leaves (leaf)	Average number of roots (root)
Control	0	10	0	0.25±0.15^{d}	0.80^{a}	1.00^{b}	0.00^{c}
BAP	0.5	10	0	0.53±0.16^{abc}	1.20^{a}	1.60^{ab}	1.40^{abc}
	1	10	0	0.77±0.18^{abc}	1.40^{a}	2.00^{ab}	1.60^{abc}
	2	10	1	0.88±0.25^{a}	2.40^{a}	3.80^{ab}	2.60^{ab}
	3	10	2	0.56±0.12^{abc}	1.80^{a}	3.00^{ab}	2.60^{ab}
	4	10	0	0.60±0.08^{abc}	1.60^{a}	3.00^{ab}	2.00^{ab}
	5	10	0	0.51±0.08^{abc}	1.20^{a}	1.80^{ab}	1.80^{ab}
TDZ	0.5	10	0	0.40±0.09^{c}	1.00^{a}	1.20^{b}	0.00^{c}
	1	10	0	0.41±0.17^{c}	1.20^{a}	2.00^{ab}	0.00^{c}
	2	10	0	0.39±0.19^{c}	1.20^{a}	2.40^{ab}	0.00^{c}
	3	10	2	0.61±0.16^{abc}	1.80^{a}	2.80^{ab}	1.40^{abc}
	4	10	2	0.49±0.16^{abc}	1.40^{a}	2.80^{ab}	2.60^{ab}
	5	10	0	0.39±0.13^{c}	1.40^{a}	2.00^{ab}	1.80^{ab}
***m*T**	0.5	10	0	0.39±0.12^{c}	1.40^{a}	2.00^{ab}	1.20^{bc}
	1	10	0	0.51±0.16^{abc}	1.40^{a}	2.40^{ab}	1.40^{abc}
	2	10	0	0.56±0.09^{abc}	1.80^{a}	3.20^{ab}	1.60^{abc}
	3	10	0	0.62±0.05^{abc}	2.00^{a}	2.80^{ab}	2.40^{ab}
	4	10	0	0.84±0.29^{ab}	2.40^{a}	4.20^{a}	2.80^{ab}
	5	10	0	0.46±0.11^{bc}	1.40^{a}	2.20^{ab}	1.60^{abc}
zeatin	0.5	10	0	0.39±0.10^{c}	1.00^{a}	1.40^{b}	1.40^{abc}
	1	10	0	0.59±0.11^{abc}	1.40^{a}	2.80^{ab}	1.80^{ab}
	2	10	0	0.83±0.20^{ab}	2.40^{a}	3.80^{ab}	3.00^{a}
	3	10	0	0.86±0.29^{ab}	1.60^{a}	3.20^{ab}	2.60^{ab}
	4	10	0	0.56±0.19^{abc}	1.40^{a}	3.00^{ab}	2.00^{ab}
	5	10	0	0.54±0.11^{abc}	1.20^{a}	2.40^{ab}	1.80^{ab}

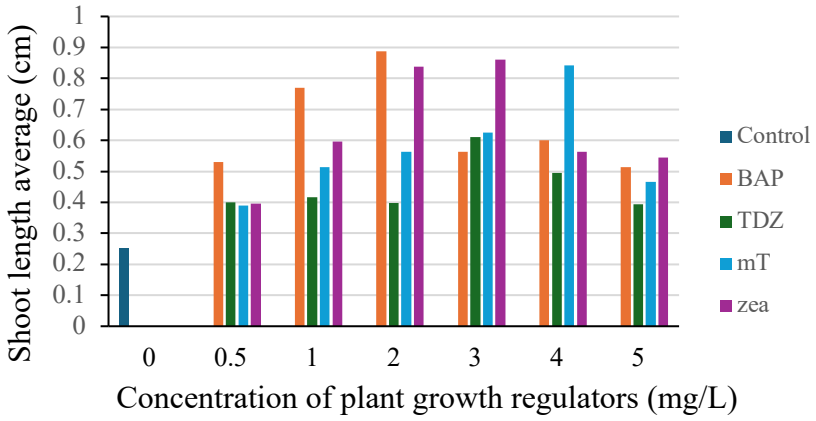

Figure 5. The average shoot length of *Rhynchostylis* following 8-week cultivation on KC medium supplemented with growth regulators BAP, TDZ, mT, and zeatin at concentrations of 0, 0.5, 1, 2, 3, 4, and 5 mg/L

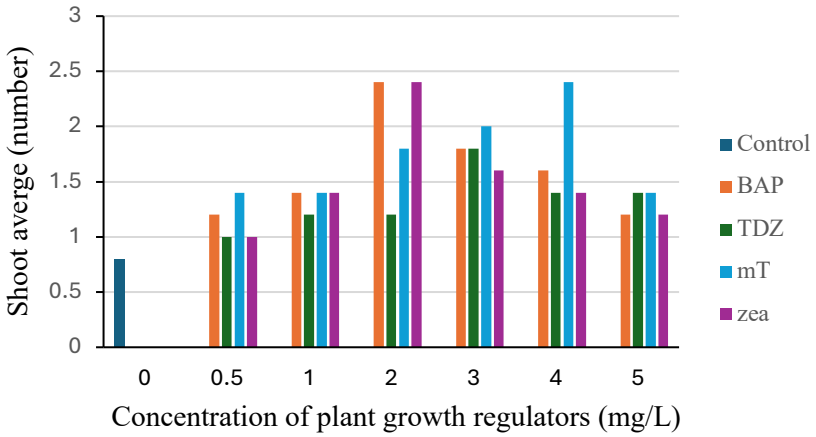

Figure 6. The average number of shoots of *Rhynchostylis* following 8-week cultivation on KC medium supplemented with growth regulators BAP, TDZ, mT, and zeatin at concentrations of 0, 0.5, 1, 2, 3, 4, and 5 mg/L

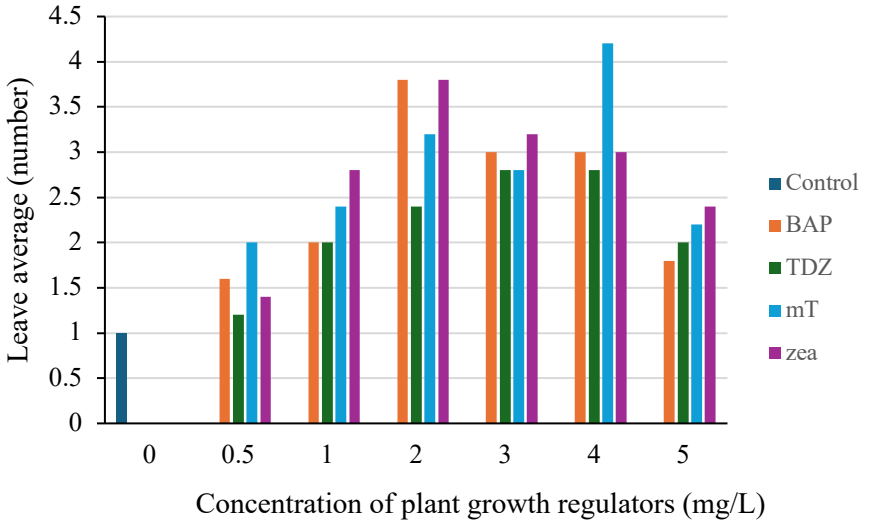

Figure 7. The average number of leaves of *Rhynchostylis* following 8-week cultivation on KC medium supplemented with growth regulators BAP, TDZ, mT, and zeatin at concentrations of 0, 0.5, 1, 2, 3, 4, and 5 mg/L

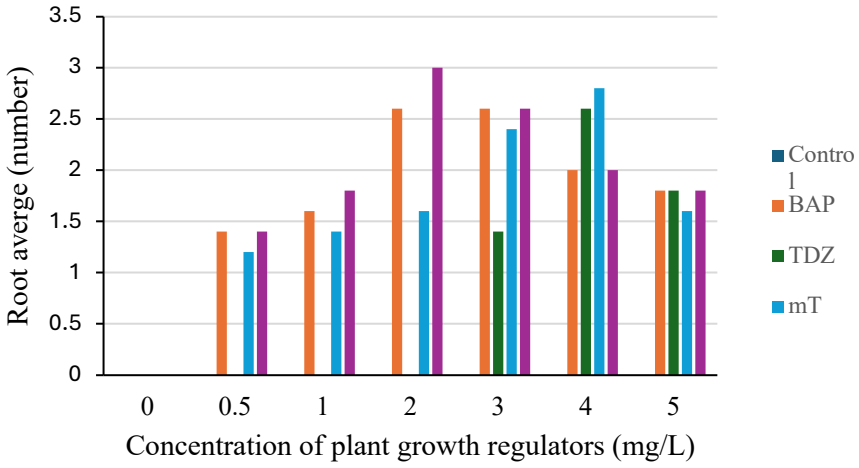

Figure 8. The average number of roots of *Rhynchostylis* following 8-week cultivation on KC medium supplemented with growth regulators BAP, TDZ, mT, and zeatin at concentrations of 0, 0.5, 1, 2, 3, 4, and 5 mg/L

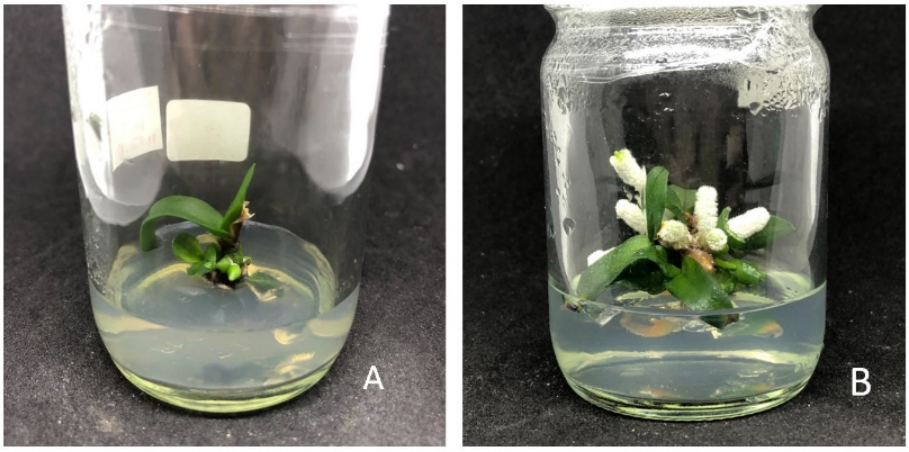

Figure 9. Following 8-week cultivation of *Rhynchostylis* nodes: A) without supplementation of growth regulators; and B) on MS medium supplemented with BAP growth regulator at 2 mg/L concentration

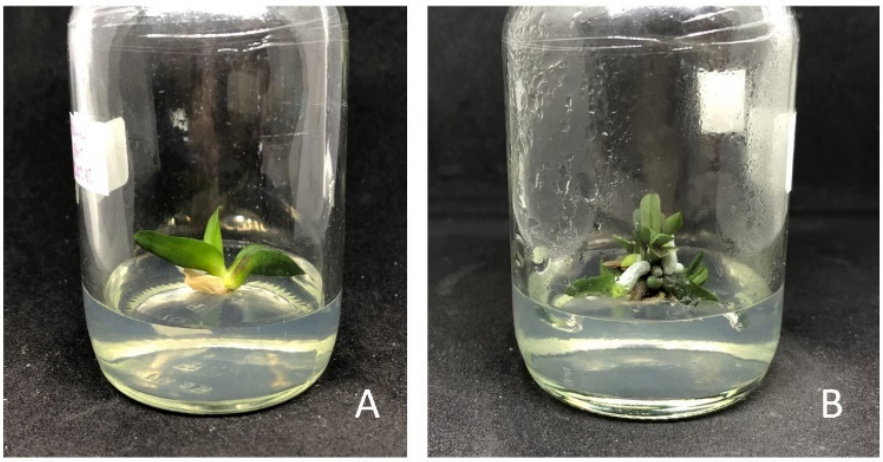

Figure 10. A. Following 8-week cultivation of *Rhynchostylis* nodes: A) without supplementation of growth regulators; and B) on KC medium supplemented with BAP growth regulator at 2 mg/L concentration

For plant acclimatization, the plantlets were transferred into pots containing a mixture of soil and coconut dust and subsequently covered with plastic bags after five weeks. The results indicated that the plants were capable of adjusting to their new environment and sustaining their growth (Figure 11).

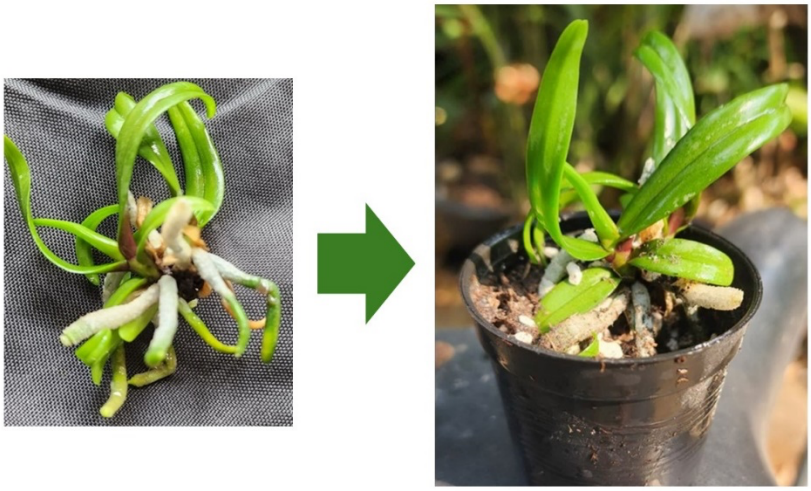

Figure 11. The plantlet was cultivated on soil and coconut dust mixture for 5 weeks

Discussion

Following an 8-week culture period of *Rhynchostylis* nodes, it was found that the cultures cultivated on synthetic MS medium exhibited noticeably better growth compared to those grown on synthetic KC medium across all measured growth parameters. This observation aligns with the findings of Suzuki *et al*. (2010) and Suzuki *et al*. (2012), who indicated that the MS medium has a higher nutritional composition and concentration than the KC medium. Consequently, the growth of *Rhynchostylis* in the MS medium was greater compared to that observed in the KC medium.

The best growth of new *Rhynchostylis* plantlets was observed in cultures grown on MS medium supplemented with the growth regulator BAP at a concentration of 2 mg/L. BAP (6-benzylaminopurine), classified as a cytokinin class, is a prominent plant growth regulator. In the context of plant tissue culture, cytokinins are commonly used to promote tissue proliferation and shoot development (Van Staden and Mooney, 1988; Mazid *et al*., 2011; Kieber and Schaller, 2018). As a result, the observed growth was the highest, characterized by the highest number of protocorms, the longest average shoot length, the highest average number of shoots per explant, as well as the highest average number of leaves and roots. This phenomenon can be attributed to the presence of distinct cytokine transit receptors in various plant cell types. Each plant cell

contains multiple cytokinin transport receptors, each exhibiting varying affinities for different cytokinin subtypes. Therefore, it's plausible that among the cytokinins tested, the plant cells possess a BAP receptor with the highest affinity (Lomin *et al*., 2012). Moreover, Rupp *et al*. (1999) also found that transgenic *Arabidopsis* plants with an enhanced cytokinin content produced more leaf cells than control plants, suggesting that cytokinin plays a crucial role in regulating leaf cell formation. Additionally, Wu *et al*. (2021) indicated that during the proliferation and expansion stages of leaf cell growth, cytokinins facilitate cell division and enhance cell expansion.

This summary encapsulates the influence of plant growth regulators (PGRs) on the tissue culture of *Rhynchostylis* orchids, highlighting that the most significant growth was achieved when nodal segments were cultured on the Murashige and Skoog (1962) medium (MS) supplemented with 2 mg/L of BAP.

Acknowledgments

This research was funded by the King Mongkut's Institute of Technology Ladkrabang Research Fund (KRIS). The authors express their gratitude to King Mongkut's Institute of Technology Ladkrabang for providing scholarships to all participants in the research program.

Conflicts of interest

The authors declare no conflict of interest.

References

Chugh, S., Guha, S. and Rao, I. U. (2009). Micropropagation of orchids: A review on the potential of different explants. Scientia Horticulturae 122:507-520.

Dutra, D., Kane, M. E. and Richardson, L. (2009). Asymbiotic seed germination and in vitro seedling development of *Cyrtopodium punctatum*: a propagation protocol for an endangered Florida native orchid. Plant Cell, Tissue and Organ Culture, 96:235-243.

Godo, T., Komori, M., Nakaoki, E., Yukawa, T. and Miyoshi, K. (2010). Germination of mature seeds of *Calanthe tricarinata* Lindl., an endangered terrestrial orchid, by asymbiotic culture *in vitro*. In Vitro Cellular & Developmental Biology-Plant 46:323-328.

Kieber, J. J. and Schaller, G. E. (2018). Cytokinin signaling in plant development. Development 145, dev 9344.

Knudson, L. (1946). A new nutrient solution for the germination of orchid seed. American Orchid Society Bulletin, 15:214-217.

Lomin, S. N., Krivosheev, D. M., Steklov, M. Y., Osolodkin, D. I. and Romanov, G. A. (2012). Receptor Properties and Features of Cytokinin Signalling. Acta Naturae, 4:31-45.

Mazid, M. A., Khan, T. A. and Mohammad, F. (2011). Cytokinins, A classical multifaceted hormone in plant system. Journal of Stress Physiology and Biochemistry, 7:347-368.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth bioassays with tobacco tissue culture. Physiologia Plantarum, 15:473-497.

Rupp, H. M., Frank, M., Werner, T., Strnad, M. and Schmulling, T. (1999). Increased steady state mRNA levels of the STM and KNAT1 homeobox genes in cytokinin overproducing *Arabidopsis thaliana* indicate a role for cytokinins in the shoot apical meristem. Plant Journal, 18:357-363.

Suphuntee, N., Tetsana, N., Poopath, M. and Tanikkool, S. (2017). Threatened Plants in Thailand (V. Chamchumroon, Ed.). Office of the Forest Herbarium, Forest and Plant Conservation Research Office, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment.

Suzuki, R. M., Almeida, V., Pescador, R. and Ferreira, W. M. (2010). In vitro germination and growth of *Cattleya bicolor* Lindley (Orchidaceae). Hoehnea, 37:731-742.

Suzuki, R. M, Moreira, V. C., Pescador, R. and Melo Ferreira, W. (2012). Asymbiotic seed germination and in vitro seedling development of the threatened orchid *Hoffmannseggella cinnabarina*. In Vitro Cellular and Developmental Biology, 48:500-511.

Thammasiri, K. (2016). Thai Orchid Genetic Resources and Their Improvement. Horticulturae 2.

Thitiprasert, W., Ratanasatien, C., Chitrakon, S., Watanesk, O., Chotechuen, S., Forrer, V. S., Sommut, W., Somsri, S., Samitaman, P. and Changtragoon, S. (2007). Country report on the state of plant genetic resources for food and agriculture in Thailand (1997-2004).

Van Staden, J. and Mooney, P. A. (1988). The effect of cytokinin preconditioning on the metabolism of adenine derivatives in soybean callus. Journal of Plant Physiology, 133:466-469.

Wu, W., Du, K., Kang, X. and Wei, H. (2021). The diverse roles of cytokinins in regulating leaf development. Horticulture Research, 8:118.

(Received: 23 December 2024, Revised: 24 June 2026, Accepted: 6 July 2026)