Research Article

Assessing the Effect of Peptone and Coconut Water in the Micropropagation of *Dendrobium enobi Purple 'Splash.'*

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Abstract | *Dendrobium enobi Purple 'Splash'* (DEPS) is a commercially valuable orchid hybrid requiring efficient in-vitro propagation. This study evaluated the effects of two natural additives, coconut water and peptone, on DEPS micropropagation. Protocorm-like bodies were cultured on half-strength Murashige and Skoog medium supplemented with selected concentrations of coconut water or peptone. Coconut water at 300 mL L^{-1} (CW3) significantly increased shoot length (31.33 ± 3.51 mm), while 400 mL L^{-1} (CW4) produced the highest shoot elongation (3.87 ± 0.12 cm). Peptone at 2.0 g L^{-1} (P2) significantly enhanced root development, yielding the highest root number (15.00 ± 1.73) and root length (1.83 ± 0.42 cm). The highest total chlorophyll content (0.36 mg g^{-1} FW) was also observed in peptone-treated cultures, specifically P4. Overall, coconut water significantly promoted shoot proliferation, while peptone promoted root growth and chlorophyll content compared with the control, indicating its potential as a cost-effective and environmentally friendly supplement for sustainable DEPS micropropagation.

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Introduction

The *Orchidaceae* family is the largest group of flowering plants, comprising about 28,000 species worldwide and nearly 4,000 in Malaysia (Go *et al.*, 2020). Orchids possess high ornamental, medicinal, and economic value, particularly genera such as *Dendrobium*, *Vanda*, and *Cymbidium*, which dominate the global cut-flower market (Yuan, 2021).

However, orchids face severe threats from habitat loss, overcollection, and climate change, making them among the most endangered plant groups globally (Besi *et al.*, 2023). Many *Dendrobium* species are listed under CITES Appendix II due to their low natural pollination, specialized fungal dependence for germination, and poor seed viability (Ma *et al.*, 2022; Zhao *et al.*, 2024). These factors highlight the need for efficient propagation strategies to ensure their conservation and commercial sustainability (Targu *et al.*, 2023).

Conventional propagation methods, such as seed sowing and division, are slow and inefficient. In

contrast, in-vitro micropropagation allows large-scale production of uniform and disease-free plants under controlled conditions (Lal and Singh, 2020; Lokesh and Madagoudra, 2021). Despite its advantages, tissue culture is often limited by high costs and the dependence on synthetic growth regulators (Pithiya *et al.*, 2022). Therefore, supplementing media with natural additives offers a more economical and sustainable alternative (Hashizume, 2023).

Among natural additives, coconut water (CW) and peptone have shown potential in promoting growth and morphogenesis. CW, rich in cytokinins, vitamins, and amino acids, enhances protocorm formation and shoot development in several orchid species, including *Phalaenopsis amabilis* (Salsabila *et al.*, 2022; Pyati *et al.*, 2002). Similarly, peptone, a protein hydrolysate rich in peptides and amino acids, stimulates root and shoot development in orchids such as *Phalaenopsis* and *Dendrobium* hybrids (Salsabila *et al.*, 2022; Shekarriz *et al.*, 2014).

Despite these findings, there is limited information on the specific effects and optimal concentrations of coconut water and peptone for the in-vitro micropropagation of DEPS; therefore, this study aimed to evaluate their individual effects on DEPS growth, with the hypothesis that selected concentrations of these natural additives would significantly enhance shoot, root, and chlorophyll development compared with the control.

Materials and Methods

Eleven culture media were evaluated. The control consisted of ½ MS medium (Duchefa Biochemie, The Netherlands) without supplementation. Five media were supplemented with different concentrations of peptone, and five with varying concentrations of coconut water (CW) (Table 1). Fresh coconuts (*Cocos nucifera* L.) were obtained from a local market in Puncak Alam, Malaysia. Coconut water was extracted and filtered three times using sterile double-folded muslin cloth before incorporation into the media.

All media were supplemented with 30 g/L sucrose (ChemAR®, Malaysia) and solidified with 3 g/L gelrite (Duchefa Biochemie, The Netherlands). Media components were dissolved in 1 L of distilled water using SCHOTT Duran® glass bottles, with water obtained from a Select Bio 160 purification system. The pH was adjusted to 5.4–5.6 before autoclaving at 121 °C and 15 psi for 20 min (Balilashaki *et al.*, 2023). Sterilized media were aseptically dispensed into sterile pill tubes under a laminar flow hood and allowed to solidify overnight.

Table 1: *Concentrations of peptone and coconut water supplemented into 1/2 MS Medium*

Treatment code	Additive type	Concentration
C (Control)	None	0
P05	Peptone	0.5 g/L
P1	Peptone	1 g/L
P2	Peptone	2 g/L
P3	Peptone	3 g/L
P4	Peptone	4 g/L
CW1	Coconut water	100 mL/L
CW2	Coconut water	200 mL/L
CW3	Coconut water	300 mL/L
CW4	Coconut water	400 mL/L
CW5	Coconut water	500 mL/L

Used for culture initiation of DEPS's PLBs.

Culture initiation

Protocorm-like bodies (PLBs) formed from seed germination that were cultured in modified MS medium after three months were utilized for culture initiation. To reduce contamination risk, the PLBs were surface sterilized before inoculation. The sterilization process involved immersing the PLBs in 70% ethanol for 30 seconds, followed by rinsing them thoroughly with sterile distilled water to remove any remaining ethanol residue (Attri, 2023). Following sterilization, the PLBs were aseptically transferred into the prepared media containing different concentrations of peptone and coconut water, as outlined in Table 1. Each treatment was replicated ten times, with one PLB around 1 cm in diameter cultured per pill tube to ensure individual monitoring of growth response.

All these procedures were carried out under sterile conditions inside a laminar flow hood to ensure contamination-free cultures. The cultured PLBs were maintained in a controlled growth room under a 16-hour light/8-hour dark photo period. Light was provided using white LED lamps with an average intensity of 1500-3000 lux, and the temperature was kept at 25 ± 2 °C (Singh 2024). Growth and proliferation rates of the PLBs were monitored over six months.

Data collection

Data was collected after 6 months. The number and length of shoots present in each pill tube were measured using a 25 cm ruler (Nelson *et al.*, 2023). Shoot count was determined based on the presence of an apical meristem or the development of visible leaves. In addition, the number and length of roots were also measured for each pill tube.

Chlorophyll content determination

Chlorophyll content was measured to evaluate the effect of different treatments on pigment accumulation in the protocorm-like bodies (PLBs). Samples were collected at the end of the culture period, and approximately 0.5 g of fresh DEPS tissue was used for each replication. The chlorophyll was extracted using 80% (v/v) acetone. The PLB tissue was homogenized in 5 mL of 80% acetone using a mortar and pestle, then centrifuged at 10,000 rpm for 10 minutes to obtain a clear supernatant (Abdouli *et al.*, 2023). The absorbance of the extract was measured at 645 nm (A645) and 660 nm (A660) using a UV-Vis spectrophotometer. Chlorophyll a, chlorophyll b, and total chlorophyll contents were calculated using the following formulas with modifications (Arnon, 1949):

- Chlorophyll a (mg/g FW) = (12.7 × A660) − (2.69 × A645) × Final volume of extract (ml)/1000 × Weight of sample taken (g)
- Chlorophyll b (mg/g FW) = (22.9 × A645) − (4.68 × A660) × Final volume of extract (ml)/1000 × Weight of sample taken (g)
- Total chlorophyll (mg/g FW) = (Chlorophyll a) + (Chlorophyll b)

Statistical analysis

Quantitative data were analysed using the Statistical Package for the Social Sciences (SPSS) software, version 29.0. The Shapiro-Wilk test was performed to assess the normality of data distributions. Based on the results, appropriate statistical tests were selected. The number of roots met the assumptions of normality and homogeneity of variances; therefore, a one-way analysis of variance (ANOVA) was conducted to examine the effect of different peptone and coconut water concentrations on the number of roots of DEPS. Given that a significant effect was observed ($p < 0.05$), Tukey's Honest Significant Difference (HSD) test was performed to determine which specific treatments showed differences. In contrast, data on shoot number, shoot length, root length, and chlorophyll content did not meet parametric assumptions. As such, these variables were analysed using the Kruskal–Wallis test, a non-parametric alternative suitable for comparing more than two groups (Silva and Borges, 2023). Since the test indicated a significant overall effect ($p < 0.05$), post hoc pairwise comparisons were conducted using Dunn's test to identify which specific treatment groups differed significantly from each other.

Figure 1: *Growth of DEPS plantlets after six months of in-vitro cultivation using 3-month- old PLB explants under different peptone treatments: (a) C, (b) P05, (c) P1, (d) P2, (e) P3, and (f) P4. The bar represents 1 cm.*

Results and Discussion

Morphology of DEPS using peptone

The development of DEPS PLBs cultured with 4 g/L peptone exhibited clear morphological changes across different growth stages, as illustrated in Figure 1. At the early stage (a), three-month-old PLBs grown on a PGR-free medium appeared pale, compact, and showed minimal signs of organogenic differentiation. After one week of culture (b), the PLBs turned green and began forming early protrusions, indicating the initiation of shoot development. By the end of one month (c), plantlets began to display several shoots, although they were loosely organized, with a few roots emerging. After two months (d), plantlets developed longer, slender leaves and thicker roots, although the root system was still moderately established. At the four-month stage (e), DEPS plantlets demonstrated vigorous growth, with multiple well-formed shoots, bright green elongated leaves, and a moderately branched root system consisting of whitish-green

roots. In the final observed stage (f), the plantlets formed dense clusters of upright shoots with darker green, broader, and thicker leaves. The roots became more numerous, longer, and thicker, exhibiting a healthy translucent greenish appearance. Overall, the progression from stage (a) to (f) indicates substantial improvements in shoot proliferation, leaf development, and root formation, suggesting the plantlets were nearing readiness for the next phase, ex-vitro acclimatization.

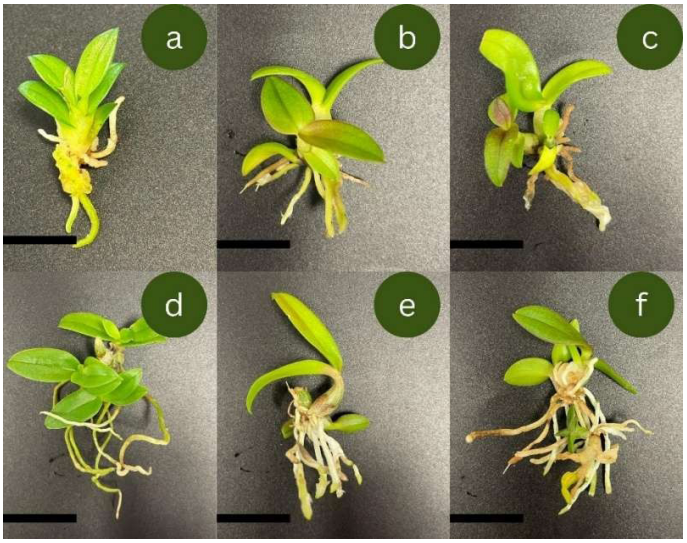

Figure 2: *Growth of DEPS plantlets after six months of in-vitro cultivation using 3-month- old PLB explants under different coconut water treatments: (a) Control, (b) CW1, (c) CW2, (d) CW3, (e) CW4, and (f) CW5. The bar represents 1 cm.*

Morphology of DEPS using coconut water

The development of DEPS PLBs exhibited distinct morphological differences between cultures supplemented with coconut water, observable across various growth stages, as shown in Figure 2. At the initial stage (a), protocorm-like bodies (PLBs) formed on a PGR- free medium appeared pale and compact, with no noticeable structural differentiation in either treatment. After one week of culture (b), PLBs in coconut water displayed a brighter green coloration with early protrusions suggestive of shoot initiation. By one month (c), plantlets exhibited faster early organogenesis, with clearer leaf emergence and the initiation of fine root structures. After two months (d), plantlets displayed broader, shorter leaves and a higher number of shoot initials, although root systems remained moderately developed. At four months (e), the morphological differences became more pronounced, in which coconut water cultures formed dense clusters of multiple leafy shoots with moderate rooting. By six months (f), plantlets achieved superior shoot proliferation, producing many well-formed shoots per explant, and developed a significantly longer but smaller number of roots.

Shoot and root proliferation of DEPS

The effects of coconut water and peptone on the micropropagation of DEPS were evaluated through shoot and root proliferation. Parameters such as the number of shoots, shoot length, number of roots, and root length were measured under different treatment conditions, as shown in Table 2.

Table 2: *Results of shoot and root proliferation of DEPS.*

Treatment	Total number of shoots per explant	Length of shoots (cm)	Total number of roots per explant	Length of roots (cm)
C (Control)	9.67±0.58[a]	0.90±0.31[a]	1.67±1.53[b]	0.33±0.29[a]
P05	13.67±1.15	1.30±0.21[a]	7.67±1.53[b]	0.73±0.25[a]
P1	18.33±0.58	1.47±0.17	12.33±1.55	1.60±0.36
P2	19.00±1.00	3.50±0.76	15.00±1.73	1.83±0.42
P3	25.67±3.05	3.70±0.62[a]	11.33±1.15	1.57±0.38
P4	22.00±1.73	2.83±0.44	11.67±0.58	1.17±0.15
CW1	14.33±1.54[a]	1.73±0.15	5.00±2.00	1.33±0.057[a]
CW2	19.00±1.73	2.17±0.12	8.00±1.00	1.27±0.057
CW3	31.33±3.51	2.67 ±0.18	8.67±1.53	1.53±0.057
CW4	18.33±2.08	3.87±0.12[a]	9.00±1.00	1.33±0.0643
CW5	20.33±0.58	2.47±0.15	11.00±1.00	1.40±0.082

[a] *indicates statistically significant differences between treatments based on Dunn's post-hoc test (P.adj < 0.05).*
[b] *indicates statistically significant differences between treatments based on Tukey HSD post-hoc test (P.adj < 0.05).*

Shoot proliferation of DEPS

Shoot proliferation of DEPS was significantly affected by supplementation with peptone (0–4 g/L) and coconut water (0–500 mL/L) after six months of culture (Figure 3). Preliminary normality testing indicated that the data were non-normally distributed; therefore, non-parametric statistical analyses were applied. Kruskal–Wallis tests revealed significant differences among the 11 treatments for both shoot number ($H = 29.542$, $p = 0.001$) and shoot length ($p < 0.05$), confirming that treatment type had a substantial influence on shoot proliferation and elongation.

For shoot number, the control (C), low coconut water concentration (CW1), and low peptone

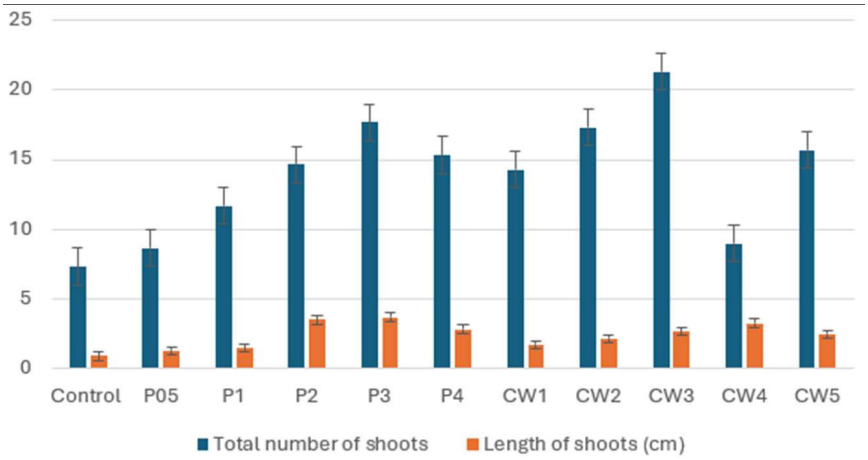

Figure 3: *Shoot proliferation of DEPS after 6 months of using peptone and coconut water.*

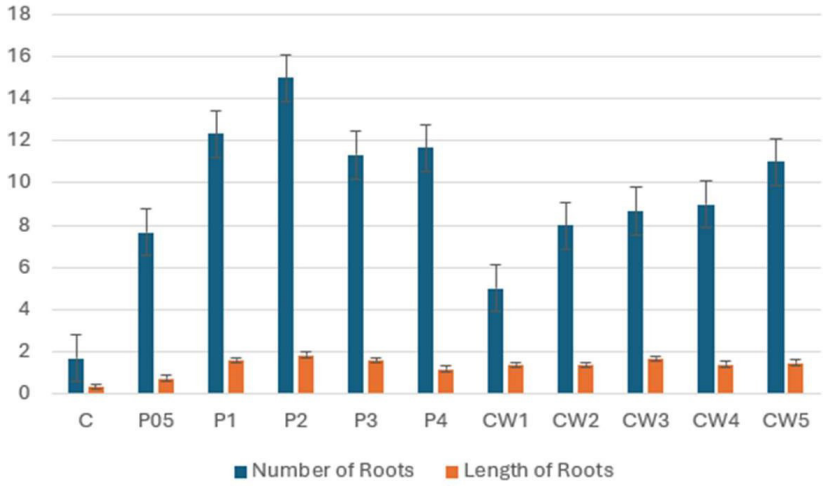

Figure 4: *Root proliferation of DEPS after 6 months of using peptone and coconut water.*

concentration (P05) consistently produced the lowest values, indicating limited stimulatory effects at these levels. In contrast, higher concentrations, particularly CW3, P3, and P4, resulted in markedly increased shoot proliferation. Post-hoc Dunn's test showed that the control produced significantly fewer shoots than P3 (Padj = 0.016) and CW3 (Padj = 0.004). Similarly, P05 (Padj = 0.029) and CW1 (Padj = 0.046) were significantly inferior to CW3. These findings clearly demonstrate that CW3 was the most effective treatment for shoot multiplication, outperforming both the control and low-concentration treatments.

A similar trend was observed for shoot length. Dunn's post-hoc analysis revealed that shoots from the control were significantly shorter than those produced under P3 (*P*adj = 0.006) and CW4 (*P*adj = 0.023), while P05 also produced significantly shorter shoots compared with P3 (*P*adj = 0.018). Among all treatments, CW4 recorded the greatest mean shoot length (3.87 ± 0.12 cm), indicating its strong promotive effect on shoot elongation. Although P3 also produced relatively long shoots, which were 3.70 ± 0.62 cm, its performance was statistically superior, mainly when compared to the control and low- concentration treatments.

The enhanced performance of coconut water treatments, particularly CW3 and CW4, may be attributed to the presence of naturally occurring cytokinins, vitamins, sugars, and amino acids, which collectively stimulate cell division and shoot development (Uyen *et al.*, 2024). Similarly, peptone supplementation at 3.0 g/L (P3) significantly improved both shoot number and length, likely due to its role as an organic nitrogen source that supports rapid cell proliferation and tissue differentiation (Salsabila *et al.*, 2022).

Root proliferation of DEPS

Root proliferation of DEPS was significantly influenced by supplementation with peptone (0–4 g/L) and coconut water (0–500 mL/L) after six months of culture (Figure 4). Both root number and root length varied among treatments; however,

different statistical approaches were applied according to data distribution.

Table 3: *Influence of peptone and coconut water on chlorophyll accumulation in DEPS.*

Treatment	Chlorophyll a	Chlorophyll b	Total chlorophyll (mg/g FW)
C	0.055 ± 0.002	0.005 ±0.004	0.061±0.003
P05	0.014±0.01	0.08±0.02	0.094±0.009
P1	0.100±0.005	0.064±0.009	0.16±0.005
P2	0.120±0.005	0.058±0.005	0.179±0.006
P3	0.126±0.02	0.124±0.03	0.25±0.006
P4	0.207±0.02	0.153±0.01	0.36±0.009
CW1	0.018±0.005	0.018±0.002	0.036±0.004[b]
CW2	0.058±0.004	0.03±0.005	0.088±0.004
CW3	0.056±0.008	0.032±0.007	0.088±0.005[b]
CW4	0.063±0.009	0.084±0.01	0.15±0.006
CW5	0.174±0.03	0.055±0.01	0.23±0.02

[b] *indicates statistically significant differences among treatments based on Dunn's post-hoc test (P.adj < 0.05).*

Root number data met parametric assumptions and were therefore analyzed using one-way ANOVA followed by Tukey's HSD post-hoc test. Most treatments significantly increased root number compared with the control ($p < 0.05$), including CW2–CW5 and all peptone treatments from P05 to P4, while CW1 did not differ significantly from the control ($p = 0.529$). Among peptone treatments, higher concentrations were generally more effective, with P4 producing the highest root number 11.67 ± 0.58 cm, representing the greatest improvement over the control. Coconut water treatments also enhanced root formation, with CW5 yielding a similarly high absolute root number 11.00 ± 1.00 cm, although it did not exceed the performance of P4.

Root length data were non-normally distributed and were analyzed using the Kruskal–Wallis test followed by Dunn's post-hoc test. The control produced significantly shorter roots compared with P3 (*P*adj = 0.016) and CW3 (*P*adj = 0.004). In addition, both P05 (*P*adj = 0.029) and CW1 (*P*adj = 0.046) resulted in significantly shorter roots than CW3. Among all treatments, P2 recorded the greatest mean root length 1.83 ± 0.42 cm, indicating that moderate peptone supplementation was particularly effective in promoting root elongation.

The enhanced root development observed under coconut water treatments may be attributed to the presence of carbohydrates, vitamins, and endogenous plant growth regulators that stimulate root initiation and elongation (Aishwarya *et al.*, 2022). Meanwhile, peptone provides readily available amino acids and organic nitrogen, which support root meristem activity and cell expansion, especially at moderate to higher concentrations.

Chlorophyll content of DEPS

The total chlorophyll content of DEPS plantlets varied across treatments and, as Table 3 shows, clearly correlated with growth performance.

The total chlorophyll content of DEPS plantlets was significantly influenced by supplementation with peptone and coconut water after six months of in-vitro culture. The control group (C) exhibited the lowest chlorophyll content (0.061 mg/g FW), reflecting

limited physiological activity and reduced pigment synthesis in the absence of organic additives. Among the peptone treatments, chlorophyll content increased progressively with rising concentrations, with P4 producing the highest accumulation (0.36 ± 0.009 mg/g), which was significantly greater than CW1 (0.036 ± 0.004 mg/g, P.adj = 0.0038) and CW3 (0.088 ± 0.005 mg/g, P.adj = 0.036). P3 (0.247 ± 0.001 mg/g) also exhibited significantly higher chlorophyll than CW1 (0.036 ± 0.004 mg/g, P.adj = 0.0188). This upward trend aligns with the enhanced shoot and root proliferation observed in these treatments, indicating that peptone supplementation supports chlorophyll biosynthesis and improves photosynthetic capacity in developing plantlets. The likely mechanism involves the supply of amino acids and peptides, which serve as precursors for chlorophyll synthesis and enhance overall metabolic activity.

Coconut water supplementation also significantly promoted chlorophyll accumulation relative to the control. Among the CW treatments, CW5 recorded the highest chlorophyll content (0.211 ± 0.001 mg/g), followed by CW4 (0.144 ± 0.001 mg/g). These improvements are consistent with the observed morphological growth, suggesting that coconut water provides vitamins, cytokinins, and other bioactive compounds that stimulate pigment production and physiological development in DEPS.

Overall, both peptone and coconut water treatments resulted in higher chlorophyll content than the control, with P4 being the most effective treatment. The superior chlorophyll accumulation under these treatments aligns with the enhanced shoot and root development,indicating a strong relationship between morphological and physiological improvements.

These findings support the role of organic additives in boosting photosynthetic efficiency and plantlet growth during in-vitro culture. Similar trends have been reported in *Dactylorhiza hatagirea*, where peptone supplementation significantly improved chlorophyll content and growth parameters (Giri *et al*., 2012), reinforcing the broader applicability of these additives in orchid micropropagation.

Conclusions and Recommendations

This study demonstrated that the incorporation of natural organic additives such as coconut water and peptone can significantly enhance the in-vitro micropropagation efficiency of DEPS. Among the treatments, 300 mL/L coconut water resulted in the highest shoot proliferation, while 400 mL/L promoted shoot elongation. Peptone at 2.0 g/L effectively supported root growth, and peptone at 4 g/L yielded the highest reading for total chlorophyll content, indicating its role as a vital nitrogen source in tissue culture. These findings confirm that coconut water and peptone are cost-effective and eco-friendly alternatives to synthetic growth regulators, offering promising solutions for sustainable commercial propagation and conservation of orchid species.

The study is limited using a single hybrid, controlled in vitro conditions, and the absence of ex vitro validation, which may affect the generalizability of the results. Future recommendations include: (1) testing the combined application of coconut water and peptone to explore potential synergistic effects, (2) optimizing their concentrations and timing of application, and (3) extending this approach to other commercially valuable and endangered orchid species to validate broader applicability and support conservation efforts.

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The authors confirm that this manuscript has not been published previously and is not under consideration for publication in any other journal. All authors have contributed significantly to this work and have approved the final version of the manuscript. All authors agree with the conditions outlined in the journal's copyright assignment. This study did not involve human participants or animals and therefore did not require ethical approval.

Novelty Statement

This study is the first to systematically evaluate the effects of peptone and coconut water at optimized concentrations on the in-vitro micropropagation of DEPS. The findings provide a practical, sustainable protocol for enhancing shoot, root, and chlorophyll development, offering a cost-effective alternative to synthetic growth regulators in orchid propagation and conservation.

Author's Contribution

Noor Anilizawatima Sulong: Conceptualization, supervision, project administration, writing the original draft, and supervision
Afiqah Atiqah Mohd Jeffri: Methodology, writing (review and editing)
Intan Hidayah Mohamad Jamal: Methodology, data col-lection, writing (review and editing)
Nurin Irdina Ismail: Laboratory work, validation, resources
Nur Fatin Amira Noor Haimay: Laboratory work, data support
Siti Suhaila A Rahman: Funding acquisition, resources and supervision

Generative AI and AI-assisted technology statement
The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

There is no conflict of interest among the authors of the manuscript.

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