ASIAN JOURNAL OF AGRICULTURE
Volume 10, Number 1, June 2026
Pages: 9

E-ISSN: 2580-4537
https://doi.org/10.13057/asianjagric/g100130

In vitro plant regeneration of *Cattleya* sp. from Protocorm-Like Bodies (PLBs) using coconut water and activated charcoal

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Manuscript received: 25 September 2025. Revision accepted: 25 february 2026.

Abstract. *Harahap F, Siregar ARS, Idramsa, Suriani C, Edi S, Panggabean NH, Daulae AH, Pohan SD, Tanjung AA, Pertiwi SI, Kairani A. 2026. In vitro plant regeneration of* Cattleya *sp. from Protocorm-Like Bodies (PLBs) using coconut water and activated charcoal. Asian J Agric 10 (1): g100130. https://doi.org/10.13057/asianjagric/g100130. Cattleya* sp. is an ornamental orchid whose seeds lack of endosperm, making natural germination and propagation difficult. In vitro culture using Protocorm-Like Bodies (PLBs) offers an alternative for mass propagation. This study aimed to evaluate the effects of coconut water and activated charcoal on the in vitro regeneration of *Cattleya* sp. PLBs. A completely randomized design was employed with varying concentrations of coconut water (0, 5, and 10%) and activated charcoal (0, 0.1, 0.2, and 0.3 g/L). Morphological parameters observed included time to shoot emergence, number of leaves, shoots, and roots, as well as shoot color. Data were analyzed using two-way ANOVA. The results showed that coconut water significantly accelerated shoot emergence with 10% of coconut water producing the fastest response (12 days after treatment). Activated charcoal enhanced shoot and leaf formation with 0.3 g/L, producing the highest number of shoot and leaf. Root formation was optimal at lower charcoal concentrations. Overall, the combination of coconut water and activated charcoal significantly influenced PLBS growth and regeneration. These findings indicate that appropriate combinations of organic additives can optimize in vitro regeneration of *Cattleya* sp. and support efficient orchid propagation.

Keywords: Activated charcoal, *Cattleya* sp., coconut water, in vitro propagation, Protocorm-Like Bodies (PLBs)

INTRODUCTION

Orchids are among the most popular ornamental plants, admired for their exceptional floral diversity and aesthetic value, which contribute to their widespread cultivation and high market-demand (Hinsley et al. 2018). The global trade of orchids as both cut flowers and potted plants is expanding, and this growth is accompanied by an increase in the overall volume of trade. In addition to their ornamental value, orchids are utilized in the medical, food, and beverage industries due to their rich content of polysaccharides, alkaloids, and other bioactive compounds (Wang et al. 2017). With the advancement of economic globalization, the global demand for orchids, both in quantity and diversity, has consistently increased each year. This growing demand has driven researchers and breeders to prioritize the development of novel orchid cultivars with distinctive aesthetics, greater resistance to stressors, and superior quality traits (Kamboj 2020).

Orchid seeds are extremely small and lack endosperm to store food reserves; therefore, although they produce large numbers of seeds, natural germination is highly limited and dependent on symbiotic fungi (Yeung 2017). If these reproductive constraints are not addressed, they may contribute to population decline and increased extinction risk among orchid species. Some alternative methods of reproduction are required to ensure the preservation of orchids and prevent their extinction. This concern is further emphasized by the inclusion of all orchid species under Appendices I and II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), which regulates international trade to prevent overexploitation (Gale et al. 2018). According to the Global International Union for Conservation of Nature (IUCN) Red List assessments conducted in 2021, approximately 46.5% of orchid species worldwide are classified under one of the three threat categories: vulnerable, endangered, and critically endangered. This percentage indicates a significant proportion of orchid species facing a high risk of extinction (IUCN 2021).

The application of tissue culture techniques is an effective strategy for orchid conservation, enabling rapid mass propagation of plantlets while reducing pressure on wild populations (Lal and Singh 2020). The latest research on in vitro cell culture is crucial as a current and up-to- date reference source to address the ongoing research requirements. Among ornamental orchids, *Cattleya* sp. holds significant commercial potential due to its wide variation in flower shape, color, and size. This genus is particularly valued for its large, brightly colored, and

visually striking flowers, earning it the common name "Queen of Orchids" (Seeja and Sreekumar 2022). The plant known as the Queen of Orchids also has a fragrant smell (Seeja and Sreekumar 2022). *Cattleya* orchids are relatively expensive when compared to other types of orchids, and cultivating them to produce flowers takes a very long time. Moreover, conventional propagation methods for *Cattleya* sp. are labor-intensive, time-consuming, and inefficient.

Using in vitro techniques (tissue culture) to grow Protocorm-Like Bodies (PLBs) or somatic embryos from selected orchid varieties is one of the solutions for plant propagation issue. It is a precise method to quickly produce many orchid seeds (Yeung and Stasolla 2024; Yusnita 2015). Therefore, this study aimed to investigate the effects of different concentrations of coconut water and activated charcoal on the in vitro regeneration of *Cattleya* sp. from PLBs, with emphasis on shoot emergence, multiplication, rooting, and overall plantlet quality. This study aimed to investigate the effects of different concentrations of coconut water and activated charcoal on the in vitro regeneration of *Cattleya* sp. from PLBs, focusing on shoots emergence, multiplication, rooting, and plantlet quality. Protocorm-Like Bodies (PLBs) are parts of orchids, like their tissues or callus that can create structures that look like protocorms when grown in a lab (Cardoso et al. 2020). The process of direct or indirect embryogenesis in orchids occurs throughout the formation of PLBs (Zhao et al. 2008; Cardoso et al. 2020; Kiaheirati et al. 2024). Given the limited number of studies reporting PLBS regeneration protocols for *Cattleya* sp., further investigation is both necessary and timely. As previous study conducted by (Yeung 2017), Protocorm-Like Bodies (PLBs)' multiplication of *Phalaenopsis* sp. was successful to increase its growth, from 1000 PLBS to 18000 PLBS in eight weeks and was ready to regenerate into new orchid plants using a temporary immersion culture bioreactor. Additionally, supplements and organic matter capable of promoting PLBS germination quickly can aid in regeneration (Utami and Hariyanto 2020).

MATERIALS AND METHODS

Study area

The study was carried out at the YAHDI Tissue Culture Laboratory, Medan, Indonesia, over a period from January to July 2025. Protocorm-Like Bodies (PLBs) of *Cattleya* sp were obtained from Department of Agriculture, Medan City and used as plant materials. Murashige and Skoog (MS) medium was employed to support the growth and development of the PLBs. Standard tissue culture equipment and laboratory instruments were used throughout the experiment Laminar Air Flow Cabinet (LAFC), culture vessels, analytical balance, pH meter, and culture racks. Environmental conditions were monitored using a thermometer, hygrometer, and lux meter. Laboratory observation sheets were prepared for systematic data collection. Media preparation materials included distilled water, macro- and micronutrients, vitamins, sucrose, coconut water, activated charcoal, agar, Plant Growth Regulators (PGRs), and sterilizing agents such as 70% and 96% ethanol.

Procedures

Protocorm-Like Bodies (PLBs) used in this study were previously obtained from in vitro-germinated *Cattleya* sp. seeds through standard orchid tissue culture procedures (Harahap et al. 2023). Protocorm-Like Bodies (PLBs) were derived from in vitro cultures of a single, uniform cultivar/clone, 8-10 week old, selected at a uniform developmental stage, characterized by spherical to oval morphology, bright green coloration, and absence of browning or necrosis. Only actively proliferating PLBs with similar size (approximately 2-4 mm in diameter) were used to minimize physiological variation. Prior to treatment, PLBs were cultured on MS medium under controlled in vitro conditions (25±2°C, 16 h photoperiod) to ensure comparable physiological status. Regeneration of PLBs from orchid seeds in tissue culture, started with sterilizing the seeds, cultured them on a Murashige and Skoog (MS) medium agar, added with plant hormones, and provided specific light/temperature conditions, allowing the seeds to germinate into protocorms which then proliferated into PLBs. Transferring PLBs involved a sterile process of moving these small plantlets from their initial growth medium to a new MS medium to encourage their organ development. For the present experiment, healthy and uniform PLBs were aseptically transferred onto MS media supplemented with coconut water at concentrations of 0%, 5%, and 10%, and activated charcoal at concentrations of 0, 0.1, 0.2, and 0.3 g L^{-1}. The combinations of treatments are presented in Table 1.

MS medium containing macro- and micro-elements of MS media supplemented with 170 mg/L NaH_2PO_4, 30 g/L sucrose, and plant growth regulators (PGR) was used for growth induction. The pH of the medium was adjusted to 5.5-6.0 with 1 N NAOH. The induced callus was sub-cultured onto similar MS medium every 6 weeks for further proliferation and development of the PLBs. The PLBs derived from seeds were used as explants to induce secondary PLBs. MS media supplemented with a combination of coconut water and activated charcoal (Table 1) were used for the induction and proliferation of the PLBs; PGR-free MS was used as the control and referred to as Ak_0A_{a0}.

Table 1. Combinations of coconut water and active charcoal in MS media

	Ak_0 (%)	**Ak_5 (%)**	**Ak_{10} (%)**
Aa (g/L)			
0	Ak_0Aa_0	Ak_5Aa_0	$Ak_{10}Aa_0$
0.1	$Ak_0Aa_{0.1}$	$Ak_5Aa_{0.1}$	$Ak_{10}Aa_{0.1}$
0.2	$Ak_0Aa_{0.2}$	$Ak_5Aa_{0.2}$	$Ak_{10}Aa_{0.2}$
0.3	$Ak_0Aa_{0.3}$	$Ak_5Aa_{0.3}$	$Ak_{10}Aa_{0.3}$

Note: Ak: Coconut water, Aa: Active charcoal

The pH of all media was adjusted to 5.5-6.0 with 1 N NaOH. Coconut Water (CW) was drawn off and filtered through a sieve before being added into the medium. Activated charcoal were first cut into sections, and then weighed according to the amount required and mixed separately with distilled water (to facilitate the blending process). Following homogenization, each of the crude organic additives was added separately to the medium. The PLBS explants were cultured on treatment medium and placed in the dark for 4 weeks at 25±2℃ and then replaced to the culture rack with continuous light and controlled temperature and humidity (25±2℃ and 80-90%, respectively). The experiments were performed in a Completely Randomized Design (CRD) with two factors and three replications.

Data analysis

Growth and regeneration were evaluated based on the following parameters: Time of shoot emergence (TSE), number of shoots (SN), number of leaves (LN), number of roots (RN), and shoot color (SC). Shoot color was assessed using the Color Grab mobile application by recording leaf color intensity values. Measurements were recorded at regular observation intervals. The mean values of the various parameters were subjected to Analysis of Variance (ANOVA) using SPSS ver. 26 software (SPSS, Chicago, IL). Prior to ANOVA, data were tested for normality using the Shapiro–Wilk test and for homogenity of variance using Levene's test. The differences between the mean of each treatment were scored using Duncan's Multiple Range Test at a p value=0.05.

RESULTS AND DISCUSSION

Coconut water and activated charcoal effect on PLBs growth

In vitro propagation of orchid through protocorm-like body formation is more challenging than other regeneration pathways due to the slow and complex developmental process involved. In the present study, coconut water, activated charcoal, and their interaction significantly influenced PLBS growth responses, although no significant effect was observed on leaf number (LN) (Table 6). Two-way ANOVA revealed that coconut water, activated charcoal, and their interaction had significant effects on time to shoot emergence (TSE), number of shoots (SN), and number of roots (RN) ($p<0.05$) (Tables 2, 4, and 7). These results confirm that the experimental factors tested were effective in modulating PLBS regeneration responses.

As shown in Table 4, in general, the addition of coconut water to 5-10% to the media seemed to hasten the emergence of shoots, particularly when activated charcoal concentrations were lower (0-0.1 g/L). On the other hand, shoot emergence was delayed when higher concentrations of activated charcoal (0.3 g/L) were added. Coconut water, activated charcoal, and their combination all had a significant impact on the number of shoot produced by PLBs (p=0.000, p=0.004, and p=0.000, respectively). The medium supplemented with 0.3 g/L activated charcoal significantly increased shoot production (35). The PLBs cultured in the media with the addition of 0.2 g/L activated charcoal produced 32.33 shoots, while with the addition of 10% coconut water alone exhibited as many as 29.33 shoots.

This study suggests that the growth-promoting qualities of coconut water may occasionally be replaced by activated charcoal and that the advantages of both substances working together are not always mutually reinforcing. Coconut water (p=0.456), activated charcoal (p=0.560), or both had no discernible effects on the number of leaves (p=0.780) (Table 6). This means that, unlike other features like shoots and roots, the growth of leaves in orchid PLBs grown in vitro might not be as affected by changes in these media supplements. Significant differences were seen in the number of roots that PLBs produced depending on coconut water (p=0.000), activated charcoal (p=0.000), and their combination (p=0.001). Moreover, the media containing 0.1 g/L activated charcoal without coconut water had the most roots (13.33), followed by the media containing 0.3 g/L activated charcoal (12.67). When combined with higher amounts of coconut water, root development generally decreased, particularly at 10% (Tables 7 and 8).

This finding highlights the potential antagonistic relationship between activated charcoal and coconut water in root formation, suggesting that too many organic additions could hinder in vitro rooting; however, this interpretation should be considered preliminary, as no mechanistic analysis conducted. The results indicate that coconut water and activated charcoal affect the growth of orchid PLBs in different ways, depending on what aspect of growth is being measured. The important interactions discovered that it's necessary to arrange the amounts of these supplements based on what researchers want to achieve, whether that's better root growth, quicker shoots development, or more shoots.

Time to shoot emergence (TSE)

The addition of coconut water, activated charcoal, and their interaction significantly affected the time to shoot emergence (TSE), with p-values of 0.019, 0.001, and 0.002, respectively (Table 2). This indicates that both individual factors and their combined application play an important role in regulating early shoot initiation. Based on Duncan's multiple range test, MS medium supplemented with 10% coconut water resulted in the fastest shoot emergence (12 days), followed closely by 5% coconut water (13 days) (Table 3). In contrast, higher concentrations of activated charcoal (0.3 g L^{-1}), particularly when combined with 10% coconut water, delayed shoot emergence.

Number of shoots (SN)

Coconut water, activated charcoal, and their interaction significantly influenced the number of shoots produced by PLBs (p=0.000, 0.004, and 0.000, respectively) (Table 4). These findings demonstrate a strong synergistic and antagonistic interaction between the two supplements, depending on concentration. The highest shoot production

(35 shoots) was recorded on MS medium supplemented with 0.3 g/L activated charcoal without coconut water ($Ak_0Aa_{0.3}$) (Table 5). Similarly, PLBs cultured on MS medium containing 0.2 g/L activated charcoal produced a high number of shoots (32.33), while the addition of 10% coconut water alone resulted in 29.33 shoots. Overall, the addition of coconut water at 5–10% tended to accelerate shoot initiation when activated charcoal concentrations were low (0-0.1 g/L), whereas higher activated charcoal concentrations favored shoot multiplication rather than rapid emergence.

Number of leaves (LN)

No significant effects of coconut water (p=0.456), activated charcoal (p=0.560), or their interaction (p=0.780) were observed on leaf number (LN) (Table 6). This suggests that leaf formation in *Cattleya* PLBs cultured in vitro is relatively stable and less responsive to changes in these organic supplements compared to shoot and root development.

Table 2. Analysis of Variance (ANOVA) showing the effect combination of Ak and Aa on TSE of orchid's PLB

Source	Type III Sum of squares	df	Mean square	F	Sig.
Corrected Model	256.556[a]	11	23.323	5.598	0.000*
Intercept	9669.444	1	9669.444	2320.667	0.000*
Coconut Water (Ak)	38.889	2	19.444	4.667	0.019*
Activated charcoal (Aa)	89.889	3	29.963	7.191	0.001*
Ak*Aa	127.778	6	21.296	5.111	0.002*
Error	100	24	4.167		
Total	10026	36			
Corrected Total	356.556	35			

Note: *: There is significant effect of treatment within groups (p<0.05) on PLB's TSE and there is interaction between two treatments (coconut water and activated charcoal)

Table 3. Duncan's posthoc test result the effect of coconut water and activated charcoal on TSE of PLB

Ak (%) / Aa (g/L)	Time to Shoot Emergence/TSE (day)				Average
	0	0.1	0.2	0.3	
0	17.33±2.5[bcd]	19.67±0.5[de]	16.00± 1[abcd]	17.00±1[bcd]	17.50±1.9
5	13.00±1[a]	14.67±0.5[abc]	15.33±2[abc]	17.00±3.6[bcd]	15.00±2.4
10	12.33±0.5[a]	14.00±1[ab]	18.33±1.5[cd]	22.00±4.3[e]	16.67±3.2
Average	14.22±2.7	16.11±2.7	16.56±1.9	18.67±3.2	

Note: Mean values followed by the same letter in the same column are not significantly different according to the Duncan's Multiple Range Test at α=0.05. Ak: Coconut water, Aa: Active charcoal

Table 4. Analysis of Variance (ANOVA) showing the effect combination of Ak and Aa on SN of orchid's PLB

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2019.639[a]	11	183.604	16.361	0.000*
Intercept	15088.028	1	15088.028	1344.478	0.000*
Coconut Water (Ak)	324.389	2	162.194	14.453	0.000*
Activated charcoal (Aa)	194.083	3	64.694	5.765	0.004*
Ak*Aa	1501.167	6	250.194	22.295	0.000*
Error	269.333	24	11.222		
Total	10026	36			
Corrected Total	356.556	35			

Note: *: There is significant effect of treatment within groups (p<0.05) on PLB's SN and there is interaction between two treatments (coconut water and activated charcoal)

Table 5. Duncan's posthoc test result the effect of coconut water and activated charcoal on SN of PLB

Ak (%) / Aa (g/L)	Shoot Number (SN)				Average
	0	0.1	0.2	0.3	
0	17.33±2.0[abc]	13.67±3.8[ab]	32.33±3.5[d]	35.00±5 [d]	24.58±10
5	21.67±2.5[c]	20.00±3[bc]	14.00±1 [ab]	14.33±4.5[ab]	17.5±4.4
10	30.33±1.5[d]	16.67[a±]1.5[bc]	18.00±5.6[abc]	12.33±2.5[a]	19.08±7.5
Average	23.11±6	16.78±3.7	21.44±8.10	20.55±11.4	

Note: Mean values followed by the same letter in the same column are not significantly different according to the Duncan's Multiple Range Test at α=0.05. Ak: Coconut water, Aa: Active charcoal

Number of roots (RN)

Root formation was significantly influenced by coconut water, activated charcoal, and their interaction (p<0.05) (Table 7). The highest number of roots (13.33) was observed in PLBs cultured on MS medium supplemented with 0.1 g/L activated charcoal without coconut water, followed by 0.3 g/L activated charcoal (12.67 roots) (Table 8). In contrast, increasing coconut water concentration to 10% generally reduced root formation, particularly when combined with higher levels of activated charcoal. This trend indicates a potential inhibitory effect of excessive organic supplementation on root induction.

These results demonstrate that coconut water and activated charcoal influence PLBS growth in a parameter-specific manner. While coconut water primarily promoted early shoot emergence, activated charcoal was more effective in enhancing shoot and root proliferation. The significant interactions observed highlight the importance of optimizing supplement concentrations based on the desired regeneration outcome.

Morphological and color quality of *Cattleya* orchid plantlets regenerated from PLBs

The supplementation of coconut water and activated charcoal to the culture media had a big impact on the morphological and color quality of *Cattleya* orchid plantlets developed from Protocorm-Like Bodies (PLBs). The Color Grab app showed that the regenerated shoots had varying color of green depending on the media treatment (Table 9). There is already a color picture from the treatment of coconut water and activated charcoal (Figure 1 and 2). In this paper, more on the emphasis of the color obtained, no measurements of magnification or scale were conducted.

The control treatment that were not supplemented with any coconut water or activated charcoal (Ak_0Aa_0) caused the shoots turn a dark green (#3D681D), which meant that chlorophyll was growing well. The dark green color was always seen in treatments with low to moderate amounts of activated charcoal (0.1-0.3 g/L), especially in $Ak_0Aa_{0.1}$ (#344C19) and $Ak_0Aa_{0.3}$ (#446F2A).

Table 6. Analysis of Variance (ANOVA) showing the effect combination of Ak and Aa on LN of orchid's PLB

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2029.639	11	184.513	0.628	0.787 ^{ns}
Intercept	37960.028	1	37960.028	129.238	0.000
Coconut Water (Ak)	476.389	2	238.194	0.811	0.456^{ns}
Activated charcoal (Aa)	618.306	3	206.102	0.702	0.560 ^{ns}
Ak*Aa	934.944	6	155.824	0.531	0.780 ^{ns}
Error	7049.333	24	293.722		
Total	47039	36			
Corrected Total	9078.972	35			

Note: *: Significant at level α=0.05, ^{ns}: There is no significant effect of treatment within groups (p<0.05) on PLB's LN

Table 7. Analysis of Variance (ANOVA) showing the effect combination of Ak and Aa on RN of orchid's PLB

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	339.556^{a}	11	30.869	11.456	0.000*
Intercept	3061.778	1	3061.778	1136.330	0.000*
Coconut Water (Ak)	112.056	2	56.028	20.794	0.000*
Activated charcoal (Aa)	128.222	3	42.741	15.863	0.000*
Ak*Aa	99.278	6	16.546	6.141	0.001*
Error	64.667	24	2.694		
Total	3466	36			
Corrected Total	404.222	35			

Note: *: There is significant effect of treatment within groups (p<0.05) on PLB's RN and there is interaction between two treatments (coconut water and activated charcoal)

Table 8. Duncan's posthoc test result the effect of coconut water and activated charcoal on RN of PLB

Ak (%) / Aa (g/L)	Root Number (RN)				Average
	0	0.1	0.2	0.3	
0	11.00±1^{cde}	13.33±1.5^{e}	7.67±0.5^{b}	11.00±1^{cde}	10.75±2.3
5	8.67±1.1^{bc}	11.00±1.7^{cde}	8.33±0.5^{bc}	12.67±3de	10.17±2.4
10	10.00±1.7^{bcd}	10.33±2.3^{bcd}	3.00±1^{a}	3.67±2^{a}	6.75±3.9
Average	9.89±1.5	11.56±2.1	6.33±2.5	9.11±4.6	

Note: Mean values followed by the same letter in the same column are not significantly different according to the Duncan's Multiple Range Test at α=0.05. Ak: Coconut water, Aa: Active charcoal

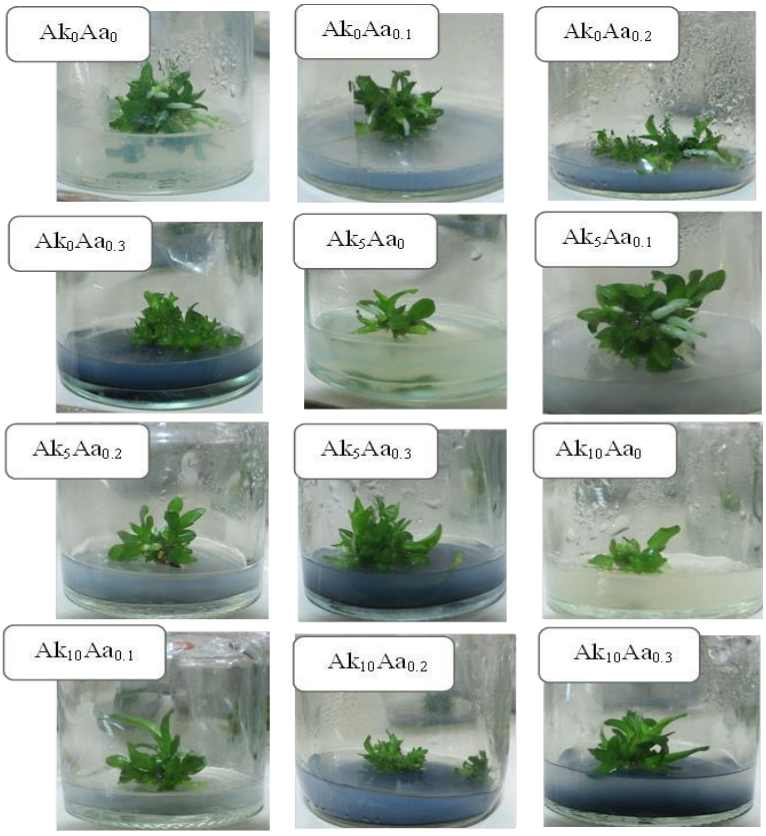

Figure 1. Performance of *Cattleya* sp. planlets 12 weeks after planting on MS medium added with activated charcoal. Note: Ak: Coconut water, Aa: Active charcoal

Table 9. Color quality of orchid planlets regenerated from PLBs of *Cattleya* sp. measured using Color Grab application

Treatments	Σ Color quality	Shoots color (PLBs)	Color specification (Hex)
Ak_0Aa_0		Dark Green	#3D681D
$Ak_0Aa_{0.1}$		Dark Green	#344C19
$Ak_0Aa_{0.2}$		Green	#5D843F
$Ak_0Aa_{0.3}$		Dark Green	#446F2A
Ak_5Aa_0		Dark Green	#355228
$Ak_5Aa_{0.1}$	4	Dark Green	#3A6227
$Ak_5Aa_{0.2}$		Dark Green	#537939
$Ak_5Aa_{0.3}$		Dark Green	#395829
$Ak_{10}Aa_0$		Green	#58833B
$Ak_{10}Aa_{0.1}$		Faded Green	#516C47
$Ak_{10}Aa_{0.2}$		Dark Faded Green	#425E3C

Note: Ak: Coconut water, Aa: Active charcoal

It was revealed that activated charcoal kept the amount of chlorophyll stable and slowed down the process of phenolic oxidation, which generally causes tissue to turn brown. Adding 5% coconut water (Ak_5) to all concentrations of activated charcoal (0-0.3 g/L) made the shoots all the same dark green color, with color values between #355228 and #537939. This means that moderate amounts of coconut water made the shoots stronger without breaking down the chlorophyll. Raising the proportion of coconut water to 10% caused the appearance of various shoot colors. The shoots in $Ak_{10}Aa_0$ were green (#58833B), whereas those in $Ak_{10}Aa_{0.1}$ were a faded green (#516C47), and those in $Ak_{10}Aa_{0.2}$ were a dark faded green (#425E3C).

Discussion

The present study demonstrated that coconut water, activated charcoal, and their interaction significantly influenced the in vitro growth and regeneration of *Cattleya* Protocorm-Like Bodies (PLBs). In particular, shoot emergence was accelerated by the addition of 5-10% coconut water in combination with low concentrations of activated charcoal (0-0.1 g/L), indicating a positive interaction between organic supplements and adsorptive agents during early shoot initiation. These findings are consistent with previous reports by Rajbahak and Rajkarnikar (2017), who showed that coconut water that combined with NAA, BAP, and kinetin enhances root and shoot development in *Dendrobium* orchids. Additionally, Gansau et al. (2016) also found that the addition of 2g/L peptone or 15% (v/v) coconut water had significantly induced 16.7% protocorms proliferation of *Dendrobium lowii*. Organic supplements such as coconut water had been used widely in orchid tissue culture due to its high content of natural cytokinins, auxins, vitamins, and amino acids that promote cell division and differentiation. The significant effects of both main factors and their interaction on shoot number further confirm that PLBS multiplication is highly responsive to medium composition.

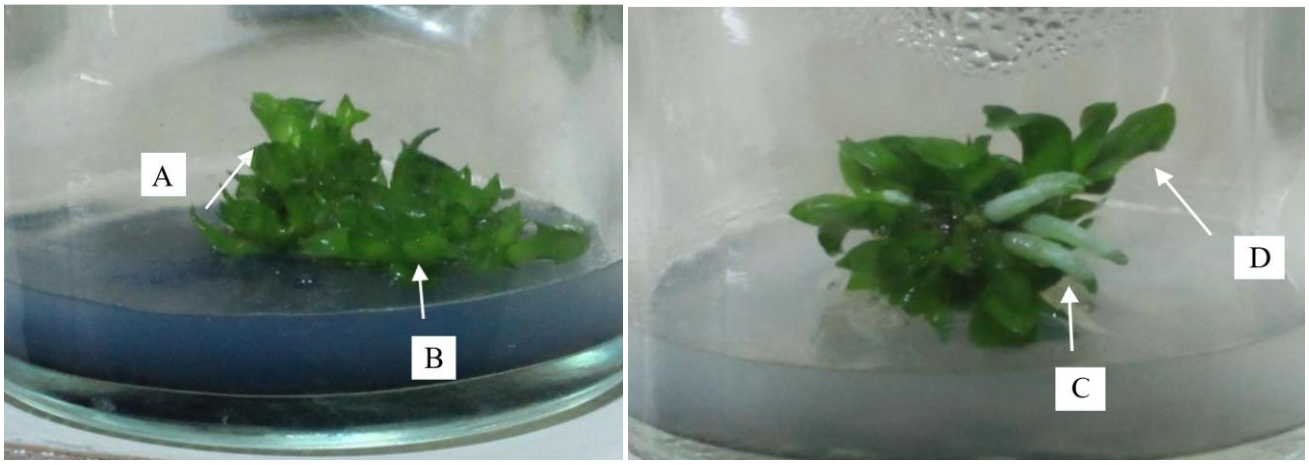

Figure 2. A. Leaves that develop from buds and are green in color ($Ak_0Aa_{0.3}$), B. New shoots are continuously formed from Protocorm-Like Bodies (PLBs) ($Ak_0Aa_{0.3}$), C. Roots emerge 12 weeks after planting ($Ak_5Aa_{0.1}$), D. Enlarged leaves show 12 weeks after planting ($Ak_5Aa_{0.1}$). Note: Ak: Coconut water, Aa: Active charcoal

The highest shoot production was observed on MS medium supplemented with 0.3 g/L activated charcoal without coconut water, followed by 0.2 g/L activated charcoal alone. Interestingly, a comparable number of shoots was also obtained with 10% coconut water in the absence of activated charcoal, suggesting that activated charcoal may partially substitute for, or synergize with, the growth-promoting effects of coconut water depending on concentration and developmental stage. This observation supports findings by Danova et al. (2023), who reported that activated charcoal enhances shoot regeneration by adsorbing phenolic compounds and toxic metabolites that accumulate in culture media and inhibit growth. Activated charcoal has been widely used in plant tissue culture due to its capacity to absorb inhibitory secondary metabolites and stabilize the physiological environment of explants (Gemechu and Amante 2021). However, excessive levels of activated charcoal may also adsorb beneficial compounds, including vitamins and plant growth regulators, thereby reducing its positive effects.

In contrast to shoot-related parameters, leaf number was not significantly affected by coconut water, activated charcoal, or their interaction. This suggests that leaf formation in orchid PLBs is less sensitive to changes in organic supplements and may be governed by intrinsic developmental controls. Similar observations were reported by Sipayung et al. (2018), who found that activated charcoal significantly enhanced shoot proliferation in *Dendrobium* but had limited influence on leaf formation. Root development, however, responded strongly to both supplements and their interaction. The highest number of roots was recorded on MS medium containing 0.1 g/L activated charcoal without coconut water, followed by treatments combining moderate coconut water and activated charcoal concentrations. In contrast, higher coconut water levels (10%) significantly reduced root formation, particularly when combined with activated charcoal. These results corroborate the findings of Yam and Arditti (2017), who reported that while coconut water supplies essential organic nutrients, excessive concentrations can disrupt hormonal balance especially through elevated cytokinin levels thereby favoring shoot formation at the expense of rooting. This highlights the importance of optimizing organic additive concentrations to achieve balanced plantlet development.

Protocorm-Like Bodies (PLBs) are unique structures in orchids and are widely regarded as functional analogues of somatic embryos. During orchid seed germination, embryos initially develop into protocorms before differentiating into shoots and roots. Under in vitro conditions, somatic tissues exposed to appropriate growth regulators and organic supplements can revert to an embryogenic state, giving rise to PLBs that resemble zygotic embryos in morphology and developmental potential. Previous studies have demonstrated that PLBS formation follows developmental stages characteristic of somatic embryogenesis (Zhao et al. 2008; Irmayanti et al. 2025). The present findings further support this concept by showing that coconut water, rich in natural cytokinins and auxins, accelerates early PLBS development and shoot initiation. Similar effects were reported by Yasmint and Harahap (2025), who observed a substantial increase in callus growth following coconut water supplementation. Depending on culture conditions and hormonal balance, PLBs may undergo direct organogenesis or indirect somatic embryogenesis. Activated charcoal, through its ability to adsorb phenolic compounds and excess growth regulators, likely alters the hormonal microenvironment surrounding PLBs, influencing developmental pathway selection. The significant interaction between coconut water and activated charcoal observed in this study reflects the complexity of hormonal regulation governing PLBS differentiation into shoots and roots. Due to their totipotent nature, PLBs are valuable for mass propagation, conservation, cryopreservation, and genetic transformation of orchids. Future studies integrating histological analysis, molecular marker identification, and hormone profiling will be essential to further confirm the embryogenic nature

of PLBs and refine regeneration protocols across diverse orchid species.

Variations in shoot color and plantlet morphology observed in this study provide additional insight into the physiological effects of organic supplements. Higher coconut water concentrations were associated with reduced chlorophyll intensity, likely due to hormonal imbalance or excessive organic matter interfering with nutrient uptake and pigment synthesis. Plantlets grown on MS medium supplemented with 0.3 g/L activated charcoal without coconut water exhibited vigorous shoot development and healthy green leaves, while continuous PLB-derived shoot formation was also observed. Root initiation became evident after 12 weeks of culture, particularly in treatments combining 5% coconut water with 0.1 g/L activated charcoal, indicating synchronized shoot and root development. Coconut water supplies natural cytokinins and amino acids that support shoot growth and chlorophyll synthesis, whereas activated charcoal improves culture conditions by adsorbing inhibitory substances and stabilizing medium composition (An et al. 2021; Wasiati et al. 2021). However, excessive organic supplementation, particularly 10% coconut water, may reduce chlorophyll stability and overall plantlet quality. Overall, the best morphological and color quality of *Cattleya* plantlets was achieved on MS medium containing 5% coconut water combined with 0.1-0.3 g/L activated charcoal, which promoted robust shoot growth, healthy leaf coloration, and effective rooting.

In conclusion, coconut water and activated charcoal significantly influenced shoot emergence, shoot multiplication, and root formation of *Cattleya* Protocorm-Like Bodies (PLBs) in vitro. The fastest shoot emergence was obtained with 10% coconut water, while activated charcoal alone, particularly at 0.3 g/L for shoots and 0.1 g/L for roots, produced the best regeneration responses. These results indicate that appropriate combinations of organic supplements are essential for optimizing PLB-based propagation. Further studies are recommended to investigate the physiological and molecular mechanisms underlying PLBS development to improve regeneration efficiency.

ACKNOWLEDGEMENTS

This research was funded by the Universitas Negeri Medan, Indonesia, under contract number 0123/UN33.8/PPKM/PPT/2025.

REFERENCES

An J, Kim PB, Park HB, Kim S, Park HJ, Lee CW, Hwang JE. 2021. Effects of different growth media on in vitro seedling development of an endangered orchid species *Sedirea japonica*. Plants 10 (6): 1193. https://doi.org/10.3390/plants10061193.

Cardoso JC, Zanello CA, Chen JT. 2020. An overview of orchid Protocorm-Like Bodies (PLBs): Mass propagation, biotechnology, molecular aspects, and breeding. Intl J Mol Sci 21 (3): 985. https://doi.org/10.3390/ijms21030985.

Danova K, Stanoeva JP, Aneva I, Alipieva K, Stefova M. 2023. Plant growth regulators and activated charcoal selectively affect phenylethanoid and flavone glycoside accumulation in *Sideritis scardica* Griseb. tissue culture. Plants 12 (13): 2541. https://doi.org/10.3390/plants12132541.

Gale SW, Fischer GA, Cribb PJ, Fay MF. 2018. Orchid conservation: Bridging the gap between science and practice. Bot J Linn Soc 186 (4): 425-434. https://doi.org/10.1093/botlinnean/boy003.

Gansau JA, Indan H, Abdullah SN, David D, Marbawi H, Jawan R. 2016. Effects of organic additives and plant growth regulators on protocorm development of *Dendrobium lowii*. Trans Sci Technol 3 (3): 462-468.

Gemechu E, Amante G. 2021. Control of browning in plant tissue culture: A review. J Sci Innov Res 10 (4): 89-93. https://doi.org/10.31254/jsir.2021.10402.

Harahap F, Hariyadi I, Silitonga M, Suryani C, Edi S, Ningsih AP. 2023. In vitro growth of *Cattleya* sp orchid from leaf explants with growth regulators. Jurnal Pembelajaran dan Biologi Nukleus 9 (1): 192-200. https://doi.org/10.36987/jpbn.v9i1.3945.

Hinsley A, De Boer HJ, Fay MF, Gale SW, Gardiner LM, Gunasekara RS, Kumar P, Masters S, Metusala D, Roberts DL, Veldman S, Wong S, Phelps J. 2018. A review of the trade in orchids and its implications for conservation. Bot J Linn Soc 186 (4): 435-455. https://doi.org/10.1093/botlinnean/box083.

Irmayanti A, Prasetiyo A, Rahayu P. 2025. Effect of young coconut water concentration on the growth of *Dendrobium* orchid seedlings. Bioscientist: Jurnal Ilmiah Biologi 13 (1): 2654-4571. https://doi.org/10.33394/bioscientist.v13i1.15056.

IUCN. 2021. The IUCN Red List of Threatened Species, Version 2021-1. https://www.iucnredlist.org.

Kamboj D. 2020. *Dendrobium* and *Vanda* Orchids as Potential Cut Flowers in North Indian Market. GINMA, Hisar.

Kiaheirati H, Hashemabadi D, Kaviani B. 2024. In vitro propagation of the orchid *Phalaenopsis circus* via organogenesis and somatic embryogenesis using protocorm and thin cell layer explants. Ital Botanist, 18: 29-50. https://doi.org/10.3897/italianbotanist.18.123376.

Lal N, Singh M. 2020. Prospects of plant tissue culture in orchid propagation: A review. Indian J Biol 7 (2): 103-110. https://doi.org/10.21088/ijb.2394.1391.7220.15.

Rajbahak S, Rajkarnikar KM. 2017. In vitro multiplication and protocorms development of *Dendrobium longicornu* Wall. ex Lindley. J Plant Res 15(1): 100-105.

Seeja G, Sreekumar S. 2022. Orchid biodiversity and genetics. In: Mérillon JM, Kodja H (eds.). Orchids Phytochemistry, Biology and Horticulture. Reference Series in Phytochemistry. Springer, Cham. https://doi.org/10.1007/978-3-030-38392-3_2.

Sipayung P, Matanari J, Lafau MB, Sulastri YS, Ginting BB, Sihombing DR, Giawa T. 2018. The effect of activated charcoal dose and benzyl amino purine concentration on the growth of orchid plantlets in murashige and skoog media in vitro. IOP Conf Ser Earth Environ Sci 205 (1): 012025. https://doi.org/10.1088/1755-1315/205/1/012025.

Utami S, Hariyanto S. 2020. Organic compounds: Contents and their role in improving seed germination and protocorm development in orchids. Intl J Agron 2020: 2795108. https://doi.org/10.1155/2020/2795108.

Wang Y, Liu L, Song S, Li Y, Shen L, Yu H. 2017. *DOFT* and *DOFTIP1* affect reproductive development in the orchid *Dendrobium* Chao Praya Smile. J Exp Bot 68 (21-22): 5759-5772. https://doi.org/10.1093/jxb/erx400.

Wasiati AR, Nugraheni IA, Setiawati Y. 2021. The combination of Murashige and Skoog (MS) media and activated charcoal on the growth of the *Vanda helvola* orchid plant in vitro. Intl J Health Sci Technol 3 (1): 159-170. https://doi.org/10.31101/ijhst.v3i1.2247.

Yam TW, Arditti J. 2017. Micropropagation of Orchids. John Wiley & Sons. https://doi.org/10.1002/9781119187080.

Yasmint M, Harahap Y. 2025. Effect of indole butyric acid (IBA) and coconut water on the growth of orchid plant shoots (*Cattleya* sp.) in vitro. Quagga: Jurnal Pendidikan dan Biologi 17 (1): 50-56. https://doi.org/10.25134/quagga.v17i1.3601.

Yeung EC. 2017. A perspective on orchid seed and protocorm development. Bot Stud 58 (1): 33. https://doi.org/10.1186/s40529-017-0188-4.

Yeung ECT, Stasolla C. 2024. Protocorm regeneration and Protocorm-Like Bodies (PLBs). In: Yeung ECT, Lee YI (eds.). Orchid propagation: The Biology and Biotechnology of the Protocorm. Springer, New York. https://doi.org/10.1007/978-1-0716-4031-9_4.

Yusnita M. 2015. Kultur Jaringan Tanaman sebagai Teknik Penting Bioteknologi untuk Menunjang Pembangunan Pertanian. Orasi Ilmiah Guru Besar Bidang Bioteknologi Pertanian, Fakultas Pertanian, Universitas Lampung. Aura Publishing, Bandar Lampung. [Indonesian]

Zhao P, Wu F, Feng FS, Wang WJ. 2008. Protocorm-Like Body (PLB) formation and plant regeneration from the callus culture of *Dendrobium candidum* Wall ex Lindl. In Vitro Cell Dev Biol-Plant 44 (3): 178-185. https://doi.org/10.1007/s11627-007-9101-2.