TROPICAL LIFE SCIENCES RESEARCH

Revival and Regeneration of Long-Term Dormant *Phalaenopsis amabilis* (L.) Blume Protocorms Using PLB Techniques: A Cost-Effective Strategy for Orchid Germplasm Conservation

Authors:

Simaa Ramziyah binti Mohd Yazid, Alvera Elka Meyrick, Eyen Khoo and Richard J. Majapun*

***Correspondence:** Richard.Majapun@sabah.gov.my

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Highlights

- Optimal regeneration of dormant PLBs of *Phalaenopsis amabilis* was achieved in ½ MS medium, yielding the highest shoot and root formation and survival rate.
- Supplementation with plant growth regulators (PGRs), organic additives, and activated charcoal did not significantly enhance regeneration.
- Reduced nutrient strength was found to be more favourable for efficient reactivation of dormant PLBs in *Phalaenopsis amabilis*.

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EARLY VIEW

Revival and Regeneration of Long-Term Dormant *Phalaenopsis amabilis* (L.) Blume Protocorms Using PLB Techniques: A Cost-Effective Strategy for Orchid Germplasm Conservation

[2]Simaa Ramziyah binti Mohd Yazid, [2]Alvera Elka Meyrick, [1]Eyen Khoo and [1]Richard J. Majapun*

[1]Forest Research Centre, Sabah Forestry Department, P.O Box 1540, 90715 Sandakan, Sabah, Malaysia
[2]Universiti Malaysia Sabah, (Faculty of Tropical Forestry) Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia

***Corresponding author:** Richard.Majapun@sabah.gov.my

Running head: Reactivating Dormant Orchid Cultures

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Abstract: *Phalaenopsis amabilis*, a highly valued orchid native to tropical Asia, is a key component of the global orchid trade. However, increasing demand and unregulated collection have placed considerable pressure on wild populations. Tissue culture is widely used for conservation and propagation, but long-term *in vitro* cultures often become dormant due to irregular maintenance, leaving valuable germplasm inactive. This study aimed to develop a revival protocol using Protocorm-Like Body (PLB) techniques to regenerate decade-old dormant cultures of *P. amabilis*, and to evaluate this approach as a technically viable and cost-effective conservation strategy for other rare, high-value orchid species as an alternative to seed banking or cryopreservation. Results showed that dormant PLBs cultured on ½ MS medium exhibited the fastest regeneration, with shoot and root formation observed as early as week 4. This medium also achieved the highest regeneration rate (40%) and survival rate (60%), together with an average of 1.45 ± 2.28 shoots and 0.8 ± 1.51 roots per explant. The superior performance of ½ MS suggests that a reduced-nutrient environment supports the

gradual reactivation of metabolic activity, promoting regeneration and survival in dormant cultures. Additionally, MS medium supplemented with 20 g/L coconut powder enhanced protocorm proliferation, producing the highest mean number of secondary PLBs (7.8±10.81). These findings highlight the value of simple, low-nutrient media in reviving long-term dormant orchid cultures and provide a potential protocol for ex situ conservation of *P. amabilis* and related species.

Keywords: *Phalaenopsis amabilis*, Dormant Explants, PLBs, Organic Additives, PGRs

Abstrak: *Phalaenopsis amabilis* iaitu orkid bernilai tinggi yang berasal dari Asia tropika, semakin terancam akibat permintaan tinggi dan pengambilan tanpa kawalan. Kultur tisu digunakan secara meluas untuk pemuliharaan dan pembiakan, namun kultur *in vitro* jangka panjang sering menjadi dorman (tidak aktif) akibat penyelenggaraan yang tidak teratur. Kajian ini bertujuan untuk membangunkan protokol pemulihan menggunakan teknik Protocorm-Like Body (PLB) bagi menjana semula kultur dorman *P. amabilis* yang telah berusia sedekad, serta menilai keberkesanan pendekatan ini sebagai strategi pemuliharaan yang kos efektif untuk spesies orkid jarang dan bernilai tinggi. PLB dorman yang dikultur pada medium ½ MS menunjukkan regenerasi terpantas, dengan pucuk dan akar terbentuk seawal minggu ke-4. Medium tersebut juga mencapai kadar regenerasi tertinggi (40%) dan kelangsungan hidup 60%, bersama purata 1.45 ± 2.28 pucuk dan 0.8 ± 1.51 akar per eksplan. Keberkesanan tertinggi pada medium ½ MS menunjukkan bahawa persekitaran rendah nutrient menyokong pengaktifan semula aktiviti metabolic secara beransur-ansur, sekali gus menggalakkan regenerasi dan kelangsungan hidup kultur dorman. Selain itu, medium MS yang ditambah dengan 20 g/L serbuk kelapa meningkatkan proliferasi protokorm, menghasilkan bilangan purata PLB sekunder tertinggi (7.8±10.81). Keputusan ini mencadangkan penggunaan medium ringkas dan rendah nutrien dalam pengaktifan semula kultur orkid dorman serta menawarkan protokol berpotensi untuk pemuliharaan ex situ *P. amabilis* dan spesies berkaitan.

Kata kunci: *Phalaenopsis amabilis*, Eksplan Dorman, PLB, Bahan Tambahan Organik, PGRs

INTRODUCTION

Phalaenopsis orchids have been dominating the global orchid market up until the present time, accounting for approximately 75 percent of orchids sold, with over 300 million young plants produced annually in countries such as Taiwan, Germany, Japan, and the United States, where Taiwan leads in global production (Yuan *et al.* 2015). Among these, *Phalaenopsis*

amabilis, commonly known as the Moon Orchid, is a highly valued species native to tropical Asia, including Borneo, and is widely cultivated for its elegant, long-lasting flowers (Griesbach 1985; Suputri *et al.* 2024). This rapid expansion of the *Phalaenopsis* industry, driven by strong international demand, has intensified commercial pressure on the species and raised concerns over overexploitation and unregulated trade, despite its inclusion in CITES Appendix II (Ningrum *et al. 2017*; Khatun *et al.* 2020). These developments resemble the illegal trade in other rare plant species such as *Nepenthes*, where public demand drives black market activity, further facilitated by tissue culture technology. Although initially introduced to address propagation challenges such as slow growth and intended to support conservation efforts, tissue culture has also become a tool that may inadvertently enable large-scale, unregulated commercial exploitation (Soetopo & Purnamaningsih 2012; Mondal & Banerjee 2017; Khatun *et al.* 2020). This dual role highlights the complex intersection between biotechnology and conservation, underscoring the need for effective regulation and monitoring.

Under the Ninth Malaysia Plan (2009–2010), the Sabah Forestry Department initiated a project to explore the ornamental potential of native plant species such as *Nepenthes, Begonia*, and orchid such as *P. amabilis*, focusing on propagation through both conventional and tissue culture methods (Majapun *et al.* 2011). After the completion of the project in 2013, some plantlets were distributed for ex-situ conservation and awareness programs, while others were preserved in tissue culture chambers at Forest Research Centre (FRC) for long-term germplasm storage. Over time, many *P. amabilis* cultures entered a dormant protocorm-like state, and some were lost due to irregular subculturing and poor growth conditions, leaving much of the germplasm stagnant for over a decade. To address this challenge, a revival initiative was initiated in early 2025 to explore the use of Protocorm-Like Body (PLB) techniques to regenerate the long-aged dormant cultures.

Protocorm-Like Bodies (PLBs) are recognized as a reliable method for propagating endangered orchid species due to their ability to regenerate directly into plantlets or form secondary PLBs for large-scale propagation (Jose & Babu 2018; Park 2021). This technique has been successfully applied to several orchid species, including *Dendrobium lowii*, *Cymbidium aloifolium,* and *Vanilla planifolia* (Gansau *et al.* 2016; Regmi *et al.* 2017; Jose & Babu 2018). In micropropagation, orchids are typically regenerated from protocorms with specialized structures that can develop shoot meristems and form symbiotic associations with fungi, making them ideal for conservation through tissue culture (Yeung 2017). Although propagation protocols for *P. amabilis* using fresh protocorms are well established (Khatun *et al.* 2020), there are currently no known strategies address the regeneration of aged, dormant cultures. These long-term *in vitro* cultures pose physiological challenges due to desiccation and nutrient depletion, requiring novel revival approaches.

Traditional ex situ conservation methods, such as cryopreservation and gene banking, are effective but often require high-cost infrastructure and specialized maintenance, making them less suitable for species that propagate vegetatively or produce recalcitrant seeds (Engelmann 2010; Reed *et al.* 2021). In contrast, the maintenance of dormant protocorms through tissue culture presents a cost-effective and accessible alternative, enabling the preservation of viable, regenerable tissues under low-input conditions (Yeung 2017; Pavlova 2025). This approach underscores the value of PLB-based revival systems for the long-term germplasm conservation of rare and economically important orchid species.

Therefore, the aim of this study is to develop a revival protocol using Protocorm-Like Body (PLB) techniques to regenerate decade-old dormant cultures of *P. amabilis*, and to evaluate this approach as a technically viable and cost-effective conservation strategy for rare, high-value orchid species that are unsuitable for seed banking or cryopreservation. This study also seeks to offer new insights into orchid conservation biotechnology by demonstrating the potential of dormant *in vitro* cultures as a living, long-term germplasm storage system. Specifically, it addresses two key questions: (1) Can aged, dormant *P. amabilis* protocorms maintained *in vitro* for over ten years be successfully revived using PLB techniques? and (2) What culture conditions best promote PLB induction and plantlet regeneration in these aged tissues?

MATERIALS AND METHODS

Explant Selection

This study was conducted over a period of 12 weeks from April to July 2025 at the Tissue Culture Laboratory under the Biotechnology Section in Forest Research Centre (FRC), Sandakan, Sabah. The explants used were *P. amabilis* protocorm-like bodies (PLBs), originally obtained from a local nursery in Sandakan under a project initiated during the Ninth Malaysia Plan (2009–2010). These PLBs had undergone repetitive subculturing and were maintained *in vitro* for over 10 years, resulting in a dormant and stagnant state. All explant selections and handling were performed under aseptic conditions inside a laminar airflow cabinet to minimize the risk of contamination. The selected PLBs were approximately 2-3 mm in height and morphologically uniform at the time of culture initiation.

Experimental Design and Data Collection

The experimental protocol was adapted from Heira *et al.* (2023) with modifications to suit the revival of long-aged *P. amabilis* protocorm-like bodies (PLBs). A total of 12 treatment

combinations were tested, as outlined in Table 1. The experiment followed a Completely Randomized Design (CRD), with each treatment replicated five times. Each replicate consisted of four PLBs, resulting in 20 explants per treatment (Figure 1). After 12 weeks of incubation, data were collected on several growth parameters, including the number of secondary PLBs, shoots, and roots formed per explant. Additionally, regeneration and survival rates were recorded to assess the effectiveness of each treatment in reviving the aged PLBs.

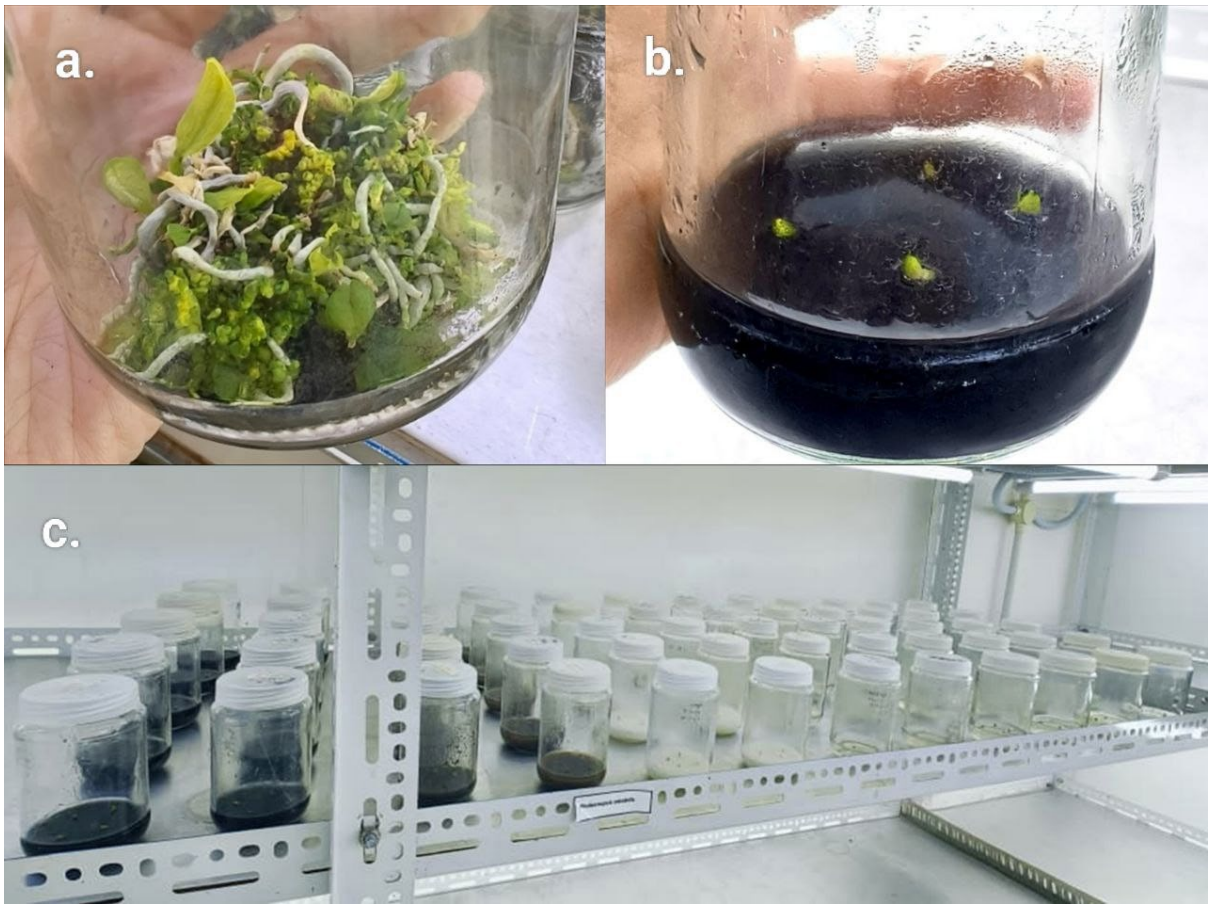

Figure 1: a. Long-stored *P. amabilis* in culture chamber in stressed condition., b. Cultured protocorms in glass jar for one replicate., c. All 12 media treatments containing cultured protocorms arranged in the growth room for observation

Media Preparation and Experimental Setup

The culture media for all treatments were prepared by thoroughly mixing essential components specific to each formulation. These included Murashige and Skoog (MS) powder (Duchefa Biochemie, The Netherlands), Orchimax powder (Duchefa Biochemie, The Netherlands), activated charcoal, organic additives such as banana powder and coconut powder (Phyto Technology Laboratories, United States), and plant growth regulators (PGRs), namely

naphthalene acetic acid (NAA) (Phyto Technology Laboratories, United States) and benzylaminopurine (BAP) (Duchefa Biochemie, The Netherlands). The pH of each medium was adjusted to 5.6 ± 0.2 to optimize nutrient availability and support plant tissue growth. Phytogel was added as the gelling agent, and the total volume was brought to one litre using distilled water. The mixture was heated in a microwave for approximately 10 minutes to fully dissolve the agar and other constituents. Following this, 50 mL of the prepared medium was dispensed into sterile glass jars, which were then autoclaved at 121°C for 20 minutes to ensure sterility. The cultured explants were incubated under controlled environmental conditions at 25 ± 2°C with a 16-hour light and 8-hour dark photoperiod. Illumination was provided by cool white, fluorescent lamps. A completely randomized design (CRD) was applied in distributing treatments, ensuring that each explant had an equal probability of receiving any of the 12 treatment combinations.

Table 1: List and composition of treatments utilized in this study

Treatment	PGR		Organic additive		Activated charcoal (g/L)
	NAA (mg/L)	BAP (mg/L)	Banana powder (g/L)	Coconut powder (g/L)	
Murashige & Skoog (MS)	-	-	-	-	-
½ Murashige & Skoog (MS)	-	-	-	-	-
Orchimax	-	-	-	-	specific amounts are unspecified
½ Orchimax	-	-	-	-	specific amounts are unspecified
Murashige & Skoog (MS)	-	-	40	-	0.7
½ Murashige & Skoog (MS)	-	-	40	-	0.7
Murashige & Skoog (MS)	-	-	-	20	-
½ Murashige & Skoog (MS)	-	-	-	20	-
Murashige & Skoog (MS)	0.5	-	-	-	-
½ Murashige & Skoog (MS)	0.5	-	-	-	-
Murashige & Skoog (MS)	-	2.0	-	-	-
½ Murashige & Skoog (MS)	-	2.0	-	-	-

Data analysis

The data were analyzed using one-way Analysis of Variance (ANOVA) to determine significant differences among treatments. Statistical analysis was performed using SPSS software (version 29.0; IBM Corporation, Armonk, New York), and results were expressed as mean ±

standard deviation (SD). Post-hoc test using Duncan's Multiple Range Test (DMRT) was applied at a 5% significance level to separate the means of secondary PLB formation, shoot, and root development.

RESULTS

Significant variation was observed among the 12 treatment combinations in their ability to revive long-aged *P. amabilis* protocorms and support subsequent plantlet development. After 12 weeks of *in vitro* culture, data on secondary PLB formation, shoot and root induction, regeneration rate, and survival rate were collected and analysed (Table 2). Graphs illustrating each parameter are shown in Figure 2, Figure 3, Figure 4, and Figure 5, respectively. Meanwhile, Appendix 1 illustrates the morphological observation of explants across 12 culture treatments.

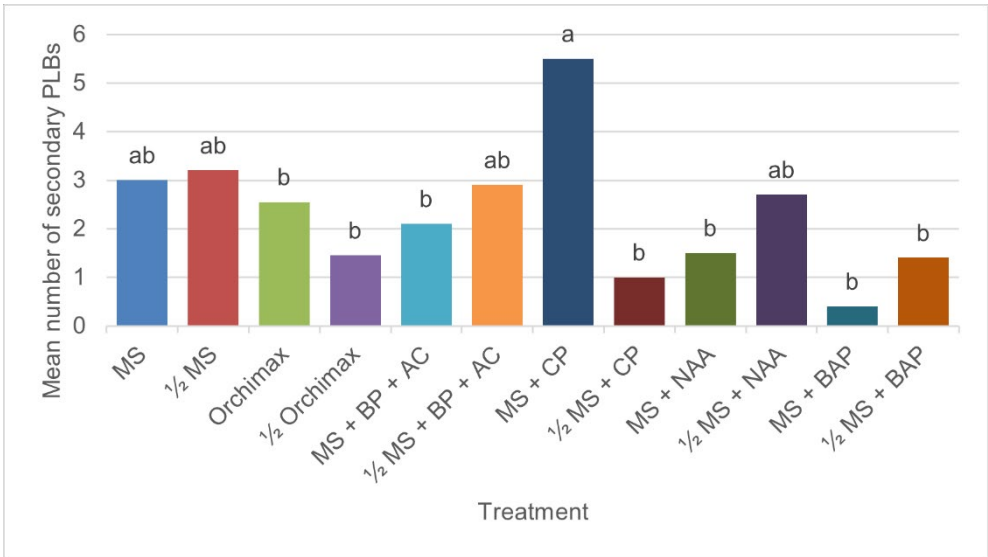

Figure 2: The effect of different treatments on the mean number of secondary PLBs of *P. amabilis* orchid. Common letters within the column indicate a non-significant difference between means using Duncan's Multiple Range Test ($p \leq 0.05$).

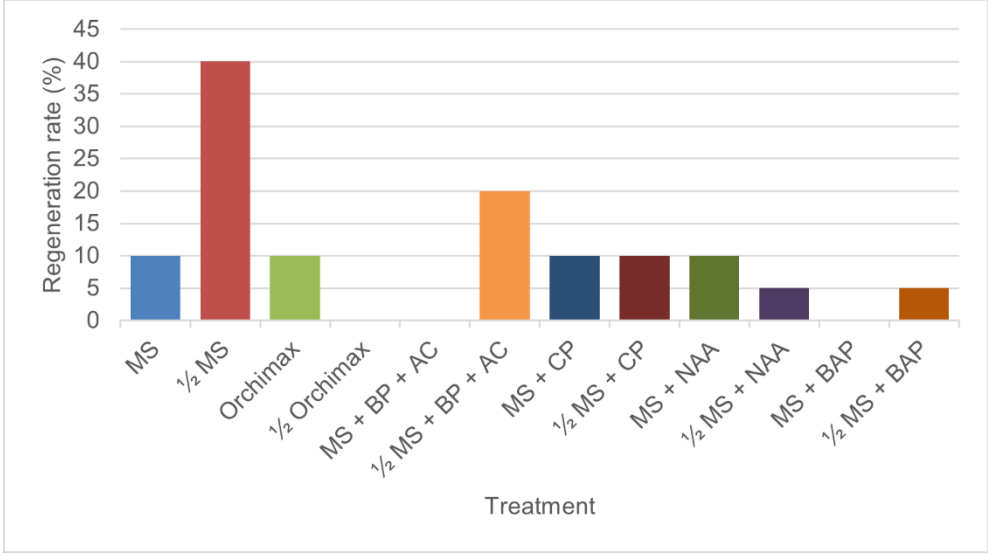

Figure 3: The effect of different treatments on the regeneration rate of *P. amabilis* orchid

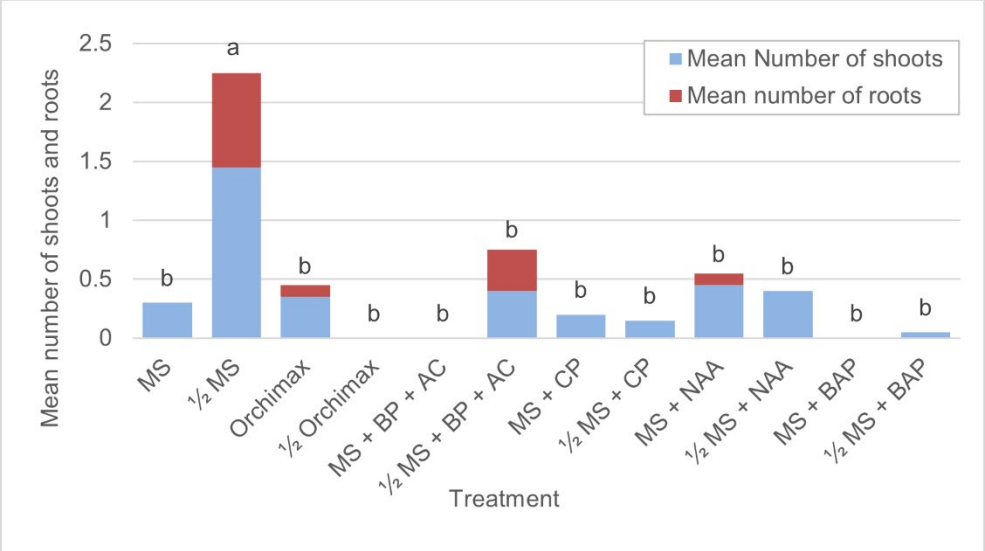

Figure 4: The effect of different treatments on the mean number of shoots and roots of *P. amabilis* orchid. Common letters within the column indicate a non-significant difference between means using Duncan's Multiple Range Test ($p \leq 0.05$)

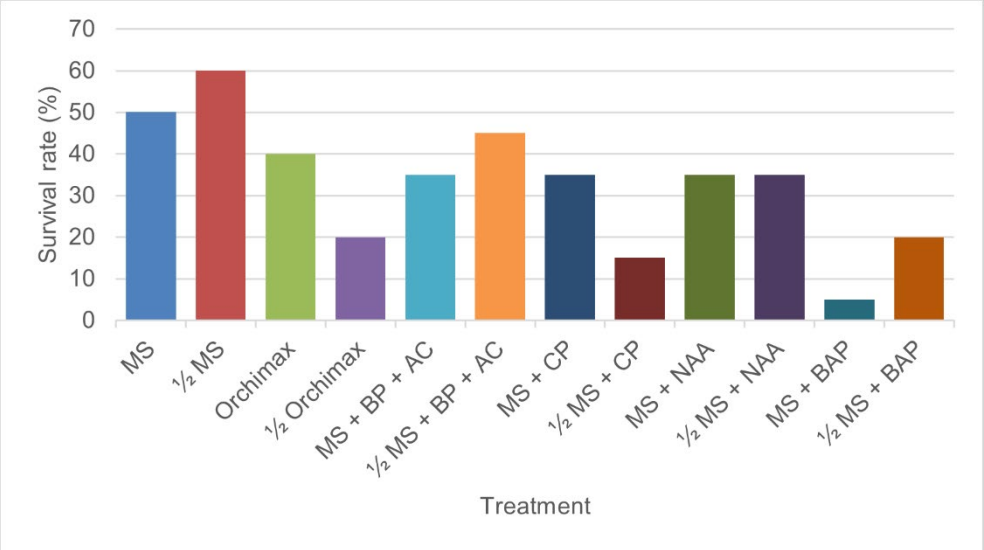

Figure 5: The effect of different treatments on the survival rate of *P. amabilis* orchid.

One-way analysis of variance (ANOVA) revealed significant differences among treatments in the mean number of secondary PLBs formed (F = 1.975, p = 0.032), with MS supplemented with coconut powder produced the highest number of secondary PLBs (7.8±10.81), as determined by Duncan's Multiple Range Test (DMRT). However, this treatment showed limited shoot and root development after 12 weeks. Morphological observations (Appendix 1) showed enlarged PLBs without complete plantlet formation.

Half-strength MS (½ MS) recorded the highest regeneration rate (40%) and survival rate (60%), as well as significantly greater number of shoots (1.45 ± 2.28) and roots (0.8 ± 1.51). ANOVA indicated significant differences among treatments for shoot formation (F = 2.687, p = 0.03) and root formation (F = 4.000, p < 0.001), with DMRT identifying ½ MS as significantly different from most other treatments. Visible shoot and root development was observed as early as week 4 (Appendix 1.). Moderate responses were recorded in ½ MS + banana powder + activated charcoal and MS + NAA treatments, which showed early organogenesis but lower regeneration and survival rates compared to ½ MS.

Other treatments including MS, Orchimax, ½ MS + Banana powder + Activated charcoal, ½ MS + Coconut powder, MS + NAA, ½ MS + NAA, and ½ MS + BAP exhibited limited development (Appendix 1). Both ½ Orchimax, and MS + Banana powder + Activated charcoal recorded low survival rates and limited regeneration (Appendix 1). Among all treatments, MS + BAP recorded the lowest response, with a mean of 0.4 ± 1.79 secondary PLBs, no complete plantlet formation, and the lowest survival rate (5%) after 12 weeks.

Table 2: Effects of different treatments on physiological parameters of *P. amabilis* protocorms after 12 weeks of *in vitro* culture

Treatment	Mean number of secondary PLBs (Mean±SD)	Regeneration rate (%)	Mean number of shoots (Mean±SD)	Mean number of roots (Mean±SD)	Survival rate (%)
MS	3.95±6.53^{ab}	10	0.3±0.92^{b}	0±0.0^{b}	50
½ MS	3.8±6.2^{ab}	40	1.45±2.28^{a}	0.8±1.51^{a}	60
Orchimax	2.55±4.92^{b}	10	0.35±1.09^{b}	0.1±0.45^{b}	40
½ Orchimax	1.45±3.19^{b}	0	0±0.0^{b}	0±0.0^{b}	20
MS + BP + AC	2.1±3.71^{b}	0	0±0.0^{b}	0±0.0^{b}	35
½ MS + BP + AC	2.9±4.06^{ab}	20	0.4±0.88^{b}	0.35±0.88^{b}	45
MS + CP	7.8±10.81^{a}	10	0.2±0.62^{b}	0±0.0^{b}	35
½ MS + CP	2.05±7.02^{b}	10	0.15±0.49^{b}	0±0.0^{b}	15
MS + NAA	1.5±2.89^{b}	10	0.45±1.47^{b}	0.1±0.45^{b}	35
½ MS + NAA	4.4±8.29^{ab}	5	0.4±1.79^{b}	0±0.0^{b}	35
MS + BAP	0.4±1.79^{b}	0	0±0.0^{b}	0±0.0^{b}	5
½ MS + BAP	2.65±7.04^{b}	5	0.05±0.22^{b}	0±0.0^{b}	20

Notes. Values are presented as mean ± standard deviation (SD). Means followed by different letters within each column indicate significant differences at $p \leq 0.05$ according to Duncan's multiple range test. MS = Murashige & Skoog, BP = Banana powder, CP = Coconut powder, AC = Activated charcoal

DISCUSSION

Half-strength MS (½ MS) medium demonstrated superior performance across all evaluated parameters, including the highest regeneration and survival rates, as well as the greatest mean number of shoots and roots formed (Table 2). This outcome is notable given that other treatments contained additional supplements such as plant growth regulators (NAA and BAP), organic additives (coconut and banana powder), and activated charcoal. Although enriched media are generally effective in orchid micropropagation (Heira *et al.* 2023; Garg *et al.* 2024), they were less suitable for long-stored, dormant *P. amabilis* PLBs in the present study.

The differential response observed among treatments may be strongly associated with the physiological state of the PLBs. PLBs maintained under prolonged *in vitro* conditions often exhibit reduced protein content and altered metabolic pathway, affecting their overall health and resilience (Wang *et al.* 2003). Under such conditions, exposure to complex or hormone-rich formulations may impose additional physiological stress (Cardoso *et al*. 2020). Nevertheless, MS supplemented with coconut powder produced the highest mean number of secondary PLBs, indicating that enhanced proliferation did not necessarily correspond to improved plantlet regeneration.

Based on the result, the highest regeneration rate, together with the mean number of shoots and roots formed of the *P. amabilis* cultured in ½ MS can be attributed to the optimal balance of nutrients and growth conditions inherent in this medium. As for the nutrient composition, the ½ MS medium contains reduced concentrations of salts and nutrients, which can prevent toxicity and promote better growth in sensitive orchid species (Maharjan *et al.* 2019) when compared to the other treatments. In terms of growth conditions, lower concentrations of growth regulators in ½ MS can minimize stress on explants, allowing for more natural growth responses (Yuniati & Isda 2024). Similar findings have been reported in other orchids, where ½ MS improved PLB development and vegetative growth in *Dendrobium* (Akter *et al.* 2007) and enhanced plantlet performance in *P. amabilis* (Sinha *et al.* 1970).

MS supplemented with coconut powder significantly increased secondary PLB formation in *P. amabilis* (Table 2), consistent with reports of enhanced protocorm or PLB proliferation in *Phalaenopsis* spp. (Santoso *et al.* 2020), *Dendrobium* spp. (Nambiar *et al.* 2012), and *Caladenia latifolia* (Bustam *et al*. 2014). The effectiveness of the coconut water or powder is attributed to its content of carbohydrates, vitamins, minerals, and plant growth regulators (Utami & Hariyanto 2020). The presence of cytokinins in coconut water or powder contributes to its growth-promoting effects, with trans-zeatin riboside identified as the major cytokinin compound (Lazim *et al.* 2015; Utami & Hariyanto 2020). Cytokinins stimulate cell division, playing a vital role in inducing PLB formation, which serves as a precursor for further plant development (Habiba *et al.* 2014). However, cytokinin-dominant environments often

favour undifferentiated proliferation over organised organogenesis (Ikeuchi *et al.* 2016). This aligns with the present findings, where MS + coconut powder increased secondary PLB formation but did not improve complete plantlet regeneration relative to ½ MS.

Apart from that, the responses to media supplemented with activated charcoal varied across treatments. Full-strength Orchimax with activated charcoal showed slightly better regeneration, whereas its half-strength form resulted in slower, poorer growth. Similarly, MS with banana powder and activated charcoal performed like half-strength Orchimax, while half-strength MS with the same additives produced responses closer to full-strength Orchimax. These differences indicate that the influence of activated charcoal depends on the nutrient composition of the medium. Previous studies reported that Orchimax media, and combinations of activated charcoal and banana powder can enhance orchid growth (Yahya *et al.* 2024; Ningrum *et al.* 2017; Djajanegara 2010), and increased charcoal levels (0.20%) have been associated with higher protocorm proliferation in *Grammatophyllum speciosum* (Rahman *et al.* 2021). However, in the present study, all activated charcoal treatments produced generally limited and slow responses compared with other formulations. This may be related to charcoal's adsorption capacity: while it can reduce phenolic oxidation and remove inhibitory compounds, it may also bind vitamins, hormones, and signalling molecules (Pan & Staden 1998), potentially reducing the availability of factors required to initiate morphogenesis in dormant tissues. This outcome may be linked to the presence of activated charcoal, which, while effective at reducing phenolic oxidation and removing inhibitory compounds in active cultures, also adsorbs vitamins, hormones, and signalling molecules (Pan & Staden 1998), thereby limiting the availability of growth regulators needed to break dormancy. Limited growth observed in these complex media containing activated charcoal may also reflect the low metabolic activity of the long-term maintained PLBs, which can restrict reactivation and subsequent development under more complex media conditions (Cardoso *et al.* 2020).

In contrast, MS + BAP consistently produced the lowest in all parameters. This poor performance observed in this treatment suggests that a high concentration of BAP may have inhibited regeneration in long aged protocorms. Although cytokinins such as BAP are commonly used to enhance somatic embryogenesis and shoot regeneration, excessive concentrations can lead to hyperhydricity, reduced root formation, and necrosis in some species (Mirabbasi & Hosseinpour 2014; Karend *et al.* 2025). Moreover, different plant species have different nutrient composition, resulting the effectiveness being highly concentration dependent and species specific (Bhowmik & Rahman 2017; Cardoso *et al.* 2020). Similar findings have been reported in orchid tissue cultures, where elevated cytokinin levels beyond the optimal range reduced PLB induction and survival due to tissue sensitivity (Chen & Chang 2004). In addition, prolonged subculturing has been linked to decreased endogenous cytokinin activity and altered auxin-cytokinin ratios, which affect the growth capacity (Gaspar *et al.* 2000;

Maylin *et al.* 2014). Under such conditions, media enriched with exogenous plant growth regulators may only cause stress to the explants. The consistently poor performance of MS+BAP suggests that long-term aged PLBs are particularly sensitive to hormonal imbalance, which requires minimal or no exogenous PGR supplementation during the reactivation phase.

The ability of dormant protocorm-like bodies (PLBs) of *P. amabilis* to regenerate into complete plantlets within a short culture (4 weeks) period represents a notable advancement in orchid micropropagation. In the present study, shoot and root development were observed as early as the fourth week of culture, particularly in ½ MS medium, which also produced the highest regeneration rate (40%) and survival rate (60%). This rapid reactivation contrasts with many previous reports in which shoot and root regeneration typically required 8–12 weeks, while several studies focused primarily on PLB induction rather than complete plantlet development (Chen & Chang 2006; Hidayah *et al.* 2023). The superior performance of ½ MS medium in this study suggests that a reduced-nutrient environment may favor metabolic reactivation and shoot initiation in dormant PLBs. Additionally, enhanced secondary PLB proliferation observed in MS supplemented with 20 g/L coconut powder further reinforces the protocol's potential in conservation and commercial propagation.

Limitations of the Study

A primary limitation of this study was the high level of microbial contamination observed during the initial culture phase, particularly within the first week of establishment. Contamination appeared to originate from the explant surface, indicating that the current sterilization protocol may require further optimization. The use of broad-spectrum antibiotics such as penicillin G (benzylpenicillin) or antimicrobial agents like Plant Preservative Mixture™ (PPM), which contains isothiazolone compounds, has been reported to effectively reduce microbial contamination without severely affecting plant tissue viability (Niedz 1998; Leifert & Cassells 2001). Incorporating such treatments during culture initiation may improve explant survival and establishment rates.

The relatively short experimental duration of the study (12 weeks) also limits assessment of long-term regeneration performance. Although early plantlet formation was observed, extended monitoring is necessary to evaluate acclimatization success and ex vitro survival. Acclimatization is a gradual process that often requires extended monitoring to ensure proper physiological adaptation, including root system development, stomatal regulation, and resistance to environmental stressors (Preece & Sutter 1991; Hazarika 2006). Future studies should therefore extend evaluation beyond in vitro regeneration to nursery establishment to fully assess plantlet viability.

Additionally, the prolonged maintenance of explants under suboptimal in vitro conditions may increase the risk of somaclonal variation. Long-term subculturing has been associated with genetic and epigenetic instability (Larkin & Scowcroft 1981). To ensure the genetic fidelity of regenerated plantlets, the use of molecular markers such as RAPD, SSR, or AFLP is recommended for routine screening of clonal integrity (Rani *et al.* 1995; Bairu *et al.* 2011). Incorporating molecular diagnostics would not only strengthen the validation of regeneration protocols but also support future conservation and commercialization efforts by ensuring true-to-type plant production.

CONCLUSION

This study shows that decade-old dormant *P. amabilis* protocorm-like bodies (PLBs) can be successfully reactivated in vitro, with half-strength MS (½ MS) medium performing best by producing the highest regeneration (40%) and survival (60%) along with greater shoot and root formation, and plantlet development observed as early as week 4. Overall, the findings support the use of simple, low-nutrient media as a practical and cost-effective approach for reviving long-term dormant orchid germplasm, offering a feasible ex situ conservation alternative for *P. amabilis* and potentially other rare, high-value orchids. Future work should focus on optimizing plant growth regulator concentrations and refining culture conditions to enhance the regeneration efficiency of long stored protocorms.

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CONFLICT OF INTEREST

The authors declare no conflict of interest relevant to this research.

AUTHORS' DECLARATION

The authors declare that the research presented is original and contributes to the advancement of the field. All sources used in this study have been properly acknowledged, and the data and materials are the property of the Sabah Forestry Department.

AUTHORS' CONTRIBUTIONS

Simaa Ramziyah binti Mohd Yazid: Study design, research supervision, laboratory work, data collection, statistical analysis, manuscript writing and editing.
Alvera Elka Meyrick: Laboratory work and data collection.
Eyen Khoo: Field support and manuscript review.
Richard J. Majapun: Conceptualisation, study design, statistical analysis, research supervision, field support, manuscript review and co-editing.

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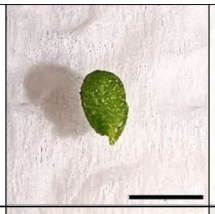
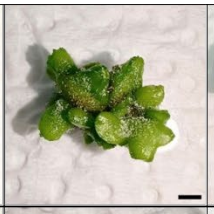
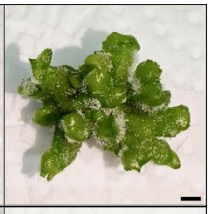
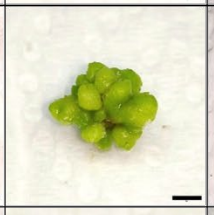
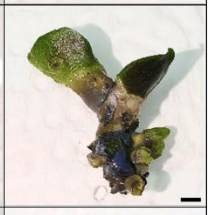
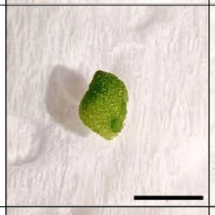
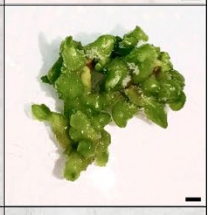
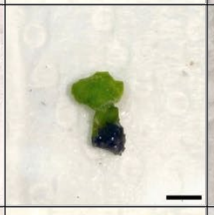
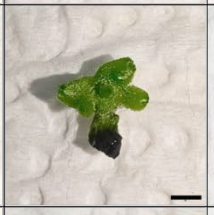
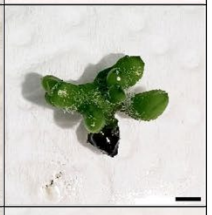
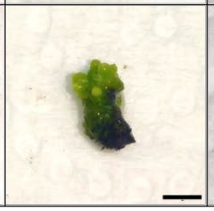
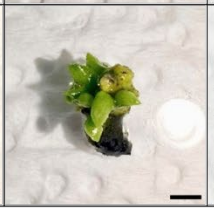
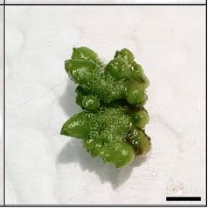
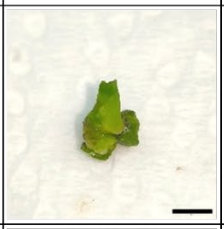
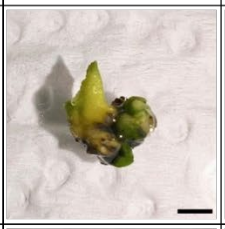
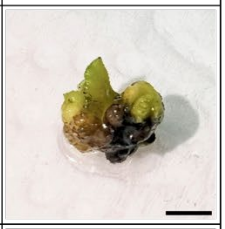
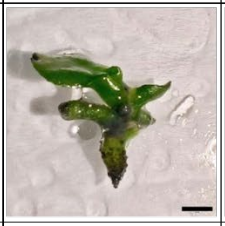
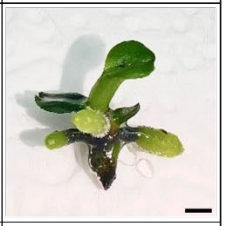
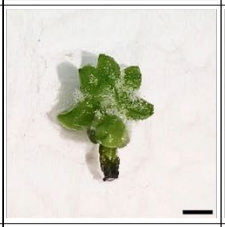
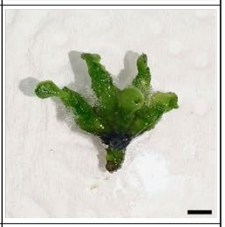
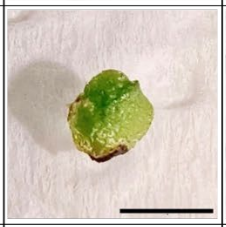
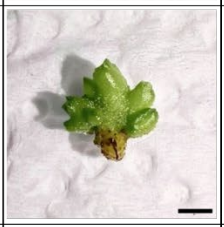
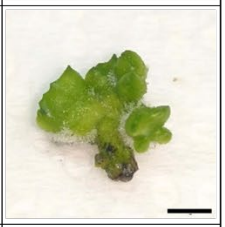
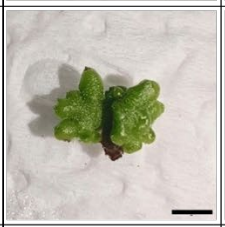
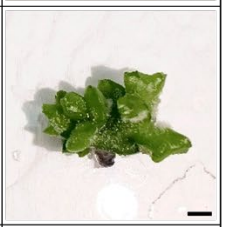
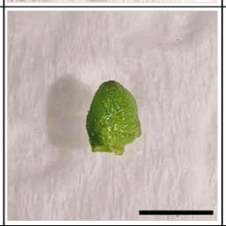
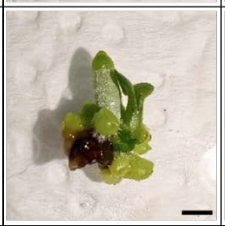
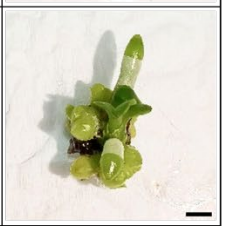
APPENDIX

Appendix 1: Morphological changes in *P. amabilis* protocorms under different treatments during 12 weeks of *in vitro* culture. Scale = 3mm.

MS + Coconut powder				
½ MS + Coconut powder				
MS + NAA				
½ MS + NAA				
MS + BAP				
½ MS + BAP				

Treatment	Weeks			
	0	4	8	12
MS				
½ MS				
Orchimax				
½ Orchimax				
MS + Banana powder + Activated charcoal				
½ MS + Banana powder + Activated charcoal				
MS + Coconut powder