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Research Article
Effect of Natural Additives Modification Medium on *in vitro* Culture of *Rhynchostylis gigantea* (Lindl.) Ridl. by Tissue Culture Technique

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Abstract

Background and Objective: *Rhynchostylis gigantea* (Lindl.) Ridl. stands out as the most popular and economically significant orchid species. Nevertheless, growing this species in natural environments is challenging due to its slow growth rate and specific ecological conditions. This study elucidated the effects of natural additives on the *in vitro* culture of *Rhynchostylis gigantea*. **Materials and Methods:** The experiments were conducted using 1/2 MS medium supplemented with various natural additives, including glucose, banana pulp, potato homogenate and coconut water, under optimal conditions at a temperature of 30°C with photoperiodic lighting (16 hrs of light: 8 hrs of dark) using fluorescent light. The 5 combinations, namely M1, M2, M3, M4 and M-Control, were considered to assess the effect of natural additives on modifying the medium in the *in vitro* culture of *Rhynchostylis gigantea* using tissue culture techniques. **Results:** The M2 exhibited the shortest duration of early protocorm formation at 22 days. The findings indicated that adding glucose and potato homogenate to the 1/2 MS medium significantly enhanced the growth and development of *Rhynchostylis gigantea*. The M2 medium containing 20 g/L of glucose and 50 g/L of potato homogenate showed the highest results, with 26 PLBs, three leaves and fully developed roots in all individual treatments at 42 days. The results of PLBs, leaves and roots on the natural additives modification mediums were found to have a statistically significant difference at a p-value of 0.05. These results suggest that glucose and potato homogenate are effective natural additives that can improve the *in vitro* culture of *Rhynchostylis gigantea*. **Conclusion:** Hence, the natural additives modification medium had the potential to be applied as *Rhynchostylis gigantea* cultured media to replace the synthetic chemical media. This discovery holds potential applications for commercially propagating this valuable orchid species.

Key words: *In vitro* culture, natural additives modification medium, *Rhynchostylis gigantea* (Lindl.) Ridl., tissue culture technique

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rhynchostylis gigantea (Lindl.) Ridl. is widely attractive among wild orchids for its exceptionally beautiful flowers[1]. This orchid species is an epiphytic that is native to Southeast Asia. It is known for its large, showy flowers ranging from white to pink to purple[2]. The cultivation of *R. gigantea* significantly impacts the economy and it is one of the most popular orchid species[3]. The World Checklist of Selected Plant Families in Cambodia records 311 native orchid species, estimating 500 species. These orchids have diverse biogeographical zones, including the Cardamom Mountains range, dry forest savannah and the Mekong Confluence. The initial checklist of Cambodia's wild orchids, listing 205 species, features prominent genera such as *Dendrobium*, *Bulbophyllum*, *Eria*, *Cleisostoma* and *Coelogyne*. Despite this richness, limited botanical research has resulted in limited information on Cambodia's wild orchids[4]. According to the previous study, *R. gigantea* is an orchid used in traditional medicine for centuries to treat various ailments, including fever, diarrhea and skin infections[5,6]. In addition, the cultivation of *R. gigantea* has potential benefits for biodiversity conservation through habitat preservation and community engagement[7]. Cultivating *R. gigantea* can help protect wild populations, provide habitat for other species, raise conservation awareness and improve rural communities' livelihoods[8]. However, *R. gigantea* has found it difficult to grow in natural environments due to its slow growth rate and its natural environment challenges specific ecological conditions[1].

Furthermore, the propagation of *R. gigantea* has been challenged by the inherently slow growth rate of the plant, making conventional methods impractical. Various approaches to growing orchids were introduced; nonetheless, the challenges of growing achievement were questioned[9]. Among the previous practices, applying *in vitro* techniques is essential in plant tissue culture in driving progress in agriculture, horticulture and biodiversity conservation for a sustainable approach to plant resource utilization[10]. *In vitro* culture techniques have emerged as a valuable alternative technology for propagating *R. gigantea*. In addition, utilizing natural additives to the culture medium in the *in vitro* cultivation of *R. gigantea* through tissue culture techniques is essential for scientific investigation[11,12]. This practice addresses nutrient deficiencies, replicates the *R. gigantea*'s native environmental conditions and induces growth-promoting effects crucial for optimal development.

In the modification medium for the cultivation of *R. gigantea*, several natural additives play crucial roles in providing essential nutrients for optimal growth and development. The presence of glucose is a primary energy source, supporting vital cellular processes[13]. Also, banana pulp contributes a complex mix of sugars, vitamins and minerals, performing as a valuable carbon source and providing additional nutrients[14]. Likewise, potato homogenate, rich in carbohydrates, proteins and minerals, adds nutritional complexity to the medium, supporting the organism's metabolic requirements[15]. Furthermore, coconut water, recognized for its vitamins, minerals and sugars, hydrates the organism and supplies essential nutrients conducive to growth[16]. These additives can enhance nutrient availability, promote growth, improve stress tolerance and reduce disease susceptibility, contributing to the overall health and productivity of *R. gigantea*[17]. The combined effect of these components aims to create a nutrient-rich environment modified to observe the specific nutritional needs of *R. gigantea* during cultivation. Therefore, this research aimed to illustrate the interactive effects of different natural additives modification mediums on PLBs proliferation and plantlet growth of *R. gigantea*. In addition, the study was carried out to develop a minimum essential and economical culture media for *R. gigantea* micropropagation. Moreover, the contamination analysis, early protocorm formed, protocorm-like bodies (PLBs) and leaf and root analysis are reported. This study also enhances the propagation of *R. gigantea* and contributes to alternative solutions for impractical regular methods.

MATERIALS AND METHODS

Study area: The experimental work of this study was carried out in the Tissue Culture Laboratory of Vitrosys Clone Technology Co., Ltd., Prek Leap National Institute of Agriculture and Department of Bioengineering, Faculty of Engineering, Royal University of Phnom Penh, Phnom Penh, Cambodia from February to July, 2018.

Plant materials and culture media: The 4-month-old intact capsules of *R. gigantea* were purchased from the local people in different districts of Ratanakiri Province (Fig. 1). The capsules were placed in an icebox for storage and transferred to the laboratory refrigerator after a day. The 1/2 MS (Murashige and Skoog) is a plant tissue culture medium that contains half of the concentration of macroelements as the original MS medium (Sigma-Aldrich,

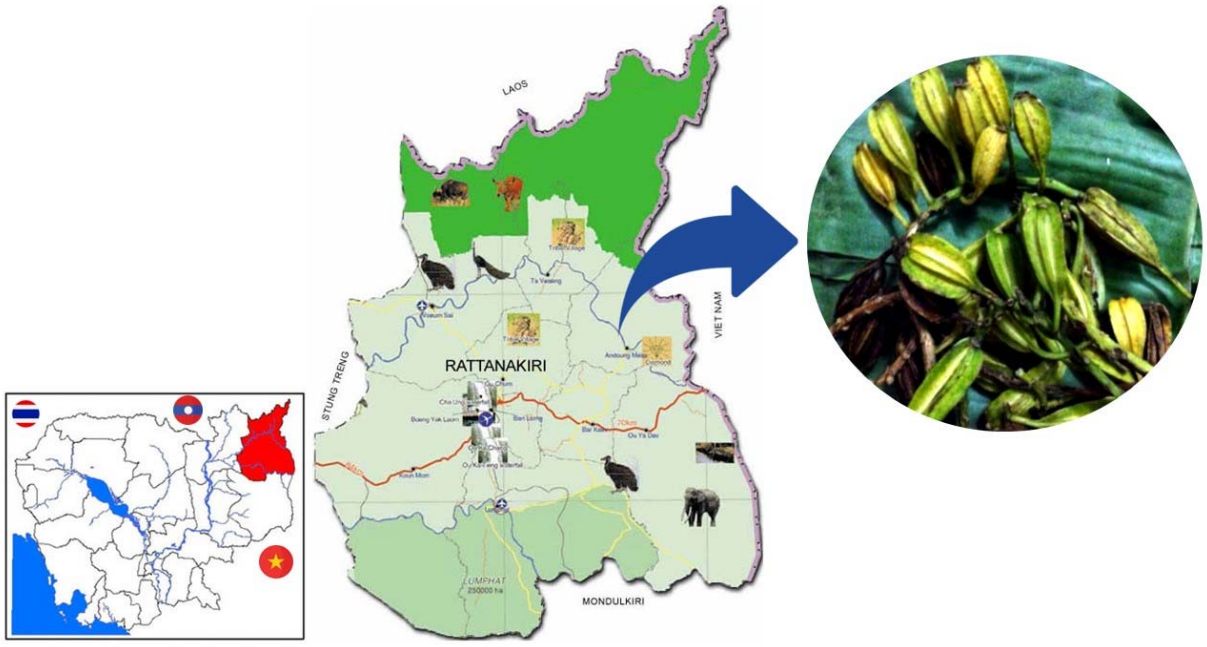

Fig. 1: Map of Ratanakiri Province and capsules of *Rhynchostylis gigantea*, map was adapted from Cambodia Community Day and ODC Cambodia.

Table 1: Composition of natural additives modification medium (g/L)

Code of medium	Natural additives modification medium (per litre)
M1	1/2MS+20 g (glucose)+50 g (banana pulp)+6 g (agar)
M2	1/2MS+20 g (glucose)+50 g (potato homogenate)+6 g (agar)
M3	1/2MS+20 g (glucose)+150 mL (coconut water)+6 g (agar)
M4	1/2MS+20 g (glucose)+50 g (banana pulp)+50 g (potato homogenate)+150 mL (coconut water)+6 g (agar)
M-Control	1/2MS+20 g (glucose)+6 g (agar)

United States of America), was used in this study. To study the effect of natural additives modification medium on *in vitro* culture, the 1/2 MS was supplemented with 20 g/L of glucose, 150 ml/L of coconut water (*Cocos nucifera*), 50 g/L of potato homogenate (*Solanum tuberosum*) and 50 g/L of banana pulp (*Musa acuminata*× *Balbisiana colla* cv. Awak).

The medium modification was prepared in a 30 mL culture bottle with a 10 mL medium. The pH of medium modification was adjusted to 5.8 before sterilization by autoclaving for 30 min at 121°C. The different natural additives modification mediums (M1, M2, M3 and M4) on the *in vitro* culture of *R. gigantea* were investigated for protocorm-like bodies (PLBs) and plantlet development at specific intervals, along with a control medium (M-Control) without natural additives supplementation (Table 1). All chemicals used for experimentation were of analytical grade.

Seed isolation for *in vitro* culture: Seed isolation for *in vitro* culture removes seeds from their fruit and prepares them for sterile culture. In the first experimental series, the capsule surfaces were disinfected by rinsing with running tap water, washing with liquid detergent for 5 min and subsequently rinsing with tap water. Following this, the capsules underwent sterilization by soaking in 70% ethanol for 30 sec, followed by three rinses with distilled water before immersion in 2% Sodium Hypochlorite (NaClO) for 20 min. After cleaning with sterile distilled water, the capsules were briefly dipped in 95% ethanol and flamed for a few sec for the final surface disinfection. Subsequently, the seeds were isolated from the capsules and transferred onto various mediums modified with natural additives (M1, M2, M3, M4 and M-Control). The cultures were maintained in the culture room at 30°C with photoperiodic lighting (16 hrs of light: 8 hrs of dark) during incubation provided by fluorescent light (Elektra FL36D/T8) at 60 µmol/m^2/sec. After 2 weeks of culturing, an early

protocorm formed was observed, which then developed into protocorm-like bodies (PLBs) at 10 week intervals. Subcultures for PLBs, leaf and root analysis were performed in 10 week intervals.

Contamination and growth analysis: In current study investigation into the *in vitro* culture of *R. gigantea*, the impact of natural additives on both contamination and growth levels was examined using tissue culture techniques. The study was conducted on individual treatments (n = 6) in a standard 1/2 MS medium without additives and with natural additives. Sterilization protocols were applied to ensure experimental accuracy and observations were systematically recorded to monitor contamination occurrences, including fungal or bacterial growth. Contamination and growth analyses were measured regularly for both control and treatment groups. Statistical analyses were performed to compare growth parameters between these groups. The contamination and growth analyses were calculated using Eq. 1 and 2, respectively:

$$\text{Contamination (\%)} = \frac{\text{Number of contaminated culture media}}{\text{Total number of culture media}} \times 100 \quad (1)$$

$$\text{Growth (\%)} = \frac{\text{Number of plant growth}}{\text{Total number of culture media}} \times 100 \quad (2)$$

Data collection: The experiments were conducted with six replications (n = 6) per individual treatment. After 2 weeks of embryo cell cultures, cell enlargement was noted and data analysis for contamination, early protocorm formed, protocorm formation and mature protocorm-like bodies formation was subsequently observed. The contamination rate and early protocorm formed were monitored initially following the mature PLBs formation. The measurements of early protocorm formation, protocorm formation and mature protocorm-like body formation were considered prohibited *in vitro* culture of *R. gigantea* by tissue culture technique due to the contamination risk.

Statistical analysis: Data were analyzed by One-way Analysis of Variance (ANOVA) to determine whether there are any statistical differences level ($p<0.05$), using SPSS (25.0). Tukey's post hoc test was performed to assess the significance of differences between pairs of group means. All results from these experiments were presented as means of replication samples ± the standard deviation and the standard error.

RESULTS AND DISCUSSION

Early protocorm formed from seeds: The daily observation of early protocorms was studied after transferring seeds onto different natural additives modification media. Seeds of *R. gigantea* play an important role in *R. gigantea* development and survival. The formation of early protocorms from seeds is a critical stage in the life cycle of *R. gigantea*. Early protocorm formed from seeds was recorded after three weeks of culturing. After three weeks of embryo cell cultures, the occurrence of protocorm formation on various modification media (M1, M2, M3, M4 and M-Control) was observed (Fig. 2a-d). Significant differences were observed among the various natural additives modification media in comparison to the control medium (M-Control). At the 4 week interval, early protocorm formation on M3 and M-Control exhibited significant differences at a 95% confidence level ($p<0.05$) compared to M1 and M2. At the same time, M4 was identified as contaminated in this study (data not shown). No statistically significant differences were observed between media M1 and M2 (Fig. 3). Early protocorm formation occurred at 26 and 22 days on M1 (glucose+banana) and M2 (glucose+potato), respectively, whereas M3 (glucose+coconut water) exhibited a longer period of 39 days, followed by M-Control (only glucose) at 52 days as the lengthiest duration of early protocorm formation. Effect of natural additives modification medium on *in vitro* culture of *R. gigantea* by tissue culture technique, such as organic sources are necessary nutrients for *R. gigantea*'s development[18]. In tissue culture, sugars are a carbohydrate, providing the energy necessary for the metabolic processes involved in cell division, growth and differentiation. It is essential for developing protocorm from embryonic cells[19]. Also, coconut water contains essential nutrients such as sugars, amino acids, vitamins and minerals. These nutrients provide a readily available source of energy and building blocks for growing protocorms, supporting their metabolic processes and overall development[20].

Furthermore, potatoes are rich in various nutrients, including carbohydrates, minerals, vitamins and growth-promoting compounds. The homogenate obtained from potatoes contains essential nutrients that support the growth and development of plant tissues[21]. The specific combination of nutrients in potatoes might be conducive to forming and maturing PLBs[22].

Additionally, the success of tissue culture techniques often depends on the precise formulation of the culture medium. Specific additives, such as coconut water and banana pulp, in addition to sugar and potato, are designed to provide

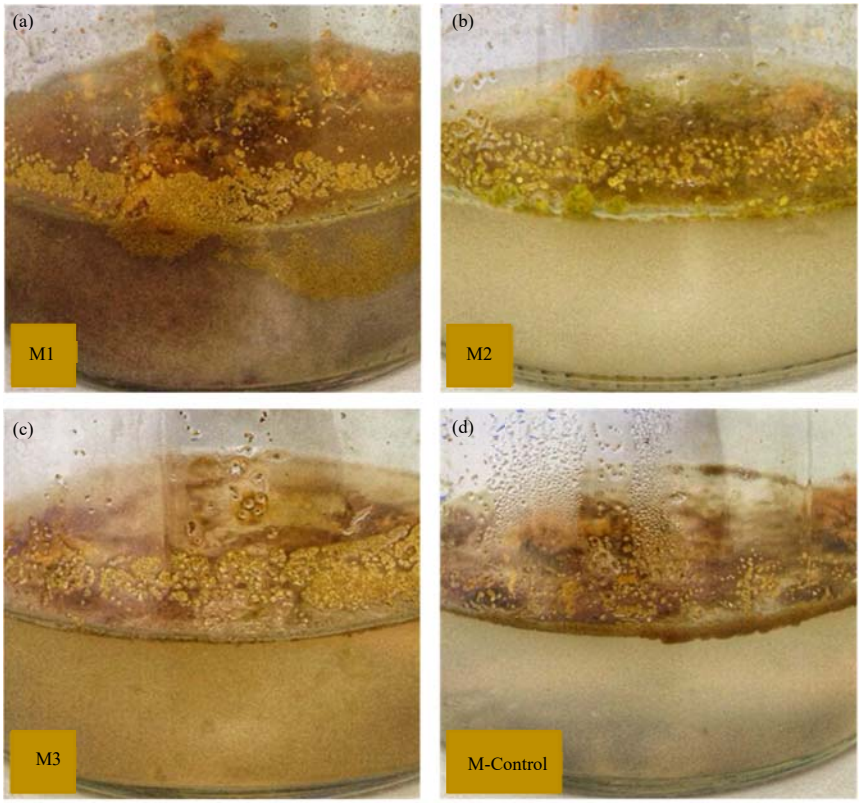

Fig. 2(a-d): Occurrence of protocorm formed on various modification media (M1, M2, M3 and M-Control), M4 was identified as contaminated (data not shown).

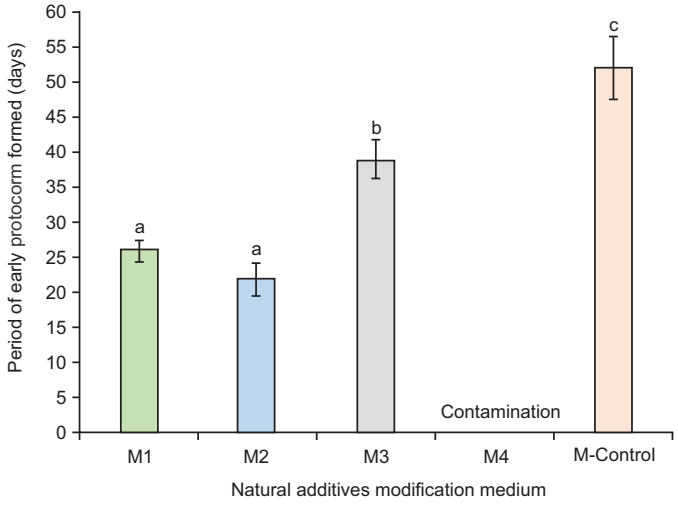

Fig. 3: Period of early protocorm formed onto the different natural additives modification mediums M1, M2, M3 and M-Control, M4 was identified as contaminated (no data recorded).
Mean difference is significant at the 0.05 level

a balanced nutrient profile that simulates the natural conditions are required for the optimal development of *R. gigantea in vitro*[23]. The exact mechanisms involved would require a more detailed analysis of the composition of the culture medium and the specific nutritional requirements of *R. gigantea* during different stages of development[24]. Therefore, M2 media was noted for the shortest duration of early protocorm formation, while M4, is considered an

optimum natural additives modification medium in this study was identified as contaminated.

Mature protocorm-like bodies (PLBs) and leaf formation: The formation of mature protocorm-like bodies (PLBs) represents a crucial stage of *R. gigantea*'s growth, especially in tissue culture. The PLBs are somatic embryos which capable of developing into complete orchid plants. The PLBs are formed through somatic embryogenesis, which involves converting differentiated somatic cells into embryonic cells[25]. The specific conditions that trigger PLB formation vary depending on the orchid species but typically include plant hormones, nutrient medium composition and temperatures[26]. As M2 revealed the shortest duration of early protocorm formation in this study, therefore, M2 was selected for the study of the formation of mature protocorm-like bodies (PLBs) and leaf formation (Fig. 4). After early protocorm formed from seeds was reported at 22 days (Fig. 5a), the protocorm formation was also recorded after 5 days of the early protocorm formed from seeds, at 27 days. Protocorm formation is an important age of *R. gigantea*'s growth related to mature protocorm-like bodies. The transformation of a tuber-like structure will eventually develop into a mature PLB. The protocorm is typically circular and has a light green or yellowish color (Fig. 5b). It is the site of storage for nutrients and energy that the *R. gigantea* will use as it grows. The protocorm also contains the meristematic tissue that will give rise to all of the *R. gigantea*'s tissues, including the roots, leaves and flowers[27]. Subsequently, the protocorm developed into mature PLBs after 17 days of the protocorm formation stage, at 44 days (Fig. 5c). The PLBs formation represents an advanced stage in *R. gigantea* tissue culture, where the protocorm-like bodies reach a more developed and mature state. PLBs are an important implement for the mass propagation of *R. gigantea*, as they can be produced in large numbers in a relatively short period. The PLBs are continually formed somatic tissues, such as leaves, stems, or roots.

In addition, leaf formation in *R. gigantea* is a main aspect of its development and it is a complex process that involves several different factors, including genetics, environmental conditions and hormonal regulation[28]. In this study, the leaf formation of *R. gigantea* was reported at 55 days or 11 days after mature PLBs formation (Fig. 5d). The development of PLBs and leaf formation is a complex processes influenced by multiple factors. Plant hormones, particularly auxins and cytokinins, play a significant role in regulating cell division, forming PLBs and leaf primordia[29]. Nutrient availability, including nitrogen, phosphorus and potassium, is essential for providing the building blocks for cell growth and development[30]. Additionally, light, temperature and humidity also play crucial role in regulating PLB development and leaf formation[31]. With current findings, applied natural additives modification medium on *in vitro* culture of *R. gigantea* is applicable for tissue culture technique. Optimizing these factors is essential for successfully propagating *R. gigantea* through tissue culture techniques.

Protocorm-like bodies (PLBs), leaf and root analysis: The analysis of PLBs and subsequent leaf development is a fundamental study of *R. gigantea*'s tissue culture. The PLBs,

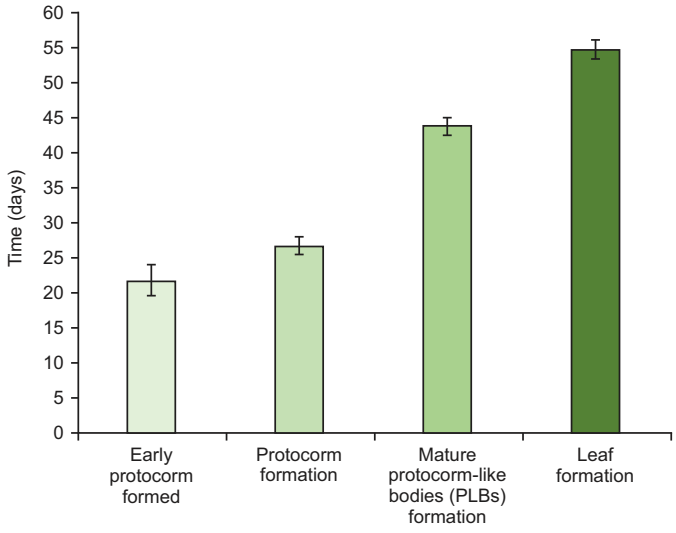

Fig. 4: Formation of early protocorm formed, protocorm formation, mature protocorm-like bodies (PLBs) and leaf formation on M2 medium.

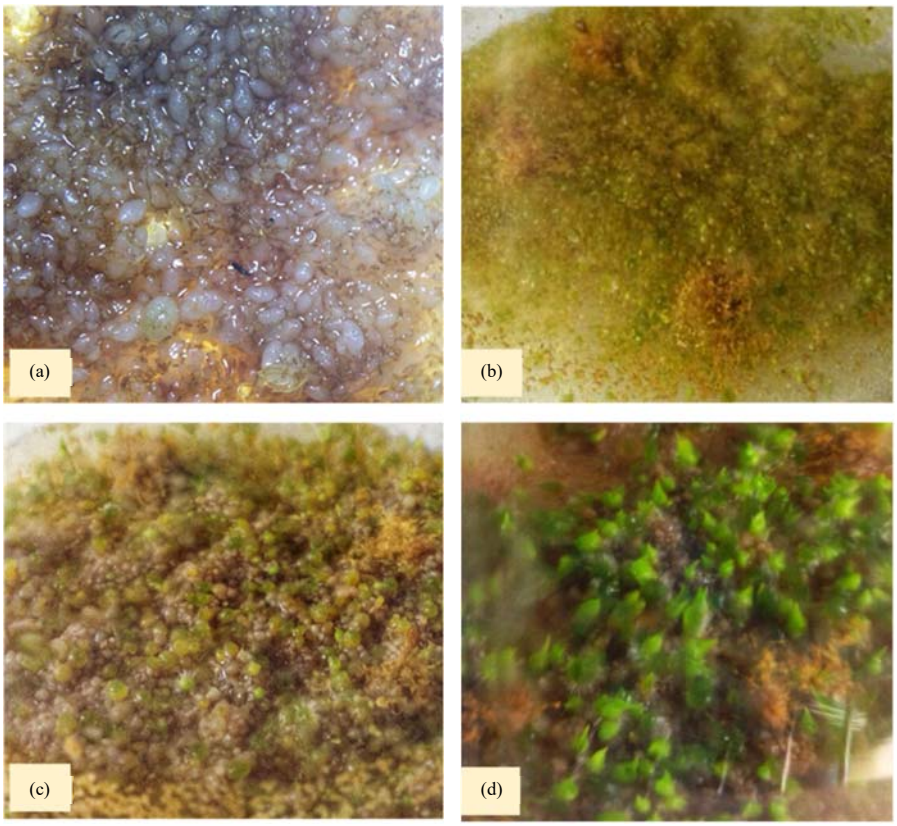

Fig. 5(a-d): Developmental stages including (a) Early protocorm formed from seeds, (b) Protocorm formation, (c) Mature protocorm-like bodies (PLBs) formation and (d) Leaf formation on M2 medium.

approaching early protocorms, serve as crucial intermediaries in the propagation processes. In this study, PLBs are likely formed from seed embryos. Once formed, PLBs can be multiplied by subculturing onto the fresh medium. The PLBs are used to rapidly propagate *R. gigantea*, as they can be easily multiplied in fresh medium and then transferred to natural habitats. The PLBs and leaf analysis of *R. gigantea* on M2 and M-Control were reported at 2, 4 and 6 weeks after subculturing, respectively. During the initial 2 weeks, M2 and M-Control presented the average PLBs at 13 and 10, respectively, while the number of leaves was reported at 2 leaves for M2 and one leaf for M-Control. At 4 weeks, M2 continually increased its PLBs to 18; likewise, there are no developed PLBs for M-Control. Furthermore, no increase in the number of leaves was recorded during the four weeks for both M2 and M-Control. However, the number of leaves was increased to 3 leaves for M2 at 6 weeks, whereas M-Control still has no increase in the number of leaves. The number of leaves from M2 increased and its PLBs dramatically increased to 26. At the same time, M-Control was recorded at 11 with no statistically significant difference from its 2 and 4 weeks. Consequently, M2 exhibited a statistically significant difference between the means of the PLBs analysis groups during the time intervals, whereas no statistically significant difference was observed between the means of the M-Control groups. The reported leave numbers for both M2 and M-Control showed no statistically significant difference at their respective time intervals. During the 6 week intervals, the root analysis was also determined as they started presenting. As a result, the average root analysis of M2 was reported as root presenting for all individual treatments (n = 6), while M-Control only reported individual treatments among 6 individuals (n = 6). The results of root analysis using One-way ANOVA showed the p-value is 0.04; since the p-value is less than 0.05, there is a statistically significant difference between the means of these groups. Therefore, the root number of M2 significantly differs from M-Control's (Fig. 6).

This study was achieved *in vitro* culture of *R. gigantea* using tissue culture techniques involving the modification of the medium with natural additives, specifically glucose and potato homogenate. These additions are vital in influencing the development of key structures such as PLBs, leaves and

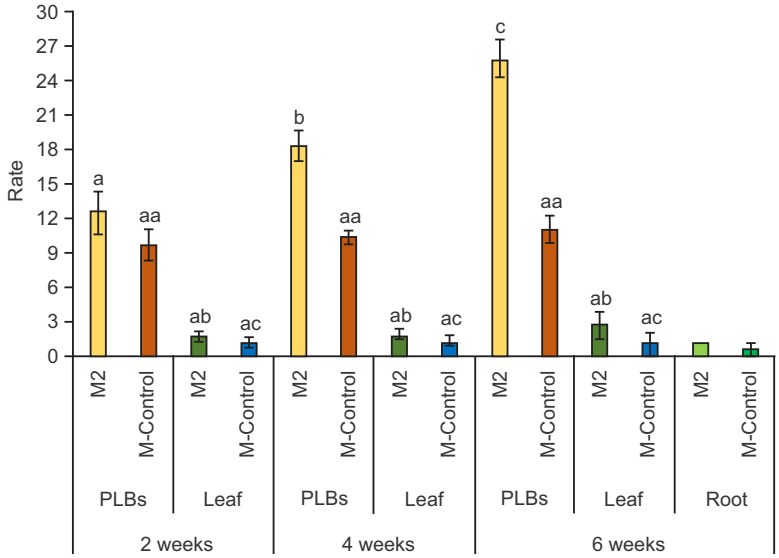

Fig. 6: Protocorm-like bodies (PLBs), leaf and root analysis reported at 2, 4 and 6 weeks.
Mean difference is significant at the 0.05 level

roots[17]. Glucose, a fundamental carbohydrate, contributes significantly to the culture medium. Its presence serves as an energy source, fostering the initiation and growth of PLBs by providing energy for cellular processes. Additionally, glucose supports leaf development by promoting photosynthesis, enhancing chlorophyll synthesis and facilitating overall leaf health. In the context of root development, glucose provides energy for cell elongation and supports the synthesis of structural components essential for a robust root system[32].

Similarly, as a natural additive, potato homogenate introduces a range of nutrients, vitamins and growth-promoting substances to the medium. This component enhances PLB initiation and growth by providing essential elements required for cellular development. The homogenate contributes to chlorophyll synthesis and overall leaf structure, promoting leaf energy and health. In addition, the homogenate's influence on root growth is significant, potentially containing compounds that stimulate root development and contribute to establishing a well-developed root system[33]. As a result, the combined effect of glucose and potato homogenate is aimed at creating a balanced and supportive environment for overall *R. gigantea*'s development. Glucose provides energy, while the potato homogenate contributes additional nutrients and growth-promoting factors. This synergistic approach is anticipated to enhance the formation of PLBs, encourages healthy leaf growth and supports the development of a root system. Likewise, the success of this natural additive modification strategy depends on careful optimization of concentrations and interactions, considering factors such as orchid species, tissue culture stage and overall medium composition.

Contamination analysis and growth rates: The analysis of contamination and growth rates is considered a main aspect of assessing the success and reliability of plant tissue culture experiments. Contamination analysis involves the systematic examination of cultures for the presence of unwanted microorganisms, such as bacteria or fungi. The analysis conducted in this study revealed contamination with strains of fungi and bacteria, as illustrated in Fig. 7a-b, where the cultured medium exhibited contamination with bacteria strains. Similarly, Fig. 7c-d demonstrate contamination with fungi strains. The appearance of contamination in plant tissue culture is diverse and the presence of contaminated cultures varies depending on the involved microorganisms. Contamination in plant tissue culture causes discoloration, browning, or darkening and the odor can range from slightly musty to foul, contingent upon the species of the contaminating microorganism. Consequently, contamination can lead to the formation of biofilms, discernible as a slimy film in the cultured medium. Moreover, contamination generates gases as metabolic byproducts, leading to the formation of gas bubbles that can affect the culture medium.

During the contamination period, fungi and bacteria strains utilize essential nutrients, thereby reducing the availability of these nutrients for plant cell growth and

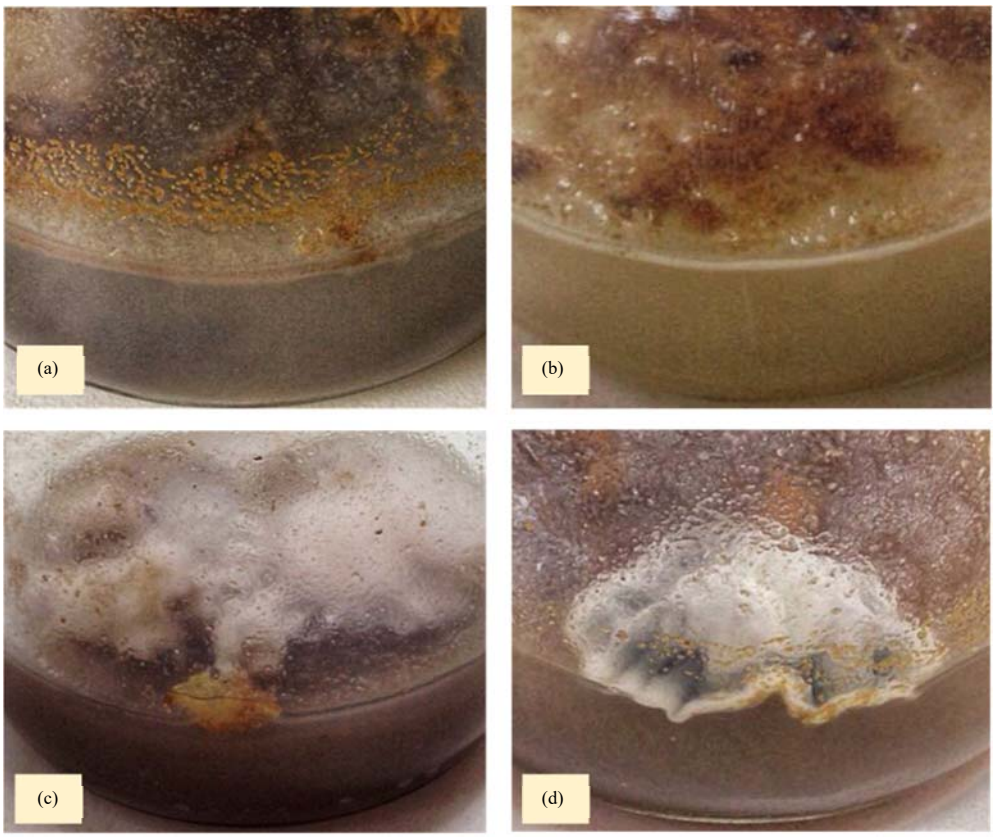

Fig. 7(a-d): Fungal and bacterial contamination on natural additives modification medium.

development[34]. Furthermore, the metabolism of the strains can produce acidic or alkaline byproducts, inducing changes in the pH of the culture medium[35]. Subsequently, this modified pH can destructively influence the growth of plant cells[36]. Some strains may produce toxins that prove detrimental to plant cells, disrupting normal cellular processes and ending in cell death[37]. Previous research has indicated that fungi and bacterial contamination can interrupt the metabolic pathways of plant cells, affecting the production of secondary metabolites and other compounds crucial for plant development. Therefore, contamination weakens the defense mechanisms of plant cells, rendering them more susceptible to infections[38,39]. This study was analyzed using a One-way Analysis of Variance (ANOVA) to determine whether there were any statistical differences between groups at a 95% confidence level ($p<0.05$). As a result of contamination analysis and growth rate assessments, no statistical significance was exhibited by M1, M2 and M3 at a significance level of 0.05. However, statistical significance was observed in M-Control when M1, M2 and M3 were compared (Fig. 8). In the experimental study, M4 exhibited contamination a few days after seed inoculation and consequently, M4 was entirely contaminated. Even though M2 was reported as having the shortest time for early protocorm formation at 22 days, it recorded a 43% contamination within a 57% growth rate, followed by M1 and M3 with a 71% contamination within a 29% growth rate.

On the other hand, M-Control, which was a controlled medium without inoculated natural additives, presented the lowest contamination rate, with 14% reported in this study. While M4 was entirely contaminated, carefully selecting quality plant material in tissue culture, specifically the avoidance of broken capsules, is a significant step in minimizing contamination rates. Broken capsules expose internal plant tissues to potential pathogens, increasing the risk of microbial infiltration. Preventing contamination in plant tissue culture involves a multi-faceted approach. Surface sterilization is important in preparing healthy and viable explants in tissue culture. Most surface contaminants can be eliminated by surface sterilization with a suitable sterilizing agent[40]. Systematic surface sterilization of plant materials and the selection of healthy, undamaged specimens reduce vulnerability to contaminants[41]. Hence, the precise

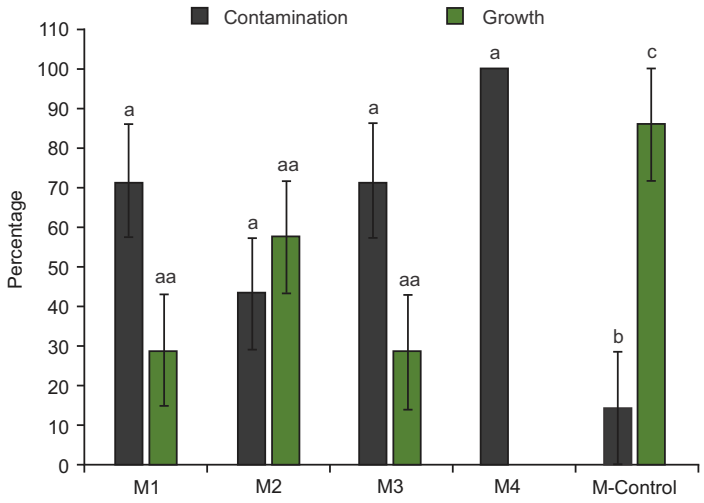

Fig. 8: Contamination analysis and growth rates.
Mean difference is significant at the 0.05 level

implementation of aseptic approaches, comprehensive sterilization practices and the wise selection of healthy plant material are necessary for limiting contamination risks in plant tissue.

CONCLUSION

This study investigated the effects of various natural additives on the *in vitro* culture of *Rhynchostylis gigantea* an orchid species of commercial importance. The experiments were conducted using 1/2 MS medium supplemented with glucose, banana pulp, potato homogenate and coconut water. The results demonstrated that adding glucose and potato homogenate to the medium significantly enhanced the growth and development of *Rhynchostylis gigantea*. Notably, an M2 medium containing 20 g/L of glucose and 50 g/L of potato homogenate produced the highest number of protocorm-like bodies (PLBs), leaves and fully developed roots. These findings suggest that glucose and potato homogenate are effective natural additives that can improve the *in vitro* culture of *Rhynchostylis gigantea*. While the M4 medium, which contained glucose, banana pulp, potato homogenate and coconut water, was considered to have the highest potential for effective modification, it was unfortunately found to be contaminated. It emphasizes the importance of strict sterilization procedures and the need for further research to optimize natural additives in *Rhynchostylis gigantea* culture. Therefore, this study contributes valuable insights into enhancing *Rhynchostylis gigantea* cultivation techniques and points out the pivotal roles of glucose and potato homogenate while highlighting the necessity for precise laboratory practices to ensure the success of natural additive modifications.

SIGNIFICANCE STATEMENT

This study was necessary due to the growing interest in sustainable and eco-friendly practices in orchid cultivation. Traditional *in vitro* culture practice often relies on synthetic chemical media, raising concerns about environmental impact and resource sustainability. Investigating the effects of natural additives on the *in vitro* culture of *Rhynchostylis gigantea*, the study offers a solution for alternative, environmentally friendly approaches in *Rhynchostylis gigantea* propagation. The unique contribution of this academic research lies in its identification and validation of specific natural additives glucose and potato homogenate that significantly enhance the growth and development of *Rhynchostylis gigantea*. The key finding not only provides a practical solution for improving *in vitro* culture conditions but also demonstrates a potential replacement for synthetic chemical media. This innovative approach aims to promote sustainability in orchid cultivation and highlights the study's significance in advancing both scientific knowledge and practical applications in horticulture.

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