Life Science and Biotechnology
Volume 2, Number 2, November 2024
Pages: 64 - 68

Life Science Biotechnology

Propagation of Dendrobium crumenatum Sw. through In Vitro Seed Culture Using Organic Complex Media

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Abstract

Dendrobium crumenatum Sw. is one of the epiphytic orchids with high ecological and economic value. This study aims to examine the effect of media containing organic compounds enriched with fertilizers for in vitro propagation of *D. crumenatum* seeds. This culture medium contains organic materials such as potatoes, bananas, coconut water, charcoal, sucrose, and foliar fertilizer. This medium was designed to provide macro, micro, and growth hormone nutrients that support seed growth. The research procedure included sterilization of orchid seeds, media preparation, seed sowing, and growth observation for about two months. The results showed that orchid seeds experienced optimal growth in media with the addition of coconut water and other organic compounds. The formulation of organic-based media with the addition of coconut water and other supporting compounds is effective in supporting the in vitro growth of orchids, providing opportunities for efficient propagation of orchid species. Explant growth reached 1-2 cm at the 68th day after sowing, with a fresh green colour was a indicator of normal growth. The even distribution of shoots indicated that the media provideed sufficient nutrients and space competition between explants. In sum, our simple tissue culture media produced from a mixture of organic materials proved reliable function and suited for growing *D. crumenatum* orchid seeds to produce plantlets, and to a further extent it would support conservation efforts.

Keywords: Conservation, organic materials, pigeon orchid, simple media, tissue culture

Introduction

Orchids are known as ornamental plants and are one of the plant commodities with high economic value (Telaumbanua, 2022). *Dendrobium* is the second largest orchid family in the Orchidaceae family and is a popular ornamental plant (Setyowati et.al., 2023). *Dendrobium crumenatum* is one of the epiphytic orchids that grow on trees and has been widely distributed in various countries in Southeast Asia. Epiphytic orchids are very dependent on other plants to live and thrive. For example, *D. crumenatum* relies on mycorrhiza to provide its nutrients and can be an inhibitor of natural regeneration if there is a disturbance in its natural habitat (Isnaini et al., 2015). The growth of *D. crumenatum* is greatly influenced by changes in temperature so that this plant can grow and flower at the same time simultaneously. Its fragrant and white flowers are very unique and attractive, shaped like a pigeon, so this orchid is nicknamed the "pigeon orchid" (Tee et al., 2010).

Dendrobium from the Orchidaceae family has been known as a traditional medicine in Asia, Europe, and Australia for centuries. *Dendrobium* was widely used for medicinal purposes in ancient China in 2800 BC. *Dendrobium crumenatum* is one of the orchids that have antimicrobial activity because it contains several bioactive compounds such as saponins, terpenoids, alkaloids, reducing sugars, and flavonoids (Sandrasagaran et al., 2016). In vitro culture is a plant propagation in a short time and produces new plants of high quality in large quantities. Plant propagation resulting from in vitro culture has the same genetic properties as its parent and can be done quickly in a controlled environment.

Media is one of the important factors in the success of in vitro culture. The media must contain all the substances and materials needed to ensure the needs of explants such as mineral salts, sources of macro and microelements, carbon sources, proteins, vitamins, and plant hormones (Markal et al., 2015). The media commonly used for orchid growth is Murashige Skoog (MS) media (Murashige & Skoog, 1962). This type of media is often used in in vitro culture because it contains many nutrients and vitamins needed by orchid explants, so it can meet the nutritional needs for orchid plant growth. The composition of MS media is sometimes still not optimal in providing the nutritional requirement of orchid plants, so organic materials can be added to optimize orchid growth.

The organic materials are often used to increase nutrition in in vitro culture media. There are many types of organic materials that can be used such as coconut water, tomato extract, banana pulp, orange

extract, and sweet corn extract and other organic materials (Ego et al., 2024). The addition of coconut water to the media can increase the growth and number of shoots in plants (Santoso et al., 2020). Report on the culture of *D. crumenatum* with simple natural media based with the addition of coconut water and charcoal can be an alternative for the propagation of *D. crumenatum* orchids (Telaumbanua, 2022). The purpose of this study was to examine the effectiveness of tissue culture media derived from organic complex for the propagation of *D. crumenatum* seeds in vitro.

Materials and Methods

This research was conducted at the Biotechnology sub-Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Jember for three months, from July to September 2024. The explants used were *D. crumenatum* orchid seeds from Sriwijaya Orchid Gallery, while the media used was a simple media consisting of the main ingredients of potatoes and bananas, with the addition of foliar fertilizer (Growmore), charcoal, and sugar.

Media preparation

The detailed ingredients for making 1 litre of simple media consist of 65 grams of potatoes, 45 grams of bananas, 8 grams of agar, 15 grams of granulated sugar, 1.5 grams of foliar fertilizer, 2.5 grams of charcoal (activated charcoal), 150 ml of coconut water. The general procedures was: First, blend potatoes, bananas, and coconut water, then add the granulated sugar. After mixing, remove the foam and adjust the pH to 5.8-6 using litmus paper. Next, put the agar into the solution and heat with stirring until boiling. Add charcoal and leaf fertilizer, then stir again until all ingredients were evenly mixed. Subsequently, sterilization of the media was carried out using an autoclave at a temperature of 121°C for 30 minutes. After the sterilization, pour the media into sterile tissue culture bottles.

Cultur in vitro

The mature seed pods of *D. crumenatum* was sterilized by soaking the pods in Clorox solution for 10 minutes then rinsed with distilled water for 30 seconds. This sterilization step was repeated three times. Afterward the orchid pods were cut and the seeds were sown into the media evenly. Subsequently, the media containing sowed seeds was tightly closed and sealed. Finally, orchid explants were growth with a photoperiod setting of 16 hours of light and 8 hours of dark and temperature 18°C.

Results and Discussion

The growth of *D. crumenatum* explants was observed at 34 and 68 days after sowing. The results showed that at 34-day-old *D. crumenatum* explants were in the early stages of growth and then experienced rapid growth at the age of 68 days after sowing with, the presence a number of explants growing on the media (Figure 1). The dominant light green colour in most plantlets indicated morphological health related to the photosynthesis occurance.

The even distribution of shoots at the base of the culture medium in the bottle indicated that there was sufficient growing space, so that each shoot can develop without any disturbance or competition (Figure 1). *D.crumenatum* at 68 days after sowing showed significant development, with indications of being in the optimal early differentiation stage. The characteristics that appeared such as green leaves, shoot formation, and no contamination indicate that environmental conditions and culture media support the growth effectively (Figure 1). According to Yasmin et al., (2018), the success rate of in vitro orchid seed germination tends to be optimal if the main requirements are met, namely aseptic conditions in orchid seeds and culture media.

Orchid seeds require aseptic conditions with a complete supply of energy and mineral nutrients in the culture medium for successful germination. Several media formulations that can be used for orchid seed germination include Murashige and Skoog, Vacin and Went, Growmore, and Gandasil media. The composition of the media plays an important role in optimizing the growth rate of protocorms and the development of orchid seedlings during the in vitro culture process. The addition of organic matter to the media can increase the effectiveness of orchid seed germination and growth (Maulida et al., 2022).

Life Science and Biotechnology
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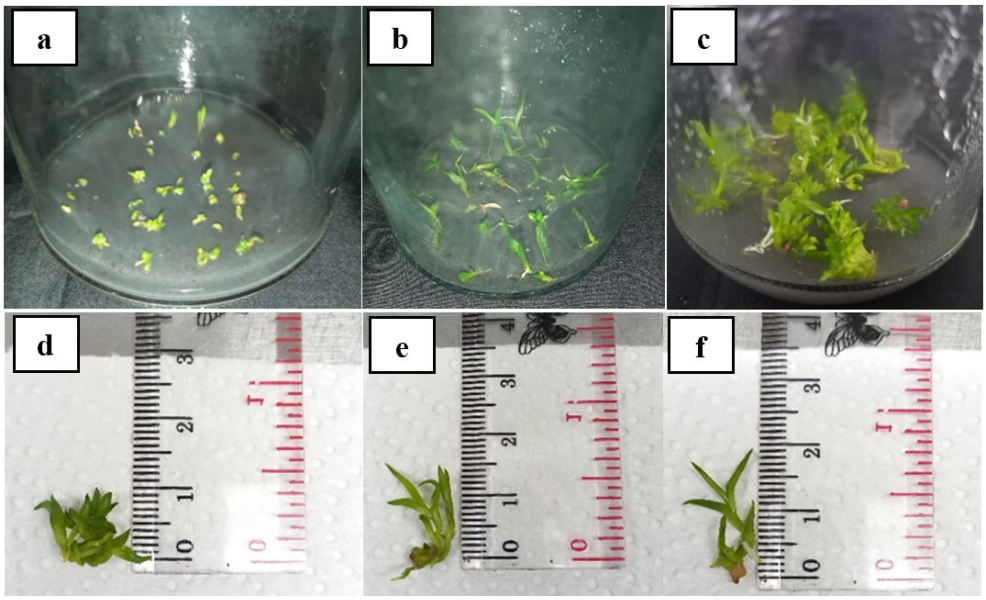

Figure 1. Morphology of *Dendrobium crumenatum* plantlets derived from simple tissue culture media. (a) 34 DAS. (b & c) 68 DAS. (d, e, & f) Plantlet measurement using a ruler at 68 DAS. DAS: days after sowing

Simple culture media consists of several ingredients that contain vitamins so that the addition of organic compounds can support orchid growth (Table 1). In this study, several organic compounds were added, one of which was coconut water. Coconut water is a natural compound that is rich in complex components, widely used in tissue culture techniques as a supporting agent for orchid plant propagation through in vitro techniques. The use of coconut water in vitro propagation is due to its natural cytokinin content, such as zeatin and ribozeatin, as well as its high IAA (Indole Acetic Acid) content. Coconut water also plays a role in stimulating the process of cell differentiation and division. The addition of coconut water to media containing essential macro and micro nutrients can accelerate germination and protocorm formation (Maulida et al., 2022). Coconut water is used as a substitute for synthetic materials, with equivalent advantages because it contains natural cytokinins that affect apical dominance. Coconut water contains carbohydrates, vitamins, minerals, proteins, and growth substances such as auxins, cytokinins, and gibberellins, which play a role in metabolism, and respiration, and stimulate cell and stem elongation (Apriliyana and Wahidah, 2021).

In this study, the addition of coconut water in the culture medium was also enriched with the addition of organic compounds such from bananas and potatoes (Table 1). The addition of bananas was aimed to accelerate the development of plantlets and roots. The nutrition content from bananas, such as carbohydrates, protein, fat, calcium, phosphorus, iron, vitamins A, B1, and C, plays a role in supporting the regeneration process. These nutrients also function as endogenous resource for the hormones of auxin, cytokinin, and gibberellin (Apriliyana and Wahidah, 2021). Bananas contain peptone which functions to induce roots and support the shoot growth process in orchids (Sandy et al., 2022). The addition of potatoes provided an accelerate the growth of plantlets due to the nutrion of carbohydrates, phosphorus, potassium, iron, and vitamins B1, B2, C, and niacin that support the development of explants (Apriliyana and Wahidah, 2021). The addition of organic compounds from potatoes, bananas and coconut water in this study supported the growth and development of orchid plantlets in the culture.

Based on the measurement results, the length of *Dendrobium crumenatum* at 68 days after sowing showed a growth of 1-2 cm (Figure 1). The fresh green colour of the plant indicates the fitness and optimal adaptation to the culture medium. The sterile environmental conditions of the culture support the growth process effectively. The challenge in optimizing the formulation of tissue culture media lies in maintaining consistency of results, especially in the use of additional ingredients such as coconut water. It is important to know the age of the coconut that is suitable and supports culture growth to obtain optimal results. The coconut water used comes from green coconuts with the characteristics that the water still fills the inside of the fruit and the flesh (endosperm) is not too thick. The ideal coconut water for tissue culture

comes from young green coconuts with an estimated age of 7-8 months. Coconut water which has been proven effective and is often used as an addition to tissue culture media is used with a concentration of 5-15% (Amalia and Hadipoentyanti, 2018). Low concentrations of coconut water (<10%) tend to produce shoots with a pale green color, while high concentrations (>15%) can have negative effects, causing stunted shoot growth and leading to death (Nugroho et al., 2019).

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Table 1. Simple media composition applied in this research and its functional roles

Materials	Function in tissue culture media	References
Foliar fertilizer	To stimulate vegetative growth	Marlina et al., 2019
Potatoes	To accelerate the growth of plantlets and support the development of explants	Apriliyana & Wahidah, 2021
Banana	To accelerate the development of plantlets and roots	Apriliyana & Wahidah, 2021
Coconut water	Plays a role in stimulating the process of cell differentiation and division, as well as accelerating germination and protocorm formation	Maulida et al., 2022
Charcoal	To maintain the stability of the media pH, stimulate root initiation by reducing the light intensity received by the plantlets, and support the morphogenesis process optimally	Warisman et al., 2024
Agar	As a gelling agent, support the plants to grow with strong structure	Asriani, 2020
Sugar	To support the growth of culture	Lestari et al., 2019

In addition to organic compounds, the orchid culture media in this study was also supplemented with activated charcoal, foliar fertilizer, sugar, and agar (Table 1). Activated charcoal acts as an agent that suppresses the release of phenolic compounds that are toxic to plants, thus supporting growth optimization and increasing the efficiency of the differentiation process. Activated charcoal has the ability to absorb toxic compounds found in the culture media and inhibitor compounds produced by plantlets. Activated charcoal plays a role in maintaining the stability of the pH of the media, stimulating root initiation by reducing the intensity of light received by the plantlets, and supporting the morphogenesis process optimally (Warisman et al., 2024).

Foliar fertilizers contain macro- and micro nutrients that can support plant growth. Application of foliar fertilizer with a concentration of 2.0 g/l shows optimal results for the growth of *Dendrobium* orchids. The nitrogen (N) content in foliar fertilizer can stimulate vegetative growth, while phosphorus supports root development and accelerates flowering. Potassium functions as a catalyst in the plant's metabolic process (Marlina et al., 2019). The planting medium will experience an increase in density, so that the plants that grow will have a stronger structure, with the addition of agar as a binding agent (Asriani, 2020). The main carbon sources used in tissue culture are sucrose and glucose, with sucrose being the most frequently used choice. White sugar meets the criteria needed to support culture growth (Lestari et al., 2019).

In conclusion, the simple tissue culture media produced from a mixture of organic materials consisting of foliar fertilizer, potatoes, banana, coconut water, sugar, and charcoal compacted using agar proved reliable function and suited for growing *D. crumenatum* orchid seeds and was able to produce reliable plantlets. In the near future, this alternative media can be applied to support the propagation practices for various orchid species and contribute to strengthening conservation efforts.

Acknowledgments

The authors thank to Sriwijaya Orchids Gallery for providing *Dendrobium* seed pods used in this research.

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