

**THE EFFECT OF GROWING MEDIUM MODIFICATION AND APPLICATION OF GROWTH REGULATORS ON THE GROWTH OF ORCHID PLANTS USING TISSUE CULTURE SYSTEM: LITERATURE REVIEW**

**Kukoh Puji Slamet Rahayu**

Pendidikan Biologi, FPMIPATI, Universitas PGRI Semarang

**Choirul Nisa Kismayanti**

Pendidikan Biologi, FPMIPATI, Universitas PGRI Semarang

**Septiana Kurniawati**

Pendidikan Biologi, FPMIPATI, Universitas PGRI Semarang

**Wima Rahayu Putri**

Pendidikan Biologi, FPMIPATI, Universitas PGRI Semarang

**Faridatul Zuhriyah**

Pendidikan Biologi, FPMIPATI, Universitas PGRI Semarang

**Alfina Damayanti**

Pendidikan Biologi, FPMIPATI, Universitas PGRI Semarang

**Atip Nurwahyunani\***

Pendidikan Biologi, FPMIPATI, Universitas PGRI Semarang

Coressponding E-mail: [atipnurwahyunan@upgris.ac.id](mailto:atipnurwahyunan@upgris.ac.id)

**Endah Rita Sulistya Dewi**

Pendidikan Biologi, FPMIPATI, Universitas PGRI Semarang

**Abstract**

*Orchids are popular ornamental plants known for their beautiful flowers and limitless varieties. In addition to choosing the right growing medium, orchid care to support their growth also requires nutrients, one of which is growth regulators. Tissue culture is a propagation technique that can rapidly multiply orchid plants in large quantities. The research method involves a literature review by analyzing several relevant articles. The literature review discusses topics such as explant contamination, growing media, and growth regulators in orchid plants. Based on the research findings, the moth orchid (*Phalaenopsis*) can thrive and grow well in both VW (Vacin and Went) and MS (Murashige and Skoog) growing media. Meanwhile, the use of MS medium with the addition of the growth regulator BAP (Benzyl Adenine Purine) in black orchids has an impact on shoot multiplication.*

**Keywords:** Orchid, Tissue Culture, Growing Medium, Growth Regulator.

## INTRODUCTION

With the advancement of time in this modern era, parallel with the development of technology in society, new innovations are created due to the push in this modern era, making every individual's work easier, both household chores and daily tasks. The rapid advancement of technology is not only felt but also applied in biotechnology. Biotechnology can be defined as the application that has basic principles of science and engineering over material processes with the help of biological agents to produce a product (Hadi, 2021).

Tissue culture is a propagation technique that can multiply plants in a short time and in large quantities (Santoso, Hardiyati, Dwiati, & Kamsinah, 2020). Tissue culture is one way to obtain pathogen-free planting materials as it produces seedlings in larger quantities in a relatively short time, free from diseases, not dependent on climate and weather, produces healthy plants, maintains the good traits of the parent, requires less land for propagation, requires minimal labor, and can multiply certain plants that are difficult to propagate conventionally.

The advantages of tissue culture include using cell culture methods that can produce bioactive compounds. The technique used is by using explants that still have actively dividing cells, making the isolation process easier and more effective. According to Collin & Edward (1998) in Puspitasari (2021), this technique does not require extensive land, production of bioactive compounds can be controlled, and it is not dependent on seasons. It is possible that cell culture or tissue culture techniques are biotechnological applications that can support the agricultural sector in Indonesia. Not only does it produce a large quantity of explants, but it can also produce new explant seedlings of higher quality. Tissue culture becomes an appropriate technique for plant propagation, providing assurance of higher-quality seeds and not depending on the season. Additionally, tissue culture has high diversity, enhances plant immunity, and can be transported over long distances with a large quantity and lower costs. Based on these factors, tissue culture becomes a suitable choice for providing plant seedlings in the current agricultural sector.

Orchids are popular ornamental plants with beautiful flowers and unlimited varieties. Sunlight is indirectly required by orchids, so they are usually found in nature as ground cover (terrestrial orchids) or under the shade (epiphytic orchids) (Figiganti, 2018). Epiphytic orchids are often found living on trees in forests from Sumatra to Papua, with a very high diversity of orchid species. Based on their stem growth, orchids are divided into two types: monopodial orchids and sympodial orchids (M, et al., 2015). The planting and care of orchids require the right growing medium.

The use of the right medium is one of the determining factors for the success of plant explant growth. MS (Murashige and Skoog) medium is commonly used for tissue culture, but for orchid culture, there is a specific medium with the right content for orchid growth, namely VW (Vacin and Went) medium. In addition to choosing the

right growing medium, orchid care to support their growth also requires nutrients, one of which is growth regulators (Wayuni et al., 2020).

According to Khan and Tabassum (2012) in Nufus et al., (2021), the types of explants used in plant parts are high meristematic parts, such as tissues in seedlings, embryos in seeds, leaves or young stems, and flowers. In the success of tissue culture plant growth or in vitro, the medium is the key to the plant's growth, especially for propagation. Growth regulators such as hormone application play a significant role in plant regeneration. Plant growth regulators commonly used for in vitro plant growth are auxin and cytokinin hormones (Hariadi et al., 2019).

## **RESEARCH METHOD**

The methodology employed in writing this article is literature review, which is a research approach utilizing the analysis of relevant literature to address research questions and gain a profound understanding of the investigated topic. According to Creswell, John. W. (as cited in Habsy, 2017), literature review is a written summary of articles from journals, books, and other documents describing theories and information, both past and present, organizing literature into topics and documents needed. The literature review methodology provides an opportunity to investigate, synthesize, and interpret previous research conducted by experts and researchers in the field.

Literature search was conducted through diverse sources, including academic databases, scientific journals, books, research reports, and other information sources related to the research topic. Subsequently, literature selection was performed. At this stage, relevant literature was chosen based on predefined inclusion and exclusion criteria. Relevant literature was then thoroughly analyzed to identify themes, patterns, similarities, differences, and contributions of each work. In this research, the literature review methodology was employed to gather, evaluate, and synthesize relevant literature to obtain a comprehensive understanding of the researched topic and to address the previously formulated research questions.

## **RESULT AND DISCUSSION**

This study is based on articles that examine research activities on tissue culture systems in orchid plants published during the period 2014-2023. Based on the results of the article review conducted, it can be explained in Table 1.

**Table 1. Selected Article Details**

No	Author	Title	Journal
1.	Selfi Aprilia Ningrum, Evria Asih, Titis Dewi Vira, Windi Putri Firdhiana, Khusna Yurdika Habsari, Praptining Rahayu(2023)	Orchid Conservation Through Tissue Culture to Foster Environmental Awareness in Students	Prosiding Webinar Biofair2023
2.	Ifa Ana Meilani, Ema Auliatusahra, Rizqi Nova Darillia, Kamila Nur Afifah, Bagus Hadi Akhsanulhaq, Praptining Rahayu (2023)	issue Culture-Oriented Education for Sustainable Development to Enhance Critical and Creative Thinking Skills	Prosiding Webinar Biofair2023
3.	Lia Risnawati, Merlly Alfina Septiana, Ade Rahma Pertiwi, Salma Najikhah, Deka Yuna Ikhtiarintyas, Praptining Rahayu (2023)	<i>Literature Review: The Potential of Tissue Culture Material Learning Through Problem- Based Learning to Improve Students' Critical Thinking Skills</i>	Prosiding Webinar Biofair2023
4.	Maharani Shintiya Putri, Hana Eldiana Yuliani, Septiana Kurniawati, WimaRahayu Putri, Haliza NurulAmin, Praptining Rahayu (2023)	<i>Literature Review: Tissue Culture Learning to Foster Science Motivation</i>	Prosiding Webinar Biofair2023
5.	Alfina Damayanti, Elsa Septiani Rintho Miharjo,Novita Anggraini, Faila Silva Solecha, Septiana Nur Kholifah, Praptining Rahayu (2023)	Analysis of the Potential Implementation of STEAM in Tissue Culture Learning in the Free Curriculum of Senior High School	Prosiding Webinar Biofair2023
6.	Dr. Endah Rita S. Dewi, M.Si., Dyah Ayu Widyastuti, M. Biotech.,Atip Nurwahyunani, M. Pd. (2021)	Biotechnology Teaching Book	Prosiding Webinar Biofair2023

7.	Desta Andriani, PebraHeriansyah (2021)	Identification of Contaminant Fungi in Various Natural Orchid Tissue Culture Explants ( <i>Bromheadia finlaysoniana</i> (Lind.) Miq)	Agro Bali : Agricultural Journal Vol. 4 No. 2: 192-199, July 2021
8.	Berlian Z. Hayati, Megalisa Siampa (2018)	Response of Black Orchid ( <i>Coeloyne Pandurata</i> ) from Tissue Culture Propagation to Various Growing Media	AgroSainT UKI Toraja Vol IX No.1
9.	Pebra Heriansyah, TrinopSagiarti, rover (2014)	The Influence of Myoinositol and Activated Charcoal on Subculture Media in Orchid Plant Tissue Culture ( <i>Dendrobium Sp</i> )	Jurnal Agroteknologi , Vol 5 No.1, Agustus 2014
10.	Ellok Dwi Sulichantini, Eliyani, Agusty Saputra, Alvera Prihatini Dewi Nazari, SusyLOWATI (2021)	The Influence of Growth Regulators and Organic Materials on the Growth of Tebu Grammatophyllum speciosum Blume Orchids through Tissue Culture	Jurnal Agroekologi Tropika LembabVolume 4, Nomor 1, Agustus 2021
11.	Nur' Anisa, Reine Suci Wulandari, Asnawati (2016)	The Effect of BAP on Shoot Multiplication of Black Orchid ( <i>Coelogyne pandaruta</i> Lindl) through Tissue Culture	Jurnal Hutan Lestari Vol. 4 (4) : 591-595
12.	Riani Ningsih, Herman Estu Eka Putra, Andre Eka Nanda (2023)	Modification of Growing Media for the Optimization of Transplanting Tissue Culture of Moon Orchids ( <i>Phalaenopsis Amabilis</i> )	Jurnal Pengembang anPotensi Laboratorium Vol.2, No.2, Agustus 2023
13.	Fetrina Oktavia (2021)	The Potential Utilization of Tissue Culture Technology in the Preparation of Superior Rubber Planting Materials	Warta Perkaretan2021, 40 (2), 75- 84
14.	Pebra Heriansyah, Elfi Indrawanis (2020)	Examination of the Contamination Level of <i>Bromheadia finlysoniana</i> L. miq Orchid Explants in In-Vitro Culture with the Addition of Tomato Extract	Jurnal Agroqua, Volume 18 No. 2 Tahun 2020
15.	Zulkaidhah, Muslimin, Andi Sahri Alam, Bau Toknok (2022)	Improvement of the Quality of Natural <i>Phalaenopsis</i> Orchids Through Crossbreeding Activities	Abditani : Jurnal Pengabdian Masyarakat 2 (1) 11-14

16.	Nurul Kamila, Sulistyono, Sidik Purnomo, NurcahyoWidyodaru, Edhi Sandra (2022)	Ethyl Methane Sulfonate (EMS) Induced Mutation on Phenotypic Appearance of Ki Aksara Orchids ( <i>Marcodes petola</i> ) In Vitro	Agrohita Jurnal Agroteknologi Fakultas Pertanian Universitas Muhammadiyah Tapanuli Selatan, Vol. 7 No.1 Tahun 2022
17.	Yan Piter B. Ziralno (2021)	Propagation Method of Purple Sweet Potato Plants ( <i>Ipomea batatas poiret</i> ) using Tissue Culture or Plantlet Stecking Techniques	JIP Jurnal Inovasi Penelitian Vol.2 No.3 Agustus 2021
18.	Andi Besse Sri Putri, Hajrah, Devi Armita, Ika Roostika Tambunan (2021)	Tissue Culture Technique for Multiplication and Conservation of Potato Plants ( <i>Solanum tuberosum L.</i> ) In Vitro	FILOGENI Jurnal Mahasiswa Biologi
19.	Herlindah Choiri, I Ketut Suada, Wayan Adiartayasa (2019)	Tissue Culture of Anthurium Plants ( <i>Anthurium andraenum</i> var. tropical) on MS Media with the Addition of Growth Regulators BAP and NAA	Jurnal Agroekoteknologi Tropika Vol.8, No.3, Juli 2019
20.	Muhammad Fauzan, Ratna Nirmala, Widi Sunaryo, Penny Pujowati (2021)	Multiplication Induction of Elephant Cassava var. ( <i>Manihot esculenta crants</i> ) Through Tissue Culture with Growth Regulators BAP and NAA	Jurnal Agroekoteknologi Tropika Lembab, Volume 3, Nomor 2, Februari 2021, Halaman 79-85

#### A. Explant Contamination

In the study by Andriani & Heriansyah (2021), the MS medium enriched with hormones 6-BAP (Benzylaminopurine) 1 mg/l; NAA (Naphthalene acetic acid) 0.5 mg/l; 100 mg myo-inositol; pyridoxine-HCl 0.5 mg/l; thiamine-HCl 0.1 mg/l; nicotinic acid 0.5 mg/l; glycine 2 mg/l; 30 g/l sucrose and 10 g/l agar was used, with pH adjustment to 5.7 using NaOH and/or HCl. In root, stem, flower stalk, and leaf explants, the highest percentage of contaminant fungi in the tissue culture of *Bromheadia finlaysoniana* orchids was found in flower stalk explants at 81%. Meanwhile, the highest number of contaminant fungus colonies grew in leaf explant cultures, specifically 28 colonies, as observed in the study by Putri et al. (2021). Explants containing many contaminant microorganisms, such as banana or

taro tubers from the soil, should be washed with flowing water for a sufficient duration. The next step is to eliminate microorganisms attached to the sample using disinfectants, particularly chemicals that are toxic to microorganisms but not harmful to plants. A commonly used disinfectant is calcium hypochlorite (chlorine) or sodium hypochlorite (usually commercially sold as bleach containing 5.25% sodium hypochlorite).

In another study by Heriansyah & Indrawanis (2020) on Contamination Levels of *Bromheadia Finlysoniana* L.Miq Orchid Explants in In-Vitro Culture with the Addition of Tomato Extract, explants were planted on solid MS medium. The medium acidity level was adjusted to pH 5.6, with the addition of 7 g/L Gelrite and 30 g/L sucrose on solid media. The medium was then sterilized in an autoclave at a temperature of 121°C with a pressure of 1 kg/cm<sup>2</sup> for 20 minutes. The results showed that the best explants for propagating tissue culture of *Bromheadia finlaysoniana* L.Miq orchids were seed and root explants, with contamination percentages of 36.81% and 37.50%, respectively. The observation for the percentage of fungal contamination showed that the best treatment for single explants was root explants with a contamination percentage of 17.36%. Meanwhile, for the best treatment combination, it was found in root and seed explants without tomato extract, with a fungal contamination percentage of 8.33%. The observation for the percentage of bacterial contamination showed that the best treatment for single explants was stem explants, with a bacterial contamination percentage of 29.17%. For the combined treatment, the best was found in seed and stem explants without tomato extract, with a fungal contamination percentage of 16.67%.

In the research conducted by Zulkaidhah et al. (2022) through the crossbreeding of *Phalaenopsis celebencis* and *Phalaenopsis venosa* orchid species, rapid propagation through tissue culture using seeds was carried out due to the absence of endosperm or very small size of the seeds (Andiani, 2008 as cited in Zulkaidhah et al., 2022). Vegetative tissue propagation produces offspring identical to the parent because its cells do not change in their genetic makeup without treatment. As a result, to prevent genetic deviations, orchid plant propagation is performed using tissue culture methods. Consequently, the offspring produced will be uniform with the parent, yielding a large number of offspring over a longer period than the parent. In the study by Ziraluo (2021), the culture percentage forming explants was 100% growth, but they could not survive due to the low percentage of explants emerging, influenced by environmental factors and insufficiently sterile equipment.

## B. Growth Medium and Plant Growth Regulators

Research by (Haryati & Siampa, 2018) on the response of black orchids resulting from tissue culture propagation to various media, using kadaka roots as a control and media such as sawdust, coconut coir, fern, and charcoal that were wet sterilized using an autoclave and soaked in fungicide, yielded results based on parameters such as the number of leaves, leaf length, number of shoots, and the percentage of live plants. The results indicated that coconut coir and sawdust planting media provided the best growth in terms of the number of leaves, number of shoots, and the percentage of live plants. This is because coconut coir contains essential nutrients such as K, P, Ca, Mg, and N needed for initial plant growth. Additionally, coconut coir has the ability to bind and retain water, maintaining the moisture of the planting medium against pests and diseases for plant growth. Meanwhile, sawdust media contains C, H, O, N, P, Ca, hemicellulose, cellulose, and lignin needed for leaf development. It also absorbs water and nutrients required by plants. Charcoal planting medium resulted in better leaf length due to its content of nitrogen, phosphorus, calcium, magnesium, silicate, copper, zinc, sodium, and iron, which can support plant growth and development.

In a study by (Pebra et al., 2014) on the Effect of Myoinositol and Activated Charcoal on Subculture Media for Tissue Culture of Orchids (*Dendrobium Sp*), the research showed that providing myoinositol alone significantly affected all observed parameters, with the best treatment being A2 (myoinositol 50 mg/l). Providing activated charcoal alone also significantly influenced all observed parameters, with the best treatment being Bo (no activated charcoal). Meanwhile, the interaction of myoinositol and activated charcoal had a significant effect on the observed parameters, with the best treatment being A2Bo (myoinositol 50 mg/l without activated charcoal). Another study by (Kamila et al., 2022) focused on the use of ethyl methane sulfonate (EMS) with different soaking durations affecting the phenotypic appearance of *Ki Aksara* orchids (*Macodes petola*). Soaking EMS at 0.2% for 30 minutes resulted in increased plant height and the highest number of roots, while soaking EMS at 0.2% for 60 minutes resulted in the highest number of leaves. Concentrations of 0.4% after 60 minutes provided the highest number of shoots in *Macodes petola*. However, concentrations of 0.6-0.8% at soaking durations of 30, 60, 90, and 120 minutes inhibited plant growth.

Research by (Sulichantini et al., 2021) on the Influence of Plant Growth Regulators and Organic Materials on the Growth of *Tebu Orchids Grammatophyllum speciosum* Blume through Tissue Culture found that the treatment VW + Kinetin 3.00 mgL<sup>-1</sup> + NAA 0.50 mgL<sup>-1</sup> + banana extract 100 gL<sup>-1</sup> yielded the best results for height and the number of leaves, attributed to the synergy between kinetin, NAA, and banana for cell division. The best treatment for the number of shoots was observed in treatments (VW + BAP 3.00 mgL<sup>-1</sup> + NAA

0.50 mgL<sup>-1</sup>) and (VW + BAP 3.00 mgL<sup>-1</sup> + NAA 0.50 mgL<sup>-1</sup> + banana extract 100 gL<sup>-1</sup>). In another study by (Choiri et al., 2019) on Tissue Culture of Anthurium Plants (*Anthurium andraeanum* var. tropical) on MS Media with the Addition of Plant Growth Regulators BAP and NAA, the best treatment for leaf explant curvature was A3B2 (5 mg/l NAA + 3 mg/l BAP). The best treatment for the in-vitro growth of shoots, leaves, and roots of anthurium was A3B3 (5 mg/l NAA + 5 mg/l BAP). The number of leaves was influenced by the addition of plant growth regulators to the medium, and the appropriate NAA concentration could stimulate root growth in plants.

Research by (Anisa et al., 2016) to determine the best BAP concentration for the multiplication of black orchid shoots through tissue culture showed that BAP significantly influenced the multiplication of *Coelogyne pandurata* shoots on MS media. The best treatments for the emergence time of shoots and the number of shoots were BAP 1.5 and 1.25 ppm, while the number of leaves was demonstrated by the treatment of 1.5 ppm BAP. Another study by (Fauzan et al., 2021) addressed the Induction of Multiple Cassava multiplication (*Manihot esculenta* crantz) Through Tissue Culture With Plant Growth Regulators BAP and NAA. The results indicated that BAP significantly affected shoot height and the number of leaves but did not significantly affect the length of cassava shoot roots. NAA significantly affected the length of the roots but did not significantly affect shoot height and the number of leaves of cassava shoots. The interaction between BAP and NAA had a significant effect on root length but had no significant effect on shoot height and the number of leaves of cassava shoots. MS media with a concentration of BAP: 1 ppm + NAA: 0.5 ppm was the best concentration for inducing the multiplication of elephant cassava shoots.

Research by (Ningsih et al., 2023) on Modification of Planting Media for the Optimization of Tissue Culture Transplanting of Moon Orchids (*Phalaenopsis Amabilis*) using four modified planting media. The best modified planting medium to optimize the transplantation of tissue culture of moon orchids (*Phalaenopsis amabilis*) was found to be modification 4, with a planting medium composition of ½ MS with the addition of banana puree (100 g/l), potato puree (60 g/l), Agroposht fertilizer (2 ml/l), Vitamin C ipi (50 mg/l), Fish oil (3 ml/l), Atonik (0.2 ml), Coconut water (150 ml/l), Vitamin B ipi (15 mg/l), Activated charcoal (0.5 g/l), Myoinositol (0.1 ml/l). Overall, the research results showed that moon orchids can live and grow well in both VW and MS media.

Research by (Fetrina, 2021) on the potential utilization of tissue culture technology in preparing superior rubber planting materials to produce rubber seedlings. Two in vitro techniques developed for multiplying rubber planting materials through tissue culture are somatic embryogenesis (SE) and organogenesis, inducing axillary buds through microcutting. Problems such as

graft incompatibility and the availability of high-quality scions can be solved with the SE method. Because the success rate of embryo induction in rubber plant explants, both directly (direct) and indirectly (indirect) through callusing, is still limited, rubber plant propagation through tissue culture combines both somatic embryogenesis and organogenesis methods. Through the organogenesis process, embryos are formed, serving as advanced explants to induce axillary buds. This technique has reportedly been successfully used in the mass production of tissue culture rubber seedlings. However, there are still some challenges to consider when mass-producing tissue culture rubber seedlings, including the planlet preparation process in the laboratory and the acclimatization process in greenhouses and fields. Various factors, including the type of explant and clone used, the type and composition of the medium, and the environmental conditions of the culture, affect the success of somatic embryogenesis induction. This process is still limited to specific clones such as PB 260, RRIM 600, PB 330, BPM 24, and IRCA 109.

## CONCLUSION

Tissue culture techniques aim to obtain pathogen-free orchid seedlings quickly and in large quantities. To support their growth, appropriate growing media and the provision of nutrients in the form of growth regulators are required. Based on research findings, the moon orchid can thrive and grow well in both VW (Vacin and Went) and MS (Murashige and Skoog) media. Meanwhile, the use of MS media with the addition of the growth regulator BAP (Benzyl Adenine Purine) in black orchids has an impact on shoot multiplication.

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