THE EFFECT OF SALICYLIC ACID ON IN-VITRO GROWTH OF DENDROBIUM MACROPHYLLUM

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ABSTRACT

Dendrobium macrophyllum, has a slow vegetative phase, posing challenges for orchid farmer and enthusiasts. In vitro tissue culture methods can overcome propagation and cultivation issues. Salicylic acid, being a phenolic compound plays a role in regulating plant growth and development in general. This research aimed to evaluate the effect of adding plant growth regulator (salicylic acid), to Vacin-Went (VW) medium on the growth of D. macrophyllum. The method used was Completely Randomized Design consisting of six salicylic acid concentration (0, 1, 2, 3, 5, 7 ppm) with 5 replications, each replication consisting of 7 plantlets. The observed parameters were plant height, number of leaves, plantlets weight, number of roots, roots length, the greenness of the leaves, the roots anatomy, and the leaves anatomy. The data analyzed with analysis of variance (Anova) at significance rate 5% level followed by Duncan's Multiple Range Test (DMRT) and regression test. The result showed that the addition of 5 ppm salicylic acid to VW medium, significantly enhancesgrowth of plant height, plantlet weight, number of roots, root length, the greenness of the leaves. Salicylic acid also effected the thickness of the anatomical structures of D. macrophyllum roots and leaves.

Keywords: *Antioxidant; Ornamental Plant; Plant Growth Regulator; Plantlets; Tissue Culture.*

INTRODUCTION

Orchidaceae, or orchids, is one of the largest families of flowering plants, with a species count ranging between 25,000 to 35,000 worldwide. Generally, orchids have a long juvenile phase that requires several years of growth before they can flower (Teixera da Silva et al. 2014). Furthermore, orchids do not possess stored food reserves for the embryonic germination process (Bhowmik and Rahman, 2020).

The emerald orchid (Dendrobium macrophyllum) is one of the orchid species known for its relatively slow growth compared to other orchid species. The emerald orchid (D. macrophyllum) categorized as an Appendix II species, indicating that is not currently endangered but faces a high risk of extinction if trade continues without proper regulations governing transactions involving this species. This is consistent with the perspective of Di Simone et al. (2021) regarding the meaning of Appendix II, which refers to regulations that control and govern trade, starting from annual trade reporting to transactions recorded in the Appendix II database, all aimed at preserving the survival of species. One of the efforts to conserve D. macrophyllum orchids includes the use of in vitro tissue culture techniques. One of the conservation efforts aimed at preserving D. macrophyllum orchid is the utilization of in vitro tissue culture techniques.

The success of in vitro tissue culture cultivation depends on several key factors, including the choice of culture medium, the selection of plantlets as the source, the aseptic environment, and the addition of Plant Growth Regulators (PGRs) into each in vitro culture treatment. Salicylic acid is a phenolic compound known to enhance a plant's defense response against diseases, extreme environmental conditions, and regulate plant growth processes (Rivas et al. 2011). Furthermore, salicylic acid plays a crucial role in regulating

many plant bioactivities, such as growth and photosynthesis processes (Al-Taey et al. 2019). Salicylic acid contains chorismate, which is a major precursor in the biosynthesis of aromatic amino acids like tryptophan, phenylalanine, and tyrosine in plants (Bagautdinova et al. 2022). Häusler et al (2014) explained that aromatic amino acids serve as precursors in the phenylpropanoid pathway, which produces phenylalanine. Phenylalanine has been shown to stimulate cytokinin hormones in plants. Based on these insights, Addition salicylic acid plays a positive role in plant growth when applied as a PGRs in tissue culture. The appropriate concentration of salicylic acid is needed to maximize its effect in the in vitro tissue culture of D. macrophyllum. The main aim of this study is to assess the effect of varying concentrations of salicylic acid on the in vitro growth of D. macrophyllum orchids.

METHODS

The study was conducted at the Plant Physiology and Biotechnology Laboratory, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta from March to December 2022. The materials used in this research including Dendrobium macrophyllum orchid plantlets, with plantlet heights ranging from 0.5 to 1 cm, salicylic acid, Vacin-Went (VW) medium, potatoes, bananas, coconut water, and activated charcoal. The essential equipment was used, such as an autoclave, a Laminar Air Flow Cabinet (LAFC), imageJ software, Soil Plant Analysis Development (SPAD) meter, and microscope trinoculer. The method used was Completely Randomized Design consisting of six salicylic acid concentration (0, 1, 2, 3, 5, 7 ppm) with 5 replications, each replication consisting of 7 plantlets.

The research consists of several stages, the preparation of stock solutions, media preparation, planting, and observation in the incubation room. The preparation of equipment and materials begins with the sterilization of the tools to be used in the tissue culture process. The sterilization process was conducted using an autoclave at a temperature of 121°C with a pressure of 1.5 atm for 45 minutes. The preparation of stock solution serves the purpose of creating VW media for the in vitro tissue culture research of D. macrophyllum orchids.

The process continues by combining the stock solution with the salicylic acid treatment using a magnetic stirrer on a hot plate to homogenize and boil the treatment media solution. Following this process, the boiled media is transferred to culture bottles for sterilization using an autoclave and is subsequently stored in the incubation room to ensure freedom from contamination. Media free from contamination is suitable for the subculture planting process under aseptic conditions using a Laminar Air Flow Cabinet (LAFC).

The observed parameters were plant height, number of leaves, plantlet weight, number of roots, root length, leaf color greenness, the roots and leaves anatomy. All recorded at the end of these research. The data analyzed with analysis of variance (Anova) at significance rate 5% level followed by Duncan's Multiple Range Test (DMRT) and regression test.

HASIL DAN PEMBAHASAN

The data resulting from the examination of the effect of salicylic acid on the growth of Dendrobium macrophyllum orchids, including variables such as plant height, number of leaves, plantlet weight, number of roots, and the greenness of the leaves is presented in Table 1.

Table 1. Plant height, number of leaves, plantlet weight, number of roots, and greenness of leaves of D. macrophyllum with salicylic acid application.

Concentrations (ppm)	Observation of variables					
	Plant height (mm)	Number of leaves (unit)	Plantlet weight (mg)	Number of roots (unit)	Root length (mm)	The greenness of leaves (SPAD)
0	38.82±5.26 bc	6.00±0.00	0.453±0.11 b	2.04±0.55 c	60.79±23.56 b	18.56±1.94 b
1	37.85±8.18 bc	4.08±0.84	0.383±0.11 b	4.04±0.89 bc	50.90±15.98 b	24.16±2.39 a
2	35.15±10.58 c	6.02±1.10	0.427±0.18 b	5.08±1.10 b	51.91±16.96 b	23.92±2.36 a
3	50.82±10.20 ab	5.06±0.55	0.614±0.06 b	6.06±2.70 b	69.10±16.50 ab	23.86±3.14 a
5	62.37±12.77 a	5.00±1.00	1.153±0.36 a	9.02±1.92 a	84.75±10.77 a	25.14±1.35 a
7	31.88±10.15 c	5.04±0.89	0.478±0.20 b	6.02±1.64 b	50.63±12.13 b	24.26±1.87 a
Sig.	0.00	0.08	0.00	0.00	0.19	0.00
CV.	32.55	16.36	55.17	44.79	31.93	12.87

Note: Numbers followed by the same letter in the same column indicate no significant difference in the DMRT at a 5% significance level. CV: Coefficient of Variant.

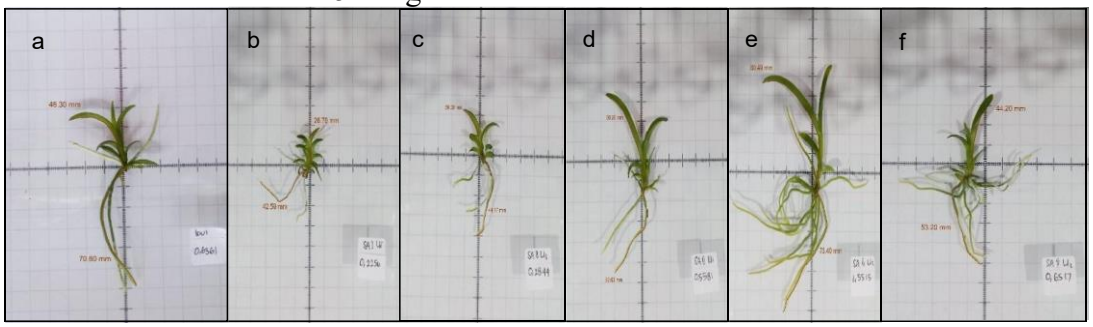


Figure 1. The results of the observation D. macrophyllum with salysilic acid treatment at (a) 0 ppm, (b) 1 ppm, (c) 2 ppm, (d) 3 ppm, (e) 5 ppm, and (f) 7 ppm.

Plant height

The application of salicylic acid can have effect of the plant height of D. macrophyllum orchids at 16 weeks after planting (Table 1). As reported by Khan et al. (2015), salicylic acid plays a role in regulating essential plant physiological processes such as photosynthesis, nitrogen metabolism, proline metabolism, antioxidant defense systems, and others. The addition of salicylic acid to the VW media at a concentration of 3 ppm yielded the most optimal result in terms of plant height. This observation is attributed to the fact that this treatment did not exhibit a significant difference in the highest concentration of salicylic acid.

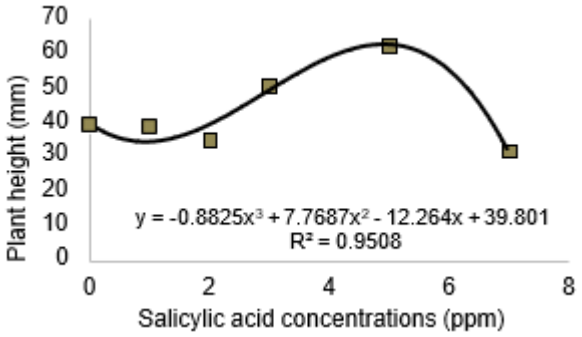


Figure 2. Plant height of D. macrophyllum with salicylic acid applications.

The addition of 1 and 2 ppm salicylic acid resulted in a decrease plant height of D. macrophyllum orchids (Figure 1). However, the application 3 ppm showed an increase in the graph, reaching its peak at a concentration of 5 ppm. After reaching this peak, the graph subsequently declines for concentrations exceeding 5 ppm salicylic acid.

Pamungkas and Puspitasari (2019) explained that plant growth regulators (PGRs)

will have a positive effect on plant growth when given in the right amount. On the other hand, if it's given in excessive amounts, PGRs will inhibit the plant's metabolic processes. This is suspected to be the reason why treatment with salicylic acid above a concentration of 5 ppm resulted in decrease in the height plant.

Number of leaves

The salicylic acid had no significant effect on the number of leaves parameter in D. macrophyllum orchids (Table 1). This is supported by significant values above 5%. These results are consistent with the research of Ortega-Macareno and Iglesias- Andreu (2021) on the effect of salicylic acid in both in vitro and in vivo vanilla cultivation. The highest number of leaves D. macrophyllum orchids was observed in the treatment with 2 ppm salicylic acid, while the lowest number of leaves was recorded in the treatment with 1 ppm salicylicacid.

Plantlet weight

The data showing that the application of salicylic acid to D. macrophyllum orchid plantlets resulted in significantly different plantlet weights, except for 5 ppm concentration (Table 1). According to Khan et al. (2015), salicylic acid plays a role in plant defense mechanism and metabolism. The application of 5 ppm salicylic acid yielded the highest plantlet weight at 1.153 mg, compared to other salicylic acid treatments. Conversely, the lowest plantlet weight was observed in 1 ppm salicylic acid treatment, which recorded 0,383 mg, and showed no significant difference was observed compared to the other treatments salicylic acid.

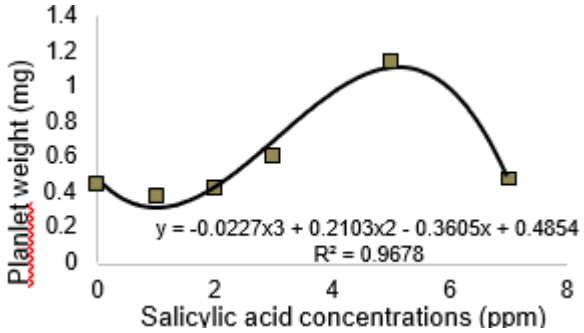


Figure 3. Plantlet weight of D. macrophyllum with salicylic acid applications.

Number of roots

There is no significant difference in the overall parameter number of roots with salicylic acid treatments, except at the 5 ppm concentration (Table 1). This can be attributedto the response of orchid plant roots to the use of salicylic acid in in vitro cultivation. According to Castillo-Pérez etal. (2021), who conducted experiments on in vitro tissue culture of Stanhopea tigrina plants in MS medium with the addition of salicylic acid as a PGR, the results showed that the appropriate concentration of salicylic acid can enhance the number of roots of Stanhopea tigrina orchids. The highest number of roots was observed in the 5 ppm salicylic acid application, while the lowest number of roots was recorded at the treatment without addition salicylic acid.

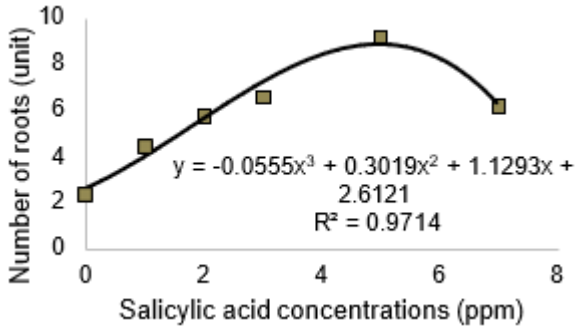


Figure 4. Number of roots of D. macrophyllum with salicylic acid applications.

The graph shows an increased number of roots of D. macrophyllum orchids when treated with salicylic acid. The application of 5 ppm salicylic acid resulted in the highest number of roots (Figure 3). Following the peak number of roots, the curve showed a decline in results, which had a negative impact on the number of roots proliferation in the plants. The polynomial regression line demonstrates a strong relationship with the distribution of the average number of roots D. macrophyllum orchids, with a value of 97.14%.

Root length

The optimal results in the parameter root length of D. macrophyllum orchids were achieved at the 3 ppm salicylic acid concentration (Table 1). The application of 3 ppm salicylic acid exhibited no significant difference compared to all other salicylic acid treatments, including the 5 ppm treatment, which yielded the longest root length. According to Castillo- Pérez et al. (2021), salicylic acid has the capability to stimulate root elongation in Stanhopea tigrina orchids.

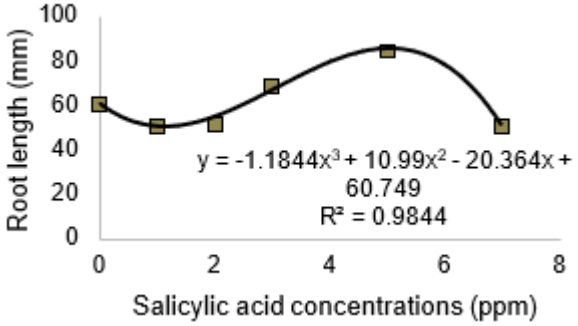


Figure 5. Root length of D. macrophyllum with salicylic acid applications

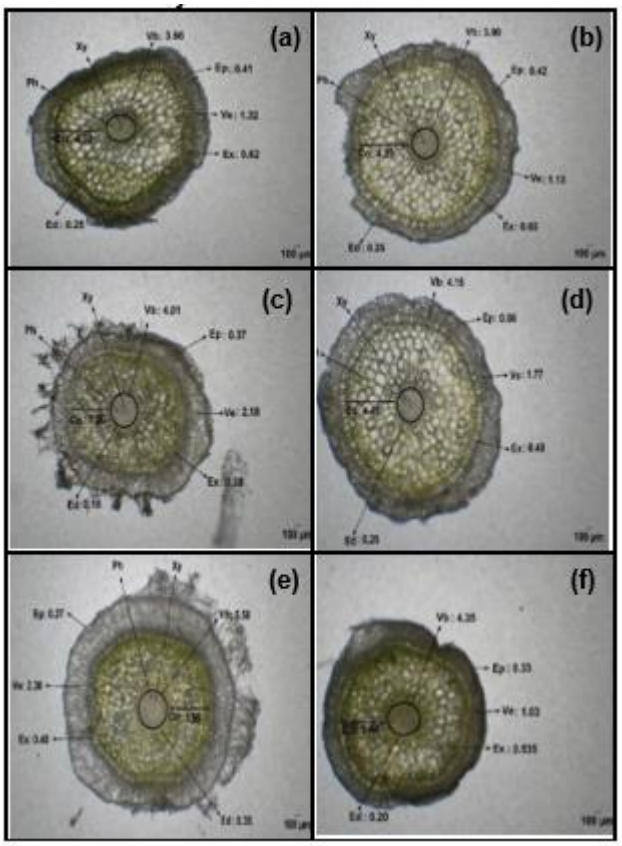
The graph formed cubic polynomial that illustrates that at the 3 ppm application, the graph shows an increase until it reaches its peak at the 5 ppm concentration (Figure 4). However, when applied at concentrations above 5 ppm, there is a decrease in the root length of D.macrophyllum orchids.

Greenness of leaves

The greenness leaves of D. macrophyllum orchids treated with salicylic acid does notexhibit a significant difference compared to the overall salicylic acid treatments added to the VW medium (Table 1). This is consistent with the research by Syahfitri and Nurcahyani (2022), who conducted an analysis of chlorophyll content and greenness leaves in Dendrobium orchids with the addition of salicylic acid. The results of their experiment showed that salicylic acid exhibited values that did not significantly differ from the overall treatments with salicylic acid. The results of this research are also consistent with Kuchlan and Kuchlan (2023), who reported an increase in chlorophyll content in plants subjected to salicylic acid.

This occurs because salicylic acid has a higher concentration of potassium and calcium ions compared to applications without the addition of salicylic acid. Barus et al (2021) explained that the application of salicylic acid can increase the concentrations of K+ and Ca2+ in plants. Both ions are known to have essential roles in chlorophyll synthesis and the regulation of stomata opening and closing in plants. The precise concentration of K+ and Ca2+ ions within the plant's metabolic system plays a vital role in influencing leaf growth and health.

Roots anatomy



Note:

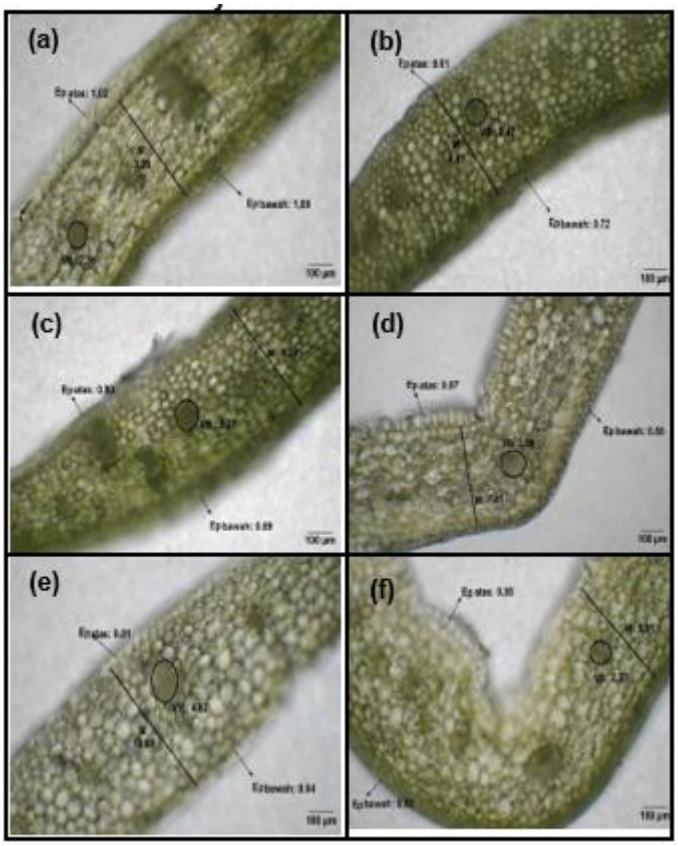
(Ep): Epidermis; (Ve): Velamen; (Ex): Exodermis; (Co): Cortex; (Ed): Endordermis; (VB): Vascular Bundle; (Ph): Phloem; (Xy): Xylem.

Figure 6. The results of the observation of the root anatomy of D. macrophyllum using a microscope under salysilic acid treatment at (a) 0 ppm, (b) 1 ppm, (c) 2 ppm, (d) 3 ppm, (e) 5 ppm, and (f) 7 ppm.

The root anatomy of D. macrophyllum treated with salicylic acid under a 4x magnification microscope revealed that this treatment did not modify the anatomical structure of D. macrophyllum roots (Figure 5). The root of D. macrophyllum is structured of the epidermis, velamen, exodermis, cortex, endodermis, and vascular bundle. The addition of salicylic acid only affects the thickness of each anatomical component of D. macrophyllum roots measured on a 100 µm scale. The thickness of the anatomical features of D. macrophyllum root specimens exhibited variable results on each concentration tested (Figure 5). The D. macrophyllum treated with salicylic acid did not show significant differences in the thickness of the epidermis, exodermis, and endodermis layers. However, the application of 5 ppm salicylic acid resulted in the greatest increase in the thickness of the velamen, vascular bundle, and endodermis, measuring 2.30, 5.58, and 0.35 µm, respectively.

According to Handini et al. (2021), the thickness of velamen serves as a facilitator for water and nutrient absorption, prevents water evaporation, and provides mechanical protection to the plant roots. Treatment with 7 ppm salicylic acid showed the smallest velamen thickness, measuring 1.03 µm. Observations of D. macrophyllum orchid root anatomy without salicylic acid treatment resulted the smallest diameter for vascular bundle thickness, measuring 3.66 µm. The thinnest endodermis layer was observed in the 2 ppm salicylic acid application, measuring 0.25 µm. A decrease in the diameter of orchid D. macrophyllum root vascular bundle can be an indicator of reduced water and mineral conductivity in the plant, while thickening of the endodermis layer may reflect the plant's adaptive response to biotic and abiotic stress.

Leaves anatomy



Note:

(Top Ep): Upper epidermis; (M): Mesophyll; (VB): Vascular Bundle; (Bottom Ep): lower epidermis.

Figure 7. The results of the observation of the root anatomy of D. macrophyllum using a microscope under salysilic acid treatment at (a) 0 ppm, (b) 1 ppm, (c) 2 ppm, (d) 3 ppm, (e) 5 ppm, and (f) 7 ppm.

The result of observing leaves anatomy of D. macrophyllum under a 10x magnification microscope with salicylic acid treatment shows that the anatomical structure of D. macrophyllum leaves consists of the upper epidermis, mesophyll, lower epidermis, and vascular bundle (Figure 6). Salicylic acid treatment influenced the thickness of the anatomical structure of D. macrophyllum leaves on a 100 µm scale. The thickness of the anatomical structure can influence the photosynthesis process of D. macrophyllum.

The salicylic acid treatment resulted in a thinning of both the upper and lower epidermis, an increase in the thickness of leaf mesophyll, and an enlargement of the diameter of the vascular budle area in D. macrophyllum. The 5 ppm salicylic acid treatment

produced the widest leaves, as evidenced by the thickness of each component of D. macrophyllum leaves. According to Nurmaeli and Tarifur (2015), the enlargement of leaf mesophyll is known to increase the chloroplasts in plants. Chloroplasts play a vital role in absorbing red and blue light, which is essential for the photosynthesis process in cultivated plants. The 5 ppm salicylic acid treatment resulted in the greatest thickening of the mesophyll layer of D. macrophyllum leaves, measuring 10.63 µm.

The largest diameter of leaf vascular bundle in D. macrophyllum orchids was found in the 5 ppm salicylic acid treatment, measuring 4.62 µm. Meanwhile, the smallest diameter of leaf vascular bundle was observed in the 7 ppm salicylic acid treatment, measuring 2.23 µm. This is believed to occur because the 7 ppm concentration is too hight for D. macrophyllum. Hartati et al. (2017) explained that excessively high concentrations of growth regulators can be toxic to plants.

CONCLUSIONS

The application of salicylic acid at a concentration of 5 ppm yielded the best results in terms of plant height, plantlet weight, number of roots, root length, and greenness of leaves of D. macrophyllum at 16 weeks after planting.

Salicylic acid application did not influence the number of leaves D. macrophyllum at 16 weeks after planting.

The 5 ppm salicylic acid treatment resulted in the thickest velamen, root vascular bundle, endodermis, mesophyll, and leaf vascular bundle components.

Suggestions

The recommendation is to use salicylic acid concentration of 5 ppm to achieve better results in terms of plant height, plantlet weight, number of roots, and the greenness of leaves of D. macrophyllum orchids. Additionally, a recommendation to pursue further research regarding the use of a 5 ppm salicylic acid concentration in the ex vitro cultivation of D. macrophyllum is highly encourage

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