JPPIPA 10(12) (2024)

Jurnal Penelitian Pendidikan IPA

Journal of Research in Science Education

http://jppipa.unram.ac.id/index.php/jppipa/index

Optimizing Thidiazuron Concentrations for Enhanced In Vitro Protocorm Growth of *Grammatophyllum stapeliiflorum* Orchid

Zozy Aneloi Noli[1*], Naura Muthiah Arli[1], Suwirmen[1]

[1] Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Andalas, Sumatera Barat, Padang, Indonesia

Received: August 26, 2024
Revised: November 04, 2024
Accepted: December 25, 2024
Published: December 31, 2024

Corresponding Author:
Zozy Aneloi Noli
zozynoli@sci.unand.ac.id

DOI: 10.29303/jppipa.v10i12.9067

Abstract: Cytokinins are growth regulators that play a crucial role in cell division and shoot growth. Thidiazuron, a potent cytokinin, was the focus of our research. The research aimed to determine the effect of several concentrations of Thidiazuron on the protocorm growth of *Grammatophyllum stapeliiflorum* and to identify the best concentration of Thidiazuron (mg/L) for the protocorm growth of *G. stapeliflorum* orchids in vitro. The research was conducted from January until March 2022 at the Plant Physiology Laboratory, Universitas Andalas, Padang. The research used a Completely Randomized Design (CRD) with 5 treatments and 5 replications. Several concentrations of Thidiazuron were added to MS media with various concentrations: 0 mg L^{-1} (A), 0.50 mg L^{-1} (B), 1 mg L^{-1} (C), 1.50 mg L^{-1}(D), and 2 mg L^{-1} (E). The parameters in this research were the percentage of life explants, day of shoot appearance, number of shoots, shoot length, number of leaves, number of roots, and root length. The results showed that Thidiazuron significantly affected the protocorm growth of the *G. stapeliiflorum* orchid compared to the control treatment. Notably, 2 mg L^{-1} Thidiazuron emerged as the best concentration for all growth parameters of protocorm *G. stapeliiflorum*, a finding that could significantly impact our understanding of orchid cultivation and plant physiology.

Keywords: *Grammatophyllum stapeliiflorum; In Vitro*; MS Media; Protocorm; Thidiazuron

Introduction

Orchids are ornamental plants that have the potential to be developed because they have commercial value with various shapes, colors, sizes, distinctive aromas, and long-lasting flower qualities (Nurcahyani et al., 2017). Orchids have 25,000 species and 800 genera, some of which are difficult to find and are nearly extinct. The Grammatophyllum genus is an orchid that is threatened with extinction. There are 12 species in the genus Grammatophyllum worldwide, one of which is *Grammatophyllum stapeliiflorum*. The *G. stapeliiflorum* orchid is a type of orchid that is slowly becoming rare and difficult to find in its natural habitat due to illegal hunting and habitat destruction through deforestation and land conversion (Isda & Fatonah, 2014; Salsabila et al., 2022). The orchid *G. stapeliiflorum* is included in the Appendix II group, a list of species that are not necessarily threatened with extinction but in which trade must be controlled in order to avoid utilization incompatible with their survival (CITES, 2021).

Regarding orchid conservation, in vitro propagation is an effective strategy to overcome poaching. Controlling illegal orchid poaching can be achieved through in vitro propagation, a technique that grows plant parts under sterile conditions. This method, which involves growing plant parts under aseptic conditions, was chosen for its numerous advantages: it requires minimal land, facilitates the propagation of plants that are difficult or slow to grow, allows for year-

How to Cite:
Noli, Z. A., Arli, N. M., & Suwirmen. (2024). Optimizing Thidiazuron Concentrations for Enhanced In Vitro Protocorm Growth of Grammatophyllum stapeliiflorum Orchid. *Jurnal Penelitian Pendidikan IPA*, *10*(12), 10886–10892. https://doi.org/10.29303/jppipa.v10i12.9067

round cultivation, produces healthier and more uniform plants, and enables long-term storage of plant stock (Kapoor & Singh, 2014). Some factors contributing to tissue culture techniques include seed maturity, genotype, media, growth regulators, carbohydrates, and explant type (Reddy et al., 2021). Medium is an important thing in tissue culture. Medium generally contains macronutrients and micronutrients in the form of organic salts in certain levels and ratios, sources of carbohydrates, water, amino acids, vitamins, and growth regulators (Chen et al., 2015).

Plant Growth Regulators (PGR) play a crucial role in the success of in vitro culture. When used in small amounts, these non-nutritive organic compounds can either support, inhibit, or alter physiological processes in plants. Their primary function is stimulating growth and morphogenesis in cell, tissue, and organ cultures (Sulichantini et al., 2020). The activities of PGRs are not only restricted to the longitudinal growth of plants, but they also affect a plethora of key processes including seed germination, defoliation, flowering, fruit formation and ripening and fruit drop, among others (Rademacher, 2015). In addition, PGRs are frequently employed in agriculture, horticulture and viticulture for various benefits, such as improving morphological structure, increasing resistance and tolerance against biotic and abiotic stresses, and for qualitatively and quantitatively enhancing yields (Naeem & Aftab, 2022).

PGR are exogenously supplied to positively modulate several biological processes in plants, such as cell division, transpiration, pho tosynthesis, ascorbate–glutathione cycle, nitrogen metabolism and antioxidant activities in both normal and stressful environ ments (Hossain et al., 2022; Malek et al., 2022; Sytar et al., 2019; Tahaei et al., 2022). PGRs are being used as external supplements for medicinal plants to increase the production of secondary metabolites, thereby improving plant defense against stresses (Jamwal et al., 2018). The most commonly used PGR are auxins, cytokinins, and gibberellins (Antala et al., 2019; Arli et al., 2023; Deli et al., 2015; Shabi et al., 2018). Cytokinin, for instance, is a PGR that promotes cell division and shoot growth in tissue culture. It comes in various types, such as BAP, Kinetin, and Thidiazuron, each with its unique function.

TDZ is one of the most widely used plant growth regulators for induction of de novo regeneration, shoot organogenesis, and somatic embryogenesis. In tissue culture systems, TDZ acts through the adenine-type cytokinin activity either by stimulating endogenous cytokinins or binding to cytokinin receptors. Thidiazuron, for example, is known for its ability to stimulate shoot formation (Restanto et al., 2018). In comparison to other PGRs, TDZ has been defined as the most effective and efficient on enhancing the levels of important metabolites in many medicinal plants (Kumari et al., 2017; Kumari et al., 2018). TDZ inhibits the leaf yellowing in different plants, such as the one occurring after pinching potted rose plants (Celikel et al., 2021). TDZ has recently shown to enhance secondary metabolites production (Debnath et al., 2018).

Based on the research conducted by Karyanti (2017), it is evident that adding thidiazuron 0.5 mg L^{-1} resulted in an average number of shoots formed, specifically 8 shoots planted in MS media on *Vanda Douglas* orchids. Similarly, the study by Widawati et al. (2020) showed that the addition of 2 mg L^{-1} thidiazuron resulted in the highest percentage of shoot formation compared to other treatments in *Vanda tricolor* orchids. These findings underscore the need for further research on the effect of thidiazuron on orchid growth.

This research aims to determine the effect of different thidiazuron concentrations on the growth of *G. stapeliiflorum* protocorm in vitro, and to identify the optimal thidiazuron concentration (mg/l) for the in vitro growth of *G. stapeliiflorum* orchid protocorm.

Method

Location and Time

The research was conducted from Januari 2022 to March 2022 at Laboratory of Plant Physiology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang.

Research Design

The research used experimental method. The research design in this study was Completely Randomized Design (CRD) with 5 treatments and 5 replications. Several concentrations of Thidiazuron were added to MS media with various concentration 0 mg L^{-1} (A); 0.50 mg L^{-1} (B); 1 mg L^{-1} (C); 1.50 mg L^{-1} (D); and 2 mg L^{-1} (E). The parameters in this research were the percentage of life explants, day of shoot appears, number of shoot, shoot length, number of leaves, number of root and root length

Materials and Tools

The materials used in this research was *Grammatophyllum stapeliiflorum*, protocorm, MS media, Thidiazuron, sugar, agar, alcohol 70%, spritus, HCl 0.1 N and NaOH 0.1 N. The tools used in this research was culture bottles, autoclaves, measuring cups 100 ml, Beaker glass 500 ml, aluminum foil, Laminar Air Flow Cabinet (LAFC), hot plates, and magnetic stirres.

Protocorm Growth Media

The media used in this research was Murashige ang Skoog (MS). MS media is made 1 liter by adding sugar

30 grams and agars 7 grams and the addition of Thidiazuron according to the concentration of the treatment. The media sterilized using an autoclave at a pressure of 17.5 psi and a temperature of 121 °C.

Subcultures

Subculture of the protocorm of the orchid *G. stapeliiflorum* is carried out by transferring the protocorm into the treatment medium. Each bottle is filled with 4 explants. Explants were incubated for 8 weeks at room temperature 24 °C

Data Analysis

Data were analyzed statistically using SPSS version 20. If the effect of the treatments was significantly different, then it was continued with Duncan's New Multiple Range Test (DNMRT) with a level of 5%.

Result and Discussion

Percentage of explant survival (%)

The result of the percentage of explant survival of protocorm *Grammatophyllum stapeliiflorum* with several concentrations of thidiazuron can be seen in Figure 1.

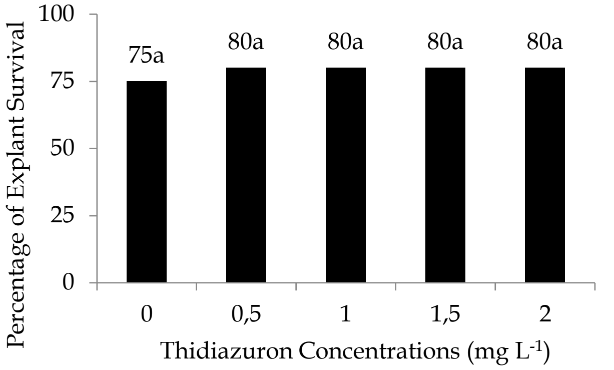

Figure 1. Percentage of explant survival of Protocorm Orchid *G. stapelliflorum* after being given several concentrations of thidiazuron during 8 weeks of culture.

The application of thidiazuron to the protocorm growth medium of *G. stapeliiforum* orchid showed no significant difference in the percentage of life explants. Adding thidiazuron 0.5 - 2.0 mg/L was not significantly different from the treatment without thidiazuron (Figure 1), this is because the concentration of thidiazuron given has not been able to significantly increase the percentage of explant survival. Eventhough the effect was not significantly different, the addition of thidiazuron with several concentrations better than control. This is related to the role of thidiazuron which can induce the growth and multiplication of protocorm. Cytokinin growth regulators can affect the percentage of explant survival. If the availability of cytokinins is very limited, cell division will be reduced and affected the explant survival (Li et al., 2022; Saifuddin, 2016).

The percentage of explant survival indicates the ability of explants to survive and adapt to the medium. The percentage of explant survival influenced by the ability of explants to absorb nutrients and growth regulators contained in the growth media (Rineksane et al., 2015; Sudheer et al., 2022). The percentage of explant survival influenced by the growth regulator used. Explants can survive due to the positive reaction of the plant to growth regulator. In in vitro culture, explants are important factors in growth that must be considered for the sterility of the explants. Explants must have high vitality and be able to grow continuously so that explants can survive (Heriansyah, 2019).

Time of Shoot Appearance (Days), Number of Shoots, Shoot Length (mm), and Number of Leaves

The result of Time of Shoot Appearance (Days), Number of Shoots, Shoot Length (mm), and Number of Leaves of protocorm *Grammatophyllum stapeliiflorum* with several concentrations of thidiazuron can be seen in Table 1.

Table 1. The average time of shoot appearance (days), number of shoots, shoot length (mm), and number of leaves of protocorm *Grammatophyllum stapeliiflorum* after being given several concentrations of thidiazuron during 8 weeks of culture

Thidiazuron concentrations (mg/L)	The average of time shoot appearance (days)	The average of number of shoots	The average of shoot length (mm)	The average of number of leaves
A. 0	20.75 c	0.90 a	8.15 a	2.55 a
B. 0.5	19.90 c	1.10 a	9.40 a	2.65 a
C. 1	15.10 b	1.25 a	11.90 b	4.35 b
D. 1.5	12.60 b	1.30 a	12.55 b	4.85 b
E. 2	9.40 a	1.80 b	15.00 c	7.80 c

Notes: The different superscript letters after the mean values (a, b, c) indicate statistically significant differences between variants at $p < 0.05$.

The application of thidiazuron to the G. stapeliiforum protocorm yielded significantly different results in terms of the average time for shoot appearance, number of shoots, shoot length, and number of leaves. Notably, the treatment with thidiazuron at 2.0 mg/L emerged as the most effective

(Table 1). It's fascinating to note that the higher the thidiazuron concentration, the faster the plant growth, particularly at the 2.0 mg/L concentration. This finding opens up exciting possibilities for accelerating plant growth, including shoots and leaves, in horticultural practices.

Thidiazuron, when used at the appropriate concentration, has a unique and impressive function. It accelerates the emergence of orchid plant shoots of G. stapeliiforum by encouraging the transformation of cytokinin ribonucleotides into more biologically active ribonucleosides. This unique function of thidiazuron, stimulating shoot formation faster than other types of cytokinins (Lyczko et al., 2022; Restanto et al., 2018). TDZ promotes plant organogenesis (shoot regeneration) and regeneration (Debnath et al., 2018). For example, TDZ induced regeneration in African violets Saintpaulia ionantha) (Padmanabhan et al., 2014; Padmanabhan et al., 2015).

Based on research conducted by Widawati et al. (2020) showed that 2.0 mg/L thidiazuron resulted in the highest percentage of shoot formation compared to other treatments on the *Vanda tricolor* orchid. According to Restanto et al. (2018), the high growth of shoots in explants is due to the proper interaction between endogenous and exogenous hormones. According to (Arli & Noli, 2024), thidiazuron was the best cytokinin for in vitroshoot inductionof D. lasianthera.

The optimum concentration of thidiazuron is very important because it can accelerate shoot formation, while a deficiency or excess can result in disrupted shoot growth (Sudiyanti et al., 2017). According to Karyanti (2017), cytokinins affect the processes of cell division, seed germination, shoot proliferation, and regulate auxin transport. Understanding these processes can inspire new strategies for plant growth and development, but it also requires careful attention to the details of the research.

Growth regulators play an important role in determining the direction of cell differentiation. The faster the shoots are formed, the more nutrients absorbed by the explants will increase, accelerating the formation of new individual explants. Because all the explants are in the culture bottle, the only source of nutrition is in the agar medium. Available nutrients are the main factor in supporting the development of explants to form new plants (Ningrum et al., 2017).

Thidiazuron with the optimum concentration can affect leaf growth. Giving Thidiazuron can stimulate cell division in explant and stimulate leaf growth. Adding Thidiazuron at the right concentration can regulate plant physiological processes to stimulate plant growth; this is related to the function of cytokinins, which can stimulate plant growth. According to Restanto et al. (2018), Thidiazuron is essential in stimulating cell division in explant tissue and the growth of shoots and leaves. The presence of cytokinin and auxin hormones in plants can increase plant growth (Ngadiani & Jayanti, 2021).

Number of Roots and Roots Length (mm)

The resulting number of roots and root length (mm) of protocorm *G. stapeliiflorum* with several concentrations of thidiazuron can be seen in Table 2.

Table 2. The average number of roots and root length (mm) of *Grammatophyllum stapeliiflorum* protocorm after being given several concentrations of thidiazuron during 8 weeks of culture

Thidiazuron concentrations (mg/l)	The average of number of roots	The average of root length (mm)
0	3.75 a	16.30 a
0.50	4.35a	17.50a
1	5.80 a	17.65a
1.50	6.00a	18.25a
2	12.25 b	22.85 b

Notes: The different superscript letters after the mean values (a,b) indicate statistically significant differences between variants at $p < 0.05$.

Thidiazuron application to the *G. stapeliiforum* orchid protocorm showed significantly different results on the average number of roots and root length. Giving Thidiazuron 2.0 mg/L was the best treatment. The higher the concentration of Thidiazuron, the faster the growth of plants, including leaves and roots (2.0 mg/L concentration) (Table 2).

The addition of Thidiazuron to the planting medium with the appropriate concentration can affect root growth. Giving Thidiazuron can stimulate cell division in explant tissue and stimulate root growth. According to Saifuddin (2016), the effect of growth regulators is closely related to their concentration. At the right concentration it can regulate plant physiological processes so that it can stimulate plant growth, including roots.

According to Restanto et al. (2018), high root growth in explants is due to the proper interaction between endogenous hormones and exogenous hormones added. PGR can stimulate plant growth which affects root formation and root length which causes plants to absorb more water and nutrients for plant growth, especially auxin. Root formation is inseparable from the process of active tissue division and differentiation and is strengthened by organic and inorganic compounds contained in the media (Kurniati et al., 2020).

Thidiazuron application to the G. stapeliiforum orchid protocorm showed significantly different results on the average number of roots and root length. Giving Thidiazuron 2.0 mg/L was the best treatment. The higher the concentration of Thidiazuron, the faster the growth of plants, including leaves and roots (2.0 mg/L concentration) (Table 2). Adding Thidiazuron to the planting medium with the appropriate concentration can significantly affect root growth. This practical application of Thidiazuron can stimulate cell division in explant tissue and promote root growth. As Saifuddin (2016) notes, the effect of growth regulators is closely related to their concentration. At the right concentration, they can regulate plant physiological processes and stimulate plant growth, including roots.

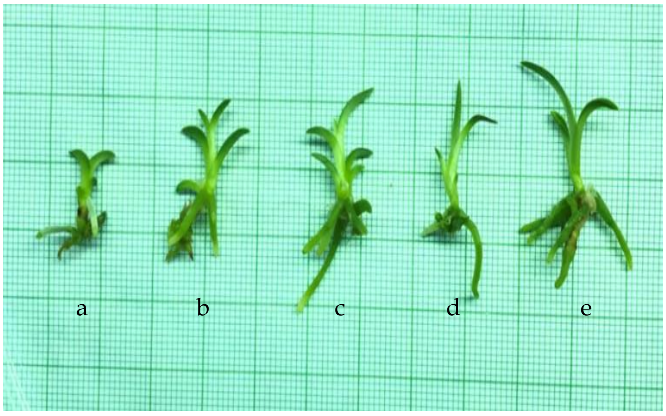

Figure 2. Effect of Several Concentrations of Thidiazuron on Protocorm Growth of *Grammatophyllum stapeliiflorum* after 8 weeks of culture (a) 0 mg L^{-1}, b) 0.50 mg L^{-1}, c) 1 mg L^{-1}, d) 1.50 mg L^{-1}, and e) 2 mg L^{-1}).

As Restanto et al. (2018) point out, the high root growth in explants is a result of the intricate interplay between endogenous hormones and the exogenous hormones added. PGR, for instance, can stimulate plant growth and influence root formation and length. This, in turn, leads to increased water and nutrient absorption for plant growth, particularly of auxin. The process of root formation, which is closely tied to active tissue division and differentiation, is further bolstered by the organic and inorganic compounds present in the media (Kurniati et al., 2020).

Conclusion

The application of varying concentrations of thidiazuron had a significant impact on the growth of *Grammatophyllum stapeliiflorum* protocorms. Among the tested concentrations, 2 mg L^{-1} thidiazuron proved to be the most effective in promoting optimal protocorm growth.

Acknowledgments

The authors are thankful to the Department of Biology, FMIPA, Andalas University for providing facilities for authors research.

Author Contributions

Conceptualization, Z.A.N. N.M.A. and S; methodology, Z.A.N.; software, N.M.A.; validation, Z.A.N. N.M.A. S.; formal analysis, N.M.A.; investigation, N.M.A.; resources, N.M.A.; data curation, N.M.A.; writing—original draft preparation, N.M.A.; writing—review and editing, Z.A.N. and S.; visualization, N.M.A.; supervision, Z.A.N. and S.; project administration, Z.A.N. N.M.A. and S.

Funding

This research received no external funding.

Conflicts of Interest

The authors declare no conflict of interest.

References

Antala, M., Sytar, O., Rastogi, A., & Brestic, M. (2019). Potential of karrikins as novel plant growth regulators in agriculture. *Plants*, *9*, 43. https://doi.org/10.3390/plants9010043

Arli, N. M., & Noli, Z. A. (2024). Shoot Induction of Dendrobium lasianthera J.J.Smith withSeveral Types of Cytokinins through In Vitro Culture. *Jurnal Penelitian Pendidikan IPA*, *10*(4), 2059–2064. https://doi.org/10.29303/jppipa.v10i4.5324

Arli, N. M., Noli, Z. A., & Idris, M. (2023). The Application of Plant Growth Regulators in Propagation of Dendrobium Orchid with Thin Cell Layer (TCL) Technique:A Review. *International Journal of Progressive Sciences And*, 416–422. https://doi.org/10.52155/ijpsat.v39.2.5505

Celikel, F. G., Zhang, Q., Zhang, Y., Reid, M. S., & Jiang, C. Z. (2021). A cytokinin analog thidiazuron suppresses shoot growth in potted rose plants via the gibberellic acid pathway. *Front. Plant Sci*, *12*, 6. https://doi.org/10.3389/fpls.2021.639717

Chen, Y., Goodale, U. M., Fan, X. L., & Gao, J. Y. (2015). Asymbiotic seed germination and in vitro seedling development of Paphiopedilum spicerianum: An orchid with an extremely small population in China. *Global Ecology and Conservation*, *3*, 367–378. https://doi.org/10.1016/j.gecco.2015.01.002

CITES. (2021). *Grammatophyllum stapeliiflorum*. https://checklist.cites.org

Debnath, S. C., Ahmad, N., & Faisal, M. (2018). *Thidiazuron: From Urea Derivative to Plant Growth Regulator*. Singapore: Springer. https://doi.org/10.1007/978-981-10-8004-3

Deli, R. N., Noli, Z. A., & Suwirmen. (2015). Respon Pertumbuhan Nodus (Artemesia vulgaris L.) pada

Medium Murashige-Skoog dengan Penambahan Beberapa Zat Pengatur Tumbuh Secara In Vitro. *Jurnal Biologi Universitas Andalas*, *4*(3), 162–168. https://doi.org/10.25077/jbioua.4.3.%25p.2015

Heriansyah, P. (2019). Multiplikasi Embrio Somatis Tanaman Anggrek (Dendrobium sp.) dengan Pemberian Kinetin dan Sukrosa secara In Vitro. *Jurnal Ilmiah Pertanian*, *15*(2), 67–78. https://doi.org/10.31849/jip.v15i2.1974

Hossain, A., Pamanick, B., Venugopalan, V. K., Ibrahimova, U., Rahman, M. A., & Siyal, A. L. (2022). Emerging roles of plant growth regulators for plants adaptation to abiotic stress–induced oxidative stress. In M. Naeem & T. Aftab (Eds.), *Emerging Plant Growth Regulators in Agriculture* (pp. 1–72). Academic Press. https://doi.org/10.1016/B978-0-323-91005-7.00010-2

Isda, M. N., & Fatonah, S. (2014). Induksi Akar pada Eksplan Tunas Anggrek (Grammatophylum scriptum var. citrinum) secara In Vitro pada Media MS dengan Penambahan NAA Dan BAP. *Al-Kauniyah Jurnal Biologi*, *7*(2), 53–57. https://doi.org/10.15408/kauniyah.v7i2.2715

Jamwal, K., Bhattacharya, S., & Puri, S. (2018). Plant growth regulator medi ated consequences of secondary metabolites in medicinal plants. *Journal of Applied Research on Medicinal and Aromatic Plants*, *9*, 26–38. https://doi.org/10.1016/j.jarmap.2017.12.003

Kapoor, M. A., & Singh, A. (2014). In Vitro Regeneration and Bublet Production in Asiatic Lilium. *Bionature*, *34*(1), 15–20. Retrieved from https://shorturl.asia/s6EgM

Karyanti. (2017). Pengaruh Beberapa Jenis Sitokinin Pada Multiplikasi Tunas Anggrek Vanda douglas Secara In Vitro. *Jurnal Bioteknologi & Biosains Indonesia*, *4*(1), 36–42. https://doi.org/10.29122/jbbi.v4i1.2200

Kumari, A., Baskaran, P., Plačková, L., Omámiková, H., Nisler, J., Doležal, K., & Staden, J. (2018). Plant growth regulator interactions in physiological processes for controlling plant regeneration and in vitro development of Tulbaghia simmleri. *Journal Plant Physiol*, *223*, 65–71. https://doi.org/10.1016/j.jplph.2018.01.005

Kumari, A., Baskaran, P., & Staden, J. (2017). In vitro propagation via organogenesis and embryogenesis of Cyrtanthus mackenii: A valuable threatened medicinal plant. *Plant Cell Tissue Organ Cult*, *131*, 407–415. https://doi.org/10.1007/s11240-017-1293-5

Kurniati, R., Khairatunnisa, F., & Indrayanti, R. (2020). Perbanyakan Lili Arumsari Menggunakan Media Generik secara In Vitro. *J. Hort. Indonesia*, *11*(2), 140–148. https://doi.org/10.29244/jhi.11.2.140-148

Li, Y. Y., Hao, Z. G., Miao, S., Zhang, X., Li, J. Q., Guo, S. X., & Lee, Y. I. (2022). Profiles of Cytokinins Metabolic Genes and Endogenous Cytokinins Dynamics during Shoot Multiplication In Vitro of Phalaenopsis. *International Journal of Molecular Sciences*, *23*(7). https://doi.org/10.3390/ijms23073755

Lyczko, J., Piotrowski, K., Kolasa, K., Galek, R., & Szummy, A. (2022). Mentha piperita L. Micropropagation and the Potential Influence of Plant Growth Regulators on Volatile Organic Compound Composition. *Molecules*, *25*(11). https://doi.org/10.3390/molecules25112652

Malek, M., Ghaderi-Far, F., Torabi, B., & Sadeghipour, H. R. (2022). Dynamics of seed dormancy and germination at high temperature stress is affected by priming and phytohormones in rapeseed (Brassica napus L. *Journal of Plant Physiology*, *269*, 153614. https://doi.org/Journal of Plant Physiology

Naeem, M., & Aftab, T. (2022). *Emerging Plant Growth Regulators in Agriculture: Roles in Stress Tolerance*. Academic Press.

Ngadiani, & Jayanti, T. (2021). Pengaruh Pemberian Hormon NAA dan BAP Pada Media MS (Murashige and Skoog) terhadap Pertumbuhan Anggrek Vanda tricolor Secara In Vitro. *Stigma*, *14*(2), 89–98. https://doi.org/10.36456/stigma.14.02.4885.89-98

Ningrum, E. F. C., Rosyidi, I. N., Puspasari, R. R., & Semiarti, E. (2017). Perkembangan Awal Protocorm Anggrek Phalaenopsis amabilis secara In Vitro setelah Penambahan Zat Pengatur Tumbuh α-Naphtalene Acetic Acid dan Thidiazuron. *Biosfera*, *34*(1), 9–14. https://doi.org/10.20884/1.mib.2017.34.1.393

Nurcahyani, E., Lande, M. L., & Noviantia, R. A. (2017). Induced Resistance of Moon Orchid Planlet (Phalaenopsis amabilis (L.) as Result of The In Vitro Salicylic Acid Selection Toward to Fusarium oxysporum. *Jurnal Penelitian Pertanian Terapan*, *17*(2), 132–137. https://doi.org/10.25181/jppt.v17i2.29

Padmanabhan, P., Murch, S. J., Sullivan, J. A., & Saxena, P. K. (2014). Development of an Efficient Protocol for High Frequency in Vitro Regeneration of a Horticultural Plant Primulina tamiana (B.L. Burtt) Mich. *Möller & A. Webber. Canadian Journal of Plant Science*, *94*(7). https://doi.org/10.4141/CJPS-2014-066

Padmanabhan, P., Murch, S. J., Sullivan, J. A., & Saxena,

P. K. (2015). Micropropagation of Primulina dryas (Dunn) Mich. *Möller & A. Webber: High Frequency Regeneration from Leaf Explants. Scientia Horticulturae*, *192*. https://doi.org/10.1016/j.scienta.2015.06.020

Rademacher, W. (2015). Plant growth regulators: backgrounds and uses in plant production. *Journal of Plant Growth Regulation*, *34*, 845–872. https://doi.org/10.1007/s00344-015-9541-6

Reddy, J., Niveshika, N., Shaju, A., Jose, A., & Betty, A. (2021). Plant Growth Regulators Used for in Vitro Micropropagation of Orchids: A Research Review. *International Journal of Biological Research*, *8*(1), 37–42. Retrieved from https://shorturl.asia/roGh4

Restanto, D. P., Kriswanto, B., Khozim, M. N., & Soeparjono, S. (2018). Kajian Thidiazuron (TDZ) dalam Induksi Plb Anggrek Phalaenopsis sp. secara In Vitro. *Agritrop*, *16*(1), 176–185. https://doi.org/10.32528/agr.v16i1.1561

Rineksane, I. A., Nurjaman, D., & Isnawan, B. H. (2015). *Kajian Penggunaan Jenis Eksplan dan Thidiazuron untuk Multiplikasi Tunas Adventif Tanaman Sarang Semut (Myrmecodia pendens Merr)*. Perry). Prosiding Seminar Nasional Forum Komunikasi Perguruan Tinggi Pertanian Indonesia. Retrieved from https://repo-dosen.ulm.ac.id/handle/123456789/12045?show=full

Saifuddin, F. (2016). Pengaruh Indole Acetic Acid (IAA) terhadap Hasil Berat Basah Akhir Planlet Kultur Jaringan Tanaman Jernang (Daemonorops draco Willd. Blume. *Jurnal Edukasi Dan Sains Biologi*, *5*(1), 14–17. Retrieved from https://www.neliti.com/publications/77472/pengaruh-indole-acetic-acid-iaa-terhadap-hasil-berat-basah-akhir-plantlet-kultur

Salsabila, S. N., Fatimah, K., Noorhazira, S., Halimatun, T. S. T. A. B., Aurifullah, M., & Suhana, Z. (2022). Effect of Coconut Water and Peptone in Micropropagation of Phalaenopsis amabilis (L.) Blume Orchid. *IOP Conference Series: Earth and Environmental Science*, *1102*(1), 12002. https://doi.org/10.1088/1755-1315/1102/1/012002

Shabi, A., Tahir, S., Wani, I., Dar, A. S., & Nisar, S. (2018). Adenine Type and Diphenyl Urea Derived Cytokinins Improve the Postharvest Performance of Iris Germanica L. *Cut Scapes. Physiology and Molecular Biology of Plants*, *24*(6). https://doi.org/10.1007/s12298-018-0554-z

Sudheer, W. N., Praveen, N., Al-Khayri, J. M., & Jain, S. M. (2022). Role of Plant Tissue Culture Medium Components. *Advances in Plant Tissue Culture*. https://doi.org/10.1016/B978-0-323-90795-8.00012-6

Sudiyanti, S., Rusbana, T. B., & Susiyanti. (2017). Inisiasi Tunas Kokoleceran (Vatica bantamensis) pada Berbagai Jenis Media Tanam dan Konsentrasi BAP (Benzyl Amino Purine Secara In Vitro. *Jurnal Agro*, *4*(1), 1–14. Retrieved from https://shorturl.asia/gBJNU

Sulichantini, E. D., Susylowati, & Ramadhan, A. (2020). Respon Morfogenesis Eksplan Pucuk Anggrek Tebu (Grammatophylum speciosum Blume) Secara In Vitro Terhadap Beberapa Konsentrasi Kinetin. *Agrifor*, *19*(2), 281–292. https://doi.org/10.31293/af.v19i2.4718

Sytar, O., Kumari, P., Yadav, S., Brestic, M., & Rastogi, A. (2019). Phytohor mone priming: regulator for heavy metal stress in plants. *Journal of Plant Growth Regulation*, *38*, 739–752. https://doi.org/10.1007/S00344-018-9886-8

Tahaei, S. A., Nasri, M., Soleymani, A., Ghooshchi, F., & Oveysi, M. (2022). Plant growth regulators affecting corn (Zea mays L.) physiology and rab17 expression under drought conditions. *Biocatalysis and Agricultural Biotechnology*, *41*, 102288. https://doi.org/10.1016/j.bcab.2022.102288

Widawati, R. A., Semiarti, E., & Widyastuti, C. T. (2020). Pengaruh Thidiazuron dan Air Kelapa terhadap Perkembangan in vitro Protokorm Vanda tricolor Lindley var. suavis. *SEMINAR NASIONAL BIOLOGI 2019 (IP2B III)*, 318–322. Retrieved from https://shorturl.asia/sLZj4