Effect of Hardening Duration and Paclobutrazol Application on Vegetative Growth of Dendrobium sutiknoi Plantlets during Acclimatization

Sari Kusuma Dewi*[1], Yuni Sri Rahayu[1], Yuliani[1], Novita Kartika Indah[1], Qiara Azzahra Putri Hariyono[1], Iftitah Naurah Salsabila Hafsa[1], Nurul Qur'ani[2]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, 60231, Indonesia

[2]Food Security and Agriculture Department Surabaya, 60233, Jawa Timur, Indonesia

***Corresponding Author**

DOI: https://doi.org/10.51244/IJRSI.2026.1311000066

Received: 25 October 2026; Accepted: 30 October 2026; Published: 23 November 2026

ABSTRACT

Hardening is a critical stage in the acclimatization of in vitro-derived orchid plantlets, determining their ability to survive and grow under ex vitro conditions. Dendrobium sutiknoi, an endemic Indonesian orchid, requires an effective hardening strategy to improve its vegetative growth during acclimatization. This study aimed to evaluate the effects of different hardening durations and paclobutrazol concentrations on the growth of D. sutiknoi plantlets. The experiment was arranged in a randomized complete block design consisting of eight treatment combinations derived from two hardening durations (2 and 4 weeks) and four paclobutrazol concentrations (0, 10, 15, and 20 ppm), with three replications. Plant height increment, number of leaves, and plantlet survival percentage were observed and analyzed using two-way analysis of variance. The combined treatments significantly affected plant height increment and leaf number ($p < 0.001$), but had no significant effect on plantlet survival ($p > 0.05$). The highest plant height increment was obtained from plantlets hardened for two weeks without paclobutrazol, whereas the highest leaf number was recorded in plantlets hardened for four weeks with 10 ppm paclobutrazol. These findings indicate that hardening duration and paclobutrazol application mainly regulate vegetative growth rather than plantlet survival and may provide useful information for developing species-specific hardening protocols to support the propagation and conservation of D. sutiknoi.

Keywords: conservation, in vitro culture, orchid propagation; plant growth regulator; vegetative growth

INTRODUCTION

Indonesia is recognized as one of the global centers of tropical orchid diversity, hosting hundreds to thousands of orchid species distributed across its archipelago, many of which possess high horticultural and conservation value (Setiowati, *et al*., 2025). Among these, the genus *Dendrobium* represents one of the largest and most ecologically diverse groups within the Orchidaceae, exhibiting wide variations in morphology, reproductive strategies, and habitat adaptation that are critical for both ex situ propagation and conservation efforts (Abduallah, *et al.*, 2026). In addition to their biological importance, orchids contribute significantly to the ornamental plant industry, with national production of potted orchids in Indonesia showing a marked increase in recent years, reflecting growing economic demand (BPS, 2024).

Despite their ecological and economic value, natural populations of orchids are experiencing rapid decline due to habitat degradation, illegal logging, land-use change, and overexploitation through trade (Fay, 2018). Climate change further exacerbates these threats by altering temperature, humidity, and precipitation regimes that are crucial for orchid growth and regeneration (Evans *et al.*, 2020). Endemic species such as *Dendrobium*

sutiknoi are particularly vulnerable, as their restricted distribution and specific physiological requirements increase extinction risk when environmental conditions deteriorate. Consequently, research-based conservation strategies are urgently needed to ensure the long-term survival of threatened orchid species in Indonesia.

Plant tissue culture has been widely applied as an effective approach for the mass propagation and conservation of orchids, enabling the production of genetically uniform and disease-free plantlets (Pyati, 2022). Various in vitro techniques, including protocorm-like body (PLB) induction and temporary immersion systems, have demonstrated high efficiency in producing large numbers of *Dendrobium* plantlets (Nongdam, *et al.*, 2023). However, the success of in vitro propagation is often constrained by low survival rates during the transition from laboratory to external environments. The acclimatization phase represents a critical bottleneck, as plantlets frequently fail to adapt to fluctuating ex vitro conditions.

Hardening, as a pre-acclimatization strategy, plays a crucial role in improving plantlet vigor and survival by gradually modifying environmental factors such as humidity, light intensity, and temperature (Putri and Ermawati, 2023). Previous studies on various orchid species have shown that appropriate hardening techniques, including media selection and the application of growth regulators such as paclobutrazol, can significantly enhance morphological performance and survival rates during acclimatization (Handini *et al.*, 2026). Nevertheless, most tissue culture studies on *Dendrobium* focus primarily on in vitro regeneration efficiency, with limited evaluation of ex vitro establishment and species-specific hardening protocols.

Paclobutrazol, a triazole-type plant growth regulator, is widely known to inhibit gibberellin biosynthesis by suppressing ent-kaurene oxidation, resulting in reduced stem elongation and modified vegetative growth patterns (Tesfahun, 2018). In addition, paclobutrazol has been reported to influence hormonal balance and photosynthetic efficiency, thereby affecting leaf development and overall plant vigor during early growth and acclimatization stages (Soumya *et al*., 2021).

To date, information regarding optimal hardening techniques for *Dendrobium sutiknoi* remains scarce, despite its potential importance for conservation and horticultural development. Given its unique physiological characteristics as a native species, the adaptation of general hardening protocols may not adequately support successful acclimatization. Therefore, this study aims to evaluate the effects of different hardening techniques on the growth of *D. sutiknoi* plantlets under in vitro conditions and to determine the most effective method for enhancing plantlet performance. The findings of this research are expected to contribute to the development of species-specific acclimatization strategies, supporting orchid conservation initiatives and aligning with Sustainable Development Goal 15 (Life on Land) through the protection of threatened plant biodiversity.

MATERIALS AND METHODS

Plantlets of *Dendrobium sutiknoi* obtained from in vitro culture were used as experimental material in this study. Uniform plantlets with comparable size and physiological condition were selected prior to the experiment. Acclimatization was conducted using a hardening technique combined with the application of the plant growth regulator paclobutrazol under controlled conditions.

The experiment was arranged in a Randomized Complete Block Design (RCBD) with three replicates per treatment. Although two experimental factors were involved, namely hardening duration and paclobutrazol concentration, both factors were combined into a single treatment technique, resulting in one experimental factor consisting of eight treatment combinations. Hardening duration consisted of H1 (2 weeks) and H2 (4 weeks), while paclobutrazol concentration consisted of P1 (0 ppm, control), P2 (10 ppm), P3 (15 ppm), and P4 (20 ppm). The combination of these factors resulted in eight treatments: H1P1, H1P2, H1P3, H1P4, H2P1, H2P2, H2P3, and H2P4, with treatments using 0 ppm paclobutrazol at both hardening durations serving as control treatments.

The dependent variables observed were plant height increment (cm), number of leaves, and plantlet survival percentage. Plant height increment was measured from the base of the plantlet to the tip of the tallest leaf, the number of leaves was counted as fully developed leaves per plantlet, and plantlet survival percentage was

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue XI November 2026

calculated as the proportion of surviving plantlets relative to the total number of plantlets at the end of the acclimatization period.

All data were analyzed using two-way analysis of variance (ANOVA) to evaluate the effect of different combined hardening techniques and paclobutrazol concentrations on the growth and survival of *D. sutiknoi* plantlets. Prior to ANOVA, data were tested for homogeneity of variance, and differences among treatment means were considered significant at $p < 0.05$.

RESULTS AND DISCUSSIONS

The experiment was conducted over a five-week observation period, consisting of one week of acclimatization followed by four weeks of hardening treatment. The treatments were arranged as a single-factor design comprising combinations of hardening duration and paclobutrazol concentration. Two levels of hardening were applied, namely two weeks (H1) and four weeks (H2), combined with four concentrations of paclobutrazol: 0 ppm (P1), 10 ppm (P2), 15 ppm (P3), and 20 ppm (P4). This resulted in eight treatment combinations: H1P1, H1P2, H1P3, H1P4, H2P1, H2P2, H2P3, and H2P4. Three replications were used for each treatment, with three plantlets per replication, resulting in a total of 72 *Dendrobium sutiknoi* plantlets. The observed variables included plant height increment, number of leaves, and percentage of surviving plantlets.

Table 1. Effect of hardening duration and paclobutrazol application results of growth *D. sutiknoi*

Growth Parameters	Source of variation	F-value	Significance
Plant height increment (cm)	Hardening (H)	0.981	ns
	PBZ (P)	195.903	**
	H x P	11.185	**
Number of leaves (leaflets)	Hardening (H)	58.656	**
	PBZ (P)	32.928	**
	H x P	7.623	**
Percentage of live plants (%)	Hardening (H)	1.036	ns
	PBZ (P)	1.459	ns
	H x P	1.459	ns

Notes:

ns = not significant ($p > 0.05$);

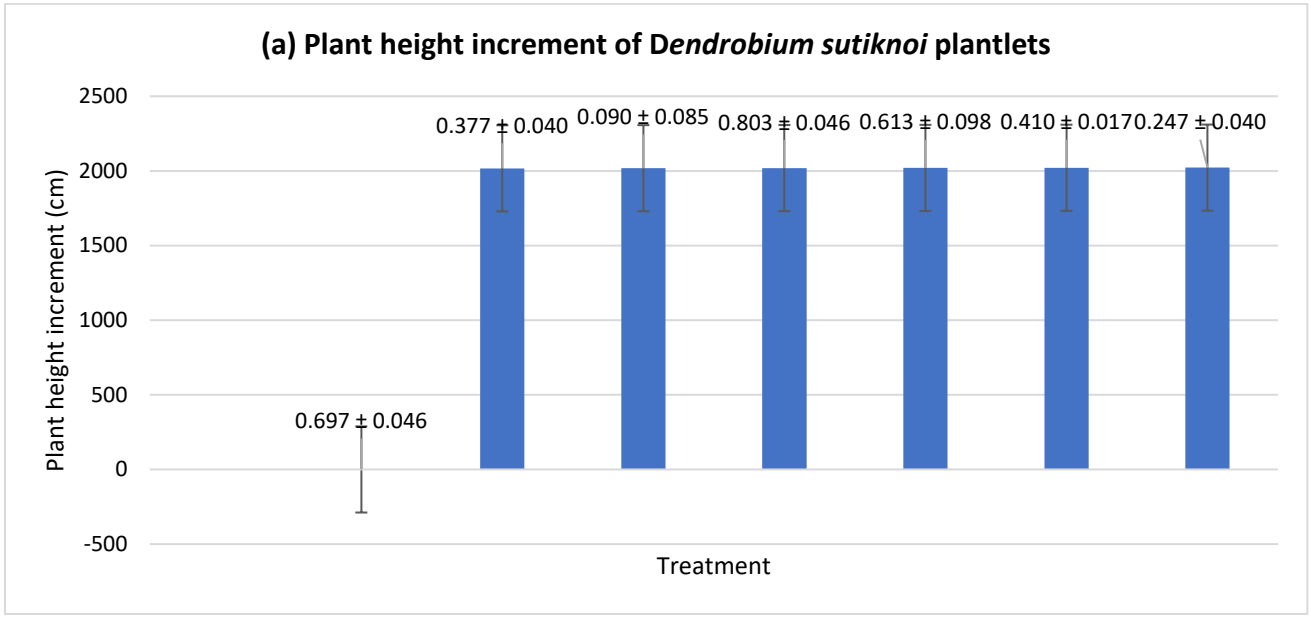
** = significant at $p < 0.01$

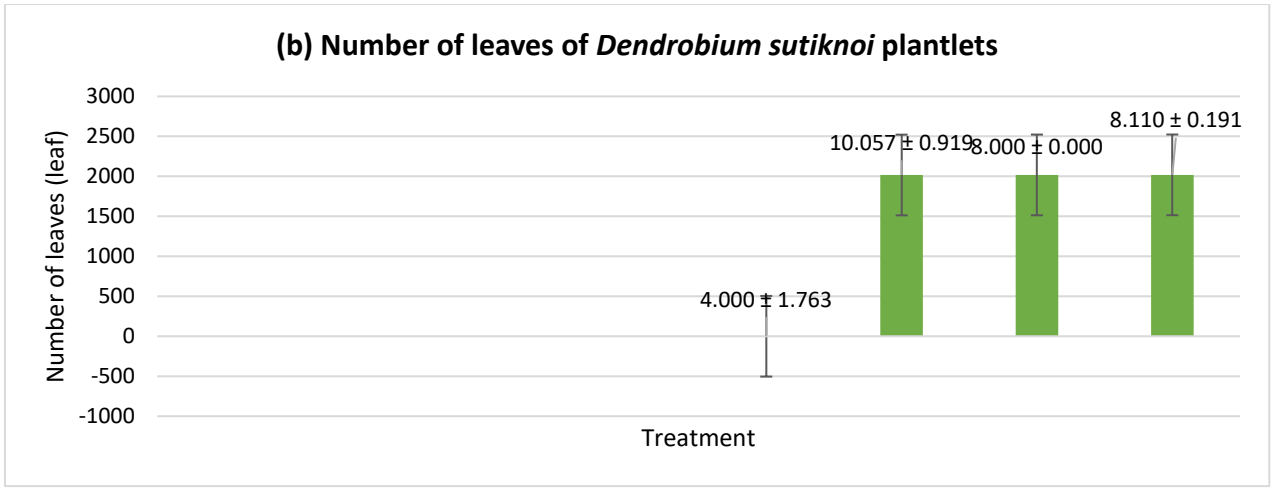
Two-way analysis of variance (ANOVA) revealed that the combined hardening and paclobutrazol treatments had a significant effect on plant height and number of leaves ($p < 0.001$). In contrast, no significant effect was observed on the percentage of surviving plantlets ($p > 0.05$). The statistical summary of the ANOVA results, including degrees of freedom, F-values, and significance levels, is presented in Table 1.

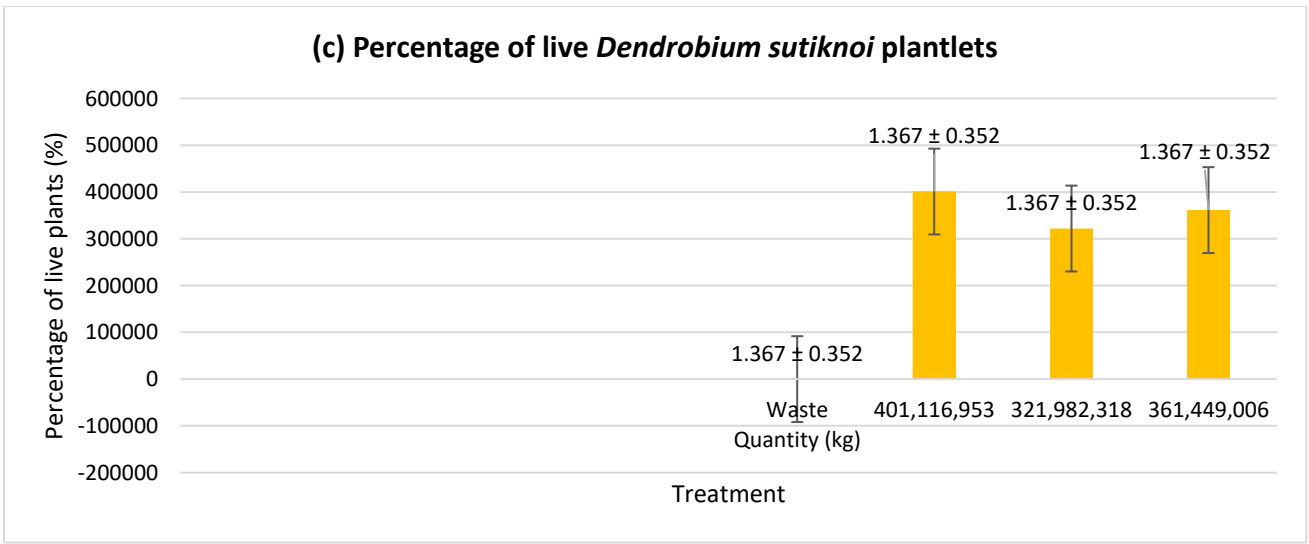
Figure 1. Effects of paclobutrazol treatments on (a) plant height increment (cm), (b) number of leaves, and (c) percentage of live *Dendrobium sutiknoi* plantlets. H1P1 = hardening for 2 weeks without paclobutrazol (0

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue XI November 2026

ppm); H1P2 = hardening for 2 weeks with 10 ppm paclobutrazol; H1P3 = hardening for 2 weeks with 15 ppm paclobutrazol; H1P4 = hardening for 2 weeks with 20 ppm paclobutrazol; H2P1 = hardening for 4 weeks without paclobutrazol (0 ppm); H2P2 = hardening for 4 weeks with 10 ppm paclobutrazol; H2P3 = hardening for 4 weeks with 15 ppm paclobutrazol; H2P4 = hardening for 4 weeks with 20 ppm paclobutrazol. Values represent mean ± SD (n = 3).







ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue XI November 2026

Based on descriptive analysis, the highest plant height increment was observed in the H1P1 treatment, while the lowest values were recorded under higher paclobutrazol concentrations, particularly in the H1P4 treatment. Regarding leaf production, the highest mean number of leaves was obtained under the H2P2 treatment, whereas lower values were generally observed in treatments without paclobutrazol application. In contrast, the percentage of surviving plantlets showed relatively uniform values across all treatments, indicating minimal variation among combinations.

The effects of hardening duration and paclobutrazol concentration on plant height increment, number of leaves, and survival percentage are illustrated in Figure 1. The figure presents the mean values ± standard deviation for each treatment combination, clearly demonstrating differences in growth responses among treatments (Figure 1). Overall, the findings show that paclobutrazol concentration and hardening time had a substantial impact on *Dendrobium sutiknoi* plantlets vegetative growth metrics, especially plant height increment and leaf count. On the other hand, the administered treatments had no discernible effect on plant survival. These results imply that *D. sutiknoi* plantlets have varied physiological reactions to the length of hardening and the use of paclobutrazol during the acclimatization stage.

As seen by the decreased plant height increment at higher PBZ concentrations relative to the control, paclobutrazol application in the hardening treatment of *Dendrobium sutiknoi* plantlets is known to hinder stem elongation. By blocking the enzyme *ent-kaurene oxidase* in the gibberellin biosynthesis pathway, paclobutrazol functions physiologically as an inhibitor of gibberellin synthesis, lowering gibberellin production and allowing plant cells to continue dividing but not elongate correctly, resulting in shorter stems (Abdalla, *et al.*, 2021; Shahzad, *et al.,* 2023). According to research by Kusumawardani et al. (2025), paclobutrazol can also lower plant height by preventing root and stem cell growth, which results in shorter internodes. This effect is commonly seen in a variety of horticultural plant species. For instance, it has been demonstrated that paclobutrazol inhibits the growth of vegetative organs in winged beans, which has a substantial effect on fruit height and other plant structures, particularly at higher concentrations that restrict cell division and expansion (Wijayanti and Dewi, 2024). Biologically, this plant's high inhibition results are linked to a decrease in gibberellin hormone, which typically contributes to stem elongation, and an accumulation of precursors in the terpenoid pathway, which can lead to an increase in abscisic acid (ABA), which is also involved in the plant's stress response. Consequently, a complicated hormonal balance is altered in addition to gibberellin inhibition in response to PBZ (Tesfahun, 2018).

According to the study's findings, applying paclobutrazol at a concentration of 10 ppm results in more *Dendrobium sutiknoi* plantlet leaves than the control. This suggests that a moderate dosage of paclobutrazol can maximize the development of vegetative organs without impairing the plant's physiological activity (Jin *et al.*, 2023). Paclobutrazol's capacity to inhibit gibberellin production, which raises the cytokinin-to-gibberellin ratio, is linked to this rise in leaf number. During the vegetative development phase, this environment is known to encourage cell proliferation and leaf tissue differentiation (Vercruysse *et al.*, 2021). Furthermore, paclobutrazol has been shown to boost chlorophyll accumulation and photosynthetic efficiency, which supports the development of new leaves by increasing the capacity of photosynthate sources in immature plants (Soumya *et al*., 2021).

The decrease in leaf number at higher paclobutrazol concentrations indicates a growth-inhibiting effect due to excessive suppression of the gibberellin biosynthesis pathway, which leads to disruption of the plant's physiological activity and vegetative organ differentiation (Desta and Amare, 2021). Additionally, high paclobutrazol concentrations are known to inhibit the production of new leaves and delay vegetative development by increasing the buildup of abscisic acid and decreasing the activity of enzymes involved in cell division (Soumya *et al*., 2021).The severe inhibition of gibberellin production, which upsets hormonal balance and shifts metabolic resources from vegetative development to stress response, is probably the cause of the decrease in leaf number at high paclobutrazol concentrations. This prevents the creation of new leaves (Soumya *et al*., 2021). Therefore, a concentration of 10 ppm can be considered as the optimum point in stimulating leaf formation, while higher concentrations reduce the efficiency of vegetative growth of plantlets.

Although the ANOVA test revealed that the paclobutrazol (PBZ) treatment had no significant impact on the percentage of plantlet survival in this study, this could be explained by the fact that PBZ is more involved in physiological changes like preventing cell elongation and altering plant energy allocation than it is in directly influencing plantlet mortality during the acclimatization phase (Soumya *et al*., 2021). As a result, there was no significant difference in the percentage of survival between the treatment and the control (Sofiana, *et al*, 2023). Several studies have reported that paclobutrazol plays a greater role in regulating hormonal balance and mild stress responses, rather than as a lethal factor that causes plant tissue death (Aini and Wicaksana, 2022).

Overall, the results indicate that the combined hardening and paclobutrazol treatments significantly affected vegetative growth parameters, particularly plant height and number of leaves, while having no significant effect on the percentage of surviving plantlets. This suggests that paclobutrazol primarily functions as a growth regulator that modifies vegetative development without adversely affecting plantlet viability during the observation period. Therefore, the study concludes that the applied treatments are more effective in controlling growth characteristics than in influencing plantlet survival.

CONCLUSION

This study demonstrated that the combination of hardening duration and paclobutrazol (PBZ) concentration influenced the vegetative growth of *Dendrobium sutiknoi* plantlets during the acclimatization stage, particularly in terms of plant height and number of leaves, but had no significant effect on plantlet survival. The highest plantlet height increment (1 cm) was recorded in the treatment involving two weeks of hardening without PBZ application (H1P1), whereas the highest number of leaves (10 leaves) was observed in the treatment involving four weeks of hardening combined with 10 ppm PBZ (H2P2). Higher PBZ concentrations tended to suppress vegetative growth, particularly stem elongation and leaf formation, indicating that the plantlets responded differently to PBZ depending on the concentration applied. These findings suggest that PBZ can be used to regulate the vegetative growth of *D. sutiknoi* plantlets during acclimatization, with 10 ppm showing potential to promote leaf development without reducing survival.

However, the optimal treatment depends on the desired growth characteristics, and further studies with longer observation periods and additional physiological parameters are needed to validate these responses and determine the most effective treatment for successful acclimatization.

ACKNOWLEDGEMENT

The authors would like to thank all the laboratory staff of Biology Laboratory, Biology Department, Mathematic and Natural Sciences Faculty, Universitas Negeri Surabaya, Plant Culture Laboratory of Food Security and Agriculture Department Surabaya and Ministry of Research Technology and Higher Education.

REFERENCES

1. Abdalla N, Taha N, Bayoumi Y, El-Ramady H, and Shalaby T. (2021). Paclobutrazol applications in agriculture, plant tissue cultures and its potential as stress ameliorant: A mini review. *EBSS*. 5(2021):245–257. https://doi.org/10.21608/jenvbs.2021.95536.1143.
2. Abduallah, A.M.A., Salem, B.I.A., Aung, T.T., Tian, C., Xue, J., Ren, X. and Zhang, X., (2026). Morphophysical Analysis and Horticultural Assessment of 10 Dendrobium Species. *New Zealand Journal of Crop and Horticultural Science*, *54*(1), p.e70016.
3. Aini, N., and Wicaksana, I. (2022). Pengaruh konsentrasi paklobutrazol terhadap karakter morfologi dan fisiologi tanaman florikultura. *Jurnal Produksi Tanaman*, 10(4), 215–222.
4. Badan Pusat Statistik. (2024). *Produksi Tanaman Hias Menurut Jenis Tanaman, 2023-2024.* Badan Pusat Statistik.
5. Desta, B., and Amare, (2021). G. Paclobutrazol as a plant growth regulator. *Chem. Biol. Technol. Agric*. 8(1). https://doi.org/10.1186/s40538-020-00199-z
6. Evans, A., Janssens, S. and Jacquemyn, H., (2020). Impact of climate change on the distribution of four closely related Orchis (*Orchidaceae*) species. *Diversity*, *12*(8), p.312.

7. Fay, M. F. (2018). Orchid conservation: how can we meet the challenges in the twenty-first century?. *Botanical Studies*, 59(1), 1-11. https://doi.org/10.1186/s40529-018-0232-z
8. Firon, N., D. LaBonte, A. Villordon, C. McGregor, Y. Kfir, and E. Pressman. (2009). Botany and physiology: storage root formation and development. p. 13–26. In Loebenstein, G., Thottappilly, G. (eds.), The Sweetpotato. Springer Netherlands.
9. Handini, A.S., Dinarti, D., Arrufitasari, P.N., Sutanto, O.P. and Hidayatullah, C.S.R., 2026. Optimasi Pertumbuhan Vegetatif Bibit *Dendrobium Lasianthera* Melalui Aplikasi Paclobutrazol Pada Fase Aklimatisasi. *Jurnal Pertanian Presisi (Journal of Precision Agriculture)*, *10*(1), pp.132-142.
10. Jin, Q., Sun, Z., Zhang, Z., Yuan, Y., Cao, Y., and Wei, S. (2023). Paclobutrazol regulates vegetative growth and improves net photosynthetic rate by regulating endogenous hormones and leaf anatomy of apple nursery stock. *Frontiers in Plant Science*, 14, 1172813.
11. Kusumawardani, A. T., Krisantini, K., Dinarti, D., and Widaningsih, I. (2025). Pengendalian tinggi tanaman hamparan Celosia dengan zat pengatur tumbuh paclobutrazol. *Buletin Agrohorti*, 13(2), 214–219
12. Kusumawati, R.D., D. Hariyono, and N. Aini. (2016). Pengaruh komposisi media tanam dan interval pemberian air sampai dengan kapasitas lapang terhadap produksi tanaman cabai rawit (*Capsicum frutescens* L.). *Plantropica 1*(2): 64–71. http://jpt.ub.ac.id/index.php/jpt/article/view/118/121
13. Nongdam, P., Nirmala, C., and Rathore, P. K. (2023). Orchid micropropagation using conventional semi-solid and temporary immersion systems: A review. Plants, 12(5), 1136. https://doi.org/10.3390/plants12051136
14. Putri, E.M. and Ermawati, N., (2023). Pengaruh teknik hardening dan konsentrasi paclobutrazol terhadap pertumbuhan planlet krisan (*Chrysanthemum* sp.) pada tahap aklimatisasi. In *agropross: national conference proceedings of agriculture* (pp. 608-615).
15. Pyati, Ashok N. (2022). In vitro propagation of orchid (*dendrobium ovatum* (l.) kraenzl.) through somatic embryogenesis. Plant Tissue Culture and Biotechnology 32(1):53–66. doi.org/10.31943/agrowiralodra.v8i1.140
16. Setiowati, F.K., Irawati, M.H., Susilo, H., Rahayu, S.E., Handayani, N., Prabaningtyas, S., Rasyid, M.A., Jamil, A.M.S. and Putra, R.S., (2025). *Eksplorasi Anggrek Sebagai Media Pembelajaran*. Idebuku. Klaten.
17. Shahzad MM, Akhtar G, Shehzad MA, Ullah S, Faried HN, Razzaq K, Nawaz F, Rajwana IA, Amin M, and Ahsan M. (2023). Paclobutrazol and maleic hydrazide-induced growth inhibition in warm season turfgrasses through structural and physiological differences. *Kuwait J Sci.* 50(4):674–680. https://doi.org/10.1016/j.kjs.2023.04.007.
18. Sofiana, N., Sulistyono, E., and Moeljani, I. (2023). Pengaruh konsentrasi paklobutrazol terhadap pertumbuhan aklimatisasi planlet anggrek *Dendrobium* sp. *Jurnal Agroteknologi Merdeka Pasuruan*, 7(1), 12–19.
19. Soumya, P. R., Kumar, P., and Pal, M. (2021). Paclobutrazol as a plant growth regulator. *Chemical and Biological Technologies in Agriculture*, 8(1), 1–15.
20. Tesfahun, W. (2018). Paclobutrazol application and its economic importance in agricultural production. *Journal of Plant Biochemistry and Physiology*, 6(3), 1–4. https://doi.org/10.1080/23311932.2018.1525169
21. Vercruysse, J., Baekelandt, A., Gonzalez, N., and Inzé, D. (2021). Molecular mechanisms regulating cell proliferation and differentiation during leaf development. *Journal of Experimental Botany*, 72(7), 2365–2384.
22. Wijayanti, J. S., and Dewi, K. (2024). Growth and development of winged bean (Psophocarpus tetragonolobus (L.) DC.) treated with paclobutrazol. *Journal of Tropical Biodiversity and Biotechnology*, 9(2), Article 86238. https://doi.org/10.22146/jtbb.86238