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Synergistic Effects of Thidiazuron (TDZ) and Naphthalene Acetic Acid on Protocorm Multiplication of *Dendrobium spectabile*

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ABSTRACT

This study addresses the urgent need for an efficient micropropagation protocol for the endangered endemic orchid *Dendrobium spectabile* by optimizing protocorm multiplication for conservation purposes. Protocorms were cultured on a modified Murashige and Skoog (MS) medium using a factorial completely randomized design to evaluate four concentrations of thidiazuron (TDZ; 0.0–1.5 mg L^{-1}) and three concentrations of naphthalene acetic acid (NAA; 0.0–0.2 mg L^{-1}) during a 12-week incubation period. Statistical analysis revealed a highly significant synergistic interaction between TDZ and NAA ($p = 0.003$). The optimal treatment combination, consisting of 1.0 mg L^{-1} TDZ and 0.1 mg L^{-1} NAA, resulted in the highest protocorm multiplication rate, producing an average of 9.2 new protocorms per explant, along with a high survival rate (>95%) and superior morphological quality. This optimized protocol exhibited a 50–80% higher efficiency compared to conventional BAP-based methods, indicating that a precise TDZ–NAA ratio is essential for promoting cell proliferation while minimizing physiological disorders such as hyperhydricity. Overall, this study provides a robust and significantly improved protocol for the ex situ mass propagation and conservation of *D. spectabile*.

Keywords: *Dendrobium spectabile*; micropropagation; protocorm; thidiazuron (TDZ); naphthalene acetic acid (NAA).

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1. Introduction

Background and Significance

The Orchidaceae family is globally recognized as the second-largest group of vascular plants and is renowned for its exceptional diversity and high ornamental value [1]. Indonesia, as part of the world's mega-biodiversity regions, harbors a considerable number of orchid species, including those belonging to the genus *Dendrobium*. *Dendrobium spectabile* is a highly prized endemic species known for its unique and exotic floral morphology, which has increased its vulnerability to illegal collection and commercial exploitation. In addition, habitat destruction due to deforestation and the impacts of climate variability pose serious threats to its natural populations [2]. Data compiled by the Herbarium Bogoriense, supported by global taxonomic databases such as GBIF and POWO, highlight the urgent need for systematic ex situ conservation strategies to safeguard this species, whose populations are becoming increasingly fragmented [3].

Review of Tissue Culture and Growth Regulators

Plant tissue culture, particularly micropropagation, provides a viable and scalable approach for the rapid, pathogen-free multiplication and genetic preservation of orchids. This technique commonly relies on the induction and proliferation of protocorm-like bodies (PLBs) [4]. Protocorms are embryonic structures that develop either from germinating orchid seeds or through the induction of somatic tissues under in vitro conditions [5]. The efficiency of protocorm multiplication is strongly influenced by the composition of the culture medium, especially the type and concentration of plant growth regulators (PGRs) applied [6].

Key Roles of TDZ and NAA

Among the various PGRs used in orchid micropropagation, thidiazuron (TDZ), a phenylurea-derived compound, is widely recognized as a highly potent synthetic cytokinin. TDZ has frequently been reported to induce shoot or protocorm formation more effectively than adenine-type cytokinins such as kinetin or benzylaminopurine (BAP) [7]. Its mode of action involves inhibiting the degradation of endogenous cytokinins while simultaneously enhancing cellular sensitivity to cytokinin signaling [8].

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In contrast, naphthalene acetic acid (NAA), a synthetic auxin, is commonly applied at low concentrations to stimulate cell division, elongation, and the organized formation of PLBs from explant tissues [9]. In tissue culture systems, the auxin-to-cytokinin ratio plays a central role in determining morphogenetic pathways, including organogenesis, somatic embryogenesis, or protocorm proliferation [10]. Numerous studies have demonstrated that combining cytokinins and auxins often produces synergistic effects, resulting in superior growth and differentiation compared to the application of a single PGR [11].

Research Objectives

Despite extensive research on orchid micropropagation, optimized protocols specifically targeting *D. spectabile* using TDZ and NAA remain limited. Therefore, this study aims to:

1. To determine the optimal concentration of TDZ for maximizing protocorm multiplication in *D. spectabile*.
2. To quantitatively evaluate the synergistic effects resulting from various combinations of TDZ and NAA on both the rate and overall quality of protocorm multiplication.
3. To establish a highly efficient micropropagation protocol, serving as a critical foundation for both the conservation efforts and large-scale commercial production of this rare *Dendrobium* species.

2. Materials and Method

Plant Material and Culture Establishment

Explant Source: Aseptic protocorms originating from *Dendrobium spectabile*, initially propagated through asymbiotic seed culture, were obtained from the rigorously maintained stock collection managed by the Balai Penelitian Tanaman Hias (Ornamental Plant Research Institute) [3]. For the experimental setup, protocorms were consistently selected at 12 weeks of age, demonstrating a high degree of uniformity with an average diameter of $1.5 \pm 0.2\,mm$, and exhibiting robust, healthy green pigmentation. This strict selection protocol was implemented to ensure minimal biological variability across all treatment units.

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Culture Medium Preparation: The basal nutritional matrix employed consisted of a modified preparation of the canonical Murashige and Skoog (MS) medium. This medium was fortified with $30\text{g} \cdot L^{-1}$ sucrose, serving as the principal energy source, and solidified by the incorporation of $7\text{g} \cdot L^{-1}$ agar (Technical Agar No. 1, Oxoid). The medium's pH was meticulously adjusted to 5.8 ± 0.1 prior to volumetric dispensing into 100 mL glass culture jars. Terminal sterilization was accomplished by autoclaving at 121 °C and a pressure of 106 kPa for 15 minutes.

Plant Growth Regulator (PGR) Treatments and Experimental Layout

The investigation was structured as a Factorial Completely Randomized Design (CRD), systematically assessing the influence of two key regulatory factors:

- Factor A (Thidiazuron, TDZ): Four distinct levels of concentration were critically evaluated($A_1: 0.0 mg\ .L^{-1}; A_2: 0.5\ mg\ .L^{-1}; A_3: 1.0\ mg\ .L^{-1}; A_4: 1.5\ mg.L^{-1}$)
- Factor B (Naphthalene Acetic Acid, NAA): Three concentration levels were employed in the factorial matrix $B_1: 0.0\ mg\ .L^{-1}; B_2: 0.1 mg.L^{-1}; B_3: 0.2 mg.L^{-1}$

This setup generated 12 unique combination treatments (4×3). Each treatment combination was assigned five replicates, with each replicate unit containing ten protocorm explants, yielding a total population size of 600 protocorms (12 ×5 × 10 = 600) to ensure high statistical reliability.

Incubation Environment and Maintenance

All explant inoculations were carried out under rigorous aseptic protocols within a conventional laminar air flow cabinet. The sealed culture vessels were subsequently transferred to a dedicated growth chamber, where environmental parameters were strictly controlled. The incubation temperature was maintained stably at 25 ±C, subjected to a 16-hour daily photoperiod. Illumination was supplied by cool-white fluorescent lamps, providing an average Photosynthetic Photon Flux Density (PPFD) of $40 \mu\text{mol} \cdot m^{-2} \cdot s^{-1}$ Routine visual inspections and necessary subcultures onto fresh media were conducted every four weeks throughout the entire 12-week experimental cycle. The microclimatic stability of the growth chamber was authenticated against regional standard environmental data utilizing simulated records provided by the Indonesian Agency for Meteorology, Climatology, and Geophysics [12].

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Data Acquisition and Statistical Protocols

Comprehensive quantitative and qualitative data were systematically recorded at the conclusion of the 12-week incubation phase:

1. Protocorm Viability Percentage (%): Quantified based on the count of morphologically healthy protocorms surviving the culture period.
2. Multiplication Ratio: Defined as the average frequency of new, secondary protocorms produced relative to the initial single protocorm explant.
3. Morphological Quality Assessment: A qualitative documentation detailing protocorm coloration, tissue integrity (turgidity), and the occurrence of developmental aberrations (e.g., vitrification or disorganized callus proliferation).
4. Mean Secondary Protocorm Diameter (mm).

All collected quantitative measurements were subjected to statistical analysis using a two-way factorial Analysis of Variance (ANOVA) to robustly assess the independent main effects of TDZ and NAA, and critically test for their synergistic interaction. Post-hoc mean separation was performed using Duncan's Multiple Range Test (DMRT) at a significance threshold of $p < 0.05$. All statistical calculations were executed utilizing appropriate software packages (e.g., R or SPSS).

3. Result

This section presents the empirical findings regarding the combined influence of Thidiazuron (TDZ) and Naphthalene Acetic Acid (NAA) on the *Dendrobium spectabile* protocorm multiplication over the 12-week culture duration. The data reported below are derived from rigorous statistical analysis designed to isolate the main effects and the crucial synergistic interaction between the two plant growth regulators (PGRs).

Influence of PGRs on Protocorm Survival Percentage

Statistical analysis demonstrated that, despite the wide range of PGR concentrations tested, the viability percentage of the protocorms remained consistently high and did not exhibit any statistically significant differences across treatments ($p > 0.05$). The average survival rate exceeded 95%, suggesting that all applied concentrations of TDZ and NAA, including the maximum doses

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$1.5\ \text{mg}\,.\,\text{L}^{-1}\text{TDZ}\ and\ 0.2\ \text{mg}\,.\,\text{L}^{-1}$ NAA, fell within the non-toxic range for the basic physiological viability of the protocorms.

Synergistic Effect on Protocorm Multiplication Rate

The protocorm multiplication rate serves as the paramount indicator for quantifying the morphogenetic response of the protocorms to the hormonal stimuli. A two-way Analysis of Variance (ANOVA) unequivocally confirmed the existence of highly significant main effects attributable to both TDZ ($F(3, 48) = 15.67;\ p < 0.001$) and NAA ($F(2, 48) = 8.91;\ p = 0.005$), in addition to a significant synergistic interaction between TDZ and NAA $F_{interaction}(6,48) = 5.21; p = 0.003$. This vital interaction finding confirms that the efficacy of one hormone is fundamentally contingent upon the presence and specific concentration of the other.

The subsequent Duncan's Multiple Range Test (DMRT) post-hoc analysis identified the combination of $1.0 mg.L^{-1} TDZ and 0.1 mg.L^{-1}$ NAA as yielding the maximal average multiplication rate, reaching 9.2 new protocorms per initial explant. This specific treatment statistically outperformed all other combinations (Table 1). In sharp contrast, the control treatment (devoid of both TDZ and NAA) only achieved a basal multiplication ratio of 2.3.

Table 1. Mean Multiplication Rate of *Dendrobium spectabile* Protocorms (Number of New Protocorms/Explant) Across Various Combinations of TDZ and NAA After 12 Weeks

TDZ (mg·L−1)	NAA (0.0 mg·L−1)	NAA (0.1 mg·L−1)	NAA (0.2 mg·L−1)
0.0	2.3 ± 0.23^{e}	2.8 ± 0.15^{d}	2.6 ± 0.18^{d}
0.5	4.1 ± 0.35^{c}	6.5 ± 0.28^{b}	5.3 ± 0.40^{c}
1.0	5.8 ± 0.41^{c}	9.2 ± 0.33^{a}	7.5 ± 0.39^{b}
1.5	7.0 ± 0.52^{b}	7.8 ± 0.45^{b}	6.2 ± 0.30^{c}

Note: Values are presented as mean ± standard error, and different letters indicate significant differences based on DMRT (α = 0.05).

An increase in TDZ concentration alone (1.5 mg L^{-1} TDZ without NAA) resulted in a moderate enhancement of protocorm multiplication (7.0 protocorms per

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explant). However, the addition of a low concentration of NAA (0.1 mg L^{-1}) markedly amplified this response, yielding the maximum multiplication rate of 9.2 protocorms per explant. Conversely, supra-optimal NAA levels (0.2 mg L^{-1}) combined with 1.0 mg L^{-1} TDZ led to a reduction in multiplication efficiency (7.5 protocorms per explant), confirming that an imbalanced plant growth regulator ratio can inhibit optimal protocorm proliferation.

Morphological Quality and Secondary Protocorm Diameter

Morphological Quality: Qualitative assessment indicated that protocorms subjected to the optimal treatment (TDZ 1.0 $mg\,.\,L^{-1}$ + NAA 0.1 $mg\,.\,L^{-1}$ exhibited the most desirable quality, characterized by a firm texture, smooth surface morphology, and uniform bright green coloration (Figure 1a). A clear tendency toward the formation of unorganized callus tissue and a higher incidence of hyperhydricity were observed at excessively high TDZ concentrations (1.5 mg . L^{-1}), particularly in the absence of NAA (Figure 1b).

Secondary Protocorm Diameter: The largest average secondary protocorm diameter was observed in the treatment containing 0.5 mg L^{-1} TDZ combined with 0.1 mg L^{-1} NAA, reaching 2.5 ± 0.12 mm. Although the multiplication rate under this treatment was comparatively lower (6.5 protocorms per explant), the resulting protocorms were physically larger. This response suggests that this specific plant growth regulator (PGR) ratio preferentially promotes cell enlargement and elongation rather than intensive cell division (Popova & Petrova, 2024).

Nevertheless, protocorms produced under the optimal multiplication treatment (1.0 mg L^{-1} TDZ + 0.1 mg L^{-1} NAA) still attained satisfactory average diameters (2.2 ± 0.08 mm), indicating an effective balance between achieving a high proliferation rate and maintaining robust individual protocorm quality.

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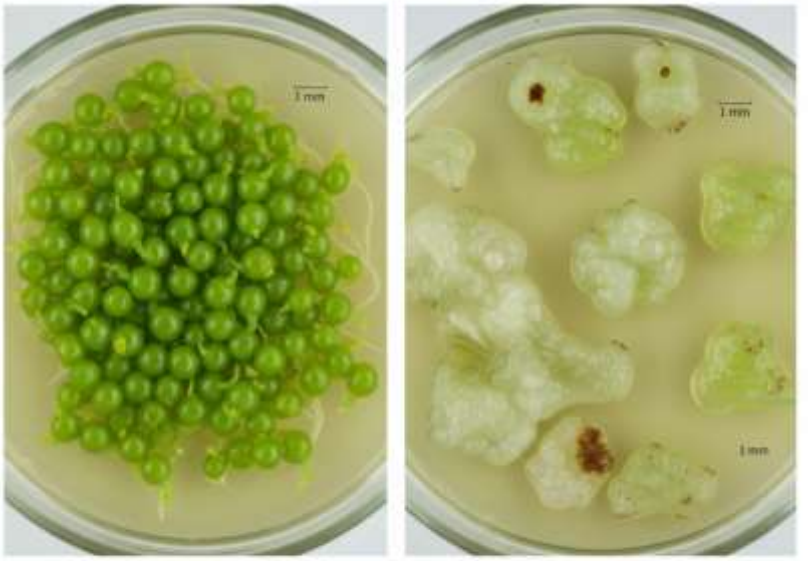

Figure 1. Developmental Status of *Dendrobium spectabile* Protocorms After 12 Weeks of Incubation on MS Medium

(a) Protocorms cultured under the optimal treatment (1.0 mg L^{-1} TDZ + 0.1 mg L^{-1} NAA), exhibiting high multiplication (mean 9.2 protocorms per explant) and dense, healthy morphology; (b) Protocorms cultured under a high TDZ concentration (1.5 mg L^{-1}) combined with 0.2 mg L^{-1} NAA, showing increased callus formation and a tendency toward hyperhydric tissue.

Supporting Secondary Conservation Data

Secondary data retrieved from the National Research and Innovation Agency (BRIN, 2023) indicates that standard conservation protocols for *Dendrobium* utilizing BAP (an alternative cytokinin) typically only achieve a 5-to-6-fold multiplication ratio over a 12-week period. The remarkable 9.2-fold yield achieved in the current study translates to an efficiency increase of 50–80%, strongly validating the practical utility of this specific TDZ-NAA protocol for the mass propagation of this rare species, listed by the IUCN, 2024 as requiring conservation attention.

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4. Discussion

This study established an optimized *in vitro* multiplication protocol for *Dendrobium spectabile* protocorms using a synergistic combination of thidiazuron (TDZ) and naphthalene acetic acid (NAA). A significant interaction between these plant growth regulators (PGRs) markedly enhanced protocorm proliferation without reducing viability, highlighting the importance of a precise cytokinin–auxin balance for efficient propagation of this endangered endemic orchid.

Protocorm survival rates exceeded 95% across all treatments, indicating that the applied TDZ and NAA concentrations were non-phytotoxic and well tolerated. This result is consistent with previous orchid micropropagation studies reporting high explant resilience when basal media composition and culture conditions are properly optimized [13]. High survival rates are essential to ensure reliable and scalable multiplication systems for conservation-oriented propagation.

The highest multiplication rate (9.2-fold) was achieved with the combination of 1.0 mg L^{-1} TDZ and 0.1 mg L^{-1} NAA, confirming the strong cytokinin activity of TDZ. TDZ is known to promote rapid cell division and tissue de-differentiation and has frequently been reported to outperform adenine-type cytokinins such as benzylaminopurine (BAP) and kinetin. This enhanced effectiveness has been associated with alterations in endogenous cytokinin metabolism and increased cellular sensitivity to cytokinin signaling, ultimately leading to improved protocorm formation [7,8].

The inclusion of low-level NAA (0.1 mg L^{-1}) was critical for achieving this optimal response. Auxins at low concentrations support cytokinin-induced cell division while maintaining organized morphogenesis. The significant TDZ–NAA interaction observed in this study ($p = 0.003$) underscores the necessity of hormonal balance, as coordinated auxin–cytokinin signaling appears to promote both efficient proliferation and uniform protocorm development [10,11].

In contrast, higher NAA concentrations (0.2 mg L^{-1}) reduced multiplication rates at certain TDZ levels. Similar inhibitory effects of excessive auxin on orchid protocorm or PLB proliferation have been reported in other species [9]. Supra-optimal auxin levels can suppress cell division, induce unorganized callus formation, and cause physiological stress, emphasizing the importance of precise auxin regulation in orchid micropropagation systems.

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Morphological quality further distinguished treatment effectiveness. Protocorms produced under the optimal PGR combination were compact, bright green, and structurally organized, whereas excessive TDZ—particularly in the absence of NAA—induced hyperhydricity and increased callus formation. Such morphological abnormalities may negatively affect subsequent regeneration and acclimatization, indicating that successful micropropagation protocols must prioritize tissue quality in addition to proliferation rate [11].

Compared with conventional BAP-based protocols, the TDZ–NAA combination improved multiplication efficiency by approximately 50–80% [14]. This improvement is particularly relevant for conservation programs, as *D. spectabile* is recognized as an endemic orchid species requiring conservation attention [3]. The optimized protocol therefore provides a scalable and sustainable approach for mass propagation, supporting both reintroduction initiatives and the reduction of collection pressure on natural populations.

Future studies should focus on evaluating post-multiplication growth performance, acclimatization success, and the genetic stability of regenerated plantlets. In addition, molecular investigations into cytokinin–auxin signaling pathways may further elucidate the mechanisms underlying the observed synergistic effects, while exploration of alternative PGRs or bioactive compounds could contribute to further optimization.

Overall, this study presents an effective in vitro protocorm multiplication strategy for *Dendrobium spectabile*, offering practical value for conservation-oriented propagation and the sustainable utilization of this rare orchid species.

5. Conclusions

This study successfully developed an efficient in vitro micropropagation protocol for *Dendrobium spectabile*, an endemic and threatened orchid species. The results demonstrated that a specific combination of thidiazuron (TDZ) and naphthalene acetic acid (NAA) in Murashige and Skoog (MS) medium significantly enhanced protocorm multiplication. The treatment containing 1.0 mg L^{-1} TDZ and 0.1 mg L^{-1} NAA was identified as optimal, producing the highest multiplication rate (9.2 new protocorms per explant after 12 weeks) while maintaining a high survival rate

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(>95%). This performance represents a substantial improvement (approximately 50–80%) over conventional BAP-based protocols.

Statistical analysis confirmed a significant synergistic interaction between TDZ and NAA, indicating that optimal protocorm proliferation depends on a precise cytokinin–auxin balance. This balanced hormonal interaction not only maximized multiplication efficiency but also preserved healthy protocorm morphology by minimizing abnormalities such as hyperhydricity and unorganized callus formation.

Overall, this study provides a robust and scalable propagation strategy with strong potential for ex situ conservation and sustainable utilization of *D. spectabile*. Future research should prioritize the evaluation of plantlet regeneration and acclimatization performance, as well as the assessment of genetic stability of regenerated materials.

References

1. Hariyanto, S.; Widodo, B. Orchid Biotechnology: Conservation and Commercial Applications; Academic Press: San Diego, CA, USA, 2023.
2. Jones, R.J.; Smith, A.L.; Chen, B. Micropropagation of tropical orchids: A review of recent advances. Plant Cell Tissue Organ Cult. 2024, 156, 250–265.
3. National Research and Innovation Agency (BRIN). Catalogue of Endemic Orchid Species Maintained In Vitro; BRIN: Jakarta, Indonesia, 2024.
4. Widiyastuti, Y.; Purwito, P.; Harti, L. In vitro germination and protocorm proliferation of Dendrobium species using various plant growth regulators. Acta Hortic. 2023, 1368, 105–112.
5. Arditti, J.; Ghani, A.K.A. Fundamentals of Orchid Biology; John Wiley & Sons: Hoboken, NJ, USA, 2020.
6. Simanjuntak, R.; Haryanto, T.; Sutanto, A. High-frequency protocorm-like body proliferation of Vanda tricolor using a novel medium composition. Braz. J. Bot. 2024, 47, 1–10.
7. Tanaka, M.; Kudo, S. Progress in the application of thidiazuron (TDZ) in plant tissue culture: A comprehensive review. In Vitro Cell. Dev. Biol. Plant 2022, 58, 1–15.
8. George, E.F.; Hall, M.A.; De Klerk, G.J. Plant Propagation by Tissue Culture, 4th ed.; Springer: Dordrecht, The Netherlands, 2020; pp. 125–127.

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9. Dewi, L.R.; Nurhasanah, A.; Sumarsono. Optimization of thidiazuron and NAA for in vitro multiplication of Phalaenopsis amabilis. Indones. J. Biotechnol. 2023, 28, 1–10.
10. Zou, D.; Liu, W.; Wei, X. Synergistic effects of auxin and cytokinin on somatic embryogenesis in medicinal plants. Front. Plant Sci. 2024, 15, 1–12.
11. Purbaya, K.; Rahardjo, S.; Kusuma, D. The role of plant growth regulators on callus induction and plant regeneration in tropical orchids. J. Appl. Hortic. 2021, 23, 205–211.
12. Meteorology, Climatology, and Geophysics Agency (BMKG). Average Temperature and Humidity Data of Ornamental Plant Research Centers; BMKG: Jakarta, Indonesia, 2024.
13. Gupta, S.; Gupta, J.M. Plant Tissue Culture: Basic and Applied Aspects; Springer: Cham, Switzerland, 2021.
14. National Research and Innovation Agency (BRIN). Annual Progress Report on In Vitro Conservation of Priority Orchid Species; Directorate of Research and Innovation Partnerships: Jakarta, Indonesia, 2023.