

## OPTIMIZING IN VITRO GROWTH OF DENDROBIUM HYBRID ORCHIDS THROUGH BASAL MEDIA FORMULATIONS AND COCONUT WATER SUPPLEMENTATION

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### ABSTRACT

*Dendrobium* orchids are among the most widely cultivated ornamental plants, yet large-scale propagation is often constrained by low multiplication rates in conventional methods. In vitro culture offers a reliable alternative, but optimization of basal media and organic supplements is required to improve efficiency. This study investigated the effects of three basal media (Murashige and Skoog, Vacin and Went, and Knudson C) and four concentrations of coconut water (0, 10, 20, and 30%) on the growth of *Dendrobium* hybrid explants. A factorial experiment arranged in a completely randomized design was conducted with three replications per treatment. Results revealed that basal medium composition significantly influenced explant performance, with MS medium supporting the greatest shoot proliferation, root development, and fresh biomass accumulation. Coconut water supplementation enhanced growth in all media, with 20% identified as the most effective concentration. At this level, explants produced an average of 4.8 shoots per explant, 6.2 roots, and 0.92 g fresh weight. Higher supplementation (30%) did not provide additional benefits and occasionally reduced growth responses, indicating that moderate supplementation is optimal. The findings confirm that MS basal medium enriched with 20% coconut water provides the most favorable conditions for in vitro propagation of *Dendrobium* orchids. This protocol offers practical value for commercial orchid production and conservation programs by enabling efficient and sustainable plantlet multiplication.

**Keywords:** *Dendrobium*, In vitro culture, Coconut water, Basal media, Micropropagation

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## 1. INTRODUCTION

Orchids represent one of the largest and most diverse families of flowering plants, with more than 28,000 species and over 100,000 hybrids distributed worldwide (Chase et al., 2022). Among these, *Dendrobium* is one of the most popular genera in the horticultural trade due to its striking floral morphology, wide range of colors, and extended vase life. In many tropical and subtropical regions, *Dendrobium* orchids are cultivated not only as ornamental plants but also as valuable export commodities. The increasing global demand for high-quality orchid cut flowers has intensified efforts to improve propagation methods to ensure sustainable production (Zhang et al., 2023).

Conventional propagation of *Dendrobium* through seeds or vegetative cuttings is often slow and inefficient. Orchid seeds are minute and lack endosperm, making them highly dependent on symbiotic fungi for germination under natural conditions (Arditti & Ghani, 2023). Vegetative propagation, such as keiki production or stem cuttings, is limited by low multiplication rates and risks of pathogen transmission. These limitations have driven the widespread adoption of plant tissue culture techniques, which enable large-scale, uniform, and disease-free plantlet production within relatively short periods. In vitro propagation, therefore, plays a central role in commercial orchid breeding, conservation, and biotechnology applications (Shukla et al., 2023).

The success of orchid micropropagation depends largely on the choice of basal medium and supplementation with appropriate organic and inorganic additives. Murashige and Skoog (MS), Vacin and Went (VW), and Knudson C (KC) are among the most widely used basal media for orchid culture, each with distinct mineral compositions. MS medium is known for its high nitrate and ammonium content, which supports rapid vegetative growth, while VW and KC media are specifically formulated for orchids, providing balanced salts that favor root and shoot differentiation (Nguyen et al., 2023). Selecting the most suitable basal medium for a given genotype is therefore critical for optimizing growth responses.

In addition to basal media, organic supplements have long been recognized as essential for successful orchid tissue culture. Coconut water (CW), a natural source of sugars, vitamins, amino acids, and phytohormones such as cytokinins and auxins, has been widely applied as a growth promoter in orchid micropropagation (Tiwari et al., 2024). Studies have demonstrated that coconut water enhances cell division, promotes callus proliferation, and stimulates both shoot and root development in several orchid species (Mishra et al., 2023). However, the optimal concentration varies considerably depending on the orchid genotype and the basal medium used, with excessive supplementation sometimes leading to abnormal growth or vitrification.

The interaction between basal medium composition and coconut water supplementation is particularly important. For instance, Nguyen et al. (2023) reported that *Phalaenopsis* explants responded differently to coconut water depending on whether MS or VW medium was used, highlighting the role of nutrient-organic interactions in morphogenesis. Similarly, experiments with *Cymbidium* and *Dendrobium* hybrids demonstrated that combining a moderately nutrient-rich medium with 10–20% coconut water yielded the highest regeneration rates (Singh et al., 2023; Putri et al., 2024). Such findings underline the necessity of tailoring medium formulations for specific species and hybrids to maximize efficiency.

Beyond practical horticultural concerns, optimizing in vitro culture also contributes to orchid conservation. Many *Dendrobium* species are threatened by habitat loss, overcollection, and climate change (Rahman et al., 2023). Efficient propagation protocols allow for ex situ conservation and reintroduction programs, ensuring genetic resources are preserved while reducing pressure on wild populations. Moreover, tissue culture provides an essential platform for advanced biotechnological interventions, including genetic transformation, secondary metabolite production, and cryopreservation (Wang et al., 2024).

Recent advances in orchid biotechnology further highlight the importance of refining conventional culture practices. For example, molecular studies have identified key regulatory genes associated with shoot apical meristem activity and hormone signaling, which are directly influenced by nutrient and organic supplement availability (Li et al., 2022). Integrating such molecular insights with empirical culture optimization may open new opportunities for precision propagation of elite hybrids.

Despite substantial progress, there remain gaps in understanding the combined effects of basal medium composition and coconut water supplementation on the growth of *Dendrobium* hybrids. While several studies have tested individual factors, few have systematically compared multiple basal media and coconut water concentrations simultaneously. Given the economic significance of *Dendrobium* orchids, developing optimized protocols tailored to hybrid genotypes is essential for enhancing propagation efficiency and supporting commercial orchid industries.

The present study was therefore undertaken to evaluate the influence of different basal media (MS, VW, and KC) and coconut water concentrations (0, 10, 20, and 30%) on the in vitro growth of *Dendrobium* hybrid explants. The specific objectives were: (i) to assess the effects of basal media on shoot and root development, (ii) to determine the optimal concentration of coconut water for promoting morphogenesis, and (iii) to identify the best medium–supplement combination for maximizing explant growth. The outcomes of this study are expected to contribute practical insights for orchid propagation industries and provide a scientific basis for further research in orchid tissue culture.

## 2. MATERIALS AND METHODS

The study was conducted using a factorial experiment arranged in a completely randomized design (CRD). Two experimental factors were tested: basal medium type and coconut water concentration. The basal media compared were Murashige and Skoog (MS), Vacin and Went (VW), and Knudson C (KC), each representing a widely used formulation in orchid tissue culture. Coconut water was added to the media at four concentrations: 0%, 10%, 20%, and 30% (v/v). Each treatment combination was replicated three times, with five explants per replicate.

Explants were obtained from healthy *Dendrobium* hybrid shoot tips that had been maintained under sterile in vitro conditions. Prior to culture, explants were trimmed to uniform size to ensure consistency across treatments. Media were prepared by supplementing the basal salts with 30 g L<sup>-1</sup> sucrose as a carbon source and 7 g L<sup>-1</sup> agar as a gelling agent. The pH of all media was adjusted to 5.8 before sterilization by autoclaving at standard conditions. After sterilization, coconut water was incorporated according to the treatment levels under aseptic conditions.

Explants were inoculated individually into culture vessels containing 20 mL of medium and incubated in a controlled growth chamber under a 16-hour photoperiod with light intensity of approximately 2,000 lux. The temperature was maintained within the optimal range for orchid culture to support explant establishment and morphogenesis.

Growth responses were evaluated after eight weeks of culture. Parameters measured included the number of shoots formed per explant, shoot length, number of roots, root length, and overall fresh weight of plantlets. Measurements were taken carefully using a digital caliper and analytical balance to ensure accuracy. Observations of morphological traits, such as leaf shape and coloration, were also recorded to identify potential abnormalities.

Data were analyzed using analysis of variance (ANOVA) appropriate for the factorial completely randomized design. When significant differences were detected, treatment means were separated using Duncan’s Multiple Range Test (DMRT) at the 5% probability level. Statistical analyses were performed using standard software packages to ensure reliability of the findings.

### 3. RESULTS

#### Effect of Basal Media and Coconut Water on Shoot Development

The type of basal medium and concentration of coconut water significantly influenced the number and length of shoots produced by Dendrobium hybrid explants (Table 1). MS medium consistently supported the highest shoot proliferation, particularly when supplemented with 20% coconut water, which yielded an average of 4.8 shoots per explant with a mean length of 2.7 cm. By contrast, VW medium without coconut water produced the lowest shoot numbers, averaging only 1.6 shoots with an average length of 1.2 cm. KC medium produced intermediate results, with shoot formation improving at higher coconut water levels but remaining below those of MS. These findings indicate that MS medium provides more balanced mineral nutrition for active shoot initiation, while coconut water further enhances cell division and elongation.

**Table 1:** Effect of Basal Media and Coconut Water on Shoot Number and Length of Dendrobium Hybrid Explants

Medium	Coconut Water (%)	Shoot Number (per explant)	Shoot Length (cm)
MS	0	2.3 b	1.8 b
MS	10	3.5 c	2.2 bc
MS	20	4.8 d	2.7 c
MS	30	3.9 cd	2.4 bc
VW	0	1.6 a	1.2 a
VW	10	2.2 ab	1.6 ab
VW	20	2.9 bc	1.9 b
VW	30	2.5 b	1.7 b
KC	0	2.0 ab	1.5 ab
KC	10	2.7 bc	1.9 b
KC	20	3.4 c	2.1 bc
KC	30	3.1 bc	2.0 b
HSD 5%	–	0.6	0.4

Note: Means followed by the same letters are not significantly different at 5% (DMRT).

#### Effect on Root Formation

Rooting responses also varied significantly across treatments (Table 2). MS medium with 20% coconut water produced the highest number of roots (6.2 per explant) with an average root length of 3.8 cm, while VW without coconut water resulted in poor rooting (1.5 roots per explant, 1.0 cm average length). KC medium supported moderate rooting, but performance improved markedly at 20% coconut water supplementation. These results suggest that while all media responded positively to coconut water addition, MS medium provided the most favorable nutrient environment for root development.

**Table 2:** Effect of Basal Media and Coconut Water on Root Number and Length of Dendrobium Hybrid Explants

Medium	Coconut Water (%)	Root Number (per explant)	Root Length (cm)
MS	0	3.1 b	2.1 b
MS	10	4.4 c	2.9 c
MS	20	6.2 d	3.8 d
MS	30	5.0 cd	3.1 c
VW	0	1.5 a	1.0 a
VW	10	2.3 ab	1.6 ab
VW	20	3.2 b	2.3 b
VW	30	2.8 b	2.0 b
KC	0	2.1 ab	1.5 ab
KC	10	3.0 b	2.1 b
KC	20	4.1 c	2.8 c
KC	30	3.7 bc	2.4 bc
HSD 5%	–	0.7	0.5

#### Effect on Fresh Weight

Fresh weight of explants, an indicator of overall biomass accumulation, was also strongly influenced by both basal medium and coconut water concentration (Table 3). MS medium with 20% coconut water again produced the highest

biomass (0.92 g per explant), while VW without coconut water recorded the lowest (0.32 g). Across all three basal media, supplementation with 20% coconut water consistently resulted in the highest fresh weights, while 30% supplementation did not provide additional benefits and sometimes caused slight reductions, likely due to osmotic stress.

**Table 3:** Effect of Basal Media and Coconut Water on Fresh Weight of Dendrobium Hybrid Explants

Medium	Coconut Water (%)	Fresh Weight (g)
MS	0	0.55 b
MS	10	0.72 c
MS	20	0.92 d
MS	30	0.79 c
VW	0	0.32 a
VW	10	0.48 ab
VW	20	0.61 b
VW	30	0.55 b
KC	0	0.40 ab
KC	10	0.58 b
KC	20	0.73 c
KC	30	0.67 bc
HSD 5%	–	0.12

Overall, MS basal medium consistently outperformed VW and KC across all measured parameters, with 20% coconut water identified as the most effective supplement level. Excessive coconut water (30%) tended to reduce performance, suggesting that moderate supplementation provides the optimal balance of organic growth factors and osmotic conditions.

shoot proliferation and elongation. This can be attributed to the relatively high nitrate and ammonium content in MS, which stimulates cell division and chlorophyll synthesis. Recent studies have confirmed that nitrogen availability is a key determinant of shoot initiation and elongation in orchids, as it directly influences amino acid and protein biosynthesis (Haque et al., 2023). Furthermore, the balanced supply of macronutrients in MS medium facilitates the maintenance of osmotic stability, which is critical for explant viability under in vitro conditions (Chen et al., 2024).

By contrast, VW medium resulted in the lowest shoot formation. Although VW was originally designed for orchid culture, its relatively lower macronutrient concentrations may limit its suitability for hybrid Dendrobium explants, which often require higher nutrient input due to their vigorous growth characteristics. Similar observations were reported in *Phalaenopsis* and *Oncidium* hybrids, where MS medium consistently produced superior shoot multiplication compared with VW and KC formulations (Zhou et al., 2023; Park et al., 2024). KC medium yielded intermediate results, suggesting that while it can support orchid growth, it may not provide the optimal balance of minerals required for shoot proliferation in Dendrobium hybrids.

Coconut water has long been recognized as a valuable supplement in plant tissue culture, largely due to its complex composition of sugars, vitamins, amino acids, and phytohormones. In this study, coconut water supplementation enhanced shoot formation across all basal media, with 20% identified as the optimal concentration. At this level, explants exhibited both higher shoot numbers and greater shoot length, indicating that coconut water provides the necessary growth regulators to stimulate meristematic activity. Similar findings were reported in *Cymbidium* and *Paphiopedilum* cultures, where moderate coconut water supplementation improved shoot differentiation and vigor (Kumari et al., 2023; Srinivasan et al., 2024).

However, supplementation at 30% did not yield further improvements and in some cases reduced growth responses. This decline can be explained by osmotic stress caused by high concentrations of organic solutes, which may disrupt water and nutrient uptake. Excess cytokinins and auxins from coconut water can also induce physiological imbalances, leading to abnormal morphogenesis or vitrification (Tan et al., 2022). Thus, the concentration of natural supplements must be carefully calibrated to balance benefits against potential inhibitory effects.

Root formation is a crucial stage in orchid micropropagation, as it determines the ability of plantlets to acclimatize successfully after transfer to ex vitro conditions. The present study showed that MS medium combined with 20% coconut water produced the highest root number and longest roots. These results suggest that a combination of balanced mineral nutrients and natural growth regulators is required for effective root induction. Nitrogen and potassium in MS medium promote root initiation and elongation, while the cytokinins and auxins in coconut water enhance cell differentiation in root meristems. Comparable outcomes were observed in *Vanda* and *Dendrobium* orchids, where supplementation with natural organic additives improved rooting efficiency (Patil et al., 2023; Lestari et al., 2024).

VW and KC media produced significantly fewer roots, even when supplemented with coconut water. This may be due to insufficient mineral composition, particularly of nitrate and phosphate, which are critical for root

#### 4. DISCUSSION

The in vitro propagation of *Dendrobium* orchids continues to be an essential strategy for ensuring large-scale, uniform, and disease-free planting materials for both commercial and conservation purposes. The present study demonstrated that both basal medium type and coconut water supplementation significantly influenced explant growth. The results highlight that Murashige and Skoog (MS) basal medium consistently outperformed Vacin and Went (VW) and Knudson C (KC), particularly when combined with 20% coconut water. This combination promoted greater shoot and root formation as well as higher fresh biomass accumulation. These findings underscore the importance of optimizing the interaction between mineral nutrition and organic additives in orchid micropropagation.

Basal media provide the fundamental mineral framework required for in vitro culture, and their composition greatly affects morphogenesis. In this study, MS medium consistently supported the highest

development. Moreover, the buffering capacity of MS medium may have interacted synergistically with coconut water to create more favorable rhizogenic conditions. The superiority of MS for rooting has also been demonstrated in other orchid genera, including *Aranda* and *Cattleya*, confirming its broad applicability for orchid propagation (Nambiar et al., 2023; Kim et al., 2024).

Fresh weight of explants is an integrative measure of overall growth and biomass accumulation. In this study, the highest biomass was observed in MS medium with 20% coconut water. This combination likely provided optimal mineral nutrition for metabolic activity while simultaneously supplying natural vitamins and phytohormones that promote photosynthesis and carbohydrate metabolism. Studies on *Phalaenopsis* and *Oncidium* orchids have also linked coconut water supplementation to enhanced carbohydrate accumulation, leading to higher biomass gain (Santos et al., 2023; Ahmad et al., 2024).

Interestingly, biomass accumulation plateaued or slightly declined at 30% coconut water supplementation across all basal media. This suggests that excessive organic supplementation can impose osmotic constraints or hormonal imbalances that reduce growth efficiency. Therefore, while natural supplements are valuable, their concentrations must be optimized to avoid negative trade-offs.

The results of this study have direct implications for orchid industries, particularly in regions where coconut water is readily available. Identifying MS medium supplemented with 20% coconut water as the most effective treatment provides a practical and cost-efficient protocol for large-scale propagation of *Dendrobium* hybrids. Unlike synthetic growth regulators, coconut water is inexpensive, easily accessible, and environmentally friendly, making it suitable for commercial laboratories and small-scale nurseries alike. Furthermore, the integration of natural additives aligns with current trends in sustainable horticulture and the demand for eco-friendly propagation techniques (Gupta et al., 2023).

While this study focused on shoot, root, and biomass growth, future research should expand to include physiological and biochemical markers such as chlorophyll content, photosynthetic efficiency, and antioxidant enzyme activity. These parameters would provide deeper insights into how basal media and coconut water interact at the metabolic level. Moreover, the molecular basis of coconut water's effects on hormone signaling and gene expression in orchids remains poorly understood. Recent advances in transcriptomic and proteomic analyses could be applied to elucidate these mechanisms (Mei et al., 2024).

It is also important to consider acclimatization success in *ex vitro* conditions. Plantlets with higher root number and biomass are more likely to survive transplanting into soil. Thus, evaluating survival rates after acclimatization would provide practical validation of the identified optimal treatment. Additionally, combining coconut water with other natural additives, such as banana homogenate or peptone, could further enhance plantlet quality (Arulmozhiyan et al., 2024).

Finally, conservation programs for threatened orchid species may also benefit from the optimized protocols established for hybrids. The cost-effectiveness and accessibility of coconut water supplementation make it an attractive option for large-scale multiplication of rare or endangered *Dendrobium* species, contributing to *ex situ* conservation and biodiversity restoration efforts (Sivakumar et al., 2023).

## Conclusion

The present study demonstrated that both basal medium composition and coconut water supplementation play critical roles in the *in vitro* propagation of *Dendrobium* hybrid orchids. Among the treatments tested, Murashige and Skoog (MS) basal medium consistently produced the best results, supporting superior shoot initiation, root development, and biomass accumulation compared with Vacin and Went (VW) and Knudson C (KC) media. The addition of coconut water significantly enhanced explant growth across all media, with 20% identified as the optimal concentration. At this level, explants displayed the highest shoot numbers, longest roots, and greatest fresh weight, indicating a synergistic effect between mineral nutrition and natural organic supplements.

Excessive supplementation with 30% coconut water did not further improve growth and in some cases reduced performance, likely due to osmotic stress or hormonal imbalance. These findings highlight the importance of balancing mineral and organic inputs in orchid tissue culture. The optimized protocol of MS basal medium with 20% coconut water offers a cost-effective and sustainable method for large-scale propagation of *Dendrobium* hybrids. Beyond commercial applications, such protocols can support conservation programs by enabling efficient *ex situ* multiplication of valuable germplasm. Future research should focus on molecular and physiological mechanisms underlying the observed responses, as well as evaluating acclimatization success under *ex vitro* conditions.

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