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(RESEARCH ARTICLE)

Effect of modifying basal medium strength on the in vitro propagation of *Dendrobium mussauense*

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Abstract

Dendrobium mussauense an orchid species endemic to specific regions, is increasingly rare in its natural habitat. This study aimed to evaluated the effects of Murashige and Skoog (MS) medium modification on the in vitro propagation of *D. mussauense*. The experiment was conducted using Completely Randomized Design (CRD), consisting of two MS medium types (full-strenght and half-strenght) with nine replications. Data were analyzed using Analysis of Variance (ANOVA), followed by Duncan's Multiple Range Test (DNMRT) at a 5% significance level. The result indicated that the of half strength MS medium significantly enhanced shoot induction. Specifically, shoot number increased by 380% and shoot length by 630%. These findings suggest that half-strenght MS medium is the most effective formulation for promoting shoot proliferation in *D. mussauense* micropropagation.

keywords: Orchid Epiphytic; In Vitro Culture; MS Medium; Modification; Shoot Proliferation

1. Introduction

Dendrobium mussauense is an endemic orchid species whose natural populations are becoming increasingly difficult to find in the wild. As a member of the genus *Dendrobium*, this species possesses considerable ornamental value due to its attractive morphology and potential for commercial cultivation. However, habitat destruction, environmental disturbances, and uncontrolled collection from natural habitats have contributed to a continuous decline in its population [1]. In addition, orchids generally exhibit slow natural propagation because their seeds are extremely small, lack endosperm, and require symbiotic associations with mycorrhizal fungi for germination [2]. These limitations reduce the success rate of natural regeneration and threaten the long-term sustainability of endemic orchid populations. According to the International Union for Conservation of Nature (IUCN), *D. mussauense* is categorized as Vulnerable [3]. Furthermore, the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) includes this species in Appendix II, indicating that its international trade must be regulated to prevent overexploitation [4]. These conditions highlight the urgent need for effective conservation strategies that are capable of supporting large-scale propagation while maintaining plant quality and genetic uniformity.

In vitro propagation has been widely recognized as an efficient approach for the conservation and mass propagation of rare and endangered orchids. This technique enables the aseptic culture of plant tissues, organs, or cells under controlled environmental conditions, allowing rapid production of uniform plantlets in relatively large quantities [5]. Nevertheless, the success of in vitro culture is strongly influenced by the composition of the culture medium, particularly the concentration of macro- and micronutrients, vitamins, carbon sources, and growth regulators [6]. Among various basal media, Murashige and Skoog (MS) medium is one of the most commonly used formulations because of its comprehensive nutrient composition and high mineral salt content [7]. However, several studies have reported that

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full-strength MS medium is not always optimal for epiphytic orchids, which naturally grow under relatively low nutrient availability. [8] demonstrated that half-strength MS (½ MS) medium improved seed germination and seedling development in *Phalaenopsis amboinensis*. Similarly, [9] reported that half-strength basal media promoted better vegetative growth in *Dendrobium moniliforme*. Comparable findings were also observed in other orchid species, indicating that nutrient reduction may create a more favorable osmotic and physiological environment for orchid development in vitro.

Despite numerous studies on orchid micropropagation, information regarding the appropriate basal medium composition for *Dendrobium mussauense* remains limited. Basal nutrient composition plays an important role in regulating in vitro growth, while excessive mineral concentrations may inhibit organ development, particularly in epiphytic orchids adapted to nutrient-poor environments [10,11]. Therefore, this study evaluated the use of full-strength MS and half-strength MS (½ MS) media as a practical and cost-effective approach to improve organ development and plantlet vigor in *D. mussauense.*

2. Method

This research was conducted from November 2024 to January 2025 at the Plant Physiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Andalas, Padang. The study employed a Completely Randomized Design (CRD) with one treatment factors. The factor was the type of culture medium: full-strength Murashige and Skoog (MS) and half-strength MS (½ MS). This experiment consisted of nine replicates and a total of 18 experimental units. The materials used included *D. mussauense* plantlets obtained from the Robiquetia Garden Lab, standard MS stock solutions, granulated sugar, agar, and activated charcoal. Essential equipment included culture bottles, autoclave, analytical balance, magnetic stirrer, hot plate, beakers, pH meter, sterilization tools, and Laminar Air Flow Cabinet (LAFC) for aseptic handling.

The samples used in this study were *D. mussauense* shoot explants, which served as a tissue source for propagation. In vitro propagation was conducted using MS and ½ MS media. The explants were incubated at room temperature (24–25°C) for approximately 30 days. The observed variables in this study were: explant survival rate (%), time to shoot emergence, number of shoots, shoot length, time to leaf emergence, number of leaves, leaf length, time to root emergence, number of roots, root length, and planlet height.

Culture media were modification by MS in appropriate proportions into 1000 mL of distilled water. Sucrose (30 g/L), agar (7 g/L), and activated charcoal (1 g/L) were added. The pH was adjusted to 5.8–6.0 and autoclaved at 121°C for 15 minutes. The media were then stored for three days to monitor contamination before use. All tools were sterilized prior to use by immersion in bleach for 24 hours, followed by washing, drying, and autoclaving. The LAFC workspace was sterilized using 70% ethanol and UV light exposure for two hours before each inoculation session. The plantlets were gently removed from their culture vessels, and healthy shoots approximately 1 cm in length were transplanted onto fresh media according to their respective treatments (two explants per bottle). All cultures were maintained at room temperature (24–26°C) in a culture room under constant conditions.

Daily maintenance included spraying 70% ethanol on the culture bottles and promptly removing any contaminated cultures. Observations were made for eight weeks and included eleven growth parameters: survival percentage, shoot emergence time, shoot length, number of shoots, time to leaf emergence, number of leaves, leaf length, root emergence time, root length, number of roots, and plantlet height. Measurements were taken under sterile conditions in the LAFC, using millimeter paper when necessary. The data were analyzed statistically using Analysis of Variance (ANOVA) in SPSS version 24 Differences among treatments were further evaluated using Duncan's New Multiple Range Test (DNMRT) at a 5% significance level.

3. Result and Discussion

3.1. Percentage of explant survival, contamination and browning (%)

The application of various different media types (MS and ½ MS) (Table 1). All treatments were able to maintain explants in a viable state with a healthy green appearance, indicating that the media used sufficiently met the basic nutritional requirements to support planlet survival.

Table 1 Survival, contaminating, and browning rates of *Dendrobium mussauense* explants (%)

Parameters	Media composition treatment	
	MS	½ MS
Percentage of explants survival	100	100
Percentage of contaminating	0	0
Percentage of browning	0	0

Explants cultured on both MS and ½ MS media achieved a 100% survival rate, without any contamination or browning throughout the observation period. The explants maintained a healthy green appearance, indicating that both media formulations successfully provided the necessary nutrients for initial adaptation and viability of *Dendrobium mussauense* plantlets in vitro. The absence of contamination across all treatments confirms the efficacy of the in vitro explant. In plant tissue culture, establishing aseptic conditions is crucial, as microbial growth can severely disrupt nutrient uptake, hinder explant development, and lead to culture mortality [12]. The 0% contaminating rate achieved here demonstrates that the sterilization method used is highly suitable for *D. mussauense* explants.

Furthermore, no browning symptoms were observed in either treatment. Tissue browning typically occurs when phenolic compounds, released from wounded tissues during cutting, undergo oxidation. The lack of browning suggests that the explants experienced minimal physiological stress and maintained cellular stability during culture initiation. This outcome is likely attributed to the selection of healthy donor material and an optimal media composition that minimized phenolic exudation [10].

At this early stage, both full-strength and half-strength MS media proved equally effective in supporting explant survival. While full MS medium offers a higher concentration of macro and micronutrients [13], the results show that *D. mussauense* explants can adapt and survive just as well under the reduced salt concentrations of ½ MS. This response aligns with the characteristic behavior of many orchid species, which often thrive on diluted media due to their low nutrient requirements during the initial phases of in vitro development [14,15]. This finding is consistent with previous studies, who reported a 100% survival rate in *Phalaenopsis amabilis* explants cultured on MS medium [16]. Similarly, study reported 100% survival in *Dendrobium* explants treated with potato extract and coconut water in culture media [17,18].

3.2. Effect of Medium Composition

Plant tissue culture media serve as an essential source of nutrients and as a regulator of the growth environment for explants. The Murashige and Skoog (MS) formulation is widely recognized in plant tissue culture due to its comprehensive and relatively high mineral salt content; however, in certain species particularly epiphytic orchids these concentrations may exceed the optimal requirements for growth [7]. The use of reduced-salt-strength media, such as half-strength MS (½ MS), has often been reported to better match nutrient availability with the physiological needs of plants that naturally grow in nutrient-limited environments [8]. The results of this study demonstrated differential growth responses of *D. mussauense* between full-strength MS and ½ MS media, as reflected in organ initiation, organ number, and morphological size parameters (Table 2).

Table 2 Effect of media composition on the propagation of *Dendrobium mussauense*

Parameters	Media composition treatment (A)	
	MS	½ MS
Shoot emergence time (days)	33.06 ± 10.32^{b}	22.56 ± 6.99^{a}
Number of shoots	1.81 ± 0.75^{a}	6.88 ± 4.17^{b}
Shoot length (mm)	3.20 ± 1.75^{a}	4.90 ± 1.24^{b}
Leaf emergence time (days)	35.35 ± 12.68^{b}	28.88 ± 4.39^{a}
Number of leaves	3.19 ± 2.07^{a}	10.06 ± 5.84^{b}
Leaf length (mm)	3.80 ± 1.96^{a}	6.51 ± 1.36^{b}

Root emergence time (days)	36.31 ± 13.81[b]	23.81 ± 6.08[a]
Number of roots	2.50 ± 1.78[a]	6.44 ± 3.70[b]
Root length (mm)	3.60 ± 3.10[a]	9.50 ± 4.64[b]
Planlet height (mm)	10.02 ± 5.34[a]	20.92 ± 4.84[b]

Notes : Numbers followed by the same letters are not significantly different based on the DNMRT test at the 5% level.

For the parameter of shoot emergence time, ½ MS medium tended to accelerate initiation compared with full-strength MS. This phenomenon can be explained by the lower osmotic and ionic potential in ½ MS, creating a more favorable water gradient from the medium to the cells, thereby maintaining turgor [19]. Optimal turgor pressure facilitates cell wall expansion mediated by enzymes such as expansins and pectin methylesterases, which are involved in early cell enlargement at the shoot primordia [20]. In contrast, excessive ions such as ammonium (NH_4^+) and nitrate (NO_3^-) in full-strength MS may induce physiological stress, increase endogenous abscisic acid (ABA) accumulation, and slow cell division in the shoot meristem [12].

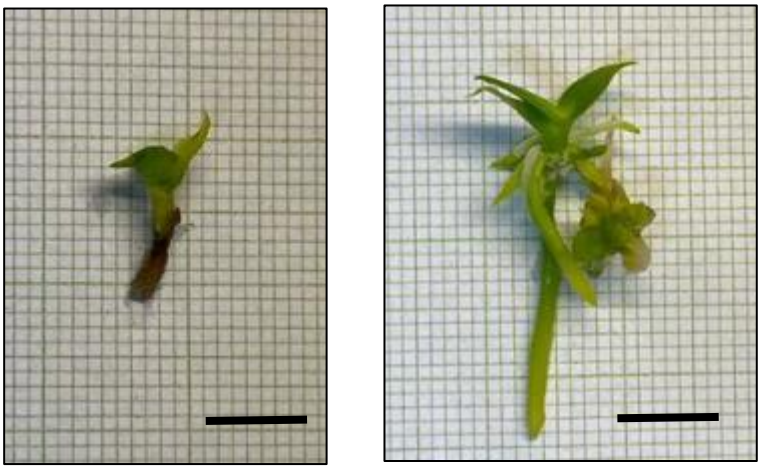
The number of shoots produced was also higher in the low-salt medium. This similar is consistent with the report, that ½ MS promoted shoot proliferation in several *Dendrobium* species [2]. Physiologically, excessive nitrogen and mineral salt levels can disrupt the auxin–cytokinin ratio within tissues, thereby inhibiting the formation of new shoots [21]. The ½ MS medium provides a nutrient balance more suited to epiphytic orchids, which in their natural habitat are adapted to low but balanced nutrient supply.

Greater shoot length observed in ½ MS indicates that this medium also better supports cell elongation processes. Elongation requires a combination of stable turgor pressure, cell wall plasticity, and energy supply for the synthesis of structural components [20]. The more moderate ionic content in ½ MS helps maintain osmotic homeostasis, thereby avoiding inhibition of cell division and elongation. In contrast, high-salt media may cause partial plasmolysis or metabolic disturbances that impede elongation.

The parameter of leaf emergence time showed a similar trend to shoots, with ½ MS promoting earlier leaf initiation. The development of new leaves depends heavily on shoot apical meristem activity and tissue differentiation, processes that require a balanced supply of carbohydrates and hormonal signals [22]. Low-salinity media can reduce oxidative stress accumulation that would otherwise inhibit leaf initiation. In addition, such media tend to maintain an auxin–cytokinin ratio that supports the formation of new vegetative organs.

The higher number of leaves produced in ½ MS suggests that this medium sustains continuous vegetative growth. Leaves are the primary organs of photosynthesis; thus, an increase in leaf number indirectly enhances the photosynthetic capacity and energy supply for plantlet growth [9]. [11] also reported that reduced-salt media promoted greater leaf production in *Phalaenopsis* compared with standard formulations. Longer leaf size in ½ MS further indicates that moderate ionic and osmotic conditions facilitate foliar tissue expansion. Leaf length growth requires integration of cell turgor, mesophyll tissue expansion, and epidermal cell elongation [23]. Such conditions are optimal in media with adequate but not excessive nutrient availability, preventing ion toxicity or metabolic impairment.

Root emergence time was also faster in ½ MS compared with full-strength MS. Root formation is highly sensitive to the auxin–cytokinin balance. The high nitrogen content in full-strength MS may elevate endogenous cytokinin levels, which can suppress root initiation [7]. The lower nitrogen content in ½ MS provides a hormonal environment more favorable for root differentiation. A greater number of roots in ½ MS indicates that this medium supports the proliferation of belowground organs. This is important for the acclimatization phase, as a robust root system improves the plantlet's ability to absorb water and nutrients after transfer to ex vitro conditions. Additionally, the greater root length in ½ MS suggests that this medium facilitates root elongation, possibly through an increased auxin–cytokinin ratio conducive to lateral and primary root growth [21].



(a) (b)

Figure 1 Morphological response of Dendrobium mussauense explants to different of MS modification, (a) full-strenght MS and (b) half-strenght MS

Overall plantlet height reflects the cumulative growth of shoots, leaves, and roots. The difference in plantlet height between the two media indicates integration of these parameters (figure 1). The low-salt medium supported balanced organ growth, resulting in plantlets with better vigor and more uniform morphology [24]. These findings reinforce the view that adjusting basal medium strength is an effective strategy for improving in vitro culture success in species sensitive to medium salinity.

4. Conclusion

Modification of the culture medium by reducing the macronutrient concentration to half-strength MS (½ MS) resulted in a statistically significant improvement in the development of shoots, roots, and leaves, with shoot proliferation increasing. Specifically, shoot number increased by 380% and shoot length by 630% compared to the full-strength MS medium.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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