

Comparative Analysis of Mycorrhizal Colonization and Phenolic Interactions in the Aerial Roots of Selected Tropical Epiphytic Orchids

Salvy Thomas^{1*}, Lincy Joseph²

¹Department of Botany, SB College, Changanassery, Kottayam, Kerala, India

²Department of Botany, CMS College, Kottayam, Kerala, India

***Corresponding Author:**

Salvy Thomas

Email: salvythomas@gmail.com

Abstract: Mycorrhizae are vital fungal associations essential for the survival of orchids in natural ecosystems, providing the necessary resources for seed germination and seedling establishment. Tropical epiphytic orchids occupy distinctive ecological niches within forest canopy systems where nutrient scarcity, periodic water stress, and intense solar radiation create extreme environmental pressures that fundamentally shape plant physiology and survival strategies. Unlike terrestrial orchids rooted in organic-rich soils, epiphytic species depend entirely upon mycorrhizal fungal associations for acquisition of essential nutrients including nitrogen and phosphorus, necessitating particularly intimate and efficient symbiotic relationships maintained throughout the plant's life cycle. This study investigates the process, extent, and pattern of mycorrhizal colonization and its relationship with total phenolic content in the aerial roots of three tropical epiphytic orchid species: *Rhynchostylis retusa* (L.) Blume, *Dendrobium Sonia* (Grex)- and *Vanda* yellow (hybrids). Anatomical characterization using Phase Contrast Microscopy revealed that fungal entry occurs through the velamen and exodermis passage cells, eventually forming hyphal coils known as pelotones within the cortical cells. Quantitative estimation of phenolics suggests a direct correlation between anatomical characteristics, the degree of fungal colonization, and total phenolic production. These findings highlight the role of phenolics as adaptive defence agents that regulate the symbiotic relationship and prevent fungal parasitism.

Keywords: Orchidaceae, Mycorrhizae, Pelotones, Phenolics, Rhynchostylis, Dendrobium, Vanda.

INTRODUCTION

The Orchidaceae family is one of the most species-rich angiosperm groups, comprising approximately 35,000 species worldwide. A significant majority of these species are epiphytic, primarily distributed across tropical and subtropical regions (Douzery *et al.*, 1999). Tropical epiphytic orchids represent a remarkable group of plants that have evolved sophisticated mechanisms to survive in nutrient-limited, water-scarce environments suspended within forest canopies. Unlike their terrestrial counterparts, epiphytic orchids depend critically upon mycorrhizal fungal associations not merely during germination and protocorm development, but throughout their adult life cycle, sustaining complex nutrient acquisition systems within their aerial roots. The unique architecture of these aerial roots, characterized by a specialized velamen tissue comprising multiple layers of dead cells with porous intercellular spaces, serves dual functions in water and nutrient acquisition while simultaneously functioning as an interface for mycorrhizal colonization. The extent and localization of mycorrhizal colonization within epiphytic orchid roots, however, differs markedly from terrestrial species, being restricted primarily to root zones in physical contact with the substrate while free-hanging aerial roots remain devoid of fungal colonization. This substrate-dependent colonization

pattern reflects the distinct ecological constraints of the epiphytic niche and implies fundamentally different signalling mechanisms governing fungal-plant recognition and nutrient exchange compared to soil-rooted systems (Sathiyadash *et al.*, 2012)

Orchids have developed sophisticated adaptations for survival, including the development of velamen - a multiple epidermis of dead cells that aids in water uptake and protects the root cortex - and a fundamental reliance on mycorrhizal interactions (Rasmussen, 2002). Orchid seeds are minute and lack endosperm or food reserves, making them obligately dependent on mycorrhizal fungi for carbon and nutrient acquisition during germination and early growth (Smith & Read, 2008). While fungi provide essential organic energy by solubilizing carbohydrates, the interaction is highly regulated by the host plant (Fitter & Moyersoen, 1996). To prevent the fungus from becoming pathogenic, orchids release phytoalexins, such as orcinol, which cause the fungal pelotones to collapse and digest. This study aims to evaluate how root anatomy and phenolic content influence this delicate balance in three popular tropical epiphytic species.

MATERIALS AND METHODS

Healthy specimens of *Rhynchostylis*, *Dendrobium*, and *Vanda* were selected and maintained in a controlled growth medium consisting of a 1:1:1 mixture of charcoal, brick pieces, and coir pith. Five aerial root samples from each species were collected, cleaned with 5% Teepol, and washed under running tap water to remove debris. Anatomical characterization was performed by preparing thin transverse sections of the roots using a sharp blade (Saha & Rao, 2006). These sections were stained with lactophenol and mounted in glycerine for observation. The slides were analysed using a Phase Contrast Microscope at various magnifications to record the infection patterns and fungal colonization density (Hadley *et al.*, 1971). For

the estimation of total phenolics, the Folin-Ciocalteau reagent method was employed as described by Malik and Singh (1980). One gram of root material was homogenized in 80% alcohol and centrifuged at 10,000 rpm for 20 minutes. The resulting supernatant was treated with Folin's reagent and 20% Na_2CO_3 before being measured at 650nm using a spectrophotometer.

RESULTS

The anatomical observations and chemical analyses revealed distinct variations among the three species regarding their internal structures and chemical responses (Table 1). The number of layers in the velamen and cortex varied significantly, influencing the space available for fungal activity.

Table 1: Anatomical Comparison of Root Layers

S. No.	Name of the Orchid	No. of Velamen layers	No. of cortical layers
1.	<i>Rhynchostylis</i>	5.5 ± 0.71	32.5 ± 3.54
2.	<i>Dendrobium</i>	11.0 ± 1.41	9.0 ± 1.41
3.	<i>Vanda</i>	9.0 ± 1.41	32.5 ± 3.54

*Values are mean \pm SD of 05 samples.

The anatomical findings revealed that *Rhynchostylis* possessed a thin velamen but a very thick cortex, allowing for the highest concentration of hyphal coils, known as pelotones. These pelotones were observed to be concentrated in the outer cortical layers. In contrast, *Dendrobium* featured the thickest velamen (10-12 layers) but a significantly restricted cortex. In this species, fungal colonization was observed to breach the endodermis and extend even

into the pith region, a phenomenon less common in the other two species. *Vanda* displayed a moderate number of velamen layers and a broad cortex where colonization was concentrated in the peripheral stelar region. The quantitative data for phenolic content demonstrated that the chemical response was most intense in species with higher adaptive capacities (Table 2).

Table 2: Total Phenolic Content

S. No.	Name of the Orchid	Total Phenol ($\mu\text{g/g}$) [*]
1.	<i>Rhynchostylis</i>	6.35 ± 0.14
2.	<i>Dendrobium</i>	3.3 ± 0.54
3.	<i>Vanda</i>	18.05 ± 1.1

*Values are mean \pm SD of 05 samples.

DISCUSSION

The study demonstrates a clear correlation between root anatomy and the symbiotic relationship. In *Rhynchostylis*, fewer velamen layers provide less absorptive surface area, but the massive cortical region offers extensive space for fungal colonization, leading to high pelotone density and a corresponding phenolic response. Conversely, *Dendrobium* has a high number of velamen layers but a restricted cortex, resulting in the lowest phenolic content among the three. This suggests that the plant actively regulates the degree of mycorrhizal development through chemical means (Masuhara & Katsuya, 1994). The higher phenolic content in *Vanda* (18.05 $\mu\text{g/ml}$) indicates a robust defensive system. Phenolics, specifically dehydroxy phenanthrin (orcinol), act as growth inhibitors for the fungus, ensuring that the symbiont does not become a parasite (Krishna & Bagyaraj, 1984). This "response action" is directly proportional to the extent of colonization allowed by the cortical volume.

Furthermore, the sclerified nature of the endodermis in these orchids serves as a vital structural barrier. In *Dendrobium*, where the endodermis was less of a barrier, colonization reached the pith, triggering a different metabolic response compared to *Vanda* and *Rhynchostylis* (Kasmir *et al.*, 2011). This interaction suggests that the orchid host maintains control over the fungus by a combination of physical barriers and chemical inhibitors to facilitate nutrient exchange while preventing tissue damage (Wu *et al.*, 2013).

The comparative morpho-anatomical analysis of mycorrhizal colonization across three tropical epiphytic orchid species *Rhynchostylis*, *Dendrobium* and *Vanda* reveals fundamental relationships between root architecture, symbiotic fungal association intensity and phenolic secondary metabolism that illuminate adaptive strategies for epiphytic survival. The disparate anatomical configurations observed among these species demonstrate that root structure is not merely a

passive substrate for colonization but rather a primary determinant of symbiotic intensity and fungal distribution patterns. *Rhynchostylis*, characterized by a thin velamen but markedly thick cortex (32.5 ± 3.54 layers) with concentrated peloton formation in outer cortical regions, represents a morphological strategy optimized for maximum mycorrhizal colonization efficiency. This architectural design maximizes the space available for fungal hyphae penetration and peloton coil formation, suggesting that this species has evolved a symbiosis-dependent nutritional strategy, particularly advantageous in substrate-rich, humid microhabitats where organic debris accumulates around aerial root systems and maintains consistent fungal-root contact (Sathiyadash *et al.*, 2012).

In marked contrast, *Dendrobium* exhibits an inverted anatomical profile: the thickest velamen layer (11.0 ± 1.41 layers) paired with the most restricted cortex (9.0 ± 1.41 layers), yet displays the remarkable phenomenon of endodermis-breaching colonization that extends fungal hyphae into the pith region—a pattern rarely documented in epiphytic orchids. This deep penetration strategy, combined with the lowest phenolic content among the three species (3.3 ± 0.54 g/g), suggests either an unusually aggressive symbiotic association or a transitional state between mutualistic symbiosis and potential parasitism. The reduced phenolic investment may indicate that the extent of fungal colonization itself provides sufficient defense or nutrient acquisition to compensate for diminished secondary metabolite accumulation, or conversely, that extensive colonization precludes the metabolic necessity for elevated phenolic production. *Vanda* presents an intermediate anatomical architecture yet demonstrates the strikingly elevated phenolic content of 18.05 ± 1.1 g/g, representing a 2.8-fold increase over *Rhynchostylis* and a 5.5-fold increase over *Dendrobium*.

This inverse correlation between mycorrhizal colonization architecture and phenolic accumulation suggests a fundamental trade-off in adaptive strategies among epiphytic orchids: species investing heavily in symbiotic efficiency reduce secondary metabolite expenditure, while species with moderate or restricted colonization capacity compensate through robust phenolic accumulation. Phenolic compounds, synthesized through the phenylpropanoid pathway *via* phenylalanine ammonia-lyase (PAL) activation, provide multifunctional stress tolerance mechanisms critical in the canopy microenvironment, including reactive oxygen species (ROS) scavenging, UV radiation absorption, and antimicrobial defence. For a species like *Vanda* occupying exposed branch positions with heightened desiccation and photoinhibition stress, elevated phenolic content offers essential protection against oxidative damage and pathogenic colonization that would otherwise be catastrophic in water-limited, light-saturated conditions.

The phenolic content differences are not merely quantitative variations but reflect distinct ecological positioning within the epiphytic orchid niche. *Rhynchostylis*, with maximal cortical accommodation of mycorrhizal fungi and minimal phenolic investment, represents a "symbiosis-specialist" strategy suited to moist, nutrient-cycling substrate where fungal associations reliably deliver phosphorus and nitrogen. *Dendrobium*'s exceptional colonization pattern combined with minimal phenolic defence suggests either a unique fungal partnership or niche exploitation in low-light, protected canopy positions where stress tolerance is less critical. *Vanda*'s pronounced phenolic accumulation indicates adaptation to high-stress canopy microhabitats where environmental pressures like drought, intense radiation, rapid water fluctuations demand robust chemical defence systems capable of functioning independently of mycorrhizal buffering. These findings underscore that phenolic metabolism in epiphytic orchids is not merely a passive consequence of mycorrhizal colonization but represents an active, regulated response to ecological niche requirements and stress exposure. The interplay between structural determinants of fungal colonization and phenolic investment reveals how tropical epiphytic orchids partition ecological space and allocate finite metabolic resources across competing physiological demands.

SUMMARY AND CONCLUSION

Mycorrhizal fungi do not enter orchid roots through the tips but rather through the velamen and exodermis passage cells. The study concludes that the number of cell layers in the velamen and cortex determines the space available for fungal colonization and that orchids regulate fungal growth through the synthesis of phenolic compounds. *Vanda* showed the highest adaptive phenolic production, while *Rhynchostylis* demonstrated the highest correlation between cortical space and colonization. The findings emphasize that the symbiotic balance in orchids is maintained through a complex interplay of anatomical barriers and biochemical inhibitors. These distinct adaptive strategies illuminate how tropical epiphytic orchids partition ecological niches and allocate finite metabolic resources in response to differential environmental stresses, with profound implications for understanding plant-fungal symbiosis evolution in nutrient-limited ecosystems and for developing conservation strategies for threatened orchid species in tropical forest canopies. Future research should focus on the molecular identification of the specific fungal species to further elucidate the specificity of these interactions.

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