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Intracellular peloton colonization and mycorrhizal associations in epiphytic orchid roots

Disanayaka D. M. H. A., Thathsarani Y. K. D. D., Edirisinghe P. and Senanayake S. P.*

[1]Department of Plant and Molecular Biology, University of Kelaniya, Sri Lanka.
priyangi@kln.ac.lk*

Orchids are highly valued for their esthetic beauty and economic significance, playing a vital role in the global floriculture industry. In their natural habitats, orchids heavily depend on mycorrhizal associations, which influence seed germination, protocorm growth, and nutrition. Despite their importance, understanding these associations remains a challenge. In Sri Lanka, there is limited knowledge exists on the root-associated fungal species interacting with epiphytic orchids and their ecological roles. Exploring the endophytic fungal microbiome in these orchids helps in identifying fungi that form mycorrhizae in epiphytic habitats, paving the way for effective cultivation and developing novel conservation strategies. Intracellular entangled hyphal coils that colonize within the cortex of the roots of epiphytic orchids are known as pelotons. Knowledge of the presence and the distribution of pelotons is crucial for understanding the symbiotic relationships between orchids and mycorrhizal fungi. Therefore, the objective of this study was to investigate the distribution of pelotons in the roots of selected epiphytic orchids. In the present study, sixteen wild-grown and cultivated epiphytic orchid roots belonging to genera, i.e. *Dendrobium*, *Arachnis*, and *Vanilla* were randomly collected from four study sites in tropical lowland, wet and intermediate zones in Sri Lanka. Microscopic imaging of microtome sections was employed to observe the peloton colonization within the randomly sampled orchid roots. Longitudinal and cross-sectional analyses of six orchid root samples revealed sparse colonization of fungal hyphae. Extensive peloton colonization was observed only in the roots of two specimens of *Dendrobium* sp. and in one specimen of each *Arachnis* sp. and *Vanilla* sp. indicating the presence of mycorrhizal fungi in the roots. However, peloton colonization was not found in all the sampled roots of the studied genera. Furthermore, microscopic observations showed that intracellular pelotons were predominantly colonized in the cortex at approximately two centimeters beyond the tip of the roots that are adhered to the substrate. Pelotons were observed as thin hyphal masses ranging from circular and irregular to elongated shapes with hyphae often knotted or spiraled in various dimensions. The study highlighted that wild *Dendrobium* sp. from Avissawella, Western province, exhibited the highest peloton colonization (5–6 per root cortical cell) with both circular and elongated shapes. In contrast, *Arachnis* sp. had one irregularly shaped peloton per root cortical cell. *Dendrobium* sp. from Mahawa, Northwestern province showed both circular and irregular pelotons while *Vanilla* sp. from Kelaniya, Western province displayed the lowest colonization with irregular pelotons. Irregular-shaped pelotons are predominantly found except in *Dendrobium* sp. from Mahawa, Northwestern province underscoring geographic and genus-specific influences on peloton morphology. These findings enhance the understanding of mycorrhizal associations in orchids and provide valuable information for future research on orchid-mycorrhizal symbioses and their ecological significance.

Keywords: Epiphytic orchids, Microscopic imaging, Mycorrhizal associations, Pelotons