ANATOMICAL PROFILING OF MYCORRHIZA IN TWELVE DOMESTICATED ORCHID SPECIES

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ABSTRACT:* *Roots of orchids have an important role in the survival, adaptation, water and nutrient absorption, and as a place of symbiosis with endophytic fungi. Endophtic fungi provide the necessary resources needed to promote seed germination, seedling establishment and survival in Orchids. The present work explores the anatomy of the root and process, extent and pattern of fungal colonization in the roots of twelve orchid species namely, Spathoglottis plicata Blume, Peristeria elata Hook, Oncidium flexuosum Sims, Dendrobium crumenatum Sw., Dendrobium var. sonia, Epidendrum sp., Arachnis flosaeris(L.)Rchb.f., Vanilla sp., Acampe sp., Vanda sp., Phalaenopsis equestris (Schauer)Rchb.f., and Phalaenopsis sp.

I. INTRODUCTION

The Orchidaceae is one of the largest plant families with close to 35000 species according to the world conservation monitoring centre. Orchids exhibit a circum global distribution. Out of the numerous genera and species, 88% are epiphytic in nature and the remaining 12% are terrestrial. Orchids attract considerable attention largely because of their extraordinary diversity. Orchids are distributed throughout all moist habitats, and a few are found in deserts. Species are found in soil, in litter, on the ground and attached to plant surfaces as epiphytes. All species form very small seeds, and require a fungus for seed germination and nourish seedlings in the wild. They are unique in forms, colors and flower structure. However, the vegetative organization is variable among the species. In general, they are composed of adventitious roots, rhizome, stem, leaves, floral ramets and flowers. During their evolutionary process, the orchids have become adapted to distinct environments, so they can be epiphytes, terrestrials, saprophytes or lithophytes. These environmental variations contribute for structural alterations in vegetative organs.

It is also one of the most advanced plant families with many adaptations enabling long term survival. One adaptive mechanism is that the orchid roots, especially the tropical species have a multiple epidermis of dead cells called velamen that protects the root cortex from excessive drying and aids in water uptake. The epiphytic orchids constitute approximately 75% of all the other Orchidaceae species. They have aerial roots with the function of fixation on substrate, and absorption of water and minerals. The terrestrial species absorb their nutrients direct from the soil, and are likely to have three kinds of roots, one adapted to absorption, and fixation; a tuber adapted to storage of nutritive substances and one specialized in storage, absorption, and fixation.

Another adaptive feature is the close relationship of orchid flowers with insect pollinators enabling species growing in close proximity and has the potential to interbreed and form hybrids. They can each be pollinated by a separate insect species thus maintaining species isolation.

The third and most important adaptive feature of orchids is its reliance on fungal interactions. This adaptation has allowed orchids to persist in less than ideal habitats and has led to their occurrence worldwide. Along with root velamen, the symbiotic relationship of terrestrial orchids with soil fungi has allowed these plants to adapt to habitats as diverse as Arctic Tundra and Rainforest canopies. This mechanism also aided the colonization of the epiphytic realm that led to large and rapid species radiation. Mycorrhizal associations also allow temperate orchids to persist for many years in a dormant state as the plant feeds on its fungal symbiont.

Orchids produce the smallest seeds compared to any flowering plant. The dust like seeds is produced in large numbers, often over a million seeds per plant. Unlike other plant species, these minute seeds lack an endosperm, or food source resulting in a small embryo covered only by a thin protective wall. This lack of food reserves and protection makes the seeds extremely vulnerable to their environment, resulting in a high mortality rate unless optimum conditions are found for germination. Seeds of orchids are undifferentiated, and lack significant reserves of nutrients. Germination depends on colonisation by a specific endophytic fungus. Germination follows a similar pattern in most cases. The seed imbibes water. The fungus penetrates the testa of the seed and enters either through epidermal hairs or the suspensor of the undifferentiated embryo. After invagination of the plasma membrane the fungus forms a tight coil or peloton in the cell. The peloton remains active for some time, but then collapses. The fungus colonises further cells, and thus spreads to other cells. Initial contact between fungus and imbibed seed can have one of the three results: the fungus and plant form a functional mycorrhiza, the fungus can parasitise the seed, or the fungus remains outside the seed.

In adult orchids, a source of carbon is assumed to be organic material surrounding the plant. Mycorrhizal endophytic fungi have the potential to solubilize carbohydrates including cellulose. The fungi translocate trehalose in the hyphae and the sugar is made available at the interface. The fungus thus acquires and translocates organic energy to and from the plant. This role is significant even in adult plants, especially epiphytes that exist in shadows of the canopy.

Transfer of photosynthates from photosynthetic host to orchid via the interconnected fungus is most common. The interaction between plant and fungus is highly regulated by the plant. The plant releases Orchinol, a phytoalexin that causes the pelotones to collapse. The degree of colonization changes over the season, indicating that the orchid is controlling uptake of nutrients while preventing parasitism by the fungus.

II. RESEARCH METHODOLOGY

2.1. ANATOMICAL CHARACTERIZATION

Twelve Orchid species namely *Spathoglottis plicata* Blume, *Peristeria elata* Hook, *Oncidium flexuosum* Sims, *Dendrobium crumenatum* Sw., *Dendrobium var. sonia*, *Epidendrum sp.*, *Arachnis flosaeris* (L.)Rchb.f., *Vanilla sp.*, *Acampe sp.*, *Vanda sp.*, *Phalaenopsis equestris* (Schauer)Rchb.f., and *Phalaenopsis sp.* were taken as samples for the present study from a garden in Kottayam, Kerala.

- All the Orchid sample plants were grown in the same garden, in potting mixture which are composed of coir pith, coconut shells and ch arcoal.
- The samples were grown close to one another.
- Mature root clippings were taken from the samples under study.
- The roots were washed in Clensol and held under running tap water to remove the impurities.
- The dirt and soil from root surfaces were removed by gently scrubbing, using properly washed hands.
- Root clippings thus obtained were sectioned by free-hand sectioning.
- They were then stained in Lactophenol cotton blue for 5 to 10 minutes and washed in water.
- The stained sections were mounted in glycerine on a clean glass slide and a cover slip was placed on it.
- The sections were viewed and imaged under the light microscope; (LABOMED CX_{RIII}) at 10x, 40x and 100x magnification
- The images obtained were compared with each other for their anatomical characteristics and fungal colonization pattern.
- The perimeter (p) of the roots of each species were calculated by the formula $p = 2\pi r$.
- The radius of the roots were obtained by using a micrometer and diameter obtained by using the formula $d = 2r$.

III.RESULTS AND DISCUSSIONS

3.1.ANATOMICAL CHARACTERIZATION OF ORCHID ROOTS

The results of the present study revealed that the anatomical parameters like number of epivelamen layers, velamen layers, exodermis, cortical layers, endodermis, protoxylem archs, perimeter : protoxylem ratio, presence of passage cells in exodermis and endodermis and protoxylem archs played an important role in aiding fungal colonization of the orchid species. The study also provided information about presence of pelotons, tilosomes and fungal colonization pattern which are specific for each sample species. The most important qualitatative (Table 1) and quatitative (Table 2) anatomical characters were analysed and tabulated.

3.2.VELAMEN

Velamen is the outermost layer of orchid roots. Components of velamen cell consist of cellulose with various proportions of lignin and suberin. The main functions of velamen are protection, mechanical support, water and nutrient absorption, reduction of transpiration and water loss, and infra red reflection (Dycus and Knudson 1957; Benzing et al. 1982, 1983; Pridgeon 1986).

An epivelamen (the outward extension of velamen) is observed in all the orchids in the study viz. *Spathoglottis plicata, Peristeria elata, Oncidium flexuosum, Dendrobium crumenatum, Dendrobium var. sonia, Epidendrum sp., Arachnis flosaeris, Vanilla sp., Acampe sp., Vanda mokara, Phalaenopsis equestris* and *Phalaenopsis sp.* The other orchids in the present study have velamen with various number of velamen layers.

In the outermost velamen layer (epivelamen) of Spathoglottis, root hairs were present. This character can be attributed to a terrestrial mode of life. Highest number of velamen layers were observed in *Dendrobium var-sonia* . However, in *Spathoglottis plicata* Blume, the velamen tissue was almost absent. This can be attributed to its terrestrial nature. The differences in the number of layers of velamen indicates the adaptation of orchids to specific environments. Orchid species from arid and dry habitats were associated with multilayers of velamen, while orchid species from humid habitats were observed to lack velamen or only one layer of velamen (Dycus and Knudson 1957; Sanford and Adanlawo 1973). The least number of velamen layers were observed in Phalaenopsis species and Arachnis. The low number of velamen layers of these orchids can be attributed to a more humid habitat.

Velamen thickening were observed in Arachnis, Acampe and in Vanda. The role of velamen cell wall thickening is for mechanical support and to avoid water loss (Noel 1974; Benzing et al. 1983) from the cortex. As they grow in illuminated areas and are exposed to high intensity of light, the velamen cell wall thickening is vital to reduce root transpiration and water loss. It is therefore a structural adaptation to thrive in a dry habitat and reduce rate of transpiration. Benzing et al. (1982) believe that the orchids velamen act as a sponge, allowing the root to immobilize a temporary but rightly accessible reservoirs of moisture and minerals, however brief its contact with precipitation or canopy leachates.

3.3.EXODERMIS

Below the velamen layers, there is exodermis layer, which is the outer layer of cortex (Engard 1944). The exodermis cell had secondary cell wall thickenings and are empty and dead at maturity (Pridgeon 1986). The exodermis cell wall thickening is caused by lignin and suberin impregnation (Fahn 1990). The function of exodermis cell wall thickening is for mechanical protection against water evaporation, to retain moisture in the cortex, and to control the entrance of mycorrhizae in cortical cells (Benzing et al. 1983; Sanford and Adanlawo, 1973; Moreira and Isaiass, 2008).

The exodermis of all the orchids in the present study is uniseriate. Most orchid species have 1 layer of exodermis. The number of exodermal layers can be more than one layer, it can range from 1-4 layers . The orchids in the present study exhibited various patterns of exodermis cell wall thickenings-'O' thickening or '∩'shaped thickening(fig.1). Epidendrum , Vanilla and Dendrobium species showed '0'thickening and '∩' thickening observed in Peristeria, Acampe, Vanda, and Phalanopsis species .

3.4.PASSAGE CELLS

Between exodermis cells, there are shorter cells that are living and have thin cell wall. They are called Passage cells. All of the twelve species in the present study showed the presence of passage cells. Passage cells in the exodermis layer are important for the passing of water and nutrient, and attracting endophytic fungi (Peterson and Enstone 2006; Senthilkumar et al. 2000).

Tilosome is the extension from the innermost cell wall of velamen cells attached to the passage cells of exodermis. The function of tilosome is to protect from water loss via root transpiration (Pridgeon et al. 1983). The presence and absence of tilosome is one of key characters in the classification, systematics and phylogenetics of orchids. Tilosomes were observed in *Vanda* sp. and *Phalaenopsis* sp. (Fig.1) in the present study. This structure permits water absorption, possibly prevent the entry of pathogens (Holtzmeier et al., 1998), and reduce root transpiration (Benzing et al., 1982; Benzing et al. 1983). Benzing et al., (1982) call attention for the fact that the movements of substances beyond the velamen may be partially blocked by the presence of these fibrous bodies. Pridgeon *et.*,(1983). also considers 'Tilosomes', as an adaptive feature of the epiphytic group.

3.5.CORTEX

Cortex is a tissue beneath exodermis which is formed by thin walled parenchymatous cells with various sizes. Outer cortex layers are composed of small size cells, while inner cortex layers are formed by large size cells. Number of cortex layers varied among the orchid species in the study (Table 2).

Vanda possess highest number of corical layers while least number of cortex layers observed in Vanilla. Intercellular hyphal coils known as 'Pelotones' were exclusively found in terrestrial orchids, Spathoglottis and Peristeria. In the present study, chloroplasts were present in cortical cells of *Vanda* and Vanilla, but they were absent in cortex of others. They had a greenish tinge in the roots which were not present in the roots of other orchids. Chloroplasts contain chlorophyll that is important for photosynthesis. As the other orchid roots appear white greyish, it is evident that it may not contain chloroplasts as chloroplasts are associated with green colored parts.

3.6.ENDODERMIS

Endodermis is a layer beneath cortical cells that protect the inner parts (vascular bundles and pith). Some orchids have secondary endodermal cell wall thickening, while some others exhibit thin walled endodermis. Similar to exodermis, cell wall thickening of endodermis too is different in different orchids (Table 1).

In the present study, uniseriate layer of endodermis found in all twelve orchid species. The function of cell wall thickening in endodermis is similar to that of exodermis and velamen, as mechanical protection and prevention against water loss because of root transpiration. All except *Peristeria elata* Hook and *Spathoglottis plicata* Blume are epiphytic orchids. They have a thin walled endodermis. Seven species of epiphytic orchids, *Oncidium sp.*, *Epidendrum sp.*,*Arachnis sp.*,*Vanilla sp.*, *Acampe sp.*,*Vanda sp.*, and *Phalaenopsis equestris* (Schauer) Rchb.f. have thickened endodermal cells compared to *Peristeria elata* Hook and *Spathoglottis plicata* Blume.

3.7.VASCULAR BUNDLES

Vascular bundle is a transport system containing xylem and phloem that are important in the transport of water and nutrients. The number of archs in vascular bundles of orchids in the present study was different.

Peristeria elata Hook, *Spathoglottis plicata* Blume and *Oncidium flexuosum* Sims had 12 archs of vascular bundles,while Phalaenopsis and Vanilla had 15 archs. *Dendrobium var.sonia*, *Arachnis sp.* and *Vanda sp.* had 20, 25 and 10 archs respectively. All the species have phloem cells embedded in the sclerenchymatous tissue which alternates with the xylem strands. All of them had a similar root anatomy of vascular bundles radially arranged with exarch xylem. In all cases except *Peristeria elata* Hook and *Spathoglottis plicata* Blume, the passage cells were found in the direction of the exarch xylem, just above it.

3.8.PITH

Pith of orchid is the central part in roots and is composed of parenchyma or schlerenchyma. Except Phalaenopsis, all other orchid species in the present study had a parenchymatous pith with thin walled rounded cells. In two species of Phalaenopsis, a thickened sclerenchymatous pith is observed.

In comparison with the root anatomy of the twelve orchid species in the present study, it is observed that the entry of the endophtyic fungi is facilitated by the specific anatomical characters in the roots like epivelamen, velamen, exodermis, passage cells, intracellular hyphae in the cortex, endodermis and stele. These characteristic features can be grouped as anatomical adaptations of Orchids.

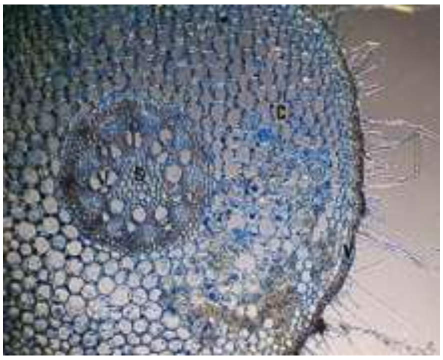
Table 1. Qualitative anatomical characters of the twelve domesticated orchid species ('+' indicates presence and '—' indicates absence of character.)

Sl No.	Name of the sample	Velamen thicken-ing	Exodermis thickening		Mycorrhizal fungal colonization pattern		Endodermis thickening	Pith	
			O	∩	Intercell-ular	Intrace-llular		Parench-ymatous	Sclerenchy-matous
1.	*Spathoglottis plicata* Blume	-	-	-	+	+	-	+	-
2.	*Peristeria sp.*	-	-	+	+	+	-		
3.	*Oncidium sp.*	-	-	-	+	-	+	+	-
4.	*Dendrobium Crumenatum* Sw.	-	+	-	+	-	-	+	-
5.	*Dendrobium var-sonia*	-	+	-	+	-	-	+	-
6.	*Epidendrum sp.*	-	+	-	+	-	+	+	-
7.	*Arachnis sp.*	+	-	-	+	-	+	+	
8.	*Vanilla sp.*	-	+	-	+	-	+	+	-
9.	*Acampe sp.*	+	-	+	+	-	+	+	-

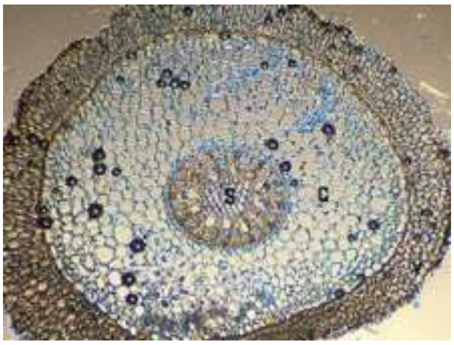
10.	*Vanda sp.*	+	-	+	+	-	+	+	-
11	*Phalaenopsis equestris* (Schauer)Rchb .f.	-	-	+	+	-	+	-	+
12.	*Phalaenopsis sp.*	-	-	+	+	-	-	-	+

TABLE 2 Quantitative root anatomical characters of twelve domesticated orchid species

Sl. No.	Name of the sample	Epivelamen layers	Velamen layers	Exodermis layers	Cortex layers	Endodermis layers
1.	*Spathoglottis plicata* Blume	1	0	1	12	1
2.	*Peristeria elata* Hook	1	5	1	10	1
3.	*Oncidium sp.*	1	6	1	7	1
4.	*Dendrobium crumenatum* Sw.	1	5	1	7	1
5.	*Dendrobium var-sonia*	1	12	1	8	1
6.	*Epidendrum sp.*	1	4	1	7	1
7.	*Arachnis sp.*	1	2	1	16	1
8.	*Vanilla sp.*	1	7	1	6	1
9.	*Acampe sp.*	1	3	1	20	1
10.	*Vanda sp.*	1	5	1	30	1
11.	*Phalaenopsis equestris* (Schauer)Rchb.f	1	2	1	14	1
12.	*Phalaenopsis sp.*	1	2	1	13	1



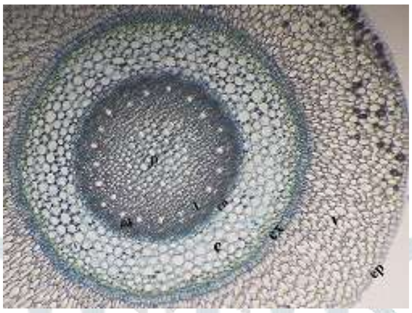
Spathoglottis plicata Blume



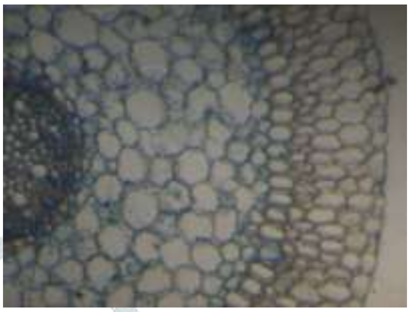
Peristeria elata Hook

Oncidium flexuosum Sims

Dendrobium crumenatum Sw.



Dendrobium var-sonia



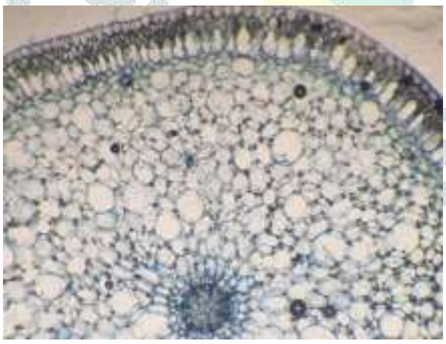
Epidendrum sp.

Arachnis sp.

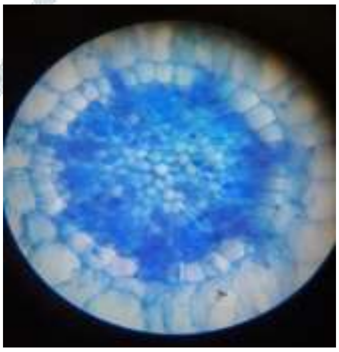
Vanilla sp.

Acampe sp.

Vanda sp.



Phalaenopsis equestris(Schauer)rchb.f.



Phalaenopsis sp.

Fig. 1.transverse sections of twelve orchid species (root hairs-rh, epivelamen-ep, velamen-V, exodermis-ex, cortex-c, passage cells-pc, xylem -x, phloem-phl, pith-p)

IV.CONCLUSION

Observations of micro preparations showed the anatomical characteristics of the orchids and the patterns of fungal colonization in the orchid mycorrhiza. The major conclusions of the study are as follows

1. The presence of root hair like structures on the epivelamen, reduced velamen tissue, absence of exodermis and the presence of inter and intracellular fungal hyphae that form knots inside the cortical cells (Pelotones) as seen in *Spathoglottis plicata* Blume and *Peristeria elata* Hook are characteristics indicative of a terrestrial lifestyle in orchids.
2. The number of layers and the nature of velamen tissue, exodermis, passage cells, cortex, endodermis, endodermal passage cells, stele and pith are the anatomical characteristics that can be considered for identifying, explaining and studying orchid mycorrhi zae, in general.
3. Tilosomes are observed to be present in Vanda and Phalaenopsis species in the present study. The function of tilosome is to pro tect from water loss via root transpiration. Therefore, it can be considered as a marker in identification of orchid species. Howe ver, an elaborate study in this respect is an immediate requirement.

4. The endodermal cells are thin walled continuous barrel shaped parenchyma cells with spaces between them that function as waypoints of fungal entry into the stele. The endodermal cells are thin or thick walled depending on their host orchids and their adaptations.
5. The pith cells of Phalaenopsis, are sclerenchymatous and the rest have a parenchymatous pith.

The characteristics of the interacting fungus and their interaction dynamics are responsible for orchid diversity to a great extent. Mycorrhizal associations during species hybridization, a potential source of speciation in the Orchidaceae, have also been examined by researchers. A dependence on narrowly specific interactions with fungi and pollinators predisposes orchids to become rare. More so, as humankind continues to have negative impacts on natural ecosystems through vegetation clearing, altered fire regimes, weed and feral animal introduction and climate change, populations of many rare orchid taxa are further on the decline. Conservation approaches for such orchids include on site protection of existing populations, ex situ storage of tissues and restoration procedures. All of these approaches require an understanding of the mycorrhizal biology of the species in question, since fungi are vital for orchid seed germination and adult vegetative life. The results of the present study therefore, offers scope for diversity studies, conservation and reintroduction programmes.

V. ACKNOWLEDGEMENT

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