MORPHOLOGICAL AND STRUCTURAL FEATURES OF MYCORRIZAL ROOTS OF *SPATHOGLOTTIS PLICATA* AND *DENDROBOIUM* SPECIES

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Abstract

Of the different types of mycorrhizae known, the orchid mycorrhizae are the least studied. In Sri Lanka, with a moderate orchid flora, mycorrhizal colonization has not been thoroughly described at the anatomical level. Structural studies have been made on some of these mycorrhizal systems.

Keywords: Pelotons, Orchid mycorrhiza and structural details.

Introduction

Roots of the members of the O*rchidaceae* are infected with mycorrhizae has been known for well over 100 years. A large number of temperate and tropical O*rchidaceae* species have been recorded for the regular occurrence of fungal colonization. Even though a substantial literature is available on the germination of orchid seeds, studies on mycorrhizal infection in adult orchids is largely neglected (Alexander 1987; Peterson and Farquhar 1994). In Sri Lanka, approximately 200 species of orchids have so far been reported. The available information does not contribute anything new to our understanding on orchid mychorrhiza. In view of this, the present investigation was undertaken, to study the structural details of mycorrhizal roots of *Spathoglottis plicata* and *Dendrobium* species.

Material and Methods

Orchid roots were collected from the Royal Botanical gardens, Peradeniya, Sri Lanka. The roots were washed thoroughly in water, cut into small pieces and fixed in Formalin Acetic Acid Alcohol (FAA) mixture of the following composition: Formalin 5ml: Acetic acid 5ml: 70% alcohol 90ml. Following overnight fixation, the root segments were transferred to 70% alcohol and preserved for further studies. Free hand sections of the roots were made for the morphological studies and infection density calculated using the formula of Hadley and Williamson (1972). For calculating the Infection Density, about 50 root sections were used. The mycorrhizal-infected roots were also subjected to cytochemical studies (Krishnamurthy 1988).

Results and Discussion

Pelotons are networks of loosely arranged fungal mycelium inside root cortical cells (Fig.3). In cross section, these appear as spherical masses of mycelia and are always formed in the parenchyma cells of the root cortex. Very rarely pelotons are seen in the dead cortical idioblastic cells (Fig.3). These idioblasts were living cells at the time of fungal infection but subsequently become dead.

It has been observed that entry of hyphae into the host root may be by means of hyphae of the free mycelium or through hyphae produced by germination of sclerotia and through intact root hairs or through velamen layers (fig.4). The entry into root hairs is mainly via the tip and sometimes the base of the root hair. A single hypha enters each root hair but occasionally two or more could be seen in single root hair (Fig.2). At the point of entry, no special vesicular or appressorial structure could be seen in the hypha. Probably enzymatic dissolution of the root hair wall by the fungus facilitates its entry through epidermal hairs of the protocorn. In the roots of some species of orchids, the root hairs serve as the sites of hyphal entry (Currah *et al* 1988; Vij and Sharma 1988). The present study also indicates that the entry of the fungus is always through the root hairs in *Spathoglottis plicata*. In other species, where the entry of the fungus could be observed either through rhizodermis or a well-developed multilayeres of velamen before entering into exodermal passage cells (Fig.5).

In the present study, as well as in earlier studies by other investigators (Clement 1988; Peterson and Currah 1990), colonization of epidermis or root hairs by fungal pelotons was never observed. It was observed in the velamen cells for first time in the *Dendrobium* species. It is likely that the cell must have the appropriate physiological conditions necessary for coil formation (Fig.4). The only instance of colonization of epidermal cells by the fungus is reported in *Goodyear repens* by Peterson and Currah (1990), but this is in the protocorm.

The entry into the cortex is always through the passage cells of the exodermis (fig.5). This is noticed not only in the orchid studied at present also in several other earlier investigations (Alcanero 1969; Esnault *et al.* 1994). In fact, the exodermal passage cells do have a control on the entry of fungi into the cortex (Peterson 1988) which remain the only living cells of the exodermis and whose cell walls do not have lignin and suberin, at least to the extent that other exodermal cells have (Esnault *et al.* 1994). Like the passage cells of the exodermis, the fungal hyphae do not settle down or form coils. This indicates that these cells act as channels for hyphal entry into the cortex and do not have the appropriate physiological conditions necessary for coil formation and hyphal degradation, although they are metabolically more active (Esnault *et al.* 1994).

The mycorrhizal fungus associated with *Spathoglottis plicata* and *Dendrobium* sp.was an anamorphic species of *Rhizoctonia.* The fungus was never observed to produce basidia and basidiospores inside the root. The identification was based on Sneh *et al.* (1991).

Hadley (1969) mentioned that the degree of infection was extensive in some green terrestrial orchids, either free for a greater part of the year or have many uninfected roots. Benzing (1982) also reported that all roots which are in contact with the substrate, were infected in the epiphytic orchids of Florida. The present study on *Spathoglottis plicata* and *Dendrobium* species, carried out only once, showed infection densities of 57.0% and 58.6% respectively but in each of these roots there were small segments not colonized by the fungus. Katiyar *et al* (1985) studied 12 species of terrestrial orchids of northeastern India and recorded an infection density range from 68.9% to 97.0% depending upon the orchid, although Hadley (1982) published results of an examination of eleven Malaysian epiphytes which showed a very low infection density. Variation of fungal colonization in epiphytes showed very low infection density. Variation of fungal colonization in epiphytic orchids may be due to endophytic specificity, seasonal change, host defence mechanism, etc., Abiotic factors such as water and nutrient availability may also influence mycorrhizal colonization.

Earlier studies on colonization by the fungus in *Spathoglottis plicata* were restricted only to the root cortex. Penetration of hyphae into the endodermis as observed by Ruinen (1953) in a species of *Dendrobium* was never observed in both taxons. However, infection could be seen up to the layer of the cortex adjoining the endodermis. Majstriks (1970) in *Dendrobium cunnignhami* reported the absence of infection in the deeper layers of the cortex. In other words, the host appears to control the growth of the fungus through its cells and restricts it to a certain region of the cortical tissue, an observation also shared by Hadley (1969) and Hadley *et al* (1971). After entry of the mycelium into the root cortex through passage cells, it grows intercellularly towards the interior layers of the cortex. Consequently, the first colonization of cortical cells was noticed in the deeper layer of the cortex and the subsequent ones gradually towards the periphery of the cortex.

According to Peterson and Currah (1990) one of most striking events in the orchid mycorrhizal association is the lysis of the pelotons (Fig.6). The oldest colonized cells were the first to act as digestion cells and cells with subsequent colonization followed this. Burgeff (1936) noticed digestion, a month after infection in *Dactylorrhiza incarnata*. Digestion was observed randomly in cortical cells and not in a defined zone of cells designated as the "digestion layers" and Williamson 1970 and Hadley 1982.

A very interesting aspect of the present study was the repeated formation of fungal coils in the same cell. A maximum of 3 generations of pelotons were noticed in the same host cell of *Spathoglottis plicata* and 2 more generations of pelotons were noticed in the same host cell of *Dendrobim* species. Successive peloton formation has been reported already by Hadley *et al* (1971), Senthilkumar and Krishnamurthy (1998),and Peterson and Currah (1990). The same host cell shows both pelotonic and non-pelotonic hyphae, the former forming bundles and the latter forming a lining layer (Fig.1) around the host cell wall, the two being connected

by cross connections. By the influence of phenol in the walls of the hyphae, The non-pelotonic hyphae help themselves to organize a fresh peloton in the same cell (Senthilkumar and Krishnamurthy 1998).

Acknowledgement

The authors are grateful to Prof. S. A. Kulasooriya, Dean, Faculty of Science, University of Peradeniya and Dr. B. Sumithrarachchi, Director, Royal Botanical Gardens, Peradeniya.

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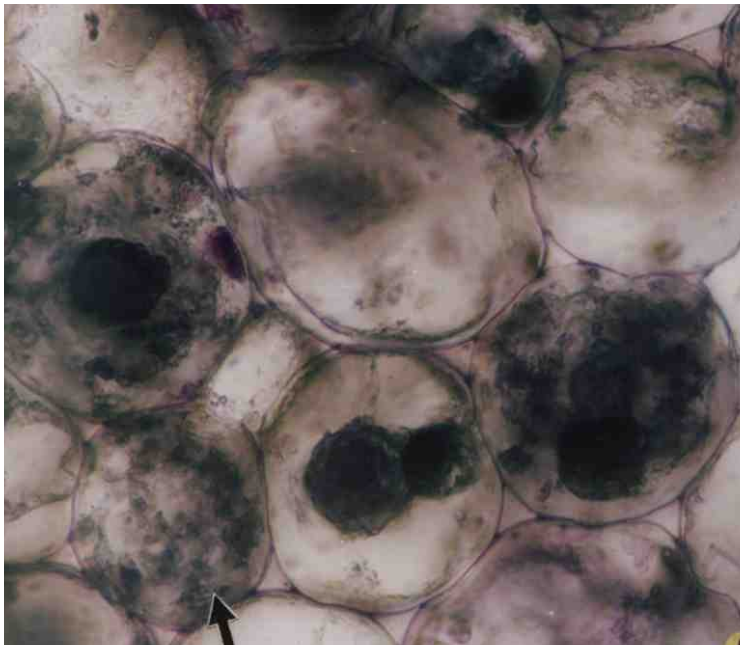

Figure 1: Successive formation of pelotons in host cells. Note that few cells show the fresh peloton (arrow) × 150

Figure 2: Behaviour of fungal hyphae in root hairs leading to the formation of chlamydospores × 650

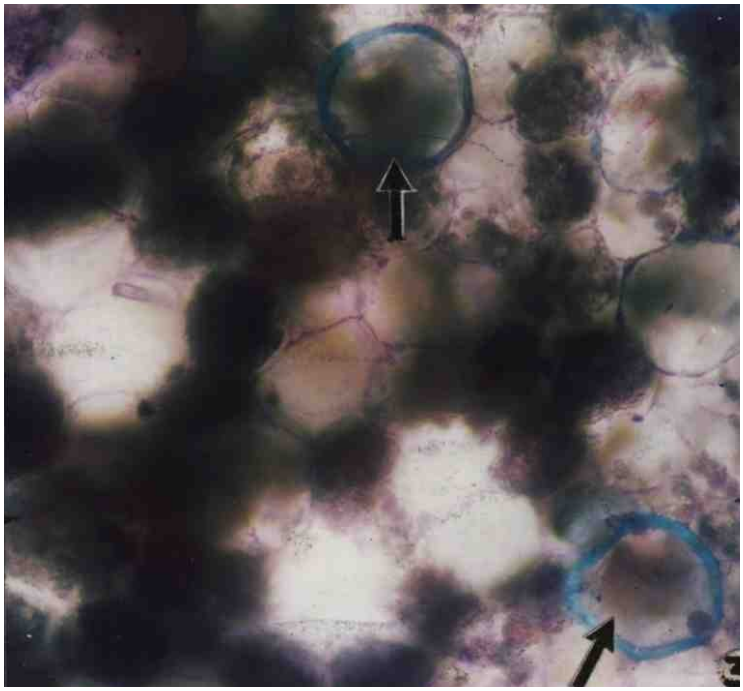

Figure 3: T.S. of root cortex showing fungal pelotons. Pelotons are seen in the ideoblastic cells of cortex (arrow) × 650

Figure 4: Peloton formation in velamen cells × 650

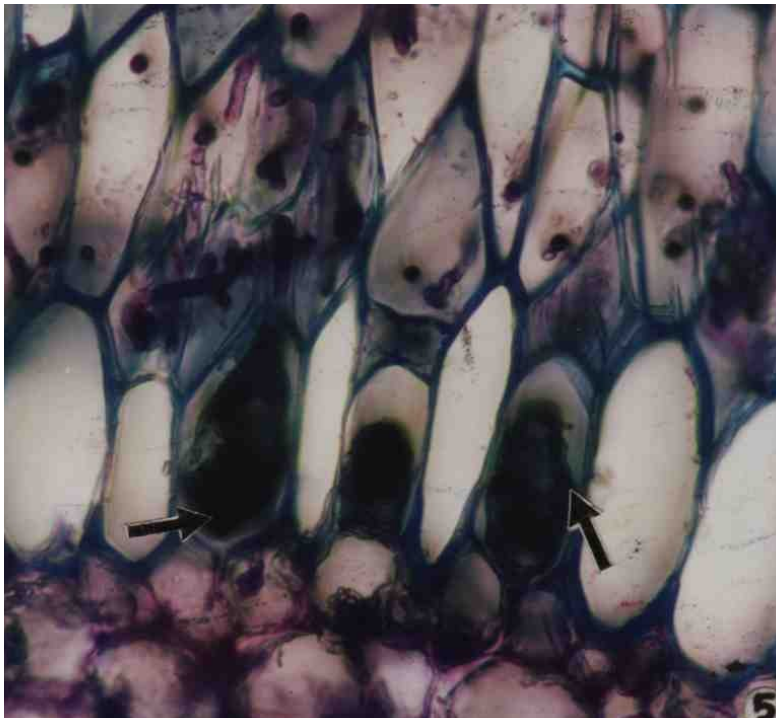

Figure 5: Fungal pelotons in the exodermal passage cells (arrow) × 150

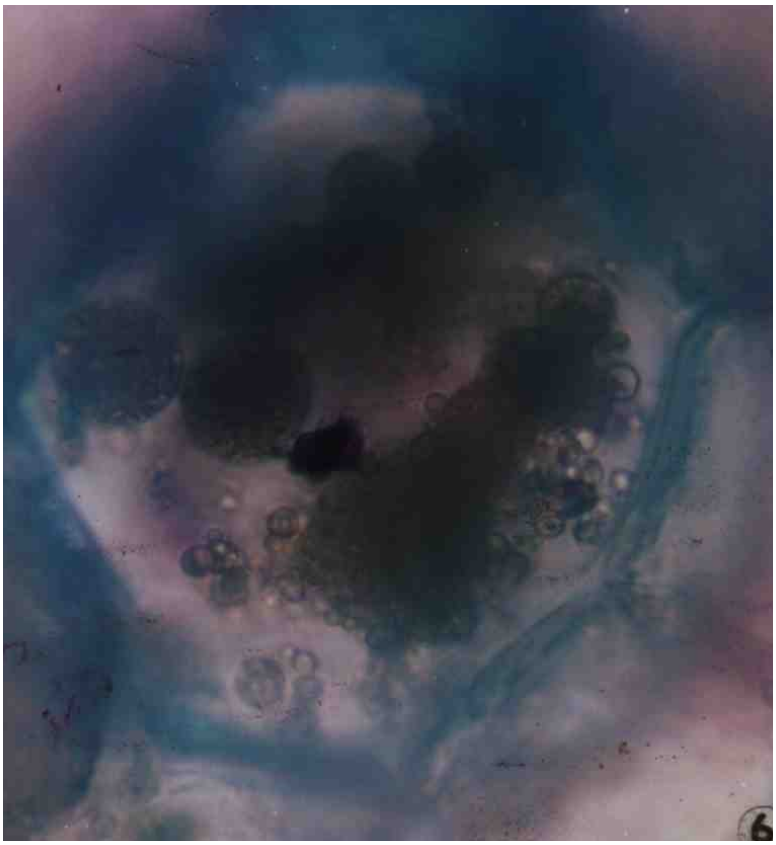

Figure 6: T.S. of cortical cells showing the degradation of pelotons × 650