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MYCORRHIZAL ASSOCIATION OF ORCHIDS IN A TROPICAL FOREST OF SOUTHERN INDIA

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RAMAN, N. & NAGARAJAN, N. 1999. Mycorrhizal association of orchids in a tropical forest of Southern India. The mycorrhizae of epiphytic and terrestrial orchids of Kodaikanal tropical forest of Western Ghats, South India, were studied. The epiphytic orchids were infected with *Rhizoctonia* sp. whereas the terrestrial orchids were infected with AM fungi. Five AM fungal species were identified, viz. *Glomus aggregatum, G. ambisporum, G. fasciculatum, G. mosseae* and *Gigaspora albida.*

Key Words: Tropical orchids - *Rhizoctonia* sp. - arbuscular mycorrhizae

RAMAN, N. & NAGARAJAN, N. 1999. Kulat mikoriza yang bersekutu dengan orkid di hutan tropika di India Selatan. Kajian dijalankan terhadap kulat mikoriza epifit dan orkid darat yang terdapat di hutan tropika Kodaikanal di Ghat Barat, India Selatan. Orkid epifit dijangkiti oleh *Rhizoctonia* sp. manakala orkid darat dijangkiti oleh kulat AM. Lima spesies kulat AM dikenal pasti, iaitu *Glomus aggregatum, G. ambisporum, G. fasciculatum, G.mosseae* dan *Gigaspora albida.*

Introduction

The orchids exert considerable fascination because of the beauty of their floral structures. Their flowers are also economically important because of their export value as well as some of their products such as vanilla. Mycorrhizae aid in the germination of seeds and establishment of orchid protocorms (Arditti 1967). The entry of fungal hyphae into the young seedlings at germination is a necessary condition for the initiation of further growth in orchids (Harley 1969). Many epiphytic and terrestrial orchids are very dependent on mycorrhizal fungi for their carbon source (Purves & Hadley 1975). Details of mycorrhizae and the fungi involved in their formation are unknown for most orchids (Currah *et al.* 1990).

Bernard (1905) isolated several species of *Rhizoctonia* in France from germinating seeds and from adult orchid roots. In Germany, Burgeff (1909) also reported the isolation of several *Rhizoctonia* species and since then the number of known orchid fungi has increased. Burgeff (1936) gave a summary of the taxonomy, physiology and specificity of the orchid fungi. Sprau (1937) found that an isolate of *Rhizoctonia* from *Orchis mascula* fruited to form a *Corticium* basidiocarp. It is now well accepted

that *Corticium solani* stimulate germination of *Orchis mascula purpurella* seeds in culture (Downie 1957). Downie (1943a) had earlier studied the distribution of *Mycelium radis goodyerae repens* in pine woods and determined the presence of this fungus by sowing the seeds of *Goodyera* on humus of different sources. Curtis (1939) and Downie (1943b) have pointed out that there is some evidence of a direct effect of soil conditions on the distribution of some of the mycorrhizal fungi of orchids. Dorr and Kollmann (1969) studied the fine structural features of the symbiotic relationship between *Neottia* and the fungus *Rhizoctonia* and found that the fungus attacking *Neottia* cells showed striking changes such as the enlargement of nucleus and the development of an extended rough surfaced endoplasmic reticulum. Blakeman *et al.* (1976) found that the infection of protocorms of *Dactylorhiza purpurella* by *Rhizoctonia* sp. led to a marked stimulation in respiration.

Warcup and Talbot (1967, 1971) successfully induced fruiting of the *Rhizoctonia* isolates obtained from terrestrial orchids in South Australia and Great Britain in culture, and found the perfect states obtained belonging to *Thanatephorus* and *Tulasnella.* Currah (1987) also isolated a species of *Thanatephorus* from mycorrhizal roots of an orchid from Canada. Studies done on the seasonal development and mycorrhizal fungi of the mycorrhizae of some orchid species of Canada indicated that pot cultured rhizoctonias are functionally mycorrhizal fungi of orchids (Currah *et al.* 1990). The occurrence of *Rhizoctonia* in pot cultures of arbuscular mycorrhizal (AM) fungi has also been established by Williams (1985). This clearly indicates the possibility of coexistence of *Rhizoctonia* in the roots of orchid along with the AM fungi.

The orchid flora from the forest region of the the Western Ghats of Southern India comprise 150 species of both epiphytic and terrestrial species. The region provides environmental conditions suitable for the luxurient growth of orchids. However, detailed information on the mycorrhizal associations and the symbionts for most Indian orchids has not been investigated. This paper therefore attempts to report the mycorrhizal status of six epiphytic and five terrestrial orchids from the Kodaikanal tropical forest of Western Ghats, India.

Materials and methods

Kodaikanal forest (10°12'–10°15'N, 77°26'–77°33'E) lies on the Palani hills of Tamil Nadu which is situated in Western Ghats. The altitude ranges from 500 to 2500 m above sea-level. Old and fresh roots of each orchid species and debris from the host bark around the vicinity of the roots of epiphytic orchids and rhizosphere soil of the terrestrial orchids were collected. Soils and plants samples were collected from the following four sites: the deciduous tropical forest of Senbaganoor and Berijam located in the lower altitude (700 m above sea- level), and from the Kodaikanal tropical forest of Pillar Rock and Mathiketan Solai located at the higher altitude (2000 m above sea-level).

The latter site is of semi-evergreen shola vegetation. Five replicate samples were collected for each orchid species.

Root samples were cleared in 10% boiling KOH and stained in lactophenol containing trypan blue (Merryweather & Fitter 1991), squashed and mounted on slides and observed under microscope at 160X magnification. In some cases, direct observation of unstained, fresh and intact roots (Arias *et al.* 1987) was made. The percentage of root infection was calculated by the gridline-intersect method (Giovannetti & Mosse 1980). The hyphae, vesicles and arbuscules were also observed under the light microscope. Root pieces were mounted on glass slides either temporarily in lactophenol or permanently in polyvinyl alcohol resin-lactophenol. The cover slip was pressed gently to flatten the roots and subsequently sealed with DPX mountant.

Bark debries and rhizosphere soil samples collected were also analysed for AM fungal spores by wet sieving and decanting, adopting the technique of Gerdemann and Nicolson (1963). One hundred gram soil was placed in one litre of luke-warm water. Organic matter and roots were removed and the suspension decanted through a 710 μm sieve. The suspension that passed through 710 μm was then decanted through 425 μm, 250 μm, 150 μm, 75 μm and 45 μm sieves. The residues were observed under a dissecting microscope for AM fungal spores. The spores were identified with the help of AM manuals (Raman & Mohankumar 1988, Schenck & Perez 1990).

The isolation and identification of endophytic fungi (other than AM fungi) were done using the techniques described by Currah (1987). Orchid root segments were surface sterilised with 30% H_2O_2 for 1 min, rinsed twice in sterile distilled water and decorticated with a sterile scalpel under the dissecting microscope. Clumps of cells were removed from the inner cortex, macerated in a drop of sterile water, and then plated on modified Melin-Norkrans medium amended with streptomycin (Raman & Mohankumar 1988). The plates were incubated at 20 ± 1°C until the hyphae became visible on the cortical cells and then grew into the medium. The hyphal tips were transferred to potato dextrose agar medium for further growth. The roots are then prepared for determination of the orientation and morphology of the fungal endophytes within the root cortical cells. Squashes of whole roots were used. Ammoniacal congo red mixed at the time of mounting with a drop of 2% aqueous phloxine was used for staining the squashes (Warcup & Talbot 1967).

Results

The roots of all the 11 orchid species were found to be mycorrhizal although percentage infection varied among the species (Table 1). The aerial roots which were not in contact with the host bark did not form mycorrhizae. All the six epiphytes showed hyphal infection with hyphal coils in the cortical cells. The old roots were more heavily infected than the young roots.

In pure culture, pale brown, submerged colonies composed of narrow, irregularly septate hyphae were observed. In older cultures, monilioid hyphae with terminal or intercalary spherical chlamydospore-like structures, about 10 µm in diameter developed. This observation proved the presence of *Rhizoctonia* sp. as endophytes in the roots of all the six epiphytic orchids examined.

The infected roots of all the five terrestrial orchids showed non-septate hyphae with arbuscules and vesicles. All efforts to isolate *Rhizoctonia*-like fungal endophytes into culture were futile. This observation clearly indicates the presence of only AM associations in the terrestrial orchid species. Five AM fungal species, *Glomus aggregatum, G. ambisporum, G. fasciculatum, G. mosseae* and *Gigaspora albida* were isolated from the rhizosphere soils of terrestrial orchids.

Statistics

Percentage infection and spore population of the different fungal species were statistically seggregated by DMRT (Table 1).

Table 1. Mycorrhizal status of orchid species of Kodaikanal tropical forest

Orchid species	Mycorrhizal infection (%)	Spore population/ 100g of soil	Hyphal infection	Arbuscules, vesicles	AM fungal species
Epiphytes					
Aerides ringens	46 gh	-	+	-	-
Dendrobium aqueum	40 i	-	+	-	-
D. herbaceum	45 h	-	+	-	-
Liparis elliptica	47 g	-	+	-	-
Oberonia verticillata	32 j	-	+	-	-
Sirhookera lanceolata	50 f	-	+	-	-
Terrestrial					
Anoectochilus elatus	74 a	480 a	+	+	*Glomus aggregatum* *G. fasciculatum*
Calanthe masuca	65 bc	225 b	+	-	*G. ambisporum* *Gigaspora albida*
Chrysoglossum maculanthum	67 b	210 b	+	-	*G. albida*
Habenaria elliptica	61 c	185 c	+	+	*Glomus aggregatum* *G. ambisporum*
Malaxis rheedi	58 d	180 c	+	-	*G. aggregatum* *G. mosseae*

In column, means followed by the same letter are not significantly different at p = 0.05.
+ : Presence of AM hyphae.

Discussion

The roots of all the epiphytic orchids examined showed presence of endophyte *Rhizoctonia* sp. Species of *Rhizoctonia* have repeatedly been isolated from the absorbing organs of green orchids and have also been confirmed to be mycorrhizal endophytes through their stimulation of seed germination and development of

orchids (Warcup & Talbot 1971, Purves & Hadley 1975). Growth stimulation of mycorrhizal infected protocorms in culture media is brought about through the supply of amino acids and other growth factors (Purves & Hadley 1976).

Five AM species were isolated in the terrestrial orchids. Successful association with AM of certain strains has been reported earlier by Hall (1976) and Katiyar *et al.* (1986). However, isolation of a single AM fungal spore from the host bark debris of epiphytic orchids was not sucessful. Other AM fungal infections were observed in the roots of epiphytic orchids. Species of *Rhizoctonia* have been observed in several terrestrial orchids (Currah *et al.* 1990). *Rhizoctonia* has also been reported to occur commonly in pot cultures of AM fungi (Williams 1985). The present study failed to isolate *Rhizoctonia* sp. from the terrestrial orchids. Further studies are therefore required to confirm the presence or absence of this fungus in the orchid species.

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