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Atypical root Endophytic Fungi of Mangrove plant Community of Sundarban and their Possible Significance as Mycorrhiza

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Roots of mangrove plants and associate vegetation inhabitating different successional stages at the Ganges rivers estuary in India showed widespread association of atypical fungal endophytes, besides the typical vesicular-arbuscular mycorrhiza (VAM), Two dark septate (DS) mycelial endophyte(s) was very widespread in distribution and formed intracellular hyphal coils and microsclerotia-like structures both in mangrove and non-mangrove roots. These two DS endophytes were isolated from infected roots and cultured. One of the isolates resembled *Rhizoctonia* sp. and the other forming pycnidia in stromatic masses in culture, remains unidentified. These two isolates of DS endophytes infected a wide variety of non-mangrove plant roots by artificial inoculation and formed intracellular structures similar to that formed in mangrove roots and comparable to that of the VAM. The isolates upon root inoculation to *Cajanas* seedlings caused significant increase in growth under nutrient stress at least partly theough mobilization of insoluble phosphate.

Key Words : Mangrove, Ecology, Mycorrhiza, Salt marsh, Fungi

INTRODUCTION

Mutualistic associations between fungi and roots of vascular plants are very diverse and in many cases are not well understood. The structure and consequence of the VA-mycorrhizal endophytes, commonest of all mutualistic associations, are now well recognized. The traditional morphological classifica-

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tion of mycorrhiza types, however, has little bearing on the physiological function or ecological reality of the diverse root-fungus associations. Besides the VA-mycorrhizal fungi, a *Rhizoctonia* type dark, septate mycelial fungus is frequently found as endophyte of many plant roots from diverse ecological habitats (Nicolson, 1959 : Read and Haselwandter, 1981 ; Venkatraman *et al.*, 1989). Evidence for mycorrhizal function of such septate mycelial endophyte(s), however, remained largely circumstantial and speculative (Russel, 1973 ; Cooke, 1977). Haselwandter and Read (1980, 1982) and Read and Haselwandter (1981) provided evidence of widespread presence of *Rhizoctania* type dark septate hyphal endophyte (DS) in the roots of alpine plant community where, in some members of the Cyperaceae, the DS type appeared to be dominant over the VA endophytes. They provided experimental evidence of symbiotic host response to such endophytes (Haselwandter and Read, 1982). Sengupta *et al.* (1989) reported extensive colonization of mangrove roots with seemingly beneficial similar dark septate mycelial endophytes. Trappe (1989) reviewed the ecological significance of the dark septate and other miscellaneous endophytes which do not form any readily recognizable structures in host roots. In the same context, details of two septate mycelial endophyte and mangrove root associations are presented here.

MATERIALS AND METHODS

Study location and sample collection

The terminal part of the deltaic drainage basin of the river Ganges in India, known as "Sundarban", (21°30′—22°30′ N ; 88°10′—89°51′E), is the home of the Indian sub-group of "old world mangroves". About 45 species of true mangroves and mangrove associates, at various stages of development, are available in present day Sundarban, parts of which have been reclaimed for agriculture and forestry. Based on physiographic characters and floristic development, the mangrove forests of Sundarban is divided into four distinct ecosuccessional stages ; (I) formative mangrove swamps, (II) developed mangrove swamps, (III) declining ridge mangroves, (IV) degraded mangroves on embankment protected highlands where agriculture and forestry have been introduced.

Histological and other investigations were carried out with root and soil samples collected from the successional stages of mangrove and other vegetation at the Ganges river estuary.

Analytical methods

Standard methods were used to analyse the physical and chemical properties of rhizosphere soils (Jackson, 1967 ; Dewis and Freitas, 1984). Root samples fixed in FAA were processed and stained by the methods of Philips and Hayman

(1970) with some modifications (Sengupta and Chaudhuri, 1990). Intensity of vesicular-arbuscular mycorrhizal root colonization was assessed by the usual slide-micrometric method (Kormanik and McGraw, 1982). For each plant sample, 5 x 100 cm root pieces taken from five randomly selected plants for each variable stage was made composite and root infection intensity estimated. Assesment of atypical endophytic colonization was difficult partieularly when such infections were simultaneous with VAM. Their colonization intensity was estimated semiquantitatively by visual examination of the same number of root pieces.

Cultural and plant growth studies

The DS type septate mycelial endophytes were isolated in Meyer's CC medium for *Macrophomina* (Meyer *et al.*, 1973) from rhizosphere soil and infected root pieces. Pure culture washed mats of the isolates were used at the rate of 5 g fresh weight per 2 kg sand as slurry inoculum for plant growth studies. *Cajanas cajan* plants were grown in sand culture under normal day-light intensity with or without inoculation for 100 days. Autoclaved culture mat was used as control inoculant. Plants were fertilized with half dilution Hoagland's solution, with or without phosphate source, every 20 days. In one treatment, Hoagland's soluble phosphate was replaced with insoluble tricaleium phosphate (GR), as a basal dose, at phosphorus level equivalent to that of the soluble phosphorus treatment.

RESULTS

Physical and chemical properties of the soil samples from the four representative stages of Sundarban mangrove ecosystem indicated that the soils were alkaline (pH 7.2 to 9.0) with high soluble salinity (3.0 to 16.0 ds m ^{1}). The soils showed high organic matter (0.460% to 1.427%) and total phosphorus (240.8 ppm to 402.7 ppm) content but low nitrogen (0.09 to 0.17%) and avilable phosphorus (8.34 to 22.11 ppm) concentrations. The values obtained were characteristic of tidollay inundated riverine saline soils.

Of the thirtyseven species of mangrove, mangrove associate and nonlittoral plants examined from these successional stages (Table 1), thirty two plant species showed presence of typical VA-mycorrhizal endophytes in their roots in varying intensities (Table 2). Four herbaceous primary plant colonizers including two members of Chenopodiaceae (*Arthocnemum indicum* and *Suaeda maritima*) were also infected by VA-mycorrhiza (Sengupta and Chaudhuri. 1990) but members of Cyperaceae were not infected.

Of the thirty seven examined plant species of the four successional stages twenty nine showed the presence of atypical fungal endophytes either alone or simul-

Table 1. Mangrove and associated plant species occurring at different successional stages of Sundarban

Successional stage	Plant species examined for endophytic fungal infection
Stage I	
Formative mangrove swamp	*Acanthus ilicifolius, Aegialitis rotundifolia Avicennia officinalis, Bruguiera* sp. *Porteresia coarctata, Rhizophora candelaria, Sonneratia apetala, Sonneratia caseolaris.*
Stage II	
Developed mangrove swamp	*Acanthus ilicifolius, Aegialitis rotundifolia, Aegiceras majus, Arthrocnemum indicum, Avicennia officinalis, Brugniera gymnorrhiza, Ceriops decandra, Ceriops tagal, Derris indica, Exoecaria agallocha, Rhizopus mucronata, Sonneratia apetala, Sonneratia caseolaris Suaeda maritima, Xylocarpus* sp.
Stage III	
Declining ridge mangrove	*Alternanthera polygonoides, Arthrocnemum indicum, Avicennia alba, Avicennia officinalis, Clerodendron inerne, Ceriops decandra, Exoecaria agallocha, Heritiera fomes, Ipomoea pescarpae, Nipa fruticans, Phoenix paludosa, Phragmitis kakra, Sesuvium portulacastrum, Thespesta populnea, Xylocarpus* sp.
Stage IV	
Degraded mangrove and embankment protected agricultural land	*Acacia auriculiformis, Acacia nilotica, Alteranthera polygonoides, Casuarina equisetifolia, Ceriops decandra, Cynodon dactylon, Cyperus rotundus, Echinocloa colona, Ipomoea pescarpae, Leptochloa filiformis, Phragmitis kakra, Prosopis juliflora, Scirpus* sp., *Sesbania* sp., *Sesuvium portulacastrum.*

Table 2 Distribution of typical VAM and other atypical endophytes in roots of mangroves and other associate plants at the Ganges river estuary

DS endophytes	VA-mycorrhiza	VAM and DS endophytes
	Clerodendron inerme (59)	*Acacia auriculiformis* (50)++
	Nipa fruticans (62)	*Acacia nilotica* (60)++
Cynodon dactylon+++	*Rhizophora candelaria* (54)	*Acanthus ilicifolius* (56)+
	Rhizophora mucronata (48)	*Aegialitis rotundifolia* (30)+
Cyperus rotundus+++	*Sonneratia apetala* (51)	*Aegiceras majus* (32)+
Echinocloa colona+++	*Sonneratia caseolaris* (58)	*Avicennia alba* (56)+
	Thespesia populnea (58)	*Avicennia marina* (50)+
Leptochloa filiformis+++	*Xylocarpus* sp. (59)	*Avicennia officinalis* (59)+
Scirpus sp.++		*Bruiguiera gymnorrhiza* (53)++
		Bruiguiera sp. (48)+
		Casuarina equisetifolia (58)++
		Ceriops decandra (45)+
		Ceriops tagal (49)+
		Derrls indica (20)++
		Exoecaria agallocha (60)+
		Heritiera fomes (82)++ +
		Ipomoea pescarpae (61)++
		Phoenix paludosa (59)+++
		Phragmitis hakra (53)+++
		Porteresia coarctata (54)+
		Prosopis juliflora (68)++
		Sesuvium portulacastrum (61)++
		Sesbania sp. (69)+++
		Suaeda maritima (48)+

Infection intensities of atypical endophytes estimated semiquantitatively as high +++ ; moderate ++ ; low +.
Figures within parenthesis indicates percentage of VAM infection.

taneously with the VA-mycorrhizal endophytes (Table 2). Morphological features and histological details of root colonisation by two septate atypical endophytes were as follows :

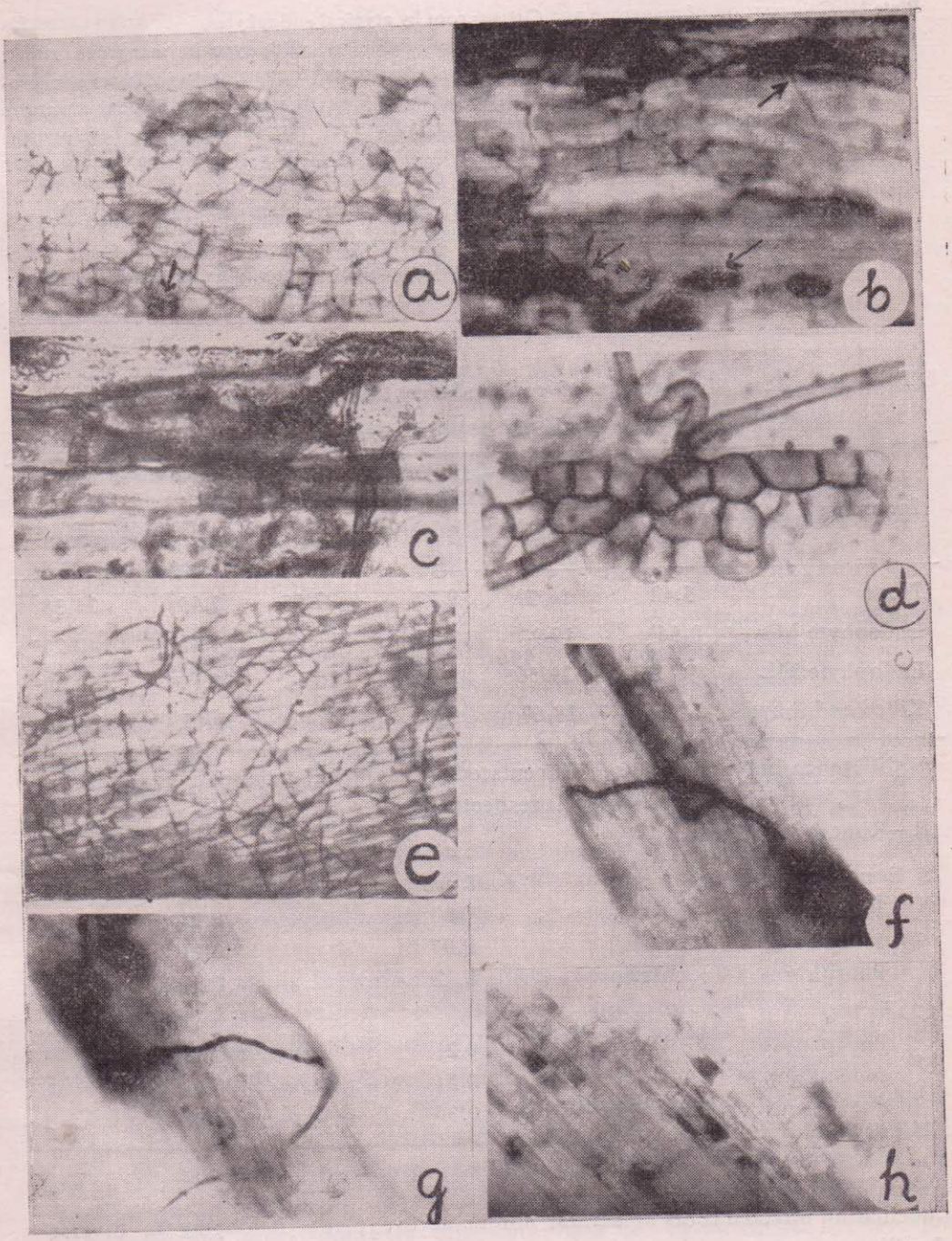
Dark brown septate mycelial, made extensive surface colonization and penetrated root surface by short hyphal branches. Hyphae developed both inter and intracellularly within the outer cortex. At lower depths, thick hyphal coils developed within a few cortical cells from penetrating branch hyphae. At surface layers. microsclerotia like structures were formed occasionally. The hyphae and hyphal coils were evidently digested at later stages (Plate 1).

The DS type endophyte was the predominat in members of Poaceae and Cyperaceae. Both frequency and intensity of root infection by the DS type of endophytes were more in plants belonging to the IIIrd and IVth successional stages and relatively infrequent in stage I and stage II swamps.

The two septate mycelial fungi were isolated in specific medium from root pieces of mangrove and associate plants with DS infections and also from their rhizosphere soils. Based on cultural and metrical characters (Table 3) one isolate (Isolate M) was identified as *Rhizoctonia* sp. The pycnidium forming isolate (isolate ML) has not been identified yet. The two isolates of DS fungi upon

Table 3. Metrical characters of dark septate (DS) mycelial endophytes in nutrient culture

DS mycelial endophyte	Metrical charaeters
Isolate M	Hyphae septate, pale brown, closely branched, 10-12 μm broad ; sclerotia deep brown to black, loosely aggregated, regular, mostly round to oval, 100-200 μm at widest points
Isolate ML	Hyphae septate, dark olivaceous brown, hyaline when young, 12-15 μm broad ; sclerotia like bodies aggregated to stromatic mass, 160-350 μm, dark brown, pycnidia dark, free or attached to stromatic mass, round, 90-160 μm, ostiolate, conidia hyaline, single celled, round, oval to rod shaped, 8-10 μm x 10-12 μm, liberated in mass



(For explanation of Figs. see page 36)

Plate I. Colonization of mangrove and *Cajanas* root by atypical endophytes.
(a—d) Stages of development of atypical endophyte (DS) type in mangrove roots: (a) Surface colonization over *Phoenix paludosa* roots (x 200). arrow indicates a microselerotium like structure ; (b) enlarged view of intracortical hyphal structures formed by the endophyte, at arrows (x 350), (c) intracellular hyphal development in root cortex (x 750) and (d) microsclerotium like structure on surface layer of *Phoenix paludosa* root (x 750).

(e—h) Stages of colonization of *Cajanas cajan* roots by the DS type mangrove root endophyte isolates M and ML ; (e) surface colonization (x 200) ; (f—g) penetration of epidermal layer and development of intracellular structures (x 400) ; (d) digestion of hyphal elements in cortical cells (x 500).

Table 4. Mean dry weight of root and shoot biomass of 100 days old *Cajanas* seedlings in sand culture upon inoculation with DS endophytes

Inoculum	Dry weight per plant (mg)					
	No P		Soluble P		Insoluble P	
	R	S	R	S	R	S
None	5.42	18.29	9.27	27.55	7.65	21.38
Endophyte M	8.56[b]	19.97[b]	11.26[a]	33.13[a]	14.47[a]	40.21[a]
Endophyte ML	9.82[a]	29.56[a]	13 05[a]	36.54[a]	16.11[a]	50.00[a]
Killed endophytes	6.11	18.67	9.84	26.88	8.45	24.26

[a, b]difference between means of inoculated and uninoculated seedlings significant at 1% and 5% probability levels respectively
R—Root, S—Shoot

S. E. m±	Root	Shoot
Phosphorus	0.07	0.21
Inoculation	0.07	0.21
Phosphorus x Inoculation	0.12	0.37
CD at 1%		
Phosphorus	0.21	0.63
Inoculation	0.21	0.63
Phosphorus x Inoculation	0.36	1.10

inoculation infected roots of several non-mangrove plants belonging to diverse families in sand or soil culture without any apparent adverse effects. Histological details of root infections caused by these fungi upon artificial inoculation of non-mangroves, were more or less comparable and similar to that in mangrove

roots (Plate 1). Both the isolates when inoculated *Cajanas cajan* test plants in sand culture gave significant benefits in terms of dry matter yield under extreme nutrient stress (Table 4). Dry matter yield of total biomass of *Cajanas* plants with isolate M increaesd over control by factors 1.2, 1.2 and 1.8 respectively for no phosphorus, soluble phosphorus and insoluble phosphorus treatments. The same for isolate ML were 1.6, 1.3 and 2.8 respectively. The difference between the effects of the two isolates were also significant in terms of growth response particularly for the insoluble phosphorus treatment. Apparently there was no host specificity of the two isolates of endophytes.

DISCUSSION

Coastal salt marsh wetlands are stressed habitats for plants due to salinity, inundation and nutrient limitation (Valiela and Teal, 1979). Mycorrhizal infection may be ecologically relevant for colonization of plant community there. Root infection by several types of fungal endophytes is a widespread feature of tree and other herbaceous members of mangrove plant community and the VA-mycorrhiza may be indeed, an ecological reality for colonization and development of mangrove also.

A characteristic feature of mangrove and associate plants was the presence of seemingly atypical innocuous fungal endophytes other than the typical VA-mycorrhiza in their roots. The dark septate mycelial endophyte(s) was as widespread as the VA-mycorrhiza, and for some species was more abundant than the latter. The two isolates of dark septate mycelial fungi obtained from the habitat formed intracellular hyphal structures comparable to that of the VAM and gave significant symbiotic host response under extreme nutritional stress, at least partly through mobilization of unavailable phosphorus. These facts would justify a mycorrhizal consequence for infections by these type of endophytes to the mangrove plant community.

The fungi which act as mycorrhizal partners for plant roots have two salient features. First, they invade the roots from soil, implying that they either have a good survival mechanism that allow them to remain viable in soil or that they either have a good survival mechanism that allow them to remain viable in soil or that they have the ability for a free living existence in soil. The saprophytic sclerotial fungi such as those isolated from the mangrove habitat seem to have both. Second, when within roots, the fungi usually penetrate host cells causing least damage to host tissue. For endomycorrhizae, minimal cell damage appears to be partly due to the ability of host cytoplasm to digest fungal hyphae and inclusion. histological evidence for which were also abundant in the present case.

Root colonizations by miscellaneous septate mycelial fungi such as those found by Haselwandter and Read (1982) in an alpine plant community (similar to those found in the present study) may be ecologically relevant for plant colonization. Trappe (1989) included these septate endophytes as mycorrhizal fungi for host dependent systems. Evidence obtained in the present study would justify that in stressed habitat of mangroves, fungal endophytes, besides the typical Endogonaceous VA-mycorrhiza, may also have mycorrhizal function and together they may determine plant adaptation there.

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