Fungal endophytes of aerial roots of *Ficus benghalensis*

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Mitosporic fungi and several sterile forms were isolated as endophytes from the leaf tissues and aerial roots of *Ficus benghalensis* (*Moraceae*). Although similar number of endophyte species was present in lamina and petiole, the endophytic fungi more densely colonized the petiole. The species composition and the colonization frequency of the endophytes were more for the aerial roots entering the soil when compared with those growing in the air since the roots recruited some endophytes from the soil. The endophyte assemblages of the leaf and aerial root and of the aerial root growing in the air and soil showed little overlap suggesting that the nature of the host tissue as well as the environment determine the endophyte composition of a host.

Key words: aerial roots, *Ficus*, fungal endophytes, root endophytes.

Introduction

A guild of fungi consisting of ascomycetes and mitosporic forms constitute the fungal endophyte assemblages of the tissues of diverse groups of plants (Stone *et al*., 2000). These fungi are in association with their host tissues without inducing any disease symptoms at least for sometime (Petrini, 1991; Cabral *et al*., 1993). Endophytic fungi have been isolated from leaves, twigs, barks and roots of several angiosperm and gymnosperm members (Stone *et al*., 2000). Although we know about the occurrence and identity of fungal endophytes (Carroll, 1986; Petrini, 1991; Bills, 1996), we know precious little about their biology (Rodriguez and Redman, 1997; Palm, 1999). For example, though there are a few studies on the changes in endophyte and saprophyte communities of intact and decomposing leaves (Sieber-Canavesi and Sieber, 1993; Okane *et al*., 1998), there are not many reports on the status of endophyte community of a plant organ as the organ grows from one environment into another. The aerial root of *Ficus benghalensis* appears to be an ideal plant organ to study this phenomenon as it originates near the crown of the tree, grows down through the air and then grows into the soil to constitute the prop root.

F. benghalensis L. (*Moraceae*), commonly known as banyan tree, is native to India. It is a very large tree with widely spreading branches, which throws out numerous large aerial roots that descend to the soil and form supports.

Materials and methods

Collection of plant samples

Leaf and aerial root tissues of *F. benghalensis* were collected from Indian Institute of Technology, Chennai. Samples were brought to the laboratory in separate sterile polythene bags and processed within 5 hours of collection.

Tissues screened for endophytes

Lamina segments (0.5 cm^2) cut from mid portion (including the midrib) of healthy mature leaves, petiole segments (0.5 cm) and segments (0.5 cm) of aerial root growing in air or soil were sampled. The aerial root segment that was screened was obtained from the subapical portion about 5 mm behind the growing tip.

In order to study the endophyte assemblage of aerial root after its growth into the soil, the following method was adopted. Approximately 1000 grams of moist soil was collected from near the trunk of the tree and was placed in sterile polythene bag. This bag was tied around the tips of the aerial roots such that the roots were buried in the soil. The bags were left undisturbed for 40 days and the roots screened as mentioned above. In another experiment, the soil samples were autoclaved (103 kPa for 20 minutes), left over for a day and autoclaved again before use (Arjuna Rao, 1971).

Surface sterilization of tissues

Three hundred segments were studied for each tissue type. The tissue segments were sterilized following the method of Fisher *et al.* (1993). The segments were dipped in 75% ethanol for 1 minute, immersed in 4% NaOCl for 3 minutes and then rinsed with 75% ethanol for 0.5 minute.

Isolation and identification of endophytes

The sterilized tissue samples were plated on Potato Dextrose Agar medium contained in Petri dishes, amended with Chloramphenicol (150 mg/l). The petri dishes were incubated at 26 C for 3 weeks in a light chamber. The light regimen was 12 hours dark: 12 hours light cycles. The tissue samples received light of 2200 lux through the Petri dish lid as measured by Lutron (Germany) Lux meter. Fungal colonies were transferred to PDA slants and identified.

To prevent the rapidly growing fungi from inhibiting other slow-growing ones, the former were removed from agar medium following isolation and identification (Bills, 1996). The sterile isolates were given code numbers based on the culture characters (Suryanarayanan *et al.*, 1998).

Analysis of results

The colonization frequency (CF%) of each endophyte was calculated following the method of Hata and Futai (1995).

$$CF = (N_{col} / N_t) \times 100$$

where N_{col} and N_t are the number of segments colonized by each endophyte and the total number of segments observed respectively.

A coefficient of similarity (Carroll and Carroll, 1978) was calculated to compare the endophyte assemblage of different organs :

$$\text{Similarity Coefficient (SC)} = 2w/(a+b)$$

where a = the sum of colonization frequency for all fungal species in a tissue and b = the similar sum for another tissue and w = the sum of lower colonization frequencies for fungal endophytes in common between the tissues. It was expressed as percentage. A Kruskal - Wallis test was performed ($P < 0.05$) to compare the number of endophyte isolates obtained from different tissues.

Results and discussion

Sporulating and sterile forms of endophytes were isolated from all the organs of *F. benghalensis* studied (Table 1). However, the overall colonization frequencies differed with different organs. The number of species occurring in the lamina and the petiole region was almost the same; yet, the petiole was more densely colonized by the endophytes as evidenced by the total CF%. Similar results have been observed for Douglas fir (Bernstein and Carroll, 1977), Oregon white oak (Wilson and Carroll, 1994) and *Azadirachta indica* (Rajagopal and Suryanarayanan, 2000). The number of endophyte taxa as well as the number of isolates was significantly low in aerial root when compared with leaf tissues. This could be due to the fact that the root tissue sample was young and exposed to the air borne endophyte inoculum for a relatively lesser time than the mature leaves. It is well known that the aged leaves harbour more endophytes than young ones (Stone, 1987; Rodrigues, 1994; Taylor *et al.*, 1999; Rajagopal and Suryanarayanan, 2000) and that such an increased

Table 1. Colonization frequencies of endophytes from different organs of *Ficus benghalensis*.

Fungus	Lamina	Petiole	Aerial root	Root in soil	Root in sterile soil
Sporormiella minima	1.7	0.7			
Lasiodiplodia theobromae		15.3	1.7		
Colletotrichum gloeosporioides	0.3				
Fusicoccum sp.	5.3				
Phoma sp.	1.0				
Phomopsis sp.1	9.7	64.7	0.3	0.7	
Phomopsis sp.2	0.7				
Phyllosticta sp.	17.0	2.7			
Aspergillus niger		1.0			
Aspergillus sp.4		0.3		1.3	
Aureobasidium pullulans				0.3	
Curvularia lunata	0.3				
Fusarium sp.1		1.7	1.3		
Fusarium sp.2		6.0		0.3	
Gliocladium roseum				2.3	
Gliocladium sp.2				1.7	
Paecilomyces sp.				3.7	
Penicillium sp.1		0.7		1.3	
Penicillium sp.2				0.3	
Phialophora sp.			0.3		
Trichoderma sp.				0.7	4.7
Sterile form 1	0.3	0.3			
Sterile form 2			0.3	0.3	
Sterile form 3			0.3		
Sterile form 4				0.3	
Sterile form 5				0.7	
Sterile form 6				0.3	
Sterile form 7					0.3

infection by endophytes is due to repeated reinfection of tissue over time (Bertoni and Cabral, 1988).

Root endophytes have been studied only recently (Fisher *et al.*, 1991a,b; Holdenrieder and Sieber, 1992; El-Morsy, 2000). In the present study, the species composition as well as CF% of the endophyte assemblage in the aerial root was found to increase significantly when it entered the soil (Table 1). Twelve of the 14 endophytes isolated from the roots growing in soil were not present in aerial roots. This suggested that these endophytes entered the roots from the soil. This was further substantiated by an experiment where roots were allowed to grow in sterilized soil. Such roots were almost free of endophytes, as only one of the 14 endophyte species could be recovered from them. Earlier studies have shown that the endophyte communities of leaf

Table 2. Similarity coefficient of endophytes isolated from *Ficus benghalensis*.

Host organs	Similarity coefficient %
Lamina - Petiole	20.66
Lamina - Aerial Root	1.47
Lamina - Root in Soil	2.76
Petiole - Aerial Root	6.75
Petiole - Root in Soil	4.82
Aerial Root - Root in soil	8.06

tissues could change with the age of the tissue and the season (Rodrigues, 1994; Suryanarayanan *et al.*, 1998; Taylor *et al.*, 1999; Rajagopal and Suryanarayanan, 2000). The present study shows that the endophyte assemblage of a plant organ such as a root changes as the organ grows from one environment to other viz. air to soil; it also shows that the soil can also serve as a reservoir for endophyte inoculum.

The species composition and frequency of endophytes are known to vary with different tissues of host plants (Rodrigues, 1994, 1996). This has prompted Petrini *et al.* (1992) to suggest that plant organs resemble distinct microhabitats with reference to endophyte infections. The results of the present study are in consonance with these observations. Similarity coefficients calculated for endophyte assemblages for *F. benghalensis* tissues showed that above 20% of endophytes are common for leaf and petiole tissues. The endophyte assemblage of aerial roots, although growing in the same environment as the leaves overlapped the endophyte assemblage of leaves by less than 2% (Table 2). In addition, the endophyte assemblages of the root growing in air and the soil overlapped by only 8% indicating that apart from the nature of the host tissue, the environment also determines the composition of endophytes of a host.

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