Endophytic fungi from *Cuscuta reflexa* and its host plants

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Cuscuta reflexa, an angiosperm parasite and seven of its angiosperm hosts were studied for their endophyte assemblages. A *Phomopsis* sp. and a sterile form (H1) were present in five of the seven host plants. A sterile form (H3) occurred in the stem of the parasite growing on six of the host plants. A *Cladosporium* sp.1 and a *Colletotrichum* sp. as well as the sterile form (H1) could be isolated from the stem of the parasite that was in association with five hosts. The composition of endophyte assemblages of *Abutilon indicum* and its parasite overlapped by 37%. In all other cases, the overlap was less than 29%. The endophyte assemblage of *Cucurbita maxima* and its parasite showed no overlap. This appears to be the first study that compared the endophyte assemblages of angiosperm hosts and their angiosperm parasites.

Key words: angiosperm parasite, *Cuscuta reflexa*, fungal endophytes, mycota.

Introduction

It is well known that certain genera of ascomycetes (sexual and asexual) and occasionally basidiomycetes and zygomycetes are able to reside within aerial tissues of vascular plants without producing any apparent symptoms. Such endophytic fungi have been reported mostly from conifers (Petrini and Carroll, 1981; Petrini, 1986). A few tropical plants including palms (Southcott and Johnson, 1997; Rodrigues, 1994), banana (Brown *et al.*, 1998) and mangroves (Suryanarayanan *et al.*, 1998) have also been studied for the presence of endophytes. However, apart from the unpublished study of Chapela (cited in Petrini *et al.*, 1992 as pers. comm.), there appears to be no comparative study on endophytes of an angiosperm host and its angiosperm parasite. The study of a host-parasite system for endophytes is expected to throw some light on host specificity of endophytes. Hence, we studied *Cuscuta reflexa*, an angiosperm parasite and seven of its angiosperm hosts belonging to different families for their endophyte assemblages.

Materials and Methods

Collection of plant samples

The host plants studied were collected in Chennai, Tamil Nadu, India and included *Achyranthes aspera* (*Amarantaceae*), *Abutilon indicum* (*Malvaceae*), *Calotropis gigantea* (*Asclepiadaceae*), *Cucurbita maxima* (*Cucurbitaceae*), *Pongamia glabra* (*Fabaceae*), *Phyllanthus reticulatus* (*Euphorbiaceae*) and *Zizyphus jujuba* (*Rhamnaceae*).

Cuscuta reflexa (*Convolvulaceae*) is a holoparasitic vine that attacks the aerial parts of many shrubs and trees and sometimes almost completely covers them. In the present study, the host and the parasite were simultaneously sampled for endophytes. For each parasitised host, the portion of its stem that was devoid of the coils of the parasite (approximately 3 cm away from the coils) as well as the stem portion of the parasite that was not in contact with the host stem were sampled.

Surface sterilization

For each host-parasite combination, three hundred 1 cm stem pieces of the host or parasite were cut and surface sterilized by the method of Fisher *et al.* (1993). The samples were washed in running water, immersed in 75% ethanol for 1 min, dipped in 4% NaOCl for 3 min and then with 75% ethanol for 30 seconds. The sterilized samples were plated on potato dextrose agar medium amended with chloramphenicol (150 mg l^{-1}) and incubated at 26 C for 21 days in a light chamber. The light was provided by three four ft Philips daylight fluorescent lamps. The light regimen was 12 h dark: 12 h light cycles. The tissue samples received about 2200 Lux of light through the Petri dish lid as measured by a Lutron Lux Meter (Germany). The Petri dishes were observed periodically and the fungi that grew out from the stem tissues were brought into pure culture and identified. To prevent the rapidly growing fungi from inhibiting the slow-growing ones, the former were removed from the agar medium following isolation (Bills, 1996). The sterile isolates that could not be identified were given code numbers based on their culture characters (Dobranic *et al.*, 1995).

Analysis of results

The colonization frequency (CF%) of an endophyte species in the stem tissue was calculated by using the formula: CF = (Ncol/Nt) × 100, where, Ncol and Nt are the number of segments colonized by each endophyte and the total number of segments observed respectively (Hata and Futai, 1995). For comparing the endophyte assemblage of each host-parasite combination, a coefficient of similarity was calculated (Carroll and Carroll, 1978). This was

calculated as follows: Similarity Coefficient = 2w/(a+b), where a = the sum of colonization frequency for all fungal species on a host and b = the similar sum for its parasite, and w = the sum of lower colonization frequencies for fungal species in common between the host and the parasite; it was expressed as %.

Results and discussion

Generally, mitosporic fungi and ascomycetes, as well as some sterile fungi were recorded as endophytes from all the host plants. The stem of *Abutilon indicum* harboured 14 fungal endophytes, while that of *Cucurbita maxima* had the least number of fungi as endophytes (Table 1). However, the total colonization frequency of the endophyte was maximum for *Zizyphus jujuba* suggesting that this plant tissue harboured more endophytes than the other plants screened; the minimum colonization frequency was seen in *Calotropis gigantea* (Table 1). A *Phomopsis* sp. and a sterile form (H1) could be isolated as endophytes from five of the seven host plants. The genus *Phomopsis* is among those that are classified as almost exclusive endophytes (Petrini, 1986; Bills and Polishook, 1992).

A total of forty fungi were isolated from the stems of *Cuscuta reflexa* (Table 2). More endophytic species were recovered from *Cuscuta reflexa* growing on *Zizyphus jujuba* (Table 2). The stem tissue of the parasite growing on *Abutilon indicum* had the least number of endophyte species. The colonization frequency of the endophytes recovered from *Cuscuta reflexa* varied from 28 to 87 (Table 2). The sterile form (H3) occurred in stem of *Cuscuta reflexa* growing on six different hosts. A *Cladosporium* sp.1 and a *Colletotrichum* sp. as well as a sterile form (H1) could be isolated from *Cuscuta reflexa* growing on five different hosts.

A computation of similarity coefficient showed that the endophyte assemblages of *Abutilon indicum* and its parasite overlapped by about 37% (Table 3). For all the other host-parasite combinations, the overlap was less than 29%. The composition of endophyte assemblage for *Cucurbita maxima* was entirely different from that of its parasite (Table 3). Although the total colonization frequency of the endophytes was maximum for *Zizyphus jujuba* stem (Table 1), and although more endophytic species could be isolated from *Cuscuta reflexa* growing on this host (Table 2), the overlap for the endophytes assemblages of this host-parasite combination was only 25.8%

The plant body of *Cuscuta reflexa* is represented by a leafless, thin, wiry stem that tightly coils around the stem of its host. It also produces haustoria that penetrate the host stem tissue and facilitate the absorption of nutrients from the host. Thus, the parasite is in close contact with its host and consequently, it is exposed virtually to the same type and load of fungal inoculum. However, in

Table 1. Colonization Frequency of endophytes in seven host plants.

Endophytes		Host						
		AA	AI	CG	CM	PG	PR	ZJ
Hyphomycetes	*Acremonium* sp.	1	-	-	-	-	-	-
	Alternaria alternata	-	-	-	-	5	1	3
	A. longissima	-	-	-	-	1	-	-
	Aspergillus flavipes	-	-	-	-	-	1	-
	A. niger	-	3	1	-	-	-	-
	A. versicolor	-	-	-	-	-	2	-
	Aureobasidium sp.	6	-	-	-	-	-	-
	Cladosporium sp. 1	3	2	2	-	-	-	-
	Cladosporium sp. 2	-	-	2	-	-	-	-
	Curvularia lunata	-	-	-	-	-	1	-
	Drechslera halodes	-	-	1	-	-	-	-
	D. hawaiiensis	-	-	-	-	1	-	4
	Fusarium sp. 1	5	-	-	9	7	-	-
	Fusarium sp. 2	3	-	-	-	2	-	-
	Fusarium sp. 3	-	-	-	-	5	1	-
	Nigrospora sp.	-	2	-	-	-	-	-
	Penicillium sp.	-	5	-	-	-	1	-
	Pithomyces sp.	-	-	-	-	-	-	1
	Thozetellopsis sp.	-	-	-	-	-	-	1
	Trichoderma sp.	-	1	-	-	-	-	-
Coelomycetes	*Botryodiplodia* sp.	-	-	-	-	-	1	-
	Colletotrichum gloeosporioides	7	-	-	-	8	3	1
	Colletotrichum sp.	8	-	-	-	-	10	12
	Pestalotiopsis sp.	-	-	-	-	-	1	2
	Phoma sp.	-	1	-	-	-	-	-
	Phomopsis sp.	2	2	-	-	2	46	75
	Phyllosticta sp.	-	3	-	-	2	-	-
	Pseudoseptoria sp.	-	-	-	-	-	-	1
	Septochyta sp.	-	-	-	-	-	1	-
Ascomycetes	*Glomerella* sp.	20	-	-	-	3	-	-
	Guignardia sp.	-	1	-	-	-	-	-
	Sporormiella minima	2	3	-	-	-	-	-
	Ascomycete H12	-	-	3	5	-	-	-
Sterile mycelia	H1	-	5	1	-	3	25	1
	H2	30	2	-	-	-	-	-
	H3	-	1	-	-	-	-	4
	H4	-	1	-	-	-	-	-

AA = *Achyranthes aspera*; AI = *Abutilon indicum*; CG = *Calotropis gigantea*; CM = *Cucurbita maxima*; PG = *Pongamia glabra*; PR = *Phyllathus reticulatus*; ZJ = *Zizyphus jujuba*.

Table 2. Colonization frequency of endophytes isolated from stem of *Cuscuta reflexa* growing on different hosts.

Endophytes		Host						
		AA	AI	CG	CM	PG	PR	ZJ
Hyphomycetes	*Acremonium* sp.	1	-	1	-	-	18	-
	Alternaria alternata	-	-	-	4	7	-	-
	Aspergillus niger	1	4	-	-	-	-	-
	A. terreus	-	-	-	-	-	4	-
	A. versicolor	-	-	-	-	-	7	-
	Cladosporium sp. 1	4	12	8	-	18	2	-
	Cladosporium sp. 2	-	-	3	1	33	-	-
	Curvularia lunata	-	-	-	-	1	-	-
	C. tuberculata	-	-	-	-	1	-	-
	Drechslera hawaiiensis	-	-	1	-	-	-	-
	Fusarium sp. 1	-	-	-	-	9	-	-
	Fusarium sp. 2	-	3	-	1	6	-	1
	Fusarium sp. 3	-	-	-	2	-	5	-
	Nodulisporium sp.	4	-	-	-	-	-	-
	Periconia sp.	-	-	-	-	-	-	1
	Penicillium sp.	-	-	-	1	-	1	-
	Pithomyces sp.	-	-	-	-	-	-	1
	Torula sp.	-	-	-	-	-	-	4
Coelomycetes	*Botryodiplodia* sp.	-	-	-	-	-	2	3
	Chaetomella sp.	-	-	-	-	-	7	-
	Colletotrichum gloeosporioides	2	-	-	-	-	-	1
	C. truncatum	-	-	-	-	1	-	-
	Colletotrichum sp.	11	-	-	8	1	5	8
	Pestalotiopsis sp.	-	-	-	-	-	-	1
	Phoma sp.	1	-	1	-	-	-	1
	Phomopsis sp.	-	1	-	-	-	-	6
	Phyllosticta sp.	4	-	-	-	-	1	2
Ascomycetes	*Chaetomium* sp.	-	-	1	-	-	-	-
	Sporormiella minima	2	-	-	-	-	-	3
	Ascomycete H12	-	-	18	-	-	-	-
Sterile mycelia	H1	16	6	-	12	-	2	16
	H2	-	-	-	-	7	-	-
	H3	3	-	1	1	2	2	2
	H5	-	2	-	-	-	-	-
	H6	-	-	3	3	-	-	-
	H7	-	-	1	-	-	-	-
	H8	-	-	-	1	-	-	-
	H9	-	-	-	1	-	-	-
	H10	-	-	1	-	-	-	-
	H11	-	-	-	-	1	-	-

AA = *Achyranthes aspera*; AI = *Abutilon indicum*; CG = *Calotropis gigantea*; CM = *Cucurbita maxima*; PG = *Pongamia glabra*; PR = *Phyllathus reticulatus*; ZJ = *Zizyphus jujuba*.

Table 3. Similarity Coefficients of the endophyte assemblages of the hosts and their parasites.

Host-Parasite	Similarity Coefficient (%)
Cucurbita maxima – Cuscuta reflexa	0
Phyllanthus reticulatus – C. reflexa	16
Pongamia glabra – C. reflexa	22.2
Achyranthes aspera – C. reflexa	23.5
Zizyphus jujuba – C. reflexa	25.8
Calotropis gigantea – C. reflexa	28.6
Abutilon indicum – C. reflexa	36.7

any given host-parasite combination in the present study, only less than 37% of the endophytic assemblages were shared by the host and the parasite. A similar study by Chapela (cited in Petrini *et al.*, 1992) showed that that the endophyte assemblages of fir tree and its mistletoe parasite overlapped by less than 15%. These results strongly suggest the existence of some degree of host specificity among fungal endophytes.

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