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ORIGINAL PAPER

Endophytic fungal communities in woody perennials of three tropical forest types of the Western Ghats, southern India

T. S. Suryanarayanan · T. S. Murali · N. Thirunavukkarasu · M. B. Govinda Rajulu · G. Venkatesan · R. Sukumar

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Abstract Fungal endophytes of tropical trees are expected to be exceptionally species rich as a consequence of high tree diversity in the tropics and the purported host restriction among the endophytes. Based on this premise, endophytes have been regarded as a focal group for estimating fungal numbers because their possible hyperdiverse nature would reflect significantly global fungal diversity. We present our consolidated ten-year work on 75 dicotyledonous tree hosts belonging to 33 families and growing in three different types of tropical forests of the NBR in the Western Ghats, southern India. We conclude that endophyte diversity in these forests is limited due to loose host affiliations among endophytes. Some endophytes have a wide host range and colonize taxonomically disparate hosts suggesting adaptations in them to counter a variety of defense chemicals in their hosts. Furthermore, such polyphagous endophytes dominate the endophyte assemblages of different tree hosts. Individual leaves may be densely colonized but only by a few endophyte species. It appears that the environment (the type of forest in this case) has a larger role in determining the endophyte assemblage of a plant host than the taxonomy of the host

T. S. Suryanarayanan (✉)
Vivekananda Institute of Tropical Mycology (VINSTROM), 70-72 P.S. Sivasamy Salai, Ramakrishna Mission Vidyapith, Chennai 600 004, India
e-mail: t_sury2002@yahoo.com

T. S. Murali · N. Thirunavukkarasu · M. B. Govinda Rajulu · G. Venkatesan
PG and Research Department of Botany, Ramakrishna Mission Vivekananda College, Chennai 600 004, India

R. Sukumar
Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560 012, India

Present Address:
T. S. Murali
Division of Biotechnology, Manipal University, Karnataka 576 104, India

Present Address:
G. Venkatesan
Department of Botany, Mannai Rajagopalaswamy Government Arts College, Mannargudi 614 001, India

plant. Thus, different tropical plant communities have to be studied for their endophyte diversity to test the generalization that endophytes are hyperdiverse in the tropics, estimate their true species richness, and use them as a predictor group for more accurate assessment of global fungal diversity.

Keywords Endophyte · Fungal endophyte · Tropical forests · Fungal diversity

Abbreviations

NBR	Nilgiri Biosphere Reserve
PCA	Principle Component Analysis
PDA	Potato dextrose agar
ID	Mean infection density
CF%	Colonization frequency (%)
MTCC	Microbial Type Culture Collection

Introduction

Fungal endophytes infect living tissues of plants and survive therein without producing any visible symptoms of their infection (Botella et al. 2010; Sakalidis et al. 2011). The horizontally transmitted endophytes have been reported from all major groups of plants including algae (Zuccaro et al. 2008; Suryanarayanan et al. 2010), lichens (Suryanarayanan et al. 2005), mosses and ferns (Petrini 1986), conifers (Giordano et al. 2009) and angiosperms (Saikkonen 2007; Tejesvi et al. 2010), and may persist even in aseptically cultured plants (Lucero et al. 2008). Their potential as biocontrol agents (Arnold et al. 2003) and producers of novel bioactive compounds (e.g., Suryanarayanan et al. 2009a; Aly et al. 2010; Xu et al. 2010) are being currently recognized, but their interactions with their plant hosts and other organisms in the ecosystem have not been studied in detail (Higgins et al. 2006; Priti et al. 2009; Saunders and Kohn 2009; Van Bael et al. 2009). Foliar endophytes may impose a significant cost on the host plant (Arnold and Engelbrecht 2007; Botella et al. 2010), alter the fitness of the host plant (Redman et al. 2002; Waller et al. 2005; Giordano et al. 2009; Botella et al. 2010), and initiate leaf litter degradation thus aiding in nutrient recycling (Kumaresan and Suryanarayanan 2002; Osono and Hirose 2009). Furthermore, endophytes are reckoned as a focal group for predicting global fungal diversity (Arnold et al. 2000; Hyde et al. 2000). The diversity of different ecological groups of fungi such as the litter fungi and endophytes are expected to peak in tropical forests as a consequence of high angiosperm diversity present in these forests (Lodge et al. 1996; Polishook et al. 1996; Fröhlich and Hyde 1999; Arnold et al. 2000; Hyde and Soytong 2008). The high endophyte diversity recorded in tropical forests in Central Panama suggested that fungal diversity is vastly underestimated currently (Arnold et al. 2000; Rodriguez et al. 2009). Considering the economic potential and ecological roles of endophytes, it is imperative that different types of tropical plant communities are screened for endophytes to facilitate realistic biodiversity estimations, appropriate bioprospecting decisions and conservation strategies. In this context, there are no comparative studies on culturable endophytes of different types of tropical forests.

We have been studying various aspects of endophyte ecology in different types of forests of southern Western Ghats of the NBR in southern India for the past 15 years as part of a broader long-term study of tropical forest dynamics (Sukumar et al. 1992, 2005), and have published results pertaining to a few tree hosts of the different forests

(Suryanarayanan et al. 2002, 2003). Here, we consolidate the results obtained in a ten-year study (from 2000 to 2009) on endophyte diversity and distribution in a dry thorn forest, dry deciduous forest, and montane evergreen forest of the NBR. The results pertain to 25 dicotyledonous tree species for each forest type (a total of 75 tree species belonging to 33 families). The aim of this study was to estimate the endophyte diversity in different types of tropical forests using several tree hosts belonging to different families. As the sampling methods, size and number of leaf segments screened, growth medium, surface sterilization procedure, incubation conditions and method of observation followed were maintained consistently throughout the study period, it was possible to compare the results across host species and forest types. We addressed host species and host family affiliations, spatial heterogeneity and colonization patterns of endophytes, and quantified colonization frequencies of endophytes for different host species.

Materials and methods

Collection sites

Twenty-five woody dicot tree species each from a tropical dry thorn forest, dry deciduous forest and a montane evergreen forest situated in the NBR (Latitude 11°32′ and 11°43′N, Long 76°22′ and 76°45′E) were studied for their leaf endophyte association. The tropical dry thorn forest covers the Sigur plateau in the rain shadow region of the Nilgiri mountains at an elevation of about 900–1,000 m above mean sea level and receives an annual rainfall of 700–1,000 mm; the annual average rainfall at Masinagudi close to the site of collection of leaf samples is 852 mm (data for 1990–2008). To its east, the dry deciduous forest is the major forest type of the Mudumalai Wildlife Sanctuary and receives about 1,000–1,400 mm of rainfall per annum, with Kargudi (where our leaf samples were collected) receiving an average of 1,217 mm per annum (data for 1989–2008).

The montane evergreen forests of the Western Ghats are at an elevation of 1,800–2,500 m above mean sea level. These forests, usually stunted in physiognomy, are largely confined to the sheltered folds of the mountains (Suresh and Sukumar 1999). While this region receives an annual rainfall of 1,300–3,000 mm, the Thaishola forest site where we collected leaf samples has an average annual rainfall of 1,433 mm (data for 2000–2006).

Sample collection

We included the most common tree species of each forest type in our screening of foliar endophytes (Table 1). For each host species, three individual trees were selected and from each individual 20 mature, healthy, symptomless leaves were collected (young leaves were avoided as they are relatively free from endophytes—Rajagopal and Suryanarayanan 2000) [total 60 leaves from each species] and screened for endophytes. Each tree host species was sampled at least once irrespective of the season. The leaf samples were processed within 24–36 h of collection.

Endophyte isolation

From each leaf, three segments (0.5 cm^2) were cut from the midrib region (including the lamina portion)—one each from the apical, middle and the basal region of the leaf. The one

Table 1 Tree hosts of tropical dry thorn, dry deciduous and montane evergreen forests of the Nilgiri Biosphere Reserve screened for their leaf endophytes

Host	Code	Family
Dry thorn forest		
Acacia ferruginea	AF	Mimosoiceae
Anogeissus latifolia	AL	Combretaceae
Bauhinia racemosa	BRC	Fabaceae
Bridelia retusa[a]	BR	Euphorbiaceae
Butea monosperma	BM	Fabaceae
Cassia fistula	CF	Fabaceae
Cordia wallichii[a]	CW	Boraginaceae
Dalbergia lanceolaria	DL	Fabaceae
Diospyros montana[a]	DM	Ebenaceae
Ehretia canarensis	EC	Boraginaceae
Elaeodendron glaucum	EG	Celastraceae
Erythroxylon monogynum	EM	Erythroxylaceae
Givotia rottleriformis	GR	Euphorbiaceae
Gmelina asiatica[a]	GA	Verbenaceae
Ixora nigricans	IN	Rubiaceae
Maytenus emarginata[a]	ME	Celastraceae
Pongamia pinnata	PP	Fabaceae
Premna tomentosa	PT	Verbenaceae
Pterocarpus marsupium[a]	PM	Fabaceae
Randia dumetorum[a]	RD	Rubiaceae
Stereospermum angustifolium	SA	Bignoniaceae
Strychnos potatorum	SP	Loganiaceae
Terminalia chebula	TC	Combretaceae
Ziziphus jujuba[a]	ZJ	Rhamnaceae
Ziziphus xylopyrus	ZX	Rhamnaceae
Dry deciduous forest		
Anogeissus latifolia	AL	Combretaceae
Careya arborea	CR	Barringtoniaceae
Casearia esculenta	CE	Samydaceae
Cassia fistula	CF	Fabaceae
Catunaregam spinosa	CS	Rubiaceae
Cordia obliqua	CO	Boraginaceae
Cordia wallichii	CW	Boraginaceae
Gmelina arborea	GAR	Verbenaceae
Grewia tiliifolia	GT	Tiliaceae
Helicteres isora	HI	Sterculiaceae
Kydia calycina	KC	Malvaceae
Lagerstroemia microcarpa	LM	Lythraceae
Lagerstroemia parviflora	LP	Lythraceae
Ougenia oojeinensis	OO	Fabaceae
Phyllanthus emblica	PE	Euphorbiaceae
Premna tomentosa	PT	Verbenaceae

Table 1 continued

Host	Code	Family
Radermachera xylocarpa	RX	Pedaliaceae
Schrebera swietenioides	SS	Oleaceae
Shorea roxburghii	SR	Dipterocarpaceae
Stereospermum personatum	SPE	Bignoniaceae
Syzygium cumini	SCU	Myrtaceae
Tectona grandis	TG	Verbenaceae
Terminalia alata	TA	Combretaceae
Terminalia crenulata	TCR	Combretaceae
Vitex altissima	VA	Verbenaceae
Montane evergreen forest		
Cinnamomum malabatrum[a]	CM	Lauraceae
Cryptocarya bourdillonii	CB	Lauraceae
Daphniphyllum neilgherrense[a]	DN	Euphorbiaceae
Euonymus angulatus	EA	Celastraceae
Eurya nitida[a]	EN	Ternstromiaceae
Glochidion zeylanicum[a]	GZ	Euphorbiaceae
Ilex denticulata	ID	Aquifoliaceae
Ilex wightiana[a]	IW	Aquifoliaceae
Isonandra candolleana	IC	Sapotaceae
Lasianthus venulosus[a]	LV	Rubiaceae
Ligustrum roxburghii	LR	Oleaceae
Litsea floribunda	LF	Lauraceae
Litsea stocksii	LS	Lauraceae
Meliosma simplicifolia	MS	Sabiaceae
Memecylon malabaricum[a]	MM	Melastomaceae
Michelia nilagirica[a]	MN	Magnoliaceae
Neolitsea zeylanica	NZ	Lauraceae
Phoebe lanceolata	PL	Lauraceae
Psychotria bisulcata[a]	PB	Rubiaceae
Rhodomyrtus tomentosa[a]	RT	Myrtaceae
Symplocos cochinchinensis	SC	Symplocaceae
Symplocos obtusa	SO	Symplocaceae
Syzygium densiflorum[a]	SD	Myrtaceae
Turpinia nepalensis	TN	Staphyleaceae
Vepris bilocularis[a]	VB	Rutaceae

[a] Also studied for endophyte distribution in entire leaf

hundred and eighty tissue segments thus obtained from 60 leaves for each host species were surface sterilized by the method of Suryanarayanan et al. (1998). From these, 150 segments were randomly selected and plated on chloramphenicol-amended PDA medium contained in 9 cm diam. Petri dishes (10 segments/dish). The Petri dishes were incubated in a light chamber with a 12 h light:12 h dark cycle for 28 days at 26 ± 1°C

(Suryanarayanan 1992). The suitability of sampling and surface sterilization procedures was determined by preliminary experiments.

The surface sterilized leaf segments were gently pressed on to antibiotic-amended PDA medium in a Petri dish and removed. The absence of the growth of any fungi from impressions of surface sterilized leaf segments on agar medium proved the efficacy of the surface sterilization protocol adopted (Schulz et al. 1998). The tissue segments were observed periodically and the fungi growing out of them were scored, isolated, cultured in PDA slants and identified. The sterile isolates could not be assigned to any taxonomic group and were given codes based on culture characteristics such as growth rate, colony surface texture and hyphal pigmentation (Suryanarayanan et al. 1998). Sterile forms with different culture characteristics were assumed to represent different taxonomic entities (Lacap et al. 2003).

Distribution of endophytes within a single leaf and construction of species area curve

Only representative tissue segments taken from a leaf were screened for endophyte presence as is the practice in studies aimed at isolating foliar endophytes (Suryanarayanan et al. 1998; Arnold et al. 2000). To overcome sampling bias arising due to this methodology, an entire leaf was sampled for a few tree species by nested quadrat method wherein endophytes from successive larger segments of a leaf were recorded (Suryanarayanan et al. 2002). Using this method, we sampled the entire leaf of eight plant species of dry thorn forest and 12 of montane evergreen forest (Table 1). Three mature green leaves were sampled for each tree species following the method of Suryanarayanan et al. (2002) and using the nested series of expanding quadrat method (Condit et al. 1996), the species area curves were constructed (Suryanarayanan et al. 2002).

Rarefaction curve, diversity estimates and measures of similarity

Rarefaction curve was used to estimate the number of species in a given sub-sample of a forest (Koellner et al. 2004). Fisher's α and Shannon–Wiener index were used to calculate the species diversity of the endophyte assemblages (Taylor 1978; Spellerberg 2008). Pielou (J') was used to measure the degree of evenness (Magurran 2004). The similarity between endophyte assemblages of the forests was calculated using both Jaccard and Sorenson similarity coefficients (Magurran 2004). Statistical softwares BiodiversityPro version 2 (The Natural History Museum and The Scottish Association for Marine Science), EstimateS version 8.0 (Colwell 2005) and GraphPad Prism version 3.00 (http://www.graphpad.com) were used for analysis.

The CF% and ID of the endophytes were calculated as follows.

$$\mathrm{CF}\% = \frac{N_{col}}{N_t} \times 100 \quad (1)$$

where, N_{col} and N_t are the number of segments colonized and the total number of segments screened respectively.

$$\mathrm{ID} = \frac{\text{Total number of isolates}}{\text{Number of segments inoculated}} \quad (2)$$

Representative endophyte cultures have been deposited in MTCC, Chandigarh, India. These include *Chaetomium* sp. (MTCC 8385), *Phyllosticta capitalensis* (MTCC 8468),

Colletotrichum gloeosporioides (MTCC 8460), *Fusarium* sp. (MTCC 8461), *Nigrospora oryzae* (MTCC 8465), *Phomopsis* sp. (MTCC 8464), *Corynespora* sp. (MTCC 8466), *Corynespora* sp. (MTCC 8467), *Colletotrichum* sp. (MTCC 8480), *Nigrospora oryzae* (MTCC 8660), *Pithomyces maydicus* (MTCC 8657), *Botrytis cinerea* (MTCC 8659), *Chaetomium* sp. (MTCC 8658), *Nodulisporium* sp. (8665), *Periconia* sp. (MTCC 8663), *Penicillium* sp. (MTCC 8664), *Trichoderma* sp. (MTCC 8662) and *Fusarium* sp. (MTCC 8666).

Results

Endophyte diversity in the forests

The number of endophyte species recovered from each tree species varied from 4 to 24 in dry thorn forest, from 6 to 25 in dry deciduous forest and from 8 to 16 in montane evergreen forest. We isolated a total of 75, 68 and 75 endophyte species from dry thorn, dry deciduous and montane evergreen forests respectively (Table 2). The adequacy of sampling was ascertained by plotting a species accumulation curve for the three forests. To avoid the influence of sample sequence on the shape of the accumulation curve, the data for each forest type were randomized 100 times using the computer software EstimateS before plotting (Fig. 1). More endophyte isolates were recovered from leaves of tree hosts from dry thorn than from dry deciduous and montane evergreen forests indicating that the trees of dry thorn forest were more densely colonized by endophytes. This was also revealed by a higher value of ID recorded for this forest (Table 2). The CF% was more than 100% in

Table 2 Endophyte status of dry thorn, dry deciduous and montane evergreen forests

	Dry thorn forest	Dry deciduous forest	Montane evergreen forest
No. of tree species screened	25	25	25
No. of tissue segments screened	3,750	3,750	3,750
No. of endophyte isolates	4,833	4,360	2,364
No. of endophyte species	75	68	75
ID	1.2	1.1	0.6
Total CF%	122.5	116.2	60.5
Annual rainfall (in mm)	700–1,000	1,000–1,400	1,300–3,000
Pielou J′	0.64	0.63	0.72
Shannon–Wiener index	2.25	2.59	2.61
Fisher's α	12.6	11.4	14.7
Dominant species (no. of host species from which isolated in parenthesis)	*Phomopsis* spp. (17) *Phyllosticta capitalensis* (5)	*Phyllosticta* spp. (12) *Phomopsis* sp. 1 (9)	*Colletotrichum* spp. (12) *Phomopsis* sp. 1 (11)
Co-dominant species (no. of host species from which isolated in parenthesis)	*Phyllosticta capitalensis* (9)	*Colletotrichum* sp. (4) *Phomopsis* sp. 2 (7) *Phyllosticta* (6)	*Colletotrichum acutatum* (10)

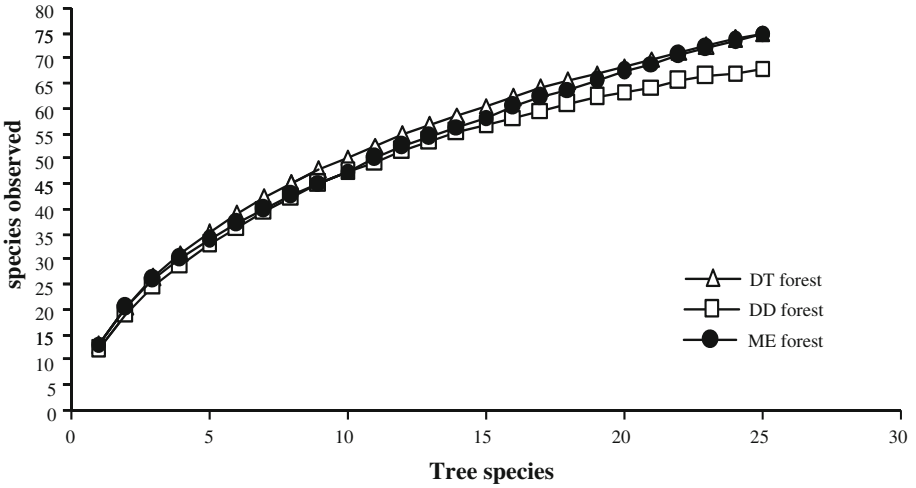


Fig. 1 Species accumulation curve for endophytes isolated from 25 different tree hosts of dry thorn (DT), dry deciduous (DD) and montane evergreen (ME) forests. Data were randomized 100 times for plotting the *graph*

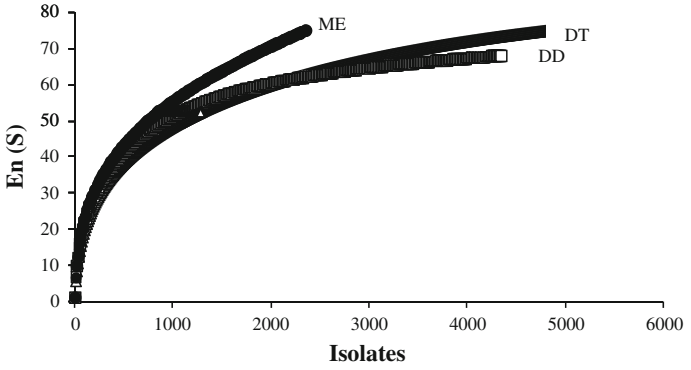


Fig. 2 Rarefaction curves for the expected number of species [En(S)] of endophytes from 25 different tree hosts in DT, DD and ME forests

dry thorn and dry deciduous forests due to multiple infections of the leaves (Table 2). The species diversity (Fisher's α and Shannon–Wiener index) for endophytes was higher for montane evergreen forest than those for dry thorn and dry deciduous forests (Table 2). Although the number of endophyte species recovered did not vary much for the three forests, the endophyte assemblage of montane evergreen forest was more diverse; the endophyte isolates of montane evergreen forests was more species rich than that of the other two forests (Table 2). A Rarefaction analyses revealed that while 2,361 endophyte isolates from montane evergreen forest captured 74 species, the same number of isolates had only 62 species in dry deciduous and dry thorn forests (Fig. 2). The limited endophyte diversity of dry thorn and dry deciduous forests when compared to that of montane evergreen forest was further substantiated by the fact that there were less number of unique species (those that occur in only one tree species irrespective of their CF%) in these two

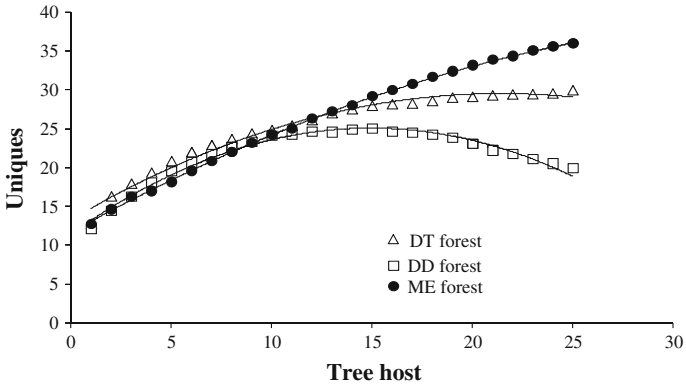


Fig. 3 Number of unique species of endophytes isolated from 25 different tree hosts in DT, DD and ME forests. Data were randomized 100 times for plotting the *graph*. The *curve* represents a polynomial trendline

forests (Fig. 3). A relatively higher evenness index (J′) of the montane evergreen forest endophyte assemblage (Table 2) also showed a more equitable distribution of endophyte species resulting in increased diversity (Longino et al. 2002).

A study of the distribution of endophytes in single leaves showed that in all the hosts (eight of dry thorn forest and 12 of montane evergreen forest), irrespective of their taxonomic affiliation or the forest in which they grew, the number of endophyte isolates increased with increasing area of leaf but the number of endophyte species increased initially with the area sampled but soon reached an asymptote.

Endophyte communities in different forests

Depending on the similarity index used, there was between 31 and 52% similarity of the endophyte assemblage between any two forests (Table 3). Thirty four endophyte species were shared between montane evergreen and dry deciduous forests, forty species were common for montane evergreen and dry thorn forests, and thirty seven species were isolated from dry deciduous and dry thorn forests. Twenty-four endophyte species were present in all the three forest types. Twenty-five species were present only in montane evergreen forest, and dry thorn and dry deciduous forests had 23 species that were unique to them. A PCA was performed to identify patterns in the data showed that the endophyte assemblages of the three forests were different enough to form three discrete clusters (Fig. 4). To discern host and site specificity of endophytes, we compared the endophyte assemblages of four tree species viz. *Anogeissus latifolia*, *Cassia fistula*, *Cordia wallichii* and *Premna tomentosa* that grew both in dry thorn and dry deciduous forest sites separated by a distance of about 15 km. A PCA analysis showed that the endophyte assemblage of conspecific tree hosts from different forests were different (Fig. 5).

Table 3 Measure of similarity between the endophyte assemblages of the forests

Forests compared	Jaccard index	Sorenson index
Dry deciduous/dry thorn	0.32	0.49
Montane evergreen/dry thorn	0.35	0.52
Montane evergreen/dry deciduous	0.31	0.48

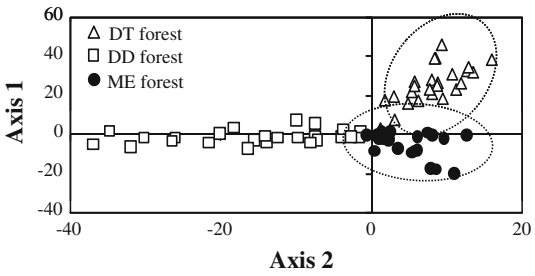
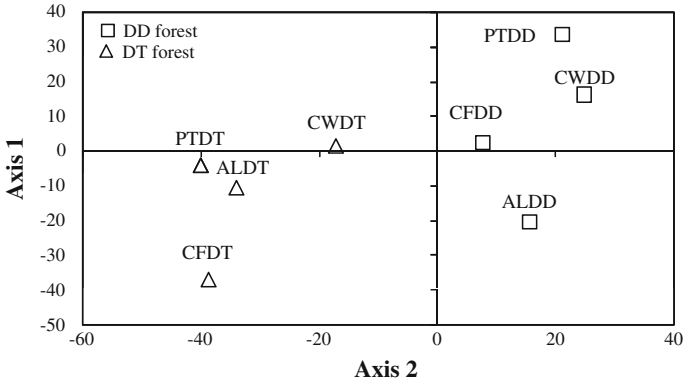


Fig. 4 Principal Component Analysis of endophyte assemblages of 25 tree hosts of DT, DD and ME forests



AL = *Anogeissus latifolia*, CF = *Cassia fistula*, CW = *Cordia wallichii*
PT = *Premna tomentosa*

Fig. 5 Principal Component Analysis of endophytes of common tree host of DT and DD forests

Discussion

The primary requisite in studies attempting to quantify diversity is establishing the adequateness of sampling. Our earlier study of the dry thorn and dry deciduous forests showed that sampling of 15 tree species in each forest type would capture a maximum of 95% of the endophyte species in these plant communities (Murali et al. 2007); therefore, we argued that sampling 25 trees for each of the three forest would be adequate for estimations. Furthermore, the flattening of both species accumulation and unique species curves for endophytes (Figs. 1, 3) indicated that the inventory was nearly complete (Longino 2000; Henderson 2003).

The higher species diversity of endophytic fungi recorded in the montane evergreen forest when compared to the dry deciduous and dry thorn forests can perhaps be attributed to the wet condition in this forest since higher moisture favours endophyte propagule dispersal and infection (Johnson and Whitney 1989; Helander et al. 1993; Bahnweg et al. 2005). However, Murali et al. (2007) in a study involving 30 different tree hosts of two of these forests (dry thorn and dry deciduous) concluded that precipitation does not affect significantly endophyte species diversity in these forests—hence, collection of leaf sample in the present study irrespective of the season does not distort the results. Therefore, in line with the argument that a higher plant host diversity will support a higher endophyte diversity (Arnold et al. 2000; Hyde et al. 2000), it is possible that the relatively higher endophyte diversity in montane evergreen forest is a result of higher tree species diversity.

The montane evergreen forest site has 66 species of woody plants (>1 cm dbh) per hectare, while the figures are 26 species and 32 species on average for the dry deciduous and dry thorn forests, respectively (H.S. Dattaraja, H.S. Suresh and R. Sukumar, unpublished data). All the three forests studied here are relatively dry forests with seasonal rainfall and distinct dry periods lasting 4–5 months when less than 50 mm rain falls each month. Such an environment could contribute to the overall lower endophyte diversity when compared to the neotropics. Continuing this line of argument, while Arnold et al. (2000) obtained 418 morphospecies of endophytes from 1,472 isolates from just two plant species in Panama, a rarefaction analysis revealed that we recovered only 54, 56 and 63 species from 1,472 isolates in dry thorn, dry deciduous and montane evergreen forest respectively (Fig. 2). Thus, lower host plant diversity and reduced levels of precipitation could be the major determinants in endophyte diversity being lower in the forests of southern India when compared to the neotropical forests (see Compant et al. 2010). Fungal species adapted to survive as endophytes under these conditions alone are selected resulting in similar endophyte species composition in leaves of different tree hosts.

Within leaf distribution

Endophytes exhibit differential distribution in a leaf. They may be more concentrated in the mid-rib region (Rajagopal and Suryanarayanan 2000; Cannon and Simmons 2002), in the lamina portion (Brown et al. 1998) or may be evenly distributed (Suryanarayanan et al. 2002). The common method of sampling a leaf for the presence of endophytes (as also used in the present study) is by analysing representative tissue segments taken from different portions of a leaf (Suryanarayanan et al. 1998; Arnold et al. 2000); this method assumes that all individuals in the sampling universe have an equal probability of being isolated. However, any species with lesser abundance or clumped distribution in the leaf are likely to be missed or recorded infrequently by this sampling method resulting in an underestimation of the diversity of endophyte community. This was not the case as sampling of entire leaves in eight tree species of dry thorn forest and 12 tree species of montane evergreen forest showed that the leaves are densely and evenly colonized but only by a few species of endophytes. These results are similar to those obtained by Suryanarayanan et al. (2002) for other tree hosts of the dry thorn and dry deciduous forests in an earlier study. Super infections of leaves by endophytes which are frequent in the tropics (Lodge et al. 1996; Arnold et al. 2000; Suryanarayanan et al. 2002) resulted in CF% being more than 100% in dry thorn and dry deciduous forests (Table 2).

Host affiliation

Our results showed that some endophyte species were common to all the forests and some were confined to one forest type. However, many of these unique species such as *Alternaria* spp., *Fusarium* spp., *Phoma* spp., *Phomopsis* spp., *Curvularia* spp., and *Lasiodiplodia* sp. are common endophyte species and could have been isolated from any of the forest hosts if we had increased our sample size. This was similar to the results obtained by Cannon and Simmons (2002) for a Guyana forest but contradictory to the observation of Arnold et al. (2000) for a forest in Panama.

When the dominant endophytes were considered, it was found that *Phomopsis* spp. (*Phomopsis* sp. 1 in particular) were universal, *Phyllosticta* spp. (especially *P. captalensis*) were confined to the more dry forest types, and *Colletotrichum* spp. were more dominant (or co-dominant) in the wetter montane evergreen forest (Table 2). Apart from the

difference in the annual rainfall, the forests differed from each other with respect to the host tree species supported by them (Fig. 4). Hence, it was also possible that the differences seen in the endophyte assemblages were a function of the tree species that harbour them. However, the endophyte assemblages of conspecific trees growing in different forests were dissimilar (Fig. 5). This observation and the fact that no distinct endophyte community was associated with any of the tree host suggested that there was a low degree of host affiliation among endophytes in these forests. Such a low host specificity has been observed for other groups of plant-associated tropical fungi such as wood rotting fungi (Lindblad 2000; Gilbert et al. 2002; Parfitt et al. 2010), ectomycorrhizal fungi (Diédhiou et al. 2010; Tedersoo et al. 2010) and arbuscular mycorrhizal fungi (Aldrich-Wolfe 2007).

Endophytes as focal group for fungal diversity—a caveat

The only practical method to estimate the global diversity of species-rich taxa such as arthropods, nematodes and fungi is through extrapolation from sampling. This involves the assessment of local species richness and determination of the distinctness of species assemblages before extrapolating the results to arrive at a reasonably correct global estimate of a taxon (Colwell and Coddington 1995). Fröhlich and Hyde (1999) and Arnold et al. (2000) extrapolated their data obtained for a few tropical plants by using plant host:endophytes ratios and concluded that the widely accepted figure of 1.5 million species of Hawksworth (2001) vastly underestimates fungal diversity. However, in the relatively dry tropical forests that we have been studying, there is little host restriction among endophytes thus cautioning against the assumption that endophytes are hyperdiverse throughout the tropics (Suryanarayanan et al. 2002, 2003, 2009b; Pandey et al. 2003; Murali et al. 2007). It has been hypothesized that host specificity in plant-dependant insects and other organisms is more frequent in temperate forests than in tropical forests owing to the disjunct distribution of plant species in the tropics (May and Beverton 1990). It would be worthwhile testing this hypothesis on endophytes and other plant-associated fungi in different types of tropical forests since, as mentioned earlier, different ecological groups of fungi appear to have low host specificity.

Endophytic fungal genera such as *Phyllosticta* (Okane et al. 2003; Pandey et al. 2003; Motohashi et al. 2009), *Pestalotiopsis* (Jeewon et al. 2004; Tejesvi et al. 2009), *Colletotrichum* (Lu et al. 2004) and *Phomopsis* (Murali et al. 2006) have loose host affiliation and colonize geographically and taxonomically separated plant hosts (see Sieber 2007; Sakalidis et al. 2011). These taxa are probably adapted to colonize unrelated hosts representing a biochemical mosaic thus depressing their species diversity in a plant community—a phenomenon similar to herbivorous insects in New Guinea lowland rainforests (Novotny et al. 2007). Such a broadening of host range, attributable to rapid evolution of host taxon preferences by the parasite or mutualist (Farrell and Sequeira 2004; Motohashi et al. 2009), has been recorded for nematodes (Subbotin et al. 2002), viruses (Knipe and Howley 2001) and beetles (Farrell and Sequeira 2004). The endophyte diversity of the forests we studied could be higher when non-culturable endophytes are considered. Furthermore, the morphological criteria used are not adequate to distinguish species of complex genera such as *Colletotrichum* (Hyde et al. 2009) and *Phyllosticta* (Wulandari et al. 2009). Molecular methods have to be used to refine the searches for species of these fungi. Thus, corrections may have to be made to the final figures of species diversity to accommodate non-culturable fungi and species that might emerge by molecular searches. These points not withstanding, the wide distribution of a few culturable endophyte species transcending host taxonomic barriers as observed in the present study also, should be taken

into account for any extrapolation exercise to avoid exaggerated diversity values (Basset et al. 1996).

To summarize, our decade long study in the forests of the Western Ghats lend credence to the hypothesis of May (1988, 1991) that, among plant-dependent organisms host specificity could be less common in the tropical forests where the diversity of plant species is high (but the dominance is low) than in the temperate forests where the converse is true. This could be due to lower densities of host species or genotypes in high-diversity communities which reduce the odds to find a host for a specialized endophyte (Saikkonen 2007). With polyphagous endophytes reported to be occurring in plant hosts belonging to different geographical locations and plant divisions (Davis et al. 2003; Mohali et al. 2005), we conclude that the global fungal diversity is likely to be less than that predicted currently.

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