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ORIGINAL PAPER

Arbuscular mycorrhizal morphology and dark septate fungal associations in medicinal and aromatic plants of Western Ghats, Southern India

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Abstract We investigated roots of 107 medicinal and aromatic plants (MAPs) in the Western Ghats region of Southern India for arbuscular mycorrhizal (AM) and dark septate endophyte (DSE) associations. Of the 107 MAPs belonging to 98 genera in 52 families examined, 79 were AM and 38 harbored a DSE association. Typical *Arum*- and *Paris*-type mycorrhizas are first reported in the presumed nonmycorrhizal family Amaranthaceae. Similarly, DSE associations are recorded for the first time in nine plant families and 37 plant species. Thirty MAPs had both AM and DSE associations. The number of MAPs having *Arum*-type mycorrhiza was greater than those having *Paris*-type. This was more prominent among herbaceous plants than in trees where the *Paris*-type was predominant. Similarly, the *Arum*-type was more prevalent in annuals than in perennials. DSE associations were more frequent in herbs and perennials compared to other MAPs.

Keywords Arbuscular mycorrhizal fungi · *Arum*-type · Dark septate endophytes · Medicinal and aromatic plants · *Paris*-type · Western Ghats

Introduction

Plants roots are colonized by numerous species of fungi with extensive, symptomless nonpathogenic endophytic or biotrophic phases in their life cycles (Sieber 2002). These include associations formed by mycorrhizal and several nonmycorrhizal fungi. In addition to the widely studied arbuscular mycorrhizal (AM) fungi (Smith and Read 1997), increased attention has recently been given to a ubiquitous group of miscellaneous fungi designated as dark septate endophytes (DSE) and characterized by melanized septate hyphae and microsclerotia. These fungi are frequent root colonizers of trees, shrubs, terrestrial orchids, and a broad range of plants in temperate and tropical habitats (Jumpponen and Trappe 1998).

AM morphology is distinguished into *Arum*-type and *Paris*-type. The *Arum*-type association is characterized by intercellular hyphal growth in the root cortex, with short lateral branches into cortical cells forming arbuscules (Smith and Smith 1997). Intracellular hyphal coils frequently having intercalary arbuscules spreading cell to cell in the cortex characterize the *Paris*-type association. The *Arum*-type has been reported to be abundant in agricultural crops, whereas, the *Paris*-type has been found to be more frequent in plants in natural ecosystems (Smith and Smith 1997; Yamato and Iwasaki 2002; Ahulu et al. 2005; Tsuyuzaki et al. 2005). Though physiological or functional disparity between *Arum*-type and *Paris*-types has not yet been fully elucidated, it has been reported that the development of *Arum*-type is faster than that of *Paris*-type (Brundrett and Kendrick 1990b; Cavagnaro et al. 2001). Anatomical characters of host roots are thought to influence the AM morphology (Brundrett and Kendrick 1988, 1990a,b). However, Kubato et al. (2005) indicated that the morphology of AM type is the result of the interaction between both the plant and the fungal species. It is therefore necessary to examine a wider range of plants growing in different habitats to fully understand the control of *Arum*- and *Paris*-type.

In contrast to the assumption that DSE associations are more frequent in cold, nutrient stressed environments,

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recent reports indicate their prevalence in arid and semiarid regions (Barrow et al. 1997; Miller et al. 1999; Muthukumar and Udaiyan 2002a). However, the ecological role of DSE fungi is currently unresolved and recent studies do indicate their potential to function as plant growth promoters under stressed environments (Newsham 1999; Barrow and Osuna 2002; Jumpponen 2001). Jumpponen and Trappe (1998) summarized the incidence of DSE associations in nearly 6,000 plants species representing about 320 genera and 100 families, but only 59 were tropical plant species (Jumpponen and Trappe 1998).

Around 35,000 to 70,000 medicinal and aromatic plants (MAPs) are used in various systems of medicine in different parts of the globe (Farnsworth and Soejarto 1991). Cultivation of MAPs is being currently carried out to meet the increasing demand for herbal drugs. In addition to conventional cultivation of MAPs, recent emphasis is on exploiting useful and appropriate soil microorganisms present in the rhizosphere of medicinal plants (Sen 1998). Although researchers (Udea et al. 1992; Muthukumar and Udaiyan 2001; Khade et al. 2002; Bukhari et al. 2003) have reported the wide spread occurrence of AM fungal associations in MAPs, there exists no information on the distribution of AM morphological types in MAPs nor on the occurrence of DSE associations in MAPs. In view of this, we analyzed the distribution of AM morphological types and the incidence of DSE associations in certain MAPs occurring in the Western Ghats region of Southern India, as well as the occurrence of AM types and DSE associations in function of plant growth forms (herbaceous, shrubs, trees, etc.).

Materials and methods

Sample collection

Roots and soil samples were collected from five individuals for 107 MAPs belonging to 98 genera in 52 families at different stages of growth (vegetative and reproductive) between December 2004 and February 2005 from different vegetation types in the Western Ghats region, Southern India (Tables 1 and 2). Care was taken during collection that roots of shrubs and tree species were positively identified. Roots were washed and stained within 24 h or preserved in FAA (formalin: glacial acetic acid: 70% ethanol, 5:5:90) up to processing. Rhizosphere soils shaken from roots of different individuals of a species were collected and were mixed to form a composite sample. These composite soil samples were used for chemical analyses and enumeration of AM fungal spores.

Determination of soil characteristics

Soil pH was determined in 1:1, soil: water (*v*:*v*), total nitrogen (N), and total phosphorus (P) were determined according to Jakson (1971), exchangeable potassium (K) was determined after extraction with ammonium acetate (Davis 1962), and soil organic matter was assessed according to Piper (1950).

Estimates of AM and DSE colonization

FAA fixed roots were washed, cleared in 2.5% KOH at 90°C (Koske and Gemma 1989), acidified with 5N HCl and stained with trypan blue (0.05% in lactoglycerol). Roots that remained dark after clearing were bleached with 3% H_2O_2 before staining. The roots were left overnight in trypan blue-lactoglycerol for staining. For observation of DSE, cleared roots were observed either directly or placed in lactoglycerol containing 0.05% trypan blue and left overnight. Fifty 1-cm long stained or unstained root samples were mounted on microscope slides in lactoglycerol and examined for AM fungal structures, melanized hyphae, and microsclerotia. Only species in which arbuscules were found were considered to be arbuscular mycorrhizal. Root length colonization by AM fungi or DSE was estimated according to the intersect method of McGonigle et al. (1990).

Enumeration and isolation of AM fungal spores

One hundred gram of soil was dispersed in 1 l water and decanted through a series of 710- to 38-μm sieves. Residues were filtered through gridded filter papers and all intact spores (noncollapsed spores with cytoplasmic contents, free from parasitic attack) were counted using a dissection microscope at ×40 magnification. Sporocarps and spore clusters were considered as one unit. Intact AM fungal spores were mounted in polyvinyl alcohol–lactoglycerol with or without Melzers reagent for identification using keys of Schenck and Perez (1990) and INVAM (http://www.invam.caf.wvu.edu). Because of the generally poor state of field material, and the low abundance of certain morphotypes, species identification was performed only with sufficient spores (minimum 25) in good condition (no sign of degradation or parasitism). Some could be identified only up to the genus.

Life history attributes and plant nomenclature

Each plant species recorded during the survey was categorized for life-form attributes as determined from the literature (Prain 1981a,b; Nair and Henry 1983; Henry et al. 1987, 1989) or field observations. Nomenclature and

authorities are as used by Nair and Henry (1983), Henry et al. (1987, 1989) for angiosperms, and Dixit (1984) and Satija and Bir (1985) for pteridophytes.

Results

Soil and AM fungal characteristics of the study sites

The soils in the study sites were slightly alkaline and low in available nutrients (Table 1). A total of twenty-three AM fungal spore morphotypes could be confidently distinguished on the basis of spore morphology, but only spores of eleven morphotypes could be identified to the species level. These included one species in *Acaulospora* (*Acaulospora scrobiculata*) and *Gigaspora* (*Gigaspora gigantea*), six in *Glomus* (*Glomus aggregatum*, *Glomus sinuosa*, *Glomus taiwanensis*, *Glomus mosseae*, *Glomus geosporum*, and *Glomus viscosum*) and three in *Scutellospora* (*Scutellospora calospora*, *Scutellospora heterogama*, *Scutellospora persica*).

Incidence of AM and DSE associations in plant species

In the present study, 79 out of 107 MAP species were colonized by AM fungi (Table 2). AM were not observed in *Crossandra infundibuliformis*, *Strobilanthes asperrimus* (Acanthaceae), *Adiantum capillus-veneris* (Adiantaceae), *Aerva lanata*, *Achyranthes aspera*, *Celosia cristata*, *Gomphrena globosa*, *Gomphrena serrata* (Amaranthaceae), *Artabotrys hexapetalus*, (Annonaceae), *Catharanthus roseus* (Apocynaceae), *Trichodesma indicum* (Boraginaceae), *Salacia chinensis* (Celastraceae), *Commelina bengahlensis* (Commelinaceae), *Cyperus rotundus* (Cyperaceae), *Mentha arvensis*

Table 1 Location, rainfall, vegetation, soil characteristics, and arbuscular mycorrhizal (AM) fungal spores numbers and species composition of the study sites

	Site		
	Coimbatore	Siruvani	Dharmapuri
Location	11°04′N and 76°93′E	10°58′N and 76°73′E	12°13′N and 78°30′E
Altitude (m asl)	426–550	500	490
Annual rainfall (mm)	500–700	800–1,500	500
Vegetation type	Forest, scrub, grassland	Forest, grassland	Forest, grassland, agricultural fields
Soil type	Sandy loam	Clay loam	Clay loam
pH	7.6±0.11[a]	7.8±0.21	7.7±1.03
Total nitrogen (mg kg^{-1})	1.0±0.01	1.6±0.10	1.2±0.25
Available phosphorus (mg kg^{-1})	1.0±0.01	1.0±0.03	1.2±0.15
Exchangeable potassium (mg kg^{-1})	7.8±0.15	2.0±0.40	5.0±0.38
Organic matter (%)	2.8±0.73	2.7±0.02	1.9±0.16
AM fungal spore numbers (spores 100 g^{-1} soil)	16.1±4.56	9.8±2.87	12.18±4.38
AM fungal species	*Acaulospora scrobiculata*, *Acaulospora* 1, *Gigaspora gigantea*, *Glomus aggregatum*, *G. sinuosa*, *G. taiwanensis*, *G. viscosum*, *Glomus* 1, *Glomus* 2, *Glomus* 3, *Glomus* 4, *Scutellospora heterogama*	*Acaulospora scrobiculata*, *Glomus mosseae*, *G. sinuosa*, *Glomus* 5, *Glomus* 6, *Glomus* 7, *Scutellospora calospora*	*Acaulospora scrobiculata*, *Acaulospora* 2, *Glomus geosporum*, *G. sinuosa*, *G. viscosum*, *Glomus* 8, *Glomus* 9, *Scutellospora calospora*, *S. persica*

[a] Mean±SE

Table 2 Arbuscular mycorrhizal (AM) types and dark septate endophyte (DSE) associations in the medicinal and aromatic plants

Family/plant names	CS	LF	LC	AM type	AM colonization (%)				DSE colonization (%)	
					RLH	RLA	RLV	RLC	Microsclerotia	Total
Acanthaceae										
Andrographis paniculata (Burm.f.) Wall.	C	H	A	*Arum*	15.22±2.97[a]	20.22±2.33	25.22±3.22	60.66±3.24		
Crossandra infundibuliformis (L.) Ness.	C	SH	P						7.83±1.15	42.14±03.03
Justica adhatoda L.	C	SH	P	*Arum*	26.16±2.97	40.37±1.75	12.79±1.85	79.33±3.49	6.73±2.08	40.89±08.90
Rostellularia procumbens (L.) Nees.	C	H	A	*Arum*	22.70±8.28	41.02±0.67	6.36±1.18	70.09±6.22	9.25±1.36	40.33±04.98
Strobilanthes asperrimus Ness.	C	H	P							
Adiantaceae										
Adiantum capillus-veneris L.	S	H	P						7.92±1.36	33.96±04.73
Amaranthaceae										
Aerva lanata (L.) Juss.	C	SH	P							
Amaranthus spinosus L.	C	H	A	*Paris*	11.08±2.01	46.10±6.23	15.98±5.15	73.17±5.03		
Amaranthus viridis L.	C	H	A	*Arum*	14.83±2.26	53.42±5.80	10.19±2.74	73.94±3.56	9.08±3.12	40.33±05.23
Achyranthes aspera L.	C	H	A							
Celosia cristata L.	C	H	A						10.15±2.18	47.63±04.76
Gomphrena globosa L.	C	H	A							
Gomphrena serrata L.	C	H	P							
Anacardiaceae										
Mangifera indica L.	C	T	P	*Paris*	5.04±4.03	34.33±4.31	25.74±6.88	65.11±4.66		
Annonaceae										
Artabotrys hexapetalus (L.f.) Bhandari.	C	SH	P	*Paris*	2.38±1.04	42.15±4.19	21.08±3.26	65.78±4.08		
Apocynaceae										
Catharanthus roseus (L.) G. Don.	C	SH	A							
Asclepiadaceae										
Calotropis gigantea (L.) R. Br.	C	SH	A	*Arum*	19.17±2.80	14.63±1.07	29.31±0.80	63.91±2.75		
Caralluma umbellata Haw.	C	H	A							
Pergularia daemia (Forssk.) Chiov.	C	CH	A	*Paris*	10.90±2.36	40.47±2.00	17.26±5.07	69.63±3.87	12.05±3.17	35.30±06.19

Table 2 (continued)

Family/plant names	CS	LF	LC	AM type	AM colonization (%)				DSE colonization (%)	
					RLH	RLA	RLV	RLC	Microsclerotia	Total
Asparagaceae										
Asparagus racemosus Willd.	C	H	P	*Arum*	19.91±4.23	20.46±6.06	26.20±4.51	66.57±4.44	15.67±5.12	56.39±06.36
Boraginaceae										
Trichodesma indicum (L.) R.Br.	C	H	A							
Caesalpiniaceae										
Cassia siamea Lam.	C	T	P	*Arum*	16.35±2.49	25.74±1.49	36.36±1.40	78.45±1.84		
Caricaceae										
Carica papaya L.	C	T	P	*Paris*	1.18±1.17	37.89±4.63	20.66±3.51	59.73±4.55		
Celastraceae										
Salacia chinensis L.	D	SH	P							
Commelinaceae										
Commelina bengahlensis L.	C	H	P							
Compositae										
Chrysanthemum cinerariifolium (Trev.) Vis.	C	H	A	*Arum*	21.56±2.72	30.49±4.72	21.19±3.24	74.05±3.47	8.10±3.85	43.96±05.42
Eclipta prostrata L.	C	H	A	*Arum*	16.42±1.79	26.19±3.11	25.19±3.11	68.81±1.95		
Emelia sonchifolia (L.) D.C.	C	H	A	*Arum*	16.52±4.02	34.59±3.22	20.48±2.94	71.00±2.46		
Sphaeranthus indicus L.	S	H	A	*Paris*	2.22±2.21	22.92±2.29	24.17±6.13	49.31±9.08		
Tagetes erecta L.	C	SH	A	*Arum*	25.56±3.12	27.03±4.03	7.03±2.96	59.93±3.31		
Tridax procumbens L.	C	H	P	*Arum*	24.31±3.42	38.20±5.13	14.04±4.86	76.75±4.41		
Vernonia cinerea (L.) Less.	C	H	P	*Paris*	8.96±3.22	41.02±7.09	10.44±3.56	60.10±8.55		
Vernonia divergens (Roxb.) Edgew.	C	SH	P	*Paris*	20.54±2.14	40.16±2.57	17.04±2.63	76.02±2.83		
Convolvulaceae										
Ipomoea batatas (L.) Lam.	C	H	P	*Paris*	19.63±1.90	39.16±1.20	6.86±3.06	78.65±7.02		
Cycadaceae										
Cycas circinalis L.	C	T	P	*Arum*	24.12±2.09	34.37±4.63	20.66±3.51	75.93±5.08		
Cyperaceae										
Cyperus rotundus L.	C	H	P						12.89±5.07	49.81±07.48
Euphorbiaceae										
Acalypha indica L.	C	H	A	*Arum*	17.21±3.04	33.84±4.95	21.14±4.69	72.19±2.84	8.36±3.45	26.67±13.56
Euphorbia hirta L.	C	H	A	*Arum*	14.67±4.30	25.57±6.27	36.54±4.06	70.82±3.83	13.07±3.98	44.79±02.55
Jatropha curcas L.	D	SH	P	*Arum*	17.52±2.31	33.94±3.11	30.93±3.89	82.40±2.88		

Table 2 (continued)

Family/plant names	CS	LF	LC	AM type	AM colonization (%)				DSE colonization (%)	
					RLH	RLA	RLV	RLC	Microsclerotia	Total
Phyllanthus emblica L.	C	T	P	*Paris*	19.44±4.26	31.88±3.78	27.33±2.75	75.08±7.80		
Phyllanthus maderspatensis L.	C	H	A	*Arum*	33.80±2.35	33.01±2.54	5.27±2.74	72.10±2.32		
Phyllanthus amarus Schum. & Thonn.	C	H	A	*Arum*	18.23±1.66	20.60±1.66	39.47±2.33	77.54±1.48		
Labiatae										
Leucas aspera (Willd.) L.	C	H	A	*Arum*	24.68±2.66	28.34±4.96	18.27±4.24	71.30±1.50		
Mentha arvensis L.	C	H	A							
Ocimum tenuiflorum L.	C	SH	P	*Arum*	21.17±3.63	40.47±2.26	12.34±1.24	73.65±4.65	8.62±3.12	31.63±09.14
Lilliaceae										
Aloe vera (L.) Burm.f	C	H	P	*Arum*	21.18±2.35	16.49±1.95	29.54±3.45	67.71±7.20	15.17±5.12	49.08±08.02
Allium cepa L.	D	H	A	*Arum*	20.05±2.34	35.25±2.56	25.22±2.36	70.52±4.52	11.45±5.08	44.16±06.76
Allium sativum L.	D	H	A	*Arum*	15.22±2.54	30.33±1.24	20.22±3.01	65.77±4.88		
Gloriosa superba L.	C	H	P	*Paris*	10.05±2.55	55.90±1.93	10.25±2.46	76.20±2.95	16.21±4.15	40.10±07.22
Lytharaceae										
Lawsonia inermis L.	C	SH	P	*Arum*	23.12±5.8	00.00± 00.00	31.95±4.77	55.08±4.73		
Magnoliaceae										
Michelia champaca L.	C	T	P	*Paris*	00.00± 00.00	45.26±3.17	24.32±1.60	69.58±2.73	8.36±3.87	41.59±03.42
Malvaceae										
Hibiscus rosa-sinensis L.	C	SH	P	Intermediate	25.84±2.34	38.31±1.76	19.30±3.26	83.46±2.66		
Sida acuta Burm. f.	C	H	P	*Paris*	00.00± 00.00	25.22±2.10	18.22±2.15	43.44±2.87		
Thespesia populnea (L.) Soland ex Correa.	C	T	P	*Paris*	2.73±1.01	49.92±1.56	18.47±2.66	71.13±5.48		
Meliaceae										
Azadirachta indica A. Juss.	C	T	P	*Arum*	20.82±3.42	34.16±3.66	20.55±3.27	75.53±1.28		
Mimosaceae										
Mimosa pudica L.	C	H	P	*Arum*	34.64±0.92	8.60±1.27	22.33±1.99	65.60±1.99	15.18±4.78	38.38±07.21
Moraceae										
Artocarpus heterophyllus Lam.	C	T	P							
Ficus benghalensis L.	C	T	P							
Moringaceae										
Moringa pterygosperma Gaertn.	C	T	P	*Paris*	2.15±1.07	26.06±1.66	21.76±1.21	49.96±1.65	8.96±3.56	42.58±03.38

Table 2 (continued)

Family/plant names	CS	LF	LC	AM type	AM colonization (%)				DSE colonization (%)	
					RLH	RLA	RLV	RLC	Microsclerotia	Total
Musaceae										
Musa paradisiaca L.	C	H	P	*Paris*	00.00± 00.00	46.84±3.19	19.91±2.66	66.75±3.48	14.91±6.14	42.92±04.52
Myrtaceae										
Psidium guajava L.	C	T	P	*Paris*	5.71±5.70	29.62±3.01	27.96±4.76	67.30±2.84		
Nephrolepidaceae										
Nephrolepis cordifolia (L.) Schott	S	H	P						4.23±1.56	38.15±02.68
Nyctanthaceae										
Nyctanthes arbor-tristis L.	C	T	P	*Paris*	21.47±2.30	39.45±2.27	21.89±1.58	82.81±1.89	12.18±4.12	48.89±07.90
Nyctaginaceae										
Mirabilis jalapa L.	C	H	P	*Paris*	1.86±00.86	25.79±4.94	22.00±2.25	48.84±5.78		
Oleaceae										
Jasminum sambac (L.) Ait.	C	CH	P	*Arum*	23.49±1.63	15.82±2.69	29.94±2.86	69.25±2.24	8.12±2.08	47.82±02.81
Ophioglosaceae										
Ophioglasum reticulatum L.	C	H	P	*Paris*	6.04±1.98	44.05±2.94	16.87±3.41	71.96±3.07	7.15±2.01	43.32±03.15
Oxalidaceae										
Biophytum longibracteatum Tad. & Jacob.	S	H	P	*Paris*	5.66±2.11	30.33±3.11	25.22±2.65	61.21±3.22		
Oxalis corniculata L.	S	H	P	*Paris*	26.68±3.57	40.58±2.78	10.85±2.90	78.01±3.78		
Oxalis corymbosa D.C.	S	H	P	*Paris*	24.94±4.99	36.52±2.89	9.61±4.51	71.08±3.44	8.74±3.56	35.46±03.05
Papilionaceae										
Canavalia gladiata (Jacq.) D.C.	C	SH	P	*Arum*	8.22±3.22	15.22±1.25	30.89±3.66	54.33±3.24		
Clitoria ternatea L.	C	CH	A	*Arum*	19.17±2.98	40.45±4.33	20.22±3.33	79.84±3.54		
Crotalaria verrucosa L.	C	H	A	*Arum*	28.89±4.12	23.67±5.26	22.62±4.21	75.13±4.69		
Pongamia pinnata (L.) Pierre.	C	T	P	*Paris*	8.29±2.75	32.00±3.63	15.70±4.13	76.27±3.56		
Pseudarthria viscida (L.) Wight & Arn.	S	SH	P	*Arum*	18.28±3.60	18.36±5.09	37.72±1.52	74.36±4.37	5.36±1.84	42.69±05.55
Periplocaceae										
Hemidesmus indicus (L.) R.Br.	C	H	A	*Arum*	14.54±5.42	45.93±3.78	17.03±2.02	76.15±4.12		
Piperaceae										
Piper longum L.	C	H	P	*Arum*	15.83±3.33	25.45±3.17	29.34±2.55	70.63±6.08		
Piper nigrum L.	C	SH	P	*Arum*	17.60±2.02	20.34±1.64	30.31±2.37	74.26±2.86		
Peperomia thomsonii Hook. f.	S	H	A	*Paris*	11.20±3.29	30.45±3.29	28.95±4.51	69.70±2.88	10.26±3.56	36.21±05.30

Table 2 (continued)

Family/plant names	CS	LF	LC	AM type	AM colonization (%)				DSE colonization (%)	
					RLH	RLA	RLV	RLC	Microsclerotia	Total
Poaceae										
Bambusa arundinacea (Retz.) Roxb.	C	H	P	*Arum*	6.73±2.74	28.35±5.33	34.93±4.21	70.01±4.67	7.12±1.08	38.03±04.68
Brachiaria ramosus (L.) Stapf	S	H	P							
Cynodon dactylon (L.). Pers.	C	H	P							
Cymbopogan caesius (Ness ex Hook & Arn.) Stapf	C	H	P						8.32±3.56	30.63±03.90
Perotis indica (L.) Kuntze.	C	H	A	*Paris*	43.70±5.05	0.79±0.78	30.12±2.48	74.67±4.15	5.12±2.15	49.42±07.29
Vetiveria zizanoides (L.) Nash.	C	H	P						12.05±5.89	41.34±01.83
Polypodiaceae										
Pleopeltis macrocarpa (Bory ex Willd.) Kaulf.	S	H	P						7.26±3.15	41.88±02.73
Punicaceae										
Punica granatum L.	C	SH	P	*Paris*	16.41±3.43	45.51±2.41	10.47±4.66	71.07±5.19	9.85±3.45	43.17±05.96
Rosaceae										
Rosa indica L.	C	SH	P	*Arum*	21.36±3.42	40.57±4.14	13.43±5.64	75.37±2.87		
Rubiaceae										
Knoxia sumatrensis (Retz.) D.C.	C	H	A	*Paris*	9.00±3.02	30.88±3.22	32.22±2.44	72.1±2.44		
Thecagonum pteritum (Blume) Babu.	C	H	A							
Rutaceae										
Aegle marmelos (L.) Correa.	D	T	P	*Arum*	31.51±2.11	31.28±3.83	9.16±5.06	72.55±3.69		
Citrus limon (L.) Burm.	C	T	P	*Arum*	22.65±3.22	30.15±2.04	30.8±3.08	82.70±3.32		
Murraya koenigii (L.) Spreng.	C	SH	P							
Santalaceae										
Santalum album L.	C	T	P	*Paris*	4.67±2.25	48.69±1.56	30.53±3.24	83.90±1.84		
Sapindaceae										
Cardiospermum halicacabum L.	C	H	A	*Arum*	17.32±2.94	19.34±6.68	14.27±7.13	64.52±6.23	10.08±3.78	43.77±03.17
Scorophulariaceae										
Scoparia dulcis L.	D	H	A	Intermediate	28.01±2.80	34.30±1.84	20.52±2.76	82.84±2.00		
Solanaceae										
Datura metel L.	C	H	A	*Arum*	26.18±1.88	29.30±1.52	13.89±3.24	69.52±1.91		

Table 2 (continued)

Family/plant names	CS	LF	LC	AM type	AM colonization (%)				DSE colonization (%)	
					RLH	RLA	RLV	RLC	Microsclerotia	Total
Lycopersicon esculentum Mill.	C	H	A	*Arum*	20.36±1.61	42.76±2.35	19.69±1.66	82.82±2.61		
Solanum melongena L.	C	H	P	*Arum*	23.17±3.11	27.76±3.88	21.13±3.76	72.07±5.54	7.26±2.54	39.95±05.54
Solanum surattense Burm.f.	C	H	A	Intermediate	14.79±1.80	22.62±3.69	17.93±3.59	55.35±7.39		
Solanum nigrum L.	C	H	A							
Umbelliferae										
Centella asiatica (L.) Urban	S	H	P	Intermediate	9.65±1.78	47.57±2.70	11.60±1.30	68.83±3.33		
Coriandrum sativum L.	D	H	A	*Paris*	15.47±1.69	26.45±3.35	37.19±2.88	79.94±2.59		
Verbenaceae										
Lantana camara L.	C	SH	P							
Vitex negundo L.	C	T	P							
Vitaceae										
Cissus quadrangularis L.	C	SH	P	*Arum*	2.47±1.59	22.71±1.61	29.56±2.59	54.71±0.67	5.36±2.36	42.66±06.05
Zingiberaceae										
Curcuma longa L.	D	H	P	*Arum*	15.75±2.12	48.46±3.08	10.56±3.01	74.78±4.06	7.12±2.56	41.83±06.64
Zingiber officinale Roscoe.	D	H	P	Intermediate	18.28±3.17	33.75±6.61	20.51±0.84	74.54±2.90	9.87±2.89	54.00±05.29

CS Collection sites *C* Coimbatore, *S* Siruvani, *D* Dharmapuri, *LF* life form, *H* herbs, *CH* climbing herbs, *SH* shrubs, *T* trees, *LC* life cycle, *A* annual, *P* perennial *RLH* root length with hyphae/hyphal coils, *RLA* root length with arbuscules/arbusculate cells, *RLV* root length with vesicles, *RLC* total colonization

[a] Mean±SE

(Labiatae), *Artocarpus heterophyllus*, *Ficus benghalensis* (Moraceae), *Brachiaria ramosus*, *Cynodon dactylon*, *Cymbopogan caesius*, *Vetiveria zizanoides* (Poaceae), *Pleopeltis macrocarpa*, *Nephrolepis cordifolia* (Nephrolepidaceae), *Thecagonum pteritum* (Rubiaceae), *Murraya koenigii* (Rutaceae), *Solanum nigrum* (Solanaceae), *Lantana camara* and *Vitex negundo* (Verbenaceae).

DSE were recorded in 38 of the 107 MAP species examined: *Acalypha indica*, *A. capillus-veneris*, *Allium cepa*, *Aloe vera*, *Amaranthus viridis*, *Asparagus racemosus*, *Bambusa arundinacea*, *Cardiospermum halicacabum*, *C. cristata*, *Chrysanthemum cinerariifolium*, *Cissus quadrangularis*, *C. infundibuliformis*, *Curcuma longa*, *C. caesius*, *Cyperus rotundus*, *Euphorbia hirta*, *Gloriosa superba*, *Jasminum sambac*, *Justica adhatoda*, *Michelia champaca*, *Mimosa pudica*, *Moringa pterygosperma*, *Musa paradisiaca*, *N. cordifolia*, *Nyctanthes arbor-tristis*, *Ocimum tenuiflorum*, *Ophioglasum reticulatum*, *Oxalis corymbosa*, *Pergularia daemia*, *Perotis indica*, *Peperomia thomsonii*, *P. macrocarpa*, *Pseudarthira viscida*, *Punica granatum*, *Rostellularia procumbens*, *Solanum melongena*, *V. zizanoides*, and *Zingiber officinale*.

Distribution of AM and DSE associations in plant families

It was possible to discriminate between *Arum*- and *Paris*-type AM at family levels only in certain cases. These were analyzed for families where three or more plant species were available (Table 2). *Arum*-type was found in Acanthaceae and Euphorbiaceae (except *Phyllanthus emblica*) and *Paris*-type was found in Oxalidaceae. Both *Paris*- and *Arum*-types were present in Amaranthaceae, Asclepiadaceae, Compositae, Lilliaceae, and Piperaceae. Similarly, both *Arum*- and intermediate types were present in

Solanaceae and Zingiberaceae. Five plant species *Hibiscus rosa-sinensis* (Malvaceae), *Scoparia dulcis* (Scrophulariaceae), *Solanum surattense* (Solanaceae), *Centella asiatica* (Umbelliferae) and *Z. officinale* (Zingiberaceae), had both intercellular hyphae, intracellular hyphal coils, inter- or intracellular vesicles, arbusculate structures or *Arum*-type arbuscules in their cortex indicating an intermediate between the *Arum*- and *Paris*-type morphologies. Plant families where AM morphology was previously unknown were: *Paris*-type in Oxalidaceae, Caricaceae, Convolvulaceae, Musaceae, Nyctanthaceae, Santalaceae, Punicaceae, Moringaceae, *Arum*-type in Acanthaceae, and *Arum*- and *Paris*-types in Piperaceae.

Of the 52 plant families examined, DSE associations were found in 27 MAP families (Table 2), which included Acanthaceae, Adiantaceae, Amaranthaceae, Asclepiadaceae, Asparagaceae, Compositae, Cyperaceae, Euphorbiaceae, Labiatae, Liliaceae, Magnoliaceae, Mimosaceae, Moringaceae, Musaceae, Nephrolepidaceae, Nyctanthaceae, Oleaceae, Ophioglosaceae, Oxalidaceae, Papilionaceae, Piperaceae, Poaceae, Polypodiaceae, Punicaceae, Sapindaceae, Solanaceae, and Zingiberaceae.

Extent and morphology of AM and DSE associations

There were large differences between patterns of colonization in MAPs (Fig. 1), and in the colonized root lengths

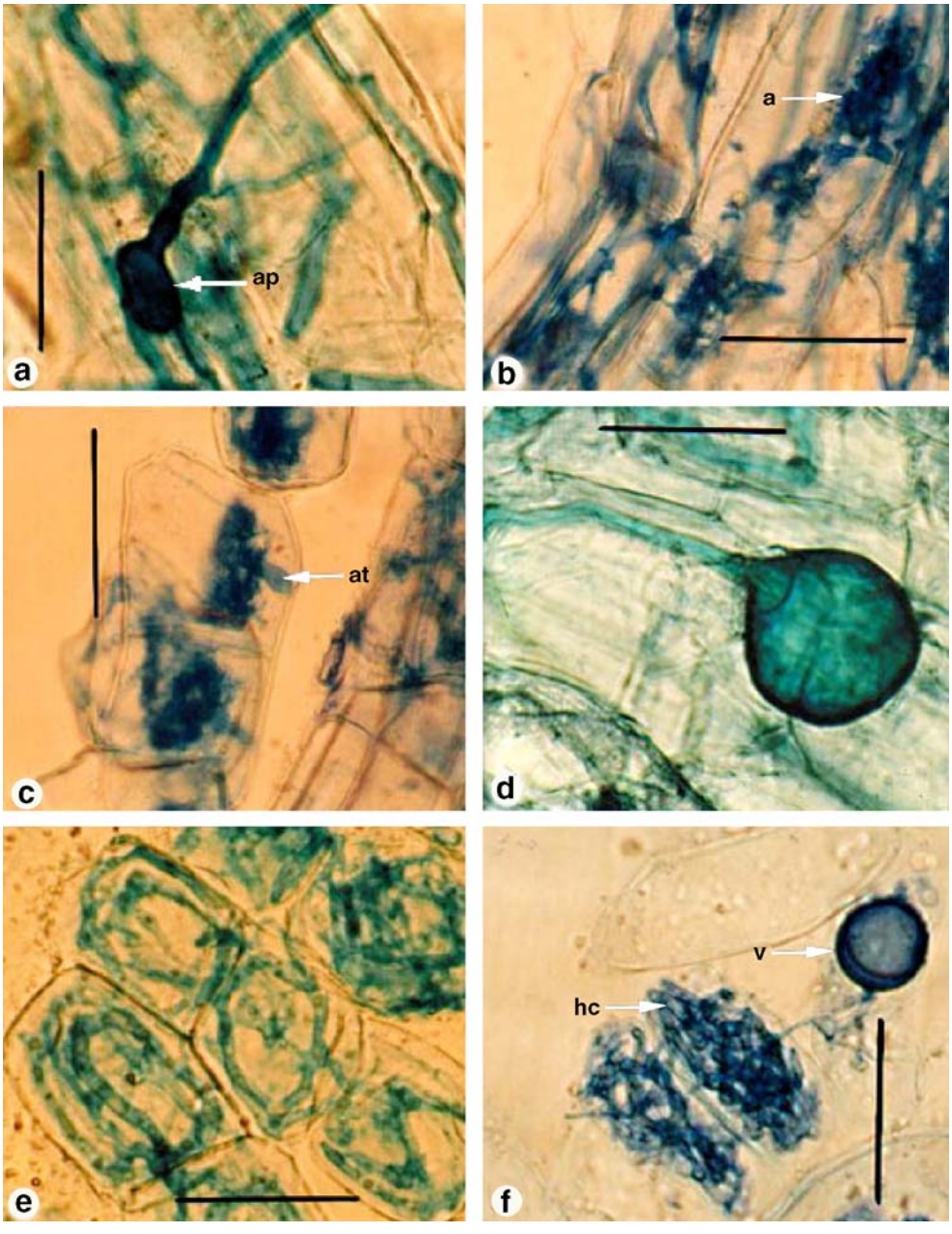

Fig. 1 Light and phase contrast micrographs showing arbuscular mycorrhizal types in medicinal and aromatic plants. **a** Appressorium (ap) on the root surface of *Nyctanthes arbor-tristis*. **b**–**d** *Arum*-type mycorrhizas in *Hibiscus rosa-sinensis* (**b**), *Amaranthus spinosus* (**c**), *Datura metel* (**d**). *Paris*-type mycorrhizas in *Gloriosa superba* (**e**) and *Santalum album* (**f**) (*a* arbuscule, at arbuscular trunk, *v* vesicle; Scale bar=50 μm)

occupied by hyphal coils, intercellular, intracellular hyphae, arbusculate coils, arbuscules, and vesicles (Table 2). The root entry by AM fungi was characterized by the formation of an appressorium (Fig. 1a). The *Arum*-type mycorrhizas were characterized by the presence of intercellular hyphae, arbuscules, and vesicles (Fig. 1b–d). Intracellular hyphal coils, arbusculate structures, and intracellular vesicles characterized the *Paris*-type (Fig. 1e,f). Intracellular hyphal coils as well as intercellular hyphae, arbuscules, and vesicles, characterized the intermediate types. The extent of colonization ranged between 49% (*Sphaeranthus indicus*, Compositae; *Mirabilis jalapa*, Nyctaginaceae) to 84% (*Santalum album*, Santalaceae).

The extent of DSE colonization ranged between 27% (*A. indica*, Euphorbiaceae) and 56% (*A. racemosus*, Asparagaceae). The pattern of DSE colonization was similar in roots of different MAPs. Among the MAP species examined, the majority (65%) had root colonization levels ranging between of 40 and 50%. Root entry by DSE was characterized by the presence of an appressorium (Fig. 2a). DSE were frequently characterized by narrow, septate, runner hyphae (2–4 μm wide) commonly occurring on the root surface and typically running parallel to the long axis of roots (Fig. 2b). Individual hyphae sometimes grew along the grooves between adjacent epidermal cells and colonized roots intercellularly. Runner hyphae on the root surface were infrequently branched at a 90° angle to the main hyphae, and occasionally bore swollen tips. The hyphae penetrated the epidermis and tended to coil in cortical cells of the outer or inner layers (Fig. 2c). Penetration through root hair was not observed. Once in the epidermis, hyphae grew from cell to cell within the epidermis parallel to the main axis of the host root, usually causing no distortion of host root. At regions the hyphae penetrated the cortical cells filling each cell with distinct sclerotia (Fig. 2d). There was no morphological distinction in intraradical and extramatrical hyphae. The root stele was not colonized in any of the plant species that had DSE fungal association and there was no evidence of damage to host root tissues arising from fungal colonization.

Extent of AM and DSE colonization in plant growth forms

The AM association was present in 78% of herbs, 58% of shrubs/undershrubs, and 89% of tree species. The extent of colonization ranged between 43%(*Sida acuta*, Malvaceae) to 83% (*S. dulcis*, Scrophulariaceae; *S. melongena*, Solanaceae) in herbs, 55% (*Lawsonia inermis*, Lythraceae; *C. quadrangularis*, Vitaceae) to 83% (*H. rosa-sinensis*, Malvaceae) in shrubs/undershrubs and 50% (*M. pterygosperma*, Moringaceae) to 84% (*S. album*, Santalaceae) in trees.

The DSE association was present in 43% of herbs, 29% of shrubs/undershrubs, and 11% of tree species. The extent

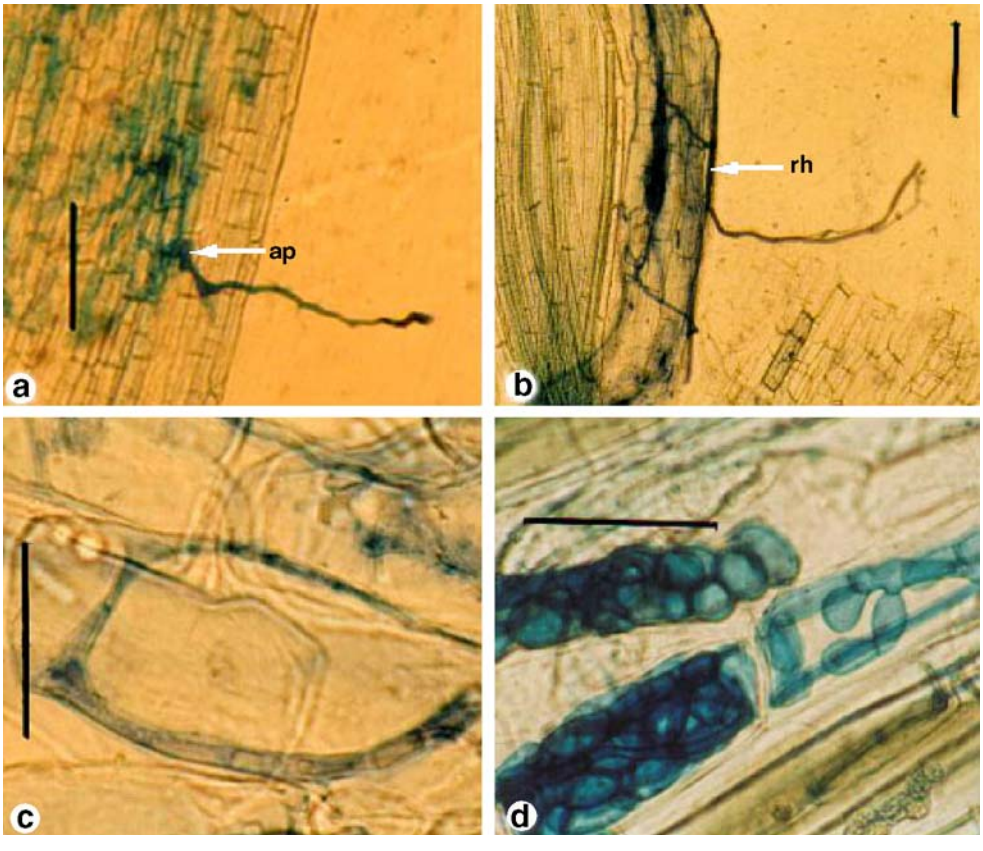

Fig. 2 Dark septate endophytic fungal association in medicinal and aromatic plants. **a** Appressorium (*ap*) on root surface in *Euphorbia hirta*. **b** Runner hyphae (*rh*) and extraradical hyphae in *Perotis indica*. **c** Intraradical hyphae in *Asparagus racemosus*. **d** Microsclerotia within root cortical cells of *Curcuma longa*. (Scale bar: **a**, **b**=100 μm, **c**, **d**=50 μm)

of DSE fungal colonization was similar, with mean colonization levels of 43% in herbs, 41% in shrubs or undershrub, and 45% in tree species. The extent of DSE colonization ranged between 27% (*A. indica*, Euphorbiaceae) to 56% (*A. racemosus*, Asparagaceae) in herbs, 32% (*O. tenuiflorum*, Labiatae) to 43% (*P. viscida*, Papilionaceae; *P. granatum*, Punicaceae; *C. quadrangularis*, Vitaceae) in shrubs, and 42% (*M. champaca*, Magnoliaceae) to 49% (*N. arbor-tristis*, Nyctanchaceae) in trees.

Plant growth forms and AM morphology

Distribution of *Arum*- and *Paris*-type AM in MAPs could be linked to plant growth characters. *Arum*-type was found in 56% of herbs, 67% of shrubs/undershrubs, and 38% of trees. *Paris*-type was found in 62% of trees, 37% of herbs, and 25% of shrubs/undershrubs. The intermediate type was not found in trees whereas 8% of herbs and shrubs/undershrubs had intermediate AM types. *Arum*-type was more prevalent in annuals than in perennials. In contrast, *Paris*- and intermediate-types were prevalent in perennial species (Fig. 3). Incidence of DSE associations was greater in perennials than in annuals.

Discussion

AM and DSE associations in this study was present in roots of, respectively, 75 and 36% of the MAPs examined. AM associations were found in *Amaranthus spinosus*, and *A. viridis*, which belong to the presumed nonmycorrhizal family Amaranthaceae (Tester et al. 1987). In the present study, frequency of mycorrhizas in different plant life forms

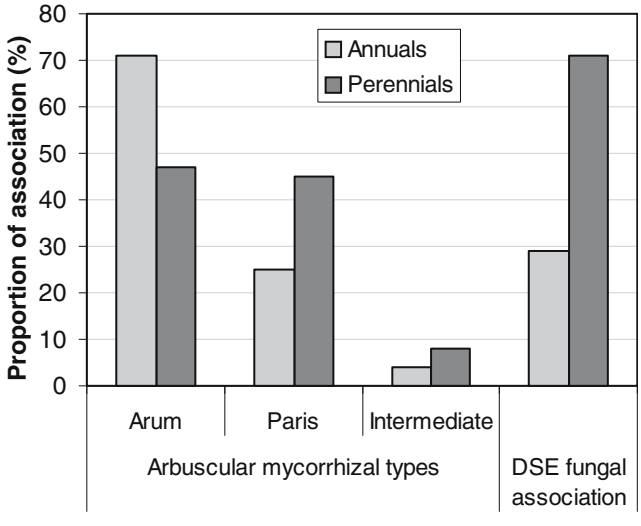

Fig. 3 Percentage medicinal and aromatic plant species of different arbuscular mycorrhizal morphological types and the incidence of dark septate fungal association in the two life-cycle classes

were in the order of trees > herbs > shrubs/undershrubs. This is in accordance with the fact that trees are more mycorrhiza-dependent than other plant life forms. The high mycorrhizal dependence of tree species is thought to arise from the fact that trees commonly have low rooting densities in soil (Baylis 1975). With the exception of two families, species within 18 plant families had the same AM morphology as in earlier descriptions (Smith and Smith 1997; Yamato and Iwasaki 2002; Muthukumar et al. 2003; Ahulu et al. 2005), indicating that AM morphology is influenced by the identity of the host plant. Studies do indicate that the same AM fungus that formed *Arum*-type mycorrhiza in a host species also formed the *Paris*-type in a different host (Gerdemann 1965; Jacquelinet-Jeanmougin and Gianinazzi-Pearson 1983). The formation of the two AM types is thought to be related to the presence of continuous longitudinal air spaces in the root cortex, so that hyphae grow along the intercellular air spaces in the *Arum*-type, and intracellularly in their absence in the *Paris*-type (Brundrett and Kendrick 1988, 1990b). Intermediate morphological types tend to occur in species with discontinuous intercellular air spaces (Smith and Smith 1997). However, Imhof and Weber (1997, 2000) noted that *Voyria obconica* formed *Paris*-type AM inspite of presence of clear intercellular spaces in the root cortex. In the present study, AM morphology of individual MAPs of the same species was consistent. This indicates that genetic factors pertaining to the host plant influence AM morphology, as more than one AM fungal species can colonize the roots of an individual plant (Van Tuinen et al. 1998; Helgason et al. 1999). Species of Oxalidaceae, Caricaceae, Amaranthaceae, Convolvulaceae, Musaceae, Acanthaceae, Nyctaginaceae, Piperaceae, Santalaceae, Punicaceae, and Moringaceae (Vijayalakshmi and Rao 1988; Neeraj et al. 1991; Muthukumar and Udaiyan 2000) have been reported to be AM but their AM morphology had not been described. In this study, it was found that Oxalidaceae, Caricaceae, Convolvulaceae, Musaceae, Nyctaginaceae, Santalaceae, Punicaceae, and Moringaceae had *Paris*-type; Acanthaceae and Sapindaceae had *Arum*-type and Amaranthaceae and Piperaceae had more than one type of AM morphology.

DSE associations were observed in roots of several plant families examined in the present study. DSE are reported for the first time in nine plant families (Amaranthaceae, Moringaceae, Punicaceae, Piperaceae, Nyctaginaceae, Zingiberaceae, Acanthaceae, Musaceae, and Asclepiadaceae) and 37 plant species. Among plant species harboring a DSE association in the present study, only DSE occurrence was known for *C. rotundus* (Cyperaceae) (Jumpponen and Trappe 1998; Muthukumar and Udaiyan 2002a). However, no DSE association was found in sixteen plant families for which this association has previously been reported. These

families include Compositae, Rubiaceae, Convolvulaceae, Rutaceae, Piperaceae, Santalaceae, Rosaceae, Myrtaceae, Anacardiaceae, Poaceae, Scrophulariaceae, Vitaceae, Apocynaceae, Boraginaceae, Caesalpiniaceae, and Cycadaceae (Jumpponen and Trappe 1998; Muthukumar and Udaiyan 2002a). Similarly four plant species *Citrus limon* (Rutaceae), *S.nigrum* (Solanaceae), *Cycas circinalis* (Cycadaceae) and *Psidium* sp. (Myrtaceae) reported to form DSE associations (Jumpponen and Trappe 1998; Muthukumar and Udaiyan 2002b), lacked the association in the present study.

The frequency of *Arum*-type mycorrhiza in each plant growth form was shrubs/undershrubs > herbs > trees, whereas it was the inverse for *Paris*-type trees > herbs > shrubs/undershrubs. Yamato and Iwasaki (2002) found *Paris*-type in nine out of 10 herbaceous understory plants of some Japanese deciduous forests, and noted that *Paris*-type was more frequent than *Arum*-type in each level of plant taxonomy from species to family. A majority of herbs examined by Ahulu et al. (2005) formed *Arum*-type and there were more *Paris*-type than *Arum*-type plant families in a mixed pine forest on a sand dune in central Hosshu, Japan. Also in the present study, only *Paris*-type mycorrhiza were found in 12 plant families compared to the *Arum*-type, which was found in eight plant families. O'Connor et al. (2001) found *Arum*-type mycorrhiza in 21 herbaceous plant species growing in an Australian desert, whereas, in the present study, both *Arum*- and *Paris*-type occurred in 56% and 37% of herbs, respectively. These differences in distribution of *Arum*- and *Paris*-types in different studies could be the result of differences in plant species composition and environmental factors operating at different sites.

DSE associations were most prevalent in herbs compared to shrubs and trees. This is in accordance with several previous studies where DSE were frequently found in herbs and infrequently in trees species (Ahlich and Sieber 1996; Ruotsalainen et al. 2002; Urcelay 2002; Barrow 2003). Only three tree species *N. arbor-tristis* (Nyctanthaceae), *M. pterygosperma* (Moringaceae), and *M. champaca* (Magnoliaceae) in the present study had DSE associations. Microsclerotia were observed in root cortical cells and their frequency of occurrence varied with the plant species. No fungal structures were found in the root stelar region of any of the plants examined which contrasts with reports by Yu et al. (2001) of occasional penetration of the vascular cylinder by *P. frontii* hyphae, and by Barrow (2003) of DSE fungi within sieve elements of *Bouteloua* spp. Root colonization by DSE has been observed simultaneously with AM or ectomycorrhizal fungi (Jumpponen and Trappe 1998). We observed such simultaneous occurrence of AM fungi and DSE in 30 plant species. This type of dual colonization by different root-associated fungi reflects a dynamic nature of the root-colonizing fungal community. DSE are known to frequently colonize older parts of the root system (Jumpponen and Trappe 1998), suggesting that they prefer ageing root tissue or that they are recycling nutrients from the senescent or dead root cells. In the present study, care was taken not to include old or dead roots for assessment and DSE was present in young roots suggesting a concurrent colonization with AM fungi. It has been proposed that DSE enhance root functions of native plants in arid ecosystems, where they are chronically exposed to very dry soils (Barrow 2003). Low soil moisture in the tropics likewise imposes problems like root desiccation, reduced mineralization, uptake of nutrients, and maintenance of adequate water relationships for plant survival. The widespread occurrence of DSE in tropical soils, as indicated in this study, emphasizes their potential to function as mutualistic fungi along with mycorrhizal fungi (Jumpponen 2001; Barrow and Osuna 2002). Further studies are ongoing to characterize the AM fungi and DSE involved and to examine their potential for promoting the growth of MAPs.

References

Ahlich K, Sieber TN (1996) The profusion of dark septate endophytic fungi in non-ectomycorrhizal fine roots of forest trees and shrubs. New Phytol 132:259–270

Ahulu EM, Nakata M, Nonaka M (2005) *Arum*- and *Paris*-type arbuscular mycorrhizas in a mixed pine forest on sand dune soil in Niigata Pretecture, central Honashu, Japan. Mycorrhiza 15:129–136

Barrow JR (2003) A typical morphology of dark-septate fungal root endophytes of *Bouteloua* in arid southwestern USA rangelands. Mycorrhiza 13:239–247

Barrow JR, Havsrtad KM, McCaslin BD (1997) Fungal root endophytes in fourwing saltbush, *Atriplex canescens* on arid rangelands of southwestern USA. Arid Soil Res Rehabil 11:177–185

Barrow JR, Osuna P (2002) Phosphorus solubilization and uptake by dark septate fungi in fourwing saltbush, *Atriplex carescens* (Pursh) Nutt. J Arid Environ 51:449–459

Baylis GTS (1975) The magnoloid mycorrhiza and mycotrophy in root systems derived from it. In: Sanders FE (ed) *Endomycorrhizas*. Academic, New York, pp 373–389

Brundrett MC, Kendrick B (1988) The mycorrhizal status, root anatomy and phenology of plants in sugar maple forest. Can J Bot 66:1153–1173

Brundrett MC, Kendrick B (1990a) The roots and mycorrhizas of herbaceous woodland plants. I. Quantitative aspects of morphology. New Phytol 114:457–468

Brundrett MC, Kendrick B (1990b) The roots and mycorrhizas of herbaceous woodland plants. II. Structural aspects of morphology. New Phytol 114:469–479

Bukhari MJ, Khade SW, Jaiswal V, Gaonkar UC, Rodrigues BF (2003) Arbuscular mycorrhizal (AM) status of tropical medicinal plants: a field survey of arbuscular mycorrhizal fungal association in herbs. Plant Arch 3:167–174

Cavagnaro TR, Gao L-L, Smith FA, Smith SE (2001) Morphology of arbuscular mycorrhizas is influenced by fungal identity. New Phytol 151:469–475

Davis DJ (1962) Emission and absorption spectrochemical methods. In: Peach K, Tracey MV (eds) Modern methods of plant analysis. Springer, Berlin Heidelberg New York, pp 1–25

Dixit RD (1984) (ed) A census of the Indian pteridophytes. Botanical Survey of India, New Delhi, India

Farnsworth NR, Soejarto DD (1991) Global importance of medicinal plants. In: Akerele O, Heywood V, Synge H (eds) The conservation of medicinal plants. Cambridge University Press, Cambridge, UK, pp 25–51

Gerdemann JW (1965) Vesicular-arbuscular mycorrhizas formed on maize and tulip tree by *Endogone fasciculata*. Mycologia 57:562–575

Helgason T, Fitter AH, Young JPW (1999) Molecular diversity of arbuscular mycorrhizal fungi colonizing *Hyacinthoides non-scripta* (blue bell) in a seminatural woodland. Mol Ecol 8:659–666

Henry AN, Kumari GR, Chitra V (eds) (1987) Flora of Tamil Nadu, India, vol 2. Botanical Survey of India, Coimbatore, India

Henry AN, Chitra V, Balakrishnan NP (eds) (1989) Flora of Tamil Nadu, India, vol 3. Botanical Survey of India, Coimbatore, India

Imhof S, Weber HC (1997) Root anatomy and mycotrophy (AM) of the achlorophyllous *Voyria truncata* (Standley) Standely & Steyermark (Gentianaceae). Acta Bot 110:127–134

Imhof S, Weber HC (2000) Root structures and mycorrhiza of the achlorophyllus *Voyria obconica* Progel (Gentianaceae). Symbiosis 29:209–211

Jacquelinet-Jeanmougin S, Gianinazzi-Pearson V (1983) Endomycorrhizas in the Gentianaceae. I. The fungus associated with *Gentiana lutea* L. New Phytol 95:663–666

Jakson ML (ed) (1971) Soil chemical analysis. Prentice Hall, New Delhi

Jumpponen A (2001) Dark septate endophytes—are they mycorrhizal? Mycorrhiza 11:207–211

Jumpponen A, Trappe JM (1998) Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. New Phytol 140:295–310

Khade SW, Bukhari MJ, Jaiswal V, Gaonkar UC, Rodeigues BF (2002) Arbuscular mycorrhizal status of medicinal plants: a field survey of AM fungal association in shrubs and trees. J Econ Taxon Bot 26:571–578

Koske RE, Gemma JN (1989) A modified procedure of for staining roots to detect VA mycorrhizas. Mycol Res 92:486–488

Kubato M, McGonigle TP, Hyakumachi M (2005) Co-occurrence of *Arum*- and *Paris*-type morphologies of arbuscular mycorrhizae in cucumber and tomato. Mycorrhiza 15:73–77

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A method which gives an objective measure of colonization of roots by vesicular–arbuscular mycorrhizal fungi. New Phytol 115:495–501

Miller RM, Smith CI, Jastrow JD, Bever VD (1999) Mycorrhizal status of the genus *Carex* (Cyperaceae). Am J Bot 86:547–553

Muthukumar T, Udaiyan K (2000) Arbuscular mycorrhizas of plants growing in the Western Ghats region, Southern India. Mycorrhiza 9:297–313

Muthukumar T, Udaiyan K (2001) Vesicular arbuscular mycorrhizal association in medicinal plants of Maruthamalai Hills, Western Ghats, Southern India. J Mycol Plant Pathol 31:180–184

Muthukumar T, Udaiyan K (2002a) Seasonality of vesicular–arbuscular mycorrhizae in sedges in a semi-arid tropical grassland. Acta Oecol 23:337–347

Muthukumar T, Udaiyan K (2002b) Arbuscular mycorrhizas in cycads of Southern India. Mycorrhiza 12:213–217

Muthukumar T, Sha L, Yang X, Cao M, Tang J, Zheng Z (2003) Mycorrhiza of plants in different vegetation types in tropical ecosystems of Xishuanbanna, southwest China. Mycorrhiza 13:289–297

Nair NC, Henry AN (eds) (1983) Flora of Tamil Nadu, India, vol 1. Botanical Survey of India, Coimbatore, India

Neeraj Shanker A, Mathew J, Varma A (1991) Occurrence of vesicular–arbuscular mycorrhizae with Amaranthaceae in soils of the Indian semi-arid region. Biol Fertil Soils 11:140–144

Newsham KK (1999) *Phialophora graminicola*, a dark septate fungus, is a beneficial associate of the grass *Vulpiaciliata* ssp. *ambigua*. New Phytol 144:517–524

O'Connor PJ, Smith SE, Smith FA (2001) Arbuscular mycorrhizal associations in the Southern Simpson Desert. Aust J Bot 49:493–499

Piper CS (ed) (1950) Soil and plant analysis. Interscience, New York, USA

Prain D (ed) (1981a) Bengal plants, vol 1. Bishen Singh Mahendra Pal Singh, Dehradun, India

Prain D (ed) (1981b) Bengal plants, vol 2. Bishen Singh Mahendra Pal Singh, Dehradun, India

Ruotsalainen AL, Väre H, Vesterg M (2002) Seasonality of root fungal colonization in low-alpine herbs. Mycorrhiza 12:29–36

Satija CK, Bir SS (eds) (1985) Polypodiaceous ferns of India. Today and Tomorrow's Printers and Publishers, New Delhi, India

Schenck NC, Perez Y (eds) (1990) Manual for the identification of VA mycorrhizal fungi. Synergistic, Gainesville, FL

Sen AN (1998) Harnessing of soil microorganisms for the benefit of medicinal plants. In: Gautam PL, Raina L, Srivastava U, Rayachaudhuri SP, Singh BB (eds) Prospects of medicinal plants. Indian Society of Plant Genetic Resources, New Delhi, pp 275–280

Sieber TN (2002) Fungal root endophytes. In: Waisel Y, Amram E, Kafkafi U (eds) Plant roots: the hidden half. Marcel Dekker Inc, New York, USA, pp 887–917

Smith SE, Read DJ (eds) (1997) Mycorrhizal symbiosis. Academic, San Diego, CA

Smith FA, Smith SE (1997) Structural diversity in (vesicular)–arbuscular mycorrhizal symbioses. New Phytol 137:373–388

Tester M, Smith SE, Smith FA (1987) The phenomenon of non-mycorrhizal plants. Can J Bot 65:419–431

Tsuyuzaki S, Hase A, Niinuma H (2005) Distribution of different mycorrhizal classes on Mount Koma, northern Japan. Mycorrhiza 15:93–100

Udea T, Husoe T, Kubo S, Nakawashi I (1992) Vesicular arbuscular mycorrhizal fungi (Glomales) in Japan II: a field survey of vesicular arbuscular mycorrhizal association with medicinal plants in Japan. Trans Mycol Soc Japan 33:77–86

Urcelay C (2002) Co-occurrence of three fungal root symbionts in *Gaultheria poeppigii* DC in Central Argentina. Mycorrhiza 12:89–92

Van Tuinen D, Jacquot E, Zhao B, Gollotte A, Gianazzi-Pearson V (1998) Characterization of root colonization profiles by microcosm community of arbuscular mycorrhizal fungi using 25S r DNA targeted nested PCR. Mol Ecol 7:879–887

Vijayalakshmi M, Rao AS (1988) Vesicular–arbuscular mycorrhizal associations of some Asteraceae and Amaranthaceae. Acta Bot Indica 16:168–174

Yamato M, Iwasaki M (2002) Morphological types of arbuscular mycorrhizal fungi in roots of understorey plants in Japanese deciduous broadleaved forests. Mycorrhiza 12:291–296

Yu T, Nassuth A, Peterson RL (2001) Characterization of the interaction between dark septate fungus *Philaocephala fortinni* and *Asparagus officinalis* roots. Can J Microbiol 47:741–753