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OCCURRENCE AND MORPHOLOGY OF ENDORHIZAL FUNGI IN CROP SPECIES

[OCURRENCIA Y MORFOLOGÍA DE HONGOS ENDORIZOS EN PLANTAS DE CULTIVO]

Tropical and Subtropical Agroecosystems

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SUMMARY

We surveyed 45 crop species in 39 genera of 21 families to explore the incidence and morphology of endorhizal fungal associations in roots. The survey indicated that 42 of the 45 crop species examined were associated with arbuscular mycorrhizal (AM) fungi. In addition, 20 of the mycorrhizal crop species were also associated with dark septate endophyte (DSE) fungi. Twenty crops had *Arum*-type and 22 had intermediate type AM morphologies. AM morphology has been described for the first time in 27 crop species. Three crop species lacked AM fungal association. *Myristica fragrans* though lacking AM association had DSE fungal association. The extent of colonization in roots ranged from 41% to 97% for AM and <1% to 61% for DSE fungi in the different crop species. Similarly, AM fungal spore numbers in the rhizosphere ranged between 4 and 60 spores 25 g $^{-1}$ of soil. Twelve AM fungal spore morphotypes belonging to *Acaulospora*, *Gigaspora*, *Glomus* and *Scutellospora* were isolated from the rhizosphere soils. Although root length colonization by AM fungi was not correlated to AM fungal spore numbers, it was significantly and negatively correlated to DSE fungal colonization. The evidence presented in this paper for the first time revealed 19 crop species to be hosts for DSE fungi.

Key words: Arbuscular mycorrhizal; crops; DSE fungi; *Arum*- type; *Glomus*

RESUMEN

Se exploraron 45 especies de plantas de cultivo que comprenden 39 géneros de 21 familias, para explorar la incidencia y morfologia de asociaciones fungicas en las raíces. Se encontró que 42 de las 45 especies estudiadas estuvieron asociadas con micorrizas arbusculares (AM). Adicionalmente, 20 de las especies de micorrizas de los cultivos estuvieron asociados con hongos endófitos septados oscuros (DSE). Veinte cultivos tuvieron hongos tipo Arum y 22 tuvieron morfologías de AM intermedias. La morfología de AM se describe por primera vez para 27 cultivos. Tres cultivos carecieron de asociación con alguna AM. *Myristica fragrans* careció de asociación con AM y tuvó asociación con DSE. El grado de colonización de las raíces fue de 41% a 97% para AM y de <1% a 61% para DSE en los diferentes cultivos. Doce morfotipod de esporas de AM pertenecientes a *Acaulospora*, *Gigaspora*, *Glomus* y *Scutellospora* fueron aislados de suelos de la rizosfera. Aunque la longitud de la raíz colonizada por AM no estuvo correlacionada con el número de esporas de AM, si fue significativa y negativamente correlacionado con la colonización por DSE. El presente trabajo es la primera evidencia presentada para 19 cultivos como hospederos de hongos tipo DSE.

Palabras clave: Micorrizas arbusculares; cultivos; hongos endófitos; tipo-*Arum*; *Glomus.*

INTRODUCTION

Most crop species are associated with endorhizal fungi. Of the different types of endorhizal fungi that associate with plants, arbuscular mycorrhizal (AM) and dark septate endophytic (DSE) fungi are the most common and widespread (Muthukumar *et al.*, 2006; Muthukumar and Prakash, 2009). Cultivated soils, like natural soils contain indigenous AM fungi that colonize crop plant roots (Piotrowski and Rilling, 2008). Most crop plants are associated with AM fungi with exceptions being in families Amaranthaceae, Chenopodiaceae and Brassicaceae. In the soil, AM fungi develop a network of extramatrical hyphae which serves as an interface between the soil and the plant root. These extramatrical hyphae are more efficient in nutrient uptake than root hairs (Allen, 2007). The increase in crop growth and yield through enhanced nutrient uptake [especially phosphorus (P)] and other mechanisms is a well-known mycorrhizal response (Akhtar and Siddiqui, 2008; Giasson *et al*., 2008; Sawers *et al*., 2008). In turn, the fungi obtain carbon from the host plant.

The morphology of AM colonization within plant roots can vary greatly among plant species. Based on the occurrence of AM fungal stuructures within the roots, AM morphology has been classified as *Arum*-, *Paris*- or intermediate-types (Dickson *et al*., 2007; Smith and Smith, 1997). In *Arum*-type, the hyphae and vesicles are intercellular with intracellular arbuscules. Intracellular hyphal coils with rudimentary arbuscules and intracellular vesicles characterize the *Paris*-type. Between these two AM morphological types are a range of morphologies termed as intermediate-types (Dickson, 2004). It has been indicated that cultivated plants have predominatly *Arum*-type morphology whereas plants in natural ecosystems have mostly *Paris*-type morphology (Smith and Smith, 1997). Although AM association has been reported in many crop species their morphologies are yet to be ascertained (Dickson *et al*., 2007; Li *et al*., 2007; Muthukumar and Prakash, 2009).

A group of melanized, septate and microsclerotia forming fungi known as DSE fungi colonizes roots of mycorrhizal and non-mycorrhizal plants. There is a debate on what role these fungal endophytes play; various experiments have shown that they can be beneficial (Narisawa *et al*., 2002; 2004; Usuki and Narisawa, 2007), while others indicate they are pathogenic (Wilcox and Wang, 1987) or act as commensals (Addy *et al*., 2005; Jumpponen, 2001; Mandyam and Jumpponen, 2005). Peterson *et al*. (2008) indicated that the DSE fungal endophytes might replace mycorrhizal fungi in certain hosts under certain conditions. DSE fungal endophytes have been shown to provide benefits to crop species of non-mycorrhizal plant families. Recently Usuki and Narisawa (2007) have shown that the DSE fungus *Heteroconicum chaetospira* transfers nitrogen (N) to Chinese cabbage (*Brassica campestris*) gaining carbon in return. In addition, DSE fungus *Phialocephala fortinii* has been reported to suppress *Verticillium* wilt in eggplant (*Solanum melongena*) but not in Chinese cabbage. Most of the DSE fungi like AM fungi, invade host cells without triggering any defense reactions (Peterson *et al*., 2008). Information on DSE fungal association in tropical crops is limited compared to temperate crops (Jumpponen and Trappe, 1998). The objectives of this study were to examine endorrhizal fungal diversity and the relationship between AM and DSE fungal endophytes in crop roots.

MATERIALS AND METHODS

Root and soil samples were collected during October 2007 from agricultural fields at Puliampatti (site-A) and Anaikatti (site-B) of Erode district (11°.30'-51º.23'N-77º.09'-35º.63', E), Tamilnadu, India. The sites have an annual mean rainfall of 500-800 mm. The soils at both locations were sandy loam. Soils at site-A had a pH of 8.45, EC of 0.06 dSm^{-1}, 18.5 mg kg^{-1} of total N, 3.23 mg kg^{-1} of available P and 26.5 mg kg^{-1} of exchangeable Potassium (K) using the procedures of Jackson (1971). Similarly, soils at site-B had a pH of 8.67, EC of 0.10 dSm^{-1}, 21.3 mg kg^{-1} of total N, 4.01 mg kg^{-1} of available P and 43.4 mg kg^{-1} of exchangeable K.

Sample collection

Samples of plant roots and soil of 45 crop species belonging to 39 genera in 21 families were collected. Five plants were sampled from each crop species. Care was taken during collection that roots of individual plants were positively identified. For this, herbs were usually dug out and sampling of non-herbs was made usually from saplings if available or the roots were traced back to the stem. Roots were gently washed and fixed in FAA (formalin-acetic acid alcohol) and transported to the laboratory for processing. Rhizosphere soil shaken from roots and adjacent to roots was collected and air-dried. Soil samples collected from individual plants of each species were divided into two halves. One-half was packed individually in polythene bags and stored at 4º C until processing for extraction and enumeration of AM fungal spores. The second half of the individual soil samples from each site were bulked to form a composite soil sample. The composite soil sample was used for establishing trap cultures and soil analysis.

AM and DSE fungal assessment

Fixed roots were washed free of FAA cut into 1-cm pieces, cleared in 2.5% KOH (Koske and Gemma, 1989), acidified with 5N HCl and stained with trypan blue or Chlorozal Black E (0.5% in lactoglycerol) overnight. Roots that remained dark after clearing were bleached in alkaline H_2O_2 prior to acidification. The stained roots were examined with an Olympus BX51 light microscope (×200 - 400) for AM fungal structures and the percentage of root length colonization was estimated according to a magnified intersection method (McGonigle *et al*., 1990).

Initiation of AM fungal trap cultures

Composite soil samples (including plant roots) from each site were placed in 15-cm diam., pots containing sterile substrate (soil: sand, 3:1 v/v; pH 7.5; 14.3 mg kg^{-1} of total N, 1.05 mg kg^{-1} of available P and 22.4 mg kg^{-1} of exchangeable K). The trap cultures were kept in shade house conditions (temperature 25 ± 6 °C, relative humidity 79 ± 8%) with sorghum [*Sorghum bicolor* (L.) Moench] as host plant. Pots were watered to the point of saturation on alternate days and after three months, spores were isolated using wet-sieving and decanting technique as described below.

Enumeration and isolation of AM fungal spores

Twenty-five grams of air-dried field or trap culture soil was dispersed in one litre water and the suspension was decanted through 710-37 µm sieves. The residues in the sieves were washed into beakers. The sievates were dispersed in water and filtered through filter papers. Each filter paper was then spread on a Petri dish and scanned under a dissection microscope at ×40 magnifications. All intact spores (non-collapsed with cytoplasmic contents and free from parasitic attack) were counted. Sporocarps and spore clusters were considered as one unit. Intact AM fungal spores were transferred using a wet needle to polyvinyl alcohol lacto glycerol with or without Melzer's reagent on a glass slide for identification. Spore identification was based on spore morphology and sub cellular characters using Olympus BX51 light microscope and by comparing to original descriptions (http://www.lrz-muenchen.de/~ schuessler/amphylo/amphylo_species.html). Spore morphology was also compared to the culture database established by INVAM (http://invam.cag.wvu.edu.).

Statistical analysis

All data were subjected to Analysis of Variance (ANOVA) to assess the significance of variations in AM and DSE fungal variables among crop species. Pearson's correlation was used to assess the relationship between AM and DSE fungal variables. Spore numbers were log transformed and percentage data on root colonization was arcsine transformed prior to analysis.

RESULTS

Occurrence and distribution of endorhizal association

Forty-two of the 45 crop species examined had AM association (Table 1). We did not observe AM fungal structures in *Beta vulgaris* (Chenopodiaceae), *Myristica fragrans* (Myristicaceae) and *Rhapanus sativus* (Brassicaceae). Roots of 20 AM crop species also contained DSE fungal structures. *Myristica fragrans* lacked AM association but had DSE fungal association.

Morphology of AM in crops

Arbuscular mycorrhizal colonization was characterized by the formation of an appressorium on the root surface and hyphal coils in few outer cortical cells near the point of entry (Fig. 1a, b). Six families had both *Arum*- and intermediate-type AM morphologies (Table 1). Twenty crop species had AM morphology of *Arum*-type and 22 had intermediate-type AM morphology. *Arum*- type morphology was characterized by intercellular hyphae and vesicles with one or two arbuscules in each cell (Fig. 1d). Intermediate-type AM morophology had a combination of intracellular hyphae with arbuscules, hyphal coils, arbusculate coils and intracellular vesicles (Fig. 1c,e,f,g). It was possible to discriminate AM morphology at family levels only in Apiaceae and Cucurbitaceae where all the species examined within each family were of *Arum*- and intermediate-type respectively.

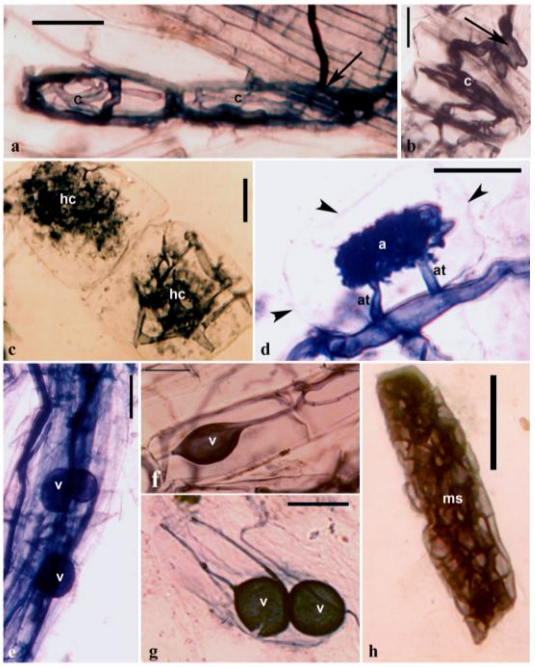

Figure 1. Endorhizal fungal association in crop species. a. Appressorium, hyphal entry point (arrow) and hyphal coils (c) of arbuscular mycorrhizal (AM) fungi in *Coffea arabica*. b. Appressorium (arrow) and coil (c) in the outer cortical cell of AM *Cucurbita maxima*. c. Intracellular arbusculate coils (hc) of AM fungi in *Arachis hypogaea*.d. *Arum*-type AM morphology in *Helianthus annus* with intercellular hyphae and two arbuscular trunks (at) bearing arbuscules (a) in a cell (arrow heads). e. Intercellular vesicles (v) and hyphae of AM fungi in *Oryza sativa*. f. Intracellular vesicle (v) of AM fungi in *Vigna radiata.* g. Intracellular vesicles (v) of AM fungi in *Amarphophallus paeniifolius*. h. Microsclerotia (ms) of dark septate endophyte fungi within *Amarphophallus paeniifolius* cell. Scale bars = 50 µm.

Table 1. Endorhizal fungal association and morphology in crop species.

Family Crop species✦	AM[a]	AM type[b]	DSE[a]	Previous reports[#] AM status[c]	AM type[b]	DSE[d]
Apiaceae						
Coriandrum sativum L.**	+	A	-	AM[11]	NR	
Daucus carota L.*	+	A	+	AM[11]	P, I[3]	NR
Araceae						
Amorphophallus paeniifolius (Dennst.) Nicolson var *campanulatus* (Blume ex Decne.) Sivadasan**	+	I	+	NR	NR	NR
Arecaceae						
Areca catechu L.*	+	I	-	AM[8]	I[8]	
Asteraceae						
Helianthus annus L.**	+	A	-	AM[6,11]	NR	Te[4]
Brassicaceae						
Raphanus sativus L.*	-	-	-	AM[1] + NM[11]	NR	
Chenopodiaceae						
Beta vulgaris L.*	-	-	-	AM[11]	NR	Te[4]
Cucurbitaceae						
Benincasa hispida (Thunb.) Cogn. **	+	I	+	NR	NR	NR
Cucurbita maxima Duchesne ex Lam.**	+	I	+	AM [1]	NR	NR
Luffa cylindrica (L.) M.Roem.*	+	I	-	NR	NR	
Euphorbiaceae						
Manihot esculenta Crantz.*	+	I	-	AM[11]	P[8]	
Ricinus communis L.**	+	A	-	AM[3]	NR	
Lamiaceae					NR	
Mentha arvensis L.*	+	A	+	AM + NM[11]	NR	NR
Lauraceae						
Cinnamomum verum Presl, Prir. Rostl.**	+	A	+	NR	NR	NR
Leguminaceae						
Arachis hypogaea L.*	+	I	-	AM[11, 7]	NR	
*Cajanus cajan (*L.) Millsp**	+	A	-	AM[6,11]	A[8]	
Cicer arietinum L.*	+	A	+	AM [7, 11]	NR	Tr[4]
Lablab purpureus (L.) Sweet.**	+	A		AM[1, 11]	NR	
Trigonella foenum-graecum L.*	+	A	+	AM[11]	NR	NR
Vigna mungo (L.) Hepper.**	+	I	+	AM[7, 11]	NR	NR
Vigna radiata (L.) Wilezek.*	+	I	-	AM[7, 10, 11]	NR	Tr[4]
Vigna trilobata (L.) Verdc.*	+	A	+	NR	NR	NR
Vigna ungiculata (L.) Walp.**	+	I	+	AM[7, 11]	P[3]	NR
Lilliaceae						
Allium cepa L.*	+	A	+	AM[11]	A[3]	Te[4]
Allium sativum L.**	+	A	+	AM[6,11]	A[3]	Te[4]
Malvaceae						
Abelmoschus esculentus (L.)Moerch.*	+	A	-	AM[1, 11]	A[3]	
Gossypium hirsutum L.**	+	I	+	AM[11]	NR	NR
Myristicaceae						
Myristica fragrans Houtt.*	-	-	+	NR		NR
Pedaliaceae						
Sesamum indicum L.*	+	A	-	AM[11]	NR	

Continue Table 1

Family Crop species[+]	AM[a]	AM type[b]	DSE[a]	Previous reports[#] AM status[c]	AM type[b]	DSE[d]
Piperaceae						
Piper nigrum L.**	+	I	+	AM[11]	A[3]	NR
Poaceae						
Oryza sativa L.*	+	I	+	AM[11, 12]	NR	NR
Pennisetum typhoides (Burm.f) Stapf & Hubbard *	+	A	-	NR	NR	
Saccharum officinarum L.**	+	I	-	AM[8, 11]	P[8]	Tr[4]
Setaria italica (L.) P. Beauv. Ess.*	+	I	-	NR	NR	
Sorghum halepense (L.) Pers.**	+	I	-	AM[11]	A, I[3]	
Zea mays L.**	+	A	-	AM[6, 11]	A, P[3]	Te[4]
Rubiaceae						
Coffea arabica L.*	+	I	+	AM[11]	P[3]	NR
Rutaceae						
Murraya koenigii (L.) Spreng. **	+	I	-	NR	P[9]	
Solanaceae						
Capsicum annum L.**	+	A	+	AM[2, 6, 11]	P[3]	NR
Nicotiana tobacum L.*	+	I	+		NR	NR
Solanum melangena L.**	+	A	+	AM[1, 11]	A[3]	NR
Solanum nigrum L.*	+	I	-	AM + NM[11]	NR	Te[4]
Solanum torvum Sw. Prodr. **	+	I	-	NM[11]	NR	NR
Zingiberaceae						
Curcuma longa L.*	+	A	-	AM[5]	A[9]	Tr[8]
Zingiber officinale Roscoe.*	+	I	-	AM[9]	I[9]	Tr[8]

[+] *Site-A; ** site-B

[a] AM, Arbuscular mycorrhizal; DSE, dark septate endophyte; +, Presence; -, absence

[b] A, *Arum*- type; I, intermediate- type; P, *Paris*- type

[c] AM, Arbuscular mycorrhizal; NM, non-mycorrhizal; NR, no report

[d] Te, Temperate; Tr, tropical; NR no report

[#] [1]Akond *et al*. (2008), [2]Castillo *et al*. (2009), [3]Dickson *et al*. (2007), [4]Jumpponen and Trappe (1998), [5]Khade and Rodrigues (2007), [6]Li *et al* (2007), [7]Molla and Solaiman (2009), [8]Muthukumar and Prakash (2009), [9]Muthukumar *et al*. (2006), [10]Valsalakumar *et al*. (2007), [11]Wang and Qiu (1996), [12]Wangiyana *et al*. (2006)

Extent of AM fungal colonization

There were large differences in the extent of colonization among crops (Table 2). The percentage of root length with hyphae (%RLH) varied significantly among crop species ($F_{44,90}$ = 3.68; P<0.01). The % RLH ranged from 33 (*Vigna trilobata*) to 81.77 (*Solanum nigrum*). The perecentage of root length with arbuscules/arbusculate coils (%RLA) ranged between 4.55 (*Cinnamomum verum*) and 41.20 (*Solanum melangena*). The %RLA varied significantly between different crops ($F_{44,90}$=9.88; P<0 001). Similarly, the percentage of root length with vesicles (%RLV) significantly varied among crops ($F_{44,90}$=6.88; P<0. 001) and ranged between <1 (*Sesamum indicum*) and 20.01 (*Allium sativum*). The percentage of root length with hyphal coils (%RLHC) varied significantly between different crop species ($F_{44,90}$ =9.88; P<0.001) and ranged between 9.03 *(Arachis hypogaea*) and 65.12 (*Zingiber officinale*). There was a significant variation in the percentage of total AM fungal colonization (%RLT) among crop species ($F_{44,90}$=5.51; P<0.001). Maximum %RLT occurred in *Solanum melangena* (96.90) and minimum %RLTC occurred in *Vigna trilobata* (41.32).

AM fungal spore numbers

AM fungal spore numbers significantly varied in the rhizospheres of different crops ($F_{44,90}$=16.67; P<0.001) and ranged from four spores $25g^{-1}$ soil (*Capsicum annum*, *Trigonella foenum-graecum*) to 60 spores $25g^{-1}$ soil (*Vigna ungiculata)* (Table 2). There was no correlation between AM fungal spore numbers and %RLTC (r = 0.209; P>0.05).

Table 2. Extent of arbuscular mycorrhizal (AM) colonization, spore numbers and dark septate endophyte (DSE) fungal colonization in field crops.

Crop species	AM colonization[a]					AM fungal spore 25 g^{-1} of soil	DSE fungal colonization[b]		
	%RLH	%RLV	%RLA	%RLHC	%RLT		%RLDH	%RLM	%RLDT
Abelmoschus esculentus	49.58 ± 3.8*	15.93 ± 2.23	28.31 ± 4.37	--	93.83 ± 1.68	26 ± 4	--	--	--
Allium cepa	53.65 ± 5.24	11.16 ± 1.93	--	--	64.82 ± 6.95	53 ± 5	4.84 ± 2.19	--	4.84 ± 2.19
Allium sativum	60.11 ± 2.57	20.01 ± 3.50	4.82 ± 0.89	--	84.88 ± 5.94	11 ± 2	1.92 ± 0.38	5.29 ± 1.67	7.22 ± 2.13
Amorphophallus paeniifolius	52.53 ± 5.90	--	20.50 ± 3.37	--	73.03 ± 3.19	47 ± 6	--	6.40 ± 2.15	6.40 ± 2.15
Arachis hypogaea	52.83 ± 6.29	--	16.07 ± 3.46	9.03 ± 3.09	77.94 ± 6.16	30 ± 4	--	--	--
Areca catechu L.		--	13.25 ± 3.21	30.05 ± 4.34	43.21 ± 6.71	8 ± 1	--	--	--
Benincasa hispida	43.92 ± 3.03	--	17.93 ± 4.91	--	61.85 ± 5.92	37 ± 4	18.03 ± 0.95	--	18.02 ± 0.95
Beta vulgaris	--	--	--	--	--	20 ± 1	--	--	--
Cajanus cajan	53.46 ± 3.09	9.28 ± 1.45	24.26 ± 2.08	--	86.89 ± 2.52	36 ± 2	--	--	--
Capsicum annum	57.10 ± 2.13	--	33.81 ± 1.88	--	90.93 ± 3.83	4 ± 1	4.93 ± 2.02	--	4.92 ± 2.02
Cicer arietinum	71.84 ± 5.49	--	22.40 ± 7.02	--	94.26 ± 1.65	21 ± 2	4.23 ± 1.25	0.41 ± 0.41	4.64 ± 1.13
Cinnamomum verum	42.46 ± 4.57	--	4.55 ± 1.82	--	47.01 ± 10.32	8 ± 1	23.38 ± 5.31	6.41 ± 5.63	29.80 ± 5.28
Coffea arabica	61.79 ± 1.97	--	6.89 ± 2.00	16.20 ± 2.81	84.89 ± 2.29	13 ± 1	1.03 ± 0.42	1.01 ± 1.01	2.04 ± 1.33
Coriandrum sativum	51.15 ± 1.58	10.44 ± 5.62	30.91 ± 4.04	--	92.78 ± 2.49	7 ± 2	--	--	--
Cucurbita maxima	57.10 ± 2.13	--	33.81 ± 1.88	--	90.92 ± 3.80	33 ± 5	4.93 ± 2.02	--	4.92 ± 2.02
Curcuma longa	61.75 ± 6.82	7.71 ± 1.01	19.35 ± 2.24	--	88.81 ± 8.33	15 ± 2	--	--	--
Daucus carota	50.18 ± 4.68	7.47 ± 2.79	6.97 ± 1.51	--	64.62 ± 5.53	6 ± 2	--	5.88 ± 1.41	5.88 ± 1.41
Gossypium hirsutum	62.83 ± 3.02	9.73 ± 3.64	10.78 ± 2.55	--	83.27 ± 5.31	30 ± 5	2.85 ± 0.10	--	2.85 ± 0.10
Helianthus annus	72.10 ± 0.56	--	14.87 ± 3.06	--	86.98 ± 2.50	47 ± 5	--	--	--
Lablab purpureus	50.97 ± 3.38	17.03 ± 0.19	14.51 ± 3.24	--	82.52 ± 0.46	39± 2	--	--	--
Luffa cylindrica	56.37 ± 1.17	--	24.14 ± 1.70	--	80.49 ± 1.05	30 ± 10	--	--	--
Manihot esculenta	40.23 ± 4.32	--	28.66 ± 7.28	--	68.90 ± 5.99	13 ± 1	--	--	--
Mentha arvensis	45.72 ± 3.11	--	30.43 ± 2.97	--	76.18 ± 5.37	5 ± 1	1.35 ± 0.92	--	1.35 ± 0.92
Murraya koenigii	69.45 ± 1.30	--	27.16 ± 0.94	--	96.61 ± 0.45	20 ± 4	--	--	--
Myristica fragrans	--	--	--	--	--	11 ± 4	61.33 ± 5.76	--	61.33 ± 5.76
Nicotiana tobacum	58.73 ± 1.46	--	--	--	58.73 ± 1.46	30 ± 9	--	27.25 ± 2.59	27.25 ± 2.59
Oryza sativa	52.96 ± 19.43	--	--	--	52.96 ± 19.43	10 ± 3	32.61 ± 8.51	10.96 ± 5.85	43.57 ± 16.27
Pennisetum hyphoides	57.87 ± 11.46	--	9.59 ± 2.43	--	67.47 ± 10.35	28 ± 5	--	--	--
Piper nigrum	56.24 ± 4.82	--	--	--	56.24 ± 4.82	7 ± 2	8.07 ± 2.78	9.33 ± 1.78	17.40 ± 4.72
Raphanus sativus	--	--	--	--	--	8 ± 3	--	--	--

Continue Table 2

Crop species	AM colonization[a]					AM fungal spore 25 g^{-1} of soil	DSE fungal colonization[b]		
	%RLH	%RLV	%RLA	%RLHC	%RLT		%RLDH	%RLM	%RLDT
Ricinus communis	57.30 ± 1.43	--	20.63 ± 3.50	--	77.94 ± 4.60	7 ± 1	--	--	--
Saccharum officinarum	63.33 ± 4.06	--	--	--	63.33 ± 4.06	23 ± 2	--	--	--
Sesamum indicum	59.31 ± 3.80	0.91 ± 0.46	30.54 ± 1.36	--	90.76 ± 0.79	26 ± 5	--	--	--
Setaria italica	75.22 ± 3.01	15.14 ±2.15	--	--	90.37 ± 2.56	41 ± 3	--	--	--
Solanum melangena	48.71 ± 0.84	6.98 ± 0.92	41.20 ± 1.89	--	96.90 ± 1.17	45 ± 2	--	0.71 ± 0.01	0.71 ± 0.01
Solanum nigrum	81.77 ± 6.10	--	6.14 ± 0.64	--	87.91 ± 6.55	25 ± 2	--	--	--
Solanum torvum	71.36 ± 10.80	--	10.09 ± 3.03	--	81.48 ± 6.32	9 ± 3	--	--	--
Sorghum halepense	53.50 ± 2.56	1.89 ± 1.89	--	--	55.38 ± 4.42	21 ± 4	--	--	--
Trigonella foenum-graecum	68.11 ± 1.31	--	16.99 ± 2.51	--	85.10 ± 1.59	4 ± 1	10.23 ± 1.21	--	10.23 ± 1.21
Vigna mungo	64.94 ± 0.95	--	28.03 ± 0.13	--	92.98 ± 0.50	59 ± 8	1.39 ± 0.65	--	1.39 ± 0.79
Vigna radiata	50.04 ± 3.65	14.19 ± 3.34	17.08 ± 2.59	--	81.31 ± 7.66	16 ± 5	--	--	--
Vigna trilobata	32.98 ± 3.78	--	8.33 ± 1.43	--	41.32 ± 3.53	41 ± 5	32.55 ± 5.42	5.26± 2.98	37.81± 5.67
Vigna ungiculata	46.27 ± 2.19	2.25 ± 1.69	19.84 ± 3.70	--	68.40 ± 3.32	60 ± 4	7.59 ± 1.20	--	7.59 ± 1.20
Zea mays	54.49 ± 1.81	--	19.42 ± 3.64	--	73.91 ± 5.41	30 ± 5	--	--	--
Zingiber officinale	--	2.45 ± 2.45	24.20 ± 4.57	65.12 ± 3.71	91.61 ± 2.85	22 ± 1	--	--	--

[a] %RLH, %RLV, %RLA, %RLHC, and %RLT, indicates root length with hyphae, vesicles, arbuscules/arbusculate cells, hyphal coils and total colonization respectively.
[b] %RLDH, %RLDM and %RLDT indicates root length with DSE fungal hyphae, microsclerotia and total colonization respectively.
*Mean ± S.E.

AM fungal species

AM fungal spores from 12 species in four genera of Acaulosporaceae, Gigasporaceae, Glomaceae and Scutellosporaceae were isolated from the crop rhizospheres and trap cultures from both sites (Table 3). These included three species in *Acaulospora*, seven in *Glomus* and one each in *Gigaspora* and *Scutellospora*. AM fungal spores of eight species occurred in site-A and seven species occurred in site-B. Spores of *Acaulospora scrobiculata*, *Glomus aggregatum*, *G. microaggregatum* and *G. geosporum* occurred at both sites. No additional species occurred in trap cultures.

Extent of DSE fungal association

Narrow (2-4 µm) diam. runner hyphae running parallel to the root surface characterized DSE fungal colonization. At times individual hyphae penetrated the epidermis and tended to coil in outer and inner cortical cells. The stelar portion was free of colonization and there was no evidence of damage to host root tissues arising from fungal colonization. There was a lack of distinction between the inter-radical and extra-matrical hyphae, which was frequently septate. Microsclerotia formation was observed in 11 crop plant species (Fig. 1h). The extent of percent DSE fungal colonization (%RLDT) ranged from <1 (*Solanum melongena*) to 61.33 (*M. fragrans*) and varied significantly among crop species ($F_{44,90}$ = 17.19; P<0.001) (Table 2). Percentage root length with DSE fungal hyphae (%RLDH) ranged from 1.03 (*Coffea arabica*) to 61.33 (*M. fragrans*). Likewise, microsclerotia colonization (%RLM) ranged between <1 (*Cicer arietinum*, *S. melongena*) and 27.25 (*Nicotiana tobacum*) and exhibited a significant variation in different crops ($F_{44, 90}$= 10.24; P< 0.01). A significant negative correlation existed between %RLDT and %RLT (r= -0.510; P<0.001)

DISCUSSION

The high incidence of crops colonized by AM fungi confirms the ubiquity of AM association in tropical agro-ecosystems. Crop species of several families examined in the present study have been previously reported AM, but their AM morphology has not been categorically studied. For example, AM morphology is known for only 16 of the 42 mycorrhizal crop species examined. Three crop species that lacked AM association in the present study belonged to plant families that have been designated as non-mycorrhizal, Brassicaceae and Chenopodiaceae, where, the third non-mycorrhizal crop species *M. fragrans* belonged to a mycorrhizal family (Tester *et al.,* 1987; Wang and Qiu, 2006). The presence of fungitoxic compounds in root cortical tissues or in root exudates may reduce susceptibility of plants to colonization. Tommerup (1984) found that *Brassica napus* produced a soluble or volatile compound that reduced the rate of germination of AM fungal spores and hyphal growth. Mustard oils are found in shoots and roots of plants from numerous families including Brassicaceae (Schreiner and Koide, 1993; Tester *et al.,* 1987). However, various cultivars of *Brassica* with different concentrations of glucosinolates in roots had no effect on the colonization of healthy roots by AM fungi (Vierheilig et al., 2000). Another reason suggested for low incidence of mycorrhizae is a lack of root exudation in weakly mycorrhizal plants (Vierheilig and Ocampo, 1990). Schwab *et al.* (1982) sprayed *Chenopodium quinoa* with the herbicide Simazine which increased root exudation and induced low levels of mycorrhizal colonization. However, AM colonization has been reported in many non-mycorrhizal crop species like *R. sativus* (Hirrel *et al.*, 1978) and *Chenopodium album* (Hirrel *et al*., 1978; Saif *et al*., 1977).

Table 3. Spores of arbuscular mycorrhizal (AM) fungal species associated with crop species.

Family	AM fungal species
Acaulosporaceae	*Acaulospora laevis* Gerd. &Trappe[1]
	Acaulospora morrowiae Spain & N.C. Schenck[2]
	Acaulospora scrobiculata Trappe[1,2]
Gigasporaceae	*Gigaspora gigantea (*T.H. Nicolson & Gerd.) Gerd. & Trappe[1]
Glomaceae	*Glomus aggregatum* N.C. Schenk & G.S. Sm. emend. Koske[1,2]
	Glomus albidum C. Walker & L.H. Rhodes.[2]
	Glomus etunicatum W.N. Becker & Gerd.[1]
	Glomus geosporum (T.H. Nicolson & Gerd.) C. Walker[1,2]
	Glomus heterosporum G.S. Sm. & N.C. Schenck[2]
	Glomus indica Manoharachary, Sharathbabu & Adholeya[1]
	Glomus microaggregatum Gemma, Koske & P.D. Olexia[1,2]
Scutellosporaceae	*Scutellospora pellucida* (T.H. Nicolson & N.C. Schenck) C. Walker & F.E. Sanders[2]

Superscript number(s) after AM fungal species names indicate site numbers (see Materials and Methods section) from which the soil samples were collected.

It is generally presumed that *Arum*-type morphology is often associated with cultivated plants which are fast-growing and light-loving (Smith and Smith, 1997), while *Paris*-type plants seems to be shade-loving and slow-growing (Brundrett and Kendrick, 1990a, 1990b; Yamato and Iwasaki, 2002). The present study also supports this view to a certain extent as 20 of the 42 AM crops had *Arum*-type morphology. Ahulu *et al.* (2005) found that the majority of the herbs in a mixed pine forest on sand dunes in Japan formed *Arum*-type. Similarly, O′Connor *et al*. (2001) found *Arum*-type AM in 21 herbaceous plant species growing in Australian desert.

AM morphology of 27 crop species is reported, to best of our knowledge, for the first time. Factors involved in determining the formation of *Arum*- and *Paris*-type morphologies, however, remain unclear. Crop species within the majority of families formed the same AM morphology in accordance with earlier observations (Dickson *et al.*, 2007; Smith and Smith, 1997) indicating that AM morphology is strongly influenced by the identity of the host plant (Table 1). Ahulu *et al*. (2006) using molecular probes demonstrated that *Rubus parvifolius* and *Hedera rhombea* formed Arum- and Paris-type AM repectively inspite of the presence of similar AM fungal fungal taxa within their roots. Gerdemann (1965) showed that the same AM fungus that formed the *Arum*-type in *Zea mays* formed *Paris*-type in *Liriodendron tulipifera*. A similar result was observed by Gianinazzi-Pearson (1984) using *Allium cepa* and *Gentiana lutea* respectively. *Glomus mosseae* and *G. viscosum*, which are known to form *Arum*-type AM in many plant species, produced *Paris*-type AM in *Smilax aspera*, which also forms *Paris*-type in nature (Bedini *et al.*, 2000). These suggests that the host plant has a significant influence on the type of AM morphology. It has been noted that differences in cell wall structure and modification produced during fungal colonization may be important in determining AM morphology in different plant species (Bonfante-Fasolo and Fontana, 1985). Brundrett and Kendrick (1990a, 1990b) suggested that the presence or absence of continuous longitudinal air spaces in the root cortex might be the factor determining formation of either the *Arum*- or *Paris*-type, respectively. However, Imhof and Weber (1997, 2000) noted that *Voyria obconica* formed *Paris*-type AM in spite of the presence of clear inter-cellular spaces in the root cortices. Cavagnaro *et al.* (2000) found both *Arum*- and *Paris*-type morphologies in *Lycopersicon esculentum* when inoculated with different AM fungal species, observing variation in each of the morphological groups, such as formation of different ratios of hyphal and arbusculate coils by pure cultures of AM fungi in the *Paris*-type AM. Cavagnaro *et al*. (2001) reasoned that if hyphal and arbusculate coils have dissimilar roles, then the differences in ratios could reflect differences in functionality. Furthermore, they noted that environmental factors, such as soil P, temperature and light as well as molecular activity at the interfaces of the morphological structures could influence AM morphology.

AM fungal spore numbers recorded in the present study was low compared to reports from other cultivated field soils. Land and Schonbeck (1991) reported a spore number range of 150-600 spores 100 g^{-1} of soil from arable soils of Germany. Similarly, Muthukumar and Udaiyan (2000) reported a spore density of 20-50 spores 10 g^{-1} of soil under cowpea (*Vigna unguiculata*) cultivation. Cuenca and Meneses (1996) reported 189-1674 spores 100 g^{-1} soil associated with cacao (*Theobroma cacao*) in Venezuela. Recently Valsalakumar *et al*. (2007) reported a spore count of 760-3920 g^{-1} of soil associated with green gram (*Phaseolus aureus*) in south India. As an array of environmental, host and fungal factors influence AM fungal spore numbers in the soils (Brundrett, 1991) the present observation is tenable.

The presence of DSE association in 21 crop species is expected as DSE fungal endophytes are assumed to be wide spread among angiosperms (Jumpponen and Trappe, 1998). In spite of this assumption, only 59 tropical plant species have been reported hosts for DSE fungi (Jumpponen and Trappe, 1998). Nineteen crop species are reported here as hosts of DSE fungi for the first time. The presence of DSE fungal association in *Allium cepa* and *Allium sativum* in the present study extends the presence of this association in the tropics as earlier reports indicate their presence in temperate agroecosystems (Jumpponen and Trappe, 1998). However, *B. vulgaris*, *C. arietinum*, *Curcuma longa*, *Helianthus annus*, *Saccharum officinarum*, *S. nigrum*, *Sorghum halepense*, *Vigna radiata*, *Z. mays* and *Z. officinale* previously reported as hosts for DSE fungi lacked the association in the present study. The functional relationship between plants and DSE fungi may be comparable to that between mycorrhizal fungi and their host. Studies have shown that DSE fungal association is mutualistic rather than parasitic. Colonization of cotton (*Gossypium hirsutum)* roots by the dematiaceous hypomecete *Cladorrhinum foecundissimum* improved biomass and shoot P concentration (Gasoni and Stegman de Gurfinkel, 1997).

It is interesting to note that all the DSE fungi colonized crops (except *M. fragrans*) also had AM association. Such a dual association of AM and DSE fungal endophytes has been previously reported by several authors (Muthukumar *et al*., 2006; Muthukumar and Udaiyan, 2002; Mandyam and Jumpponen, 2008). This type of dual colonization by

different root-associated fungi reflects a dynamic nature of root-colonizing fungal community. DSE fungal endophytes are known to frequently colonize older parts of the root system (Jumpponen and Trappe, 1998), suggesting that they prefer aging 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 the presence of DSE colonization in young roots suggests a concurrent colonization with AM fungi. It has been proposed that DSE fungal association enhance root functions of native plants in arid ecosystems, where they are exposed to very dry soils (Barrow, 2003). The wide spread occurrence of DSE fungi in crop species, as indicated in this study, emphasizes their potential to function as mutualists along with AM fungi.

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