ENDORHIZAL FUNGAL ASSOCIATION AND COLONIZATION PATTERNS IN SOLANACEAE

THANGAVELU MUTHUKUMAR¹ & RAMALINGAM SATHYA

Abstract. The Solanaceae family includes plants of high economic and medicinal value. Information on endorhizal associations in Solanaceae is limited. We investigated arbuscular mycorrhizal (AM) and dark septate endophyte (DSE) fungal associations in 20 solanaceous plant species (7 genera) taken from one or more sites, measuring the percentage of root length colonized in plants having different AM and DSE fungal structures. Root samples of all the plant species examined had AM fungal structures, and DSE fungal colonization was found in 80% of the plant species. Total AM and DSE fungal colonization and root length of plants with different fungal structures varied significantly between species. Significant between-site variation was found for root length in plants with fungal structures in *Lycopersicon esculentum* Mill., *Capsicum annuum* L., *Datura metel* L., *Solanum melongena* L., *S. nigrum* L., *S. trilobatum* L. and *S. torvum* SW. AM morphology was predominantly intermediate-type (60%), followed by Paris-type (30%). *Solanum melongena* and *S. nigrum* at different sites had Paris-type or intermediate AM morphology. DSE fungal colonization also exhibited significant between-site variation in *Capsicum frutescens*, *C. annuum*, *Datura metel*, *Solanum melongena*, *S. trilobatum* and *S. nigrum*. We found a significant negative correlation between AM and DSE fungal colonization.

Key words: arbuscular mycorrhiza, arbuscules, arbusculate coils, dark septate endophyte fungi, nightshade

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INTRODUCTION

The plant family Solanaceae, commonly known as the nightshade or potato family, is a large family with 2678 species in 115 different genera (Anonymous 2013). Members of this family are distributed in temperate and tropical regions, with a large number occurring in Australia and Central and South America. Herbs are dominant in this family, with a few shrubs and trees. A number of important agricultural crops such as potatoes, tomatoes, aubergines and peppers belong to this family (Knapp *et al.* 2004). This family also contains many popular garden ornamental plants (e.g., *Petunia* Juss., *Browallia* L., *Salpiglossis* Ruiz & Pav.) and is an important source of spices and medicinal plants. Solanaceous species are often rich in alkaloids. The toxicity of these alkaloids to humans and other animals can range from mild irritation to lethality even in small quantities (Chowański *et al.* 2016). The poisonous species in this family include

Atropa belladonna L., *Hyoscyamus niger* L. and *Datura stramonium* L. The family includes an important economic plant, tobacco (*Nicotiana tabacum* L.), which contains highly toxic alkaloids such as anabasine, anatabine, nicotine and nornicotine (Sun *et al.* 2013).

Arbuscular mycorrhizal (AM) fungi belonging to the Glomeromycota are a major component of the soil microflora of terrestrial ecosystems and play a significant role in the structure and functioning of these ecosystems (Lee *et al.* 2013). These fungi benefit plants through the uptake of immobile or diffusion-limited mineral nutrients from the soil, such as phosphorus (P), zinc (Zn) and copper (Cu), and more mobile elements such as sulfur (S), potassium (K), magnesium (Mg), calcium (Ca), sodium (Na), iron (Fe) and manganese (Mn); the extraradical hyphae of AM fungi, which can extend up to 4 cm from the root surface, absorb minerals beyond the nutrient depletion zones surrounding the roots and translocate them to the roots (Smith & Read 2008).

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Arbuscular mycorrhizal fungi also improve rooting and plant establishment, enhance plant tolerance to biotic and abiotic stresses, improve nutrient cycling and enhance plant community diversity (Lee *et al.* 2013). AM fungi can affect the water balance of both amply watered and drought-stressed host plants (Chitarra *et al.* 2016). Studies of the distribution and activity of AM fungi can help elucidate the ecological significance of AM associations (Gianinazzi *et al.* 2010). Experimental studies have shown that AM fungal associations can enhance plant growth, nutrient uptake (Diop *et al.* 2003; Manimegalai *et al.* 2011; Vosatka & Gryndler 2000; Davies *et al.* 2005; Yao *et al.* 2002; Dennett *et al.* 2011) and tolerance of salinity, heavy metals and pathogens in solanaceous crop species (Cuperman *et al.* 1996; Mohammad & Mittra 2011; Yao *et al.* 2002). Nevertheless, information on the occurrence of AM fungal associations in members of the Solanaceae is very limited. Only 25 solanaceous plant species have been assessed for their mycorrhizal status (see Wang & Qiu 2006; Sekhara Reddy *et al.* 2007; Muthukumar & Tamilselvi 2010).

After entering plant roots, AM fungi spread inter- or intracellularly within the roots. The mode of spread can vary greatly within a single plant species. Based on the distribution of fungal structures within roots, AM fungal colonization has been categorized as *Arum*, *Paris* and intermediate types. In the *Arum* type, which is frequent in field crops, linear fungal hyphae spread intracellularly, forming arbuscules on short lateral branches (Smith & Read 2008). In the *Paris* type, which is more common in plants growing naturally, the fungal hyphae spread among cells intracellularly, forming hyphal or arbusculate coils (Smith & Smith 1997; Dickson *et al.* 2007). The *Arum* and *Paris* types are generally termed 'linear' and 'coiling' in relation to the appearance of the longitudinal hyphae within the roots (Brundrett 2004). The intermediate type exhibits characteristics of both the *Arum* and *Paris* types, such as the presence of linear hyphae which may be inter- and/or intracellular hyphal and arbusculate coils. Previous studies have examined the diversity and abundance of AM fungal species (e.g., Songachan & Kayang 2012; Senés-Guerrero *et al.* 2014) but the colonization patterns of

nightshades remain largely unknown. Up to 2007, AM morphology was known only for *Datura alba* Nees, *D. metel* L., *Lycopersicon esculentum* Mill., *Nicotiana velutina* Wheeler, *Solanum ellipticum* Vel., *S. melongena* L., *S. surattense* Burm. f. and *S. verbascifolium* L. (Dickson *et al.* 2007).

Dark septate endophyte (DSE) fungi are darkly pigmented, sterile, regularly septate root-associated fungi which colonize living plant organs, especially roots, without causing any apparent negative effects (Mandyam & Jumpponen 2005). In DSE fungi-colonized roots, the fungi form a cluster of inflated, rounded, thick-walled cells within the cortical wall, called microsclerotia (Jumpponen & Trappe 1998). DSE fungi are distributed in different habitats and host plants, suggesting the absence of host specificity (Mandyam & Jumpponen 2005). The melanin present in the fungal hyphae protects DSE fungi from unfavorable or stressful conditions such as drought and extreme temperature (Mandyam & Jumpponen 2005). As with AM fungi, information on the occurrence of DSE fungal associations in Solanaceae taxa is also limited. Up to 1998 the presence of DSE associations was reported in only six tropical solanaceous plant species (Jumpponen & Trappe 1998). Later, DSE fungal associations were reported in three solanaceous plants of southern India (Muthukumar & Tamilselvi, 2010). More recently, Songachan and Kayang (2012) reported fractional colonization (<1%) of DSE fungi in roots of *Solanum khasianum* C. B. Clarke, *S. sisymbriifolium* Lam. and *S. torvum* Sw. growing naturally in Meghalaya, India.

The objective of the present study was to assess the incidence and extent of AM and DSE fungal associations in Solanaceae taxa from the Western Ghats region of southern India. We also determined AM fungal morphology and examined the influence of site and soil characteristics on AM fungal and DSE fungal associations.

MATERIALS AND METHODS

STUDY SITES AND SAMPLING

Root samples of 20 solanaceous plant species belonging to seven genera were collected during January 2012 from four different areas in Tamilnadu, India: Erode (site I),

Coimbatore (site II), Pollachi (site III) and Ooty (site IV) (Tables 1 & 2). Three plants of each species growing at a site were sampled. *Capsicum annuum* L., *Lycopersicon esculentum* Mill., *Solanum torvum* Sw. and *S. trilobatum* L. were collected from sites I and II. *Capsicum frutescens* L. was collected from sites II, III and IV. *Solanum melongena* L. was collected from sites I, II and IV. *Datura metel* L. and *Solanum nigrum* L. were collected from sites I, II, III and IV. Each root was excavated carefully without damaging it, and the fine roots were traced back to the stem to confirm that the collected roots belonged to the intended plant. Collected roots were washed free of soil and fixed in FAA (formalin: glacial acetic acid: 70% ethyl alcohol; 5:5:90; v:v:v) for processing. Five randomly picked 0–15 cm soil samples collected from each site were bulked, shade-dried at room temperature and used for soil chemistry analyses.

DETERMINATION OF SOIL CHEMICAL PROPERTIES

Soil pH and electrical conductivity (EC) were measured in 1:1 soil: water (v:v) suspensions using digital meters (ELICO, India) soon after the soil samples were brought to the laboratory. Total nitrogen (N) and total phosphorous (P) were determined according to Jackson (1971), and exchangeable potassium (K) was extracted with ammonium acetate and measured using flame photometry (Jackson 1971).

PREPARATION OF ROOTS FOR EXAMINATION OF AM AND DSE FUNGI

The fixed roots were washed free of FAA, cleared in 2.5% KOH at 90°C (Koske & Gemma 1989) for

45–80 min, acidified with 5N HCl and placed in trypan blue (0.05 % in lactoglycerol) overnight for staining. Stained fine roots mounted on microscope slides were examined with an Olympus BX 51 compound microscope (×400) for the presence AM and DSE fungal structures. The percentage of root length colonized by AM or DSE fungi was estimated according to the magnified intersection method described by McGonigle *et al.* (1990). Only roots containing arbuscules or arbusculate coils were considered to be AM. The classification of AM morphology was based on whether the fungal hyphae were present mainly as hyphae running through intercellular spaces or within cells as coils, following the descriptions of Dickson (2004). Regularly septate and melanized hyphae were deemed to indicate fungal colonization. DSE fungal colonization and structures were determined as described for AM fungi.

PLANT NOMENCLATURE AND ECONOMIC IMPORTANCE

Nomenclature and authorities for plants follow *The Plant List* (Anonymous 2013). Economic importance is given following Henry *et al.* (1987).

STATISTICAL ANALYSIS

One-way analysis of variance (ANOVA) was used to test the significance of variation of fungal variables. The paired-sample t test was performed to assess the significance of differences between means for solanaceous plant species occurring at two sites (SPSS, Windows ver. 9). Pearson’s correlation coefficient was used to assess the relationship between AM and DSE fungal

Table 1. Site and soil characteristics of the study sites.

Particulars	Sites			
	Erode (site I)	Coimbatore (site II)	Pollachi (site III)	Ooty (site IV)
Location	11°30'N and 77°14'E	11°04'N and 76°93'E	10°40'N and 77°1'E	11°24'N and 76°41'E
Altitude (m.a.s.l)	800	1800	2286	2240
Annual rainfall (mm)	800	700	823	991
Number of plant species collected	10	10	6	8
Soil type	sandy clay loam	sandy loam	sandy loam	sandy clay loam
pH*	8.16±0.01c	8.12±0.03c	7.92±0.04b	5.70±0.11a
Electrical conductivity (dSm ⁻¹)*	0.16±0.01a	0.21±0.01b	0.16±0.01a	0.25±0.01c
Total nitrogen (mg/kg)*	9.13±0.07a	9.47±0.04b	9.14±0.04a	90.03±0.04a
Available phosphorus (mg/kg)*	0.93±0.04d	0.82±0.03c	0.36±0.02a	0.61±0.03b
Exchangeable potassium (mg/kg)*	20.03±0.82bc	18.24±0.52b	16.21±0.47a	20.83±0.52c

* Means±standard error in a row followed by the same letter do not differ significantly (P>0.05) according to Duncan’s Multiple Range Test.

Table 2. Present and previous reports of the occurrence of arbuscular mycorrhizal (AM) and dark septate endophyte (DSE) fungal associations and AM morphological types in Solanaceae taxa.

Plant species	Site ^a	EI ^b	AM ^c	AM-type ^d	DSE ^c	Previous report [#]		
						AM status ^e	AM – type ^d	DSE ^f
<i>Brugmansia sanguinea</i> (Ruiz & Pav.) D. Don.	IV	O, M	+	I3	+	NR	NR	NR
<i>Capsicum frutescens</i> L.	II, III, IV	V	+	P	+/- (III)	NR	NR	NR
<i>C. annuum</i> L.	I, II	V, M	+	P	+	AM ^{1, 2, 3, 4, 13}	P ⁵ , A ⁴	Tr ^{4, 11}
<i>Datura innoxia</i> Mill.	III	M, O	+	I3	+	NR	NR	NR
<i>D. metel</i> L.	I, II, III, IV	M, O	+	I1ac	+/- (I)	AM ^{1, 6}	A ^{6,9}	NR
<i>D. stramonium</i> L.	I	M	+	I4	+	AM ¹	NR	Tr ¹¹
<i>Lycopersicon esculentum</i> Mill.	I, II	V	+	I3	+	AM ^{1, 6}	I ⁵	Tr ⁸
<i>Nicotiana tabacum</i> L.	I	M	+	I1ac	+	AM ⁴	I ⁴	Tr ⁴
<i>Physalis angulata</i> L.	III	M	+	I4	+	NR	NR	NR
<i>S. elaeagnifolium</i> Cau.	II	M	+	I3	+	NR	NR	NR
<i>Solanum melongena</i> L.	I, II, III	V	+	I3/P (II)	+/- (I)	AM ^{4,7}	A&P ⁵	Tr ⁴
<i>S. nigrum</i> L.	I, II, III, IV	V, M	+	I3/ P (IV)	+	AM+NM ¹ , AM ⁴	I ⁴ , P ⁹	Te ⁸ , NA ⁴
<i>S. pseudocapsicum</i> L.	IV	O	+	P	+	NR	NR	NR
<i>S. pubescens</i> Willd.	II	M	+	I3	–	NR	NR	NR
<i>S. sisymbirifolium</i> Lam.	IV	O	+	I4	–	AM ^{10,12}	P ¹⁰	STr ¹⁰ , Tr ¹²
<i>S. torvum</i> SW.	I, II	V, M	+	P	+	NM ¹ , AM ^{4, 9, 12}	I ⁴ , A ⁹	NA ⁴ , Tr ¹²
<i>S. trilobatum</i> L.	I, II	M	+	P	+	NR	NR	NR
<i>S. tuberosum</i> L.	IV	V	+	P	–	AM ¹	NR	Te ⁸
<i>S. viarum</i> Dunal	IV	M	+	I3	–	NR	NR	NR
<i>S. virginianum</i> L.	I	M	+	I 4	+	NR	NR	NR

^a I, II, III and IV indicates Erode, Coimbatore, Pollachi and Ooty; ^b EI – economic importance, V – vegetable; O – ornamental; M – medicinal; ^c AM – arbuscular mycorrhizal; DSE – dark septate endophyte; + – presence; – – absence; ^d A – *Arum*-type; I – intermediate-type; P – *Paris*-type; ^e AM – arbuscular mycorrhizal; NM – non-mycorrhizal; NR – no report; ^f Tr – tropical; Te – temperate; STr – subtropical; NR – no report; NA – no association.

^{#1} Wang & Qiu (2006), ² Li *et al.* (2007), ³ Castillo *et al.* (2009), ⁴ Muthukumar & Tamilselvi (2010), ⁵ Dickson *et al.* (2007), ⁶ Muthukumar *et al.* (2006), ⁷ Akond *et al.* (2008), ⁸ Jumpponen & Trappe (1998), ⁹ Muthukumar & Prakash (2009), ¹⁰ Fracchia *et al.* (2009), ¹¹ Zhang *et al.* (2011), ¹² Songachan & Kyang (2012), ¹³ Gashua *et al.* (2015).

variables. Percentage data on root colonization were arcsine-transformed prior to analysis.

RESULTS

SOIL CHARACTERISTICS

The soil was sandy loam at sites II and III, and sandy clay loam at sites I and IV (Table 2). The soil was acidic at site IV (5.70) and basic at the other sites. Electrical conductivity ranged between 0.16 dSm⁻¹ (sites I and III) and 0.24 dSm⁻¹ (sites II and IV). The soil was low in nutrients, especially phosphorus, which ranged from 3.6 kg ha⁻¹ (site III) to 9 kg ha⁻¹ (site I). Total nitrogen in soils

was similar (90 kg ha⁻¹) at all sites. Exchangeable potassium in soil was moderately high at all the sites except at site III (162 kg ha⁻¹) (Table 2).

DISTRIBUTION OF FUNGAL ASSOCIATION

All 20 solanaceous plants examined were mycorrhizal (Table 2). The mycorrhiza in Solanaceae was of AM type (Fig. 1). Entry of fungal hyphae into roots was preceded by the formation of an appressorium on the root surface, and an infection peg penetrating the rhizodermal cells (Fig. 1a–c). The hyphae from the infection peg coiled or were linear in the epidermal cells (Fig. 1c, d). Colonization in the cortical region was by means of linear hyphae

which were inter- or intracellular, and hyphal or arbusculate coils and vesicles (Fig. 1e–p). The linear hyphae sometimes bore distinct oil globules; they were smooth or contained peg-like projections (Fig. 1f, n). The vesicles were mostly intracellular and ranged in number from one to three per cell (Fig. 1q, r). Occasionally, AM fungal sporulating structures such as sporiferous saccules were seen in the cortex of *S. nigrum* and *L. esculentum* from site I (Fig. 1s).

DSE fungal colonization was found in only 16 (80%) of the 20 plant species examined (Table 2). There were no DSE fungal associations in *Solanum tuberosum*, *S. viarum*, *S. sisymbirifolium* and *S. pubescens*. *Capsicum frutescens*, *Datura metel* and *Solanum melangena* from certain sites showed no DSE fungal colonization, though root samples of these species from other sites did contain DSE fungal structures.

EXTENT OF AM COLONIZATION

The extent of AM fungal colonization and the percentage of root length with different AM fungal structures varied significantly between species (Table 3). Total colonization percentage (%RLTC) generally was high, ranging between 34.00% in *Solanum trilobatum* (site I) and 81.15% in *S. viarum* (site IV). The percentage of root length with hyphae (%RLH) ranged between 11.18% in *S. nigrum* (site I) and 26.69% in *S. viarum*. The percentage of root length with arbusculate coils (%RLAC) varied between 19.55% in *Datura metel* (site IV) and 49.53% in *S. nigrum* (site IV). The percentage of root length with hyphal coils (%RLHC) ranged from 4.43% in *S. melangena* (site I) to 34.56% in *Capsicum frutescens* (site II). The percentage of root length with vesicles (%RLV) ranged between 0.76% in *S. nigrum* (site I) and 34% in *S. trilobatum* (site I). Vesicles were absent in 13 root samples.

Capsicum annum, *Lycopersicon esculentum*, *Solanum trilobatum* and *S. torvum* from two different sites were examined (Table 2). There was no significant variation in the %RLTC or AM fungal structures in *L. esculentum*, except for %RLHC (Table 4). For *C. annum*, %RLAC was the only

AM fungal variable that exhibited significant variation between sites. %RLH was the only AM fungal variable that exhibited significant variation in *S. trilobatum* and *S. torvum*.

Capsicum frutescens showed no variation of AM fungal variables between sites. In *Solanum melongena*, the only AM fungal variables that showed significant variation between sites were %RLH and %RLHC. In *Datura metel*, only %RLAC and %RLHC exhibited significant between-site variation. In *Solanum nigrum*, %RLH, %RLAC and %RLV exhibited significant variation between sites.

Soil pH was significantly and negatively correlated with %RLH ($r = -0.361$; $p < 0.04$; $n = 33$) and %RLTC ($r = -0.464$; $p < 0.01$; $n = 33$). As %RLTC was significantly and positively correlated with soil EC ($r = -0.365$; $p < 0.04$; $n = 33$), it was significantly and negatively correlated with P ($r = -0.400$; $p < 0.02$; $n = 33$).

AM MORPHOLOGICAL TYPES

Six solanaceous species had typical *Paris*-type AM morphology, characterized by the presence of hyphal coils, with the rare occurrence of arbusculate coils and intracellular vesicles (Table 2, Fig. 1). The majority of investigated species (12) had intracellular hyphal coils or arbusculate coils with inter- or intracellular linear hyphae, characteristic of intermediate-type AM morphology. Two of these species had intermediate-type 1 (I1); intermediate-type 3 (I3) and intermediate-type 4 (I4) morphology were seen in five species each. Species with I3 morphology had intracellular linear hyphae with intracellular hyphal or arbusculate coils; in those with I4 morphology the linear hyphae were intercellular, with intracellular hyphal or arbusculate coils. In *Nicotiana tabacum* and *Datura metel*, I1 morphology was characterized by the presence of intercellular linear hyphae with intracellular arbusculate coils developing on their lateral branches (Fig. 1I, p). *Solanum melongena* had intermediate I3 type in samples from sites I and III, and *Paris*-type in samples from site II. *Solanum nigrum* had *Paris*-type morphology in material from site IV, and intermediate I3 in that

from sites I, II and III. Typical *Arum*-type AM morphology was not observed in any of the root samples examined (Table 2). *Solanum trilobatum* had *Paris*-type morphology in samples from site II, and lacked AM fungal structures except for hyphae and vesicles in samples from site I.

EXTENT OF DSE COLONIZATION

The percentage of total root length with DSE fungal hyphae (%RLDSTC) ranged between <1% in *Solanum nigrum* (site IV) and 36.42% in *S. trilobatum* (site I) (Table 4). Microsclerotia or moniliform hyphae were not observed in any of the root samples examined. %RLDST exhibited significant differences between sites in *Capsicum annuum*, *C. frutescens*, *Datura metel*, *Solanum nigrum*, *S. melongena* and *S. trilobatum*. %RLDTC was significantly and positively correlated with %RLV ($r=0.516$; $p<0.002$; $n=33$) and negatively with %RLAC ($r=-0.510$; $p<0.002$; $n=33$). We noted a significant negative correlation between %RLDSTC and %RLTC ($r=0.64$; $P<0.001$; $n=34$).

DISCUSSION

Arbuscular mycorrhizas are multifunctional in both natural systems and agroecosystems, and enhance the physical, chemical and biological quality of soils (Cardoso & Kuyper 2006). For nine of the solanaceous species we examined this is the first assessment of their mycorrhizal status. The occurrence of AM fungal associations in all the examined plant species from different sites confirms the widespread occurrence of AM fungal associations in this family. Some of the solanaceous

plants we examined, such as *Capsicum annuum*, *Nicotiana tabacum*, *Solanum melongena*, *S. nigrum*, *S. torvum*, *S. trilobatum*, *S. tuberosum*, *Datura metel*, *D. stromanium* and *Lycopersicon esculentum*, were reported to be mycorrhizal in earlier work (Ragupathy & Mahadevan, 1993; Muthukumar & Udaiyan 2000; Wang & Qiu 2006; Muthukumar *et al.* 2006; Li *et al.* 2007; Akond *et al.* 2008; Wang & Shi 2008; Muthukumar & Prakash 2009; Muthukumar & Tamilselvi 2010). Our finding of the presence of AM colonization in roots of *Datura innoxia* stands in contrast to observations by Ragupathy and Mahadevan (1993); they found it to be nonmycorrhizal in material from the tropical plains of Tamilnadu, India. Though there is no detailed information on the extent of variation of intraradical structures of different AM fungi, the available evidence suggests that in certain instances the intraradical structures produced may indicate the AM fungal taxa involved. For example, members of Gigasporaceae are known to possess intraradical hyphae with knob-like projections and inflated areas (Bentivenga & Morton 1995). The peg-like projections we observed on the intraradical hyphal surface suggests possible colonization by members of the Gigasporaceae. Similar intraradical hyphal morphology has also been reported in pteridophytes and cycads (Muthukumar & Udaiyan 2002; Muthuraja *et al.* 2014). Structures resembling the sporiferous saccules of Acaulosporaceae were also observed in roots of certain solanaceous taxa. Sporulation of Acaulosporaceae taxa within plant roots has been reported previously (e.g., Schultz *et al.* 1999).

The extent of AM colonization observed in the present study (34–81%) is within the range

Fig. 1. Arbuscular mycorrhizal (AM) and dark septate endophyte (DSE) fungal colonization in Solanaceae roots. a – appressorium (ap) on root surface and infection peg (arrow head) in *Solanum nigrum*; b & c – extraradical mycelium (em), appressorium (ap) and fungal hyphae (f) in rhizodermal cells (rc) of *S. pubescens* and *Lycopersicon esculentum*; d – intracellular linear hyphae (lh) in *Brumansia sanguine*; e – hyphal coils (hc) in cortical cells of *Capsicum frutescens*; f – intracellular hyphae with oil droplets (arrowheads) in *Datura innoxia*; g – arbuscules (a) in *Solanum nigrum*; h – arbusculate coils (ac) in *Lycopersicon esculentum*; i – arbusculate coil (ac) and intercellular hyphae (arrow heads) in *Nicotiana tabacum*; j – hyphal coil in *Solanum pseudocapsicum*; k – arbuscules in *S. pubescens*; l – intracellular hyphae (ih) bearing arbuscules in *S. viarum*; m – arbusculate coils (ac) in *Datura stramonium*; n – intracellular hyphae with peg-like projections in *Nicotiana tabacum*; o – hyphal coil (hc) in *Solanum trilobatum*; p – intercellular hyphae (arrow head) and arbusculate coil in *Datura metel*; q – intracellular vesicle (v) in *D. innoxia*; r – intracellular vesicles (v) in *Lycopersicon esculentum*; s – sporiferous saccule (ss) in cortex of *Solanum nigrum*; t & u – melanized hyphae of DSE fungi in *S. trilobatum* (t) and *S. melongena* (u). Scale bars = 25 μ m.

Table 3. Extent of arbuscular mycorrhizal (AM) and dark septate endophyte (DSE) fungal associations in members of the nightshade family.

Plant species	Site ^a	% AM colonization ^b					%DSE colonization ^c
		%RLH	%RLAC	%RLHC	%RLV	%RLTC	
<i>Brugmansia sanguinea</i>	IV	19.37±1.41 ^d	28.12±2.16	22.49±0.70	–	69.98±0.74	10.30±1.89
<i>Capsicum annuum</i>	I	–	35.89±1.29	21.04±3.86	5.76±2.91	62.68±1.53	5.26±1.40
	II	–	37.87±1.14	26.30±2.05	–	64.17±3.04	11.65±1.59
<i>C. frutescens</i>	II	–	30.83±0.32	34.56±1.58	6.37±3.36	71.76±2.55	11.42±2.15
	III	–	37.93±4.55	28.87±5.65	4.44±3.39	71.25±3.73	–
	IV	–	39.35±3.18	26.28±2.06	–	65.63±1.16	18.07±3.18
<i>Datura metel</i>	I	22.08±1.95	38.55±4.05	5.46±0.47	5.65±1.83	71.74±2.77	–
	II	18.73±1.23	21.08±1.22	23.46±2.43	8.60±0.81	71.87±1.36	7.03±0.05
	III	16.02±1.09	28.52±3.29	17.78±1.11	7.22±1.11	69.54±2.82	8.98±2.52
	IV	22.85±3.51	19.55±1.86	26.04±3.75	3.51±1.90	71.95±0.11	7.90±2.60
<i>D. stramonium</i>	I	14.19±2.00	30.47±3.57	5.45±1.93	–	50.11±1.59	10.56±2.16
<i>D. innoxia</i>	III	14.64±1.37	26.41±1.44	22.43±2.28	12.44±1.87	75.91±2.11	9.10±0.96
<i>Lycopersicon esculentum</i>	I	13.54±2.63	25.12±3.12	10.65±3.78	14.79 ±3.88	64.11±1.31	7.14±4.32
	II	16.17±1.20	28.70±3.34	17.16±2.56	3.70±2.45	65.74±0.92	6.27±0.71
<i>Nicotiana tabacum</i>	I	14.76±2.12	23.86±2.37	20.62±1.89	1.67±0.84	60.90±4.24	15.85±2.67
<i>Physalis angulata</i>	III	22.27±2.78	33.03±4.06	21.22±3.13	–	76.52±3.78	4.12±0.57
<i>Solanum melongena</i>	I	17.08±3.17	46.94±4.27	4.43±1.27	2.13±2.13	70.58±3.99	–
	II	–	38.65±2.17	29.44±3.48	–	68.10±1.98	13.44±2.19
	III	18.95±4.22	45.08±4.80	5.07±1.14	4.26±0.36	73.36±0.64	8.41±1.12
<i>S. nigrum</i>	I	11.18±0.40	47.44±2.58	11.81±1.59	0.76±0.76	71.19±3.24	2.03±0.15
	II	19.05±4.13	30.30±2.08	19.52±3.69	–	68.87±0.30	7.49±0.63
	III	18.07±1.86	24.43± 2.07	23.53±1.60	7.19±1.61	73.22±2.68	9.47±0.32
	IV	–	49.53±4.35	18.02±4.50	–	67.55±1.95	0.79±0.19
<i>S. virginianum</i>	I	22.72±3.07	44.03±2.63	13.51±3.24	–	80.26±4.74	6.08±4.07
<i>S. trilobatum</i>	I	–	–	–	34.00±6.33	34.00±6.33	36.42±2.89
	II	–	33.97±3.31	24.93±4.68	11.01±1.67	69.91±3.01	1.90±1.90
<i>S. torvum</i>	I	–	38.55±0.90	18.42±1.32	3.56±0.99	60.54±1.37	7.61±3.12
	II	–	29.49±2.60	31.41±0.64	9.33±0.84	70.23±1.37	6.91±1.96
<i>S. tuberosum</i>	IV	–	31.02±8.07	17.71±4.65	–	48.73±4.74	–
<i>S. viarum</i>	IV	26.69±2.53	33.69±3.18	20.77±1.09	–	81.15±2.26	–
<i>S. sisymbirifolium</i>	IV	23.26±5.62	23.33±1.50	24.12±6.03	9.96±0.82	80.66±2.85	–
<i>S. pseudocapsicum</i>	IV	–	39.45±2.15	32.78±5.13	–	72.23±4.17	7.63±1.95
<i>S. pubescens</i>	II	22.35±0.97	21.29±2.69	24.84±2.91	9.46±2.17	77.94±2.08	–
<i>S. elaeagnifolium</i>	II	15.34±1.10	25.35±1.60	19.72±2.36	–	60.41±1.34	9.36±0.52

^a I, II, III and IV indicates Erode, Coimbatore, Pollachi and Ooty; ^b %RLH, %RLA, %RLV, %RLHC and %RLTC indicate percentage of root length with hyphae, arbuscules/arbusculate coils, vesicles, hyphal coils and total colonization; ^c %RLDSTC indicates percentage of root length with DSE total colonization; ^d Mean ±SE.

(59–97%) reported earlier for solanaceous crop species by Muthukumar and Tamilselvi (2010). The AM colonization levels we found are higher than those reported for three solanaceous plants (35–38%) from Meghalaya, India (Songachan & Kayang 2012). Akond *et al.* (2008) also reported

low incidence of mycorrhizal associations (36%) in Solanaceae vegetable crop plants from Bangladesh. The moderate mean level of AM fungal colonization (69%) observed in the present study is consistent with findings from Muthukumar and Udaiyan (2000), Muthukumar *et al.* (2006) and

Table 4. Summary of F and t statistics for arbuscular mycorrhizal (AM) and dark septate endophyte (DSE) fungal variables for Solanaceae root samples from different sites.

Plant species	Test df	AM ^a						DSE ^b	
		%RLH	%RLA	%RLHC	%RLV	%RLTC	%RLDSTC		
<i>Capsicum annum</i>	t ₂	—	-2.9775*	-0.8997ns	1.97803ns	-0.5718ns	-4.6023*		
<i>Lycopersicon esculentum</i>	t ₂	-1.8218 ns	-0.8218	-3.5722*	1.7533ns	-0.8845ns	0.2407ns		
<i>Solanum trilobatum</i>	t ₂	—	-10.2467**	-5.3299*	2.8766*	-4.4651*	7.5393**		
<i>Solanum torvum</i>	t ₂	—	3.8876*	-9.6115**	-30.739***	-4.963*	0.5993ns		
<i>Capsicum frutescens</i>	F _{2,6}	—	2.0258ns	1.3945ns	1.4028ns	1.5948ns	16.956**		
<i>Solanum melongena</i>	F _{2,6}	11.748**	1.2325ns	40.6019***	2.9265ns	1.0289ns	22.9518**		
<i>Datura metel</i>	F _{3,8}	2.1139ns	9.3752**	15.6466**	2.1593ns	0.3072ns	5.0443*		
<i>Solanum nigrum</i>	F _{3,8}	14.9015**	18.1577***	2.4293ns	15.4594***	1.666ns	60.730***		

^a %RLH, %RLAC, %RLV, %RLHC and %RLTC indicates percentage of root length with hyphae, arbuscules/arbusculate coils, vesicles, hyphal coils and total colonization; ^b %RLDSTC indicate percentage of root length with DSE total colonization.

ns – not significant; *, **, *** – significant at P<0.05, P<0.01, and P<0.001 respectively.

Fracchia *et al.* (2009) for mean AM colonization levels in solanaceous plant species: 54% in material from the Western Ghats, and 70% in material from the Chacco Serrano Woodland, central Argentina. We found that *Solanum melongena* had 71% of its root length colonized by AM fungi, whereas Akond *et al.* (2008) reported a much lower colonization level (38%) for the same species. The high colonization levels we observed in solanaceous plant roots may be due in part to sampling time – summer, when plants tend to have high photosynthetic activity, resulting in more allocation of carbon to roots and AM fungi, resulting in higher colonization (Shamim *et al.* 1994). Also, we sampled the plants in their vegetative stage, and the soil at all the study sites had low P levels, a factor which may have also contributed to the high percentages of root length colonized by AM fungi.

Here we reported AM morphology for the first time in 12 plant species. In this study, 8 (40%) of the 20 plant species had *Paris*-type AM morphology, higher than the 15% reported for members of this family by Dickson *et al.* (2007). Our finding of the absence of typical *Arum*-type morphology in this family contrasts with findings from Fracchia *et al.* (2009), who reported *Arum*-type AM morphology in 2 of the 8 taxa they examined. Intermediate-type morphology was dominant (present in 14 of our 20 species), which is in line with findings by Muthukumar and Tamilselvi (2010): 75% of

the solanaceous crop species they examined had intermediate-type AM morphology. *Lycopersicon esculentum* (Dickson *et al.* 2007) and *Nicotiana tabacum* (Muthukumar & Tamilselvi 2010) have been shown to possess intermediate-type morphology. In intermediate-type 1 (I1) colonization, the lateral hyphae developing from intercellular linear hyphae and penetrating the cortical cells bear *Arum*-type arbuscules (Dickson 2004). In *N. tabacum* and *Datura metel*, the lateral hyphae originating from intercellular linear hyphae and penetrating the cortical cells produced intracellular arbusculate coils instead of arbuscules. This type appears to be a variant of I1 proposed by Dickson (2004). We designate it as I1ac.

In *S. nigrum* we found intermediate- and *Paris*-type morphologies in material from different sites, supporting observations by Muthukumar and Prakash (2009) and Muthukumar and Tamilselvi (2010), who noted *Paris*-type and intermediate-type morphology in material of this species from different tropical agroecosystems. *Solanum sisymbirifolium*, which Fracchia *et al.* (2009) found to possess *Paris*-type AM morphology in samples from the Chacco Serrano Woodland, had intermediate-type AM morphology in material from our site IV. Possibly the difference is due to the same host species being colonized by different fungal species at different sites, as previous work has clearly shown that AM fungal species can modify the colonization patterns of their associated plant

species to a certain extent (see Dickson *et al.* 2007 and references therein).

Our finding of a negative correlation between AM fungal colonization and soil pH is in agreement with other studies in which such a relationship was found in coconut, potato and medicinal plants (Das & Kayang 2010; Rajeshkumar *et al.* 2015; Wang & Jiang 2015). A number of soil and fungal factors are affected by soil pH. For example, germination of AM fungal spores and the development of extraradical hyphae are influenced by changes in soil pH (Hepper 1984; van Aarle *et al.* 2002). The availability of nutrients in the soil is also influenced by soil pH (Xu *et al.* 2016), and that may be part of the effect of soil pH on AM fungal colonization. In contrast to findings by Halder *et al.* (2015) and Wang and Jiang (2015), we obtained a positive correlation between soil EC and AM fungal colonization. Soil electrical conductivity, which is an excellent indicator of nutrient availability, was low in our soil samples. Although soils with EC less than 1 dS/m are considered to be nonsaline and do not affect microbial processes, soil EC in the range of 0.028–0.270 dS/m is reported to negatively influence AM fungal colonization (Halder *et al.* 2015; Wang & Jiang 2015). However, Rajeshkumar *et al.* (2015) reported a significant positive correlation between soil EC and root length colonized by AM fungi in coconut growing in soils having an EC range of 0.018–0.122 dS/m. As inherent soil and climatic factors modulate the effect of EC on biological processes, the divergent results on the influence of EC on AM fungal colonization in these studies are tenable. A number of AM fungal processes such as spore germination and mycorrhization are affected by increasing soil P, so a negative correlation between soil P and AM fungal colonization is not surprising (Liu *et al.* 2016).

Melanized, darkly pigmented sterile hyphae were observed in the root cortex of 80% of the examined solanaceous taxa, higher than the 65% figure reported for members of this family from the Chacco Serrano Woodland, Argentina (Frachia *et al.* 2009), but in agreement with previous studies reporting a high frequency of DSE fungal species in herbaceous plant species (Ruotsalainen

et al., 2002; Urcelay 2002; Barrow 2003). Solanaceous plants have a high affinity for DSE fungi. The highest number of DSE fungal isolates have frequently been obtained from roots of solanaceous plants (Diene *et al.* 2014). For example, Narisawa *et al.* (2007) showed that eggplant was a particularly effective species for baiting the DSE fungus *Heteroconium chaetospora* from the soil. Here we reported ten plant species as hosts for DSE fungi for the first time. Our finding of DSE fungal associations in *Capsicum annuum*, *Nicotiana tabacum* and *Solanum melongena* extend the presence of this association in tropical agroecosystems (Muthukumar & Tamilselvi 2010). Colonization of *S. tuberosum* and *S. nigrum* by DSE fungi, which we report here, was previously described in those species from temperate agroecosystems (Jumpponen & Trappe 1998). The effect of DSE on host plants differs depending on the host and on growth conditions. Their abundance in natural ecosystems is ubiquitous and is thought to be important in any ecosystem. We determined that the DSE-colonized plants also had AM fungal associations, as others have found (Sengupta *et al.* 1989; Muthukumar *et al.* 2006; Muthukumar & Tamilselvi 2010). Chaudhary *et al.* (2009) contended that the simultaneous occurrence of DSE and AM fungi indicates the dynamic nature of the endophyte community in natural ecosystems. During unfavorable conditions, DSE fungi can function as mutualists by taking up water and nutrients from the soil and translocating them to plant roots (Mandyam & Jumpponen 2005). The negative correlation between AM and DSE fungal colonization agrees with statements by Muthukumar and Tamilselvi (2010) who reported a similar relationship in crop plants. On the other hand, Songachan and Kayang (2012) reported a positive correlation between these fungal variables in roots of three *Solanum* species. We found that the share of AM fungal colonization in roots of solanaceous plants was 4-fold to 86-fold higher than the share of DSE fungi. A low share of root length colonization (<1%) by DSE fungi was reported by Songachan and Kayang (2012). The negative correlation between the AM and DSE fungal variables and the low percentage of root length colonization by DSE fungi clearly suggest

an interaction between these fungal types within plant roots. Generally, DSE fungi are known to act as mutualists in improving plant growth and yield only under conditions that are unfavorable for AM fungi, and the direct influence of AM fungi on DSE fungi is yet to be demonstrated as it has for the ectomycorrhizal fungi. For example, the degree of mycorrhization by the ectomycorrhizal fungus *Hebeloma crustuliniforme* has been shown to significantly reduce the biomass of the DSE fungal *Phialocephala fortinii* s.l.–*Acephala ap-planata* species complex in Norway spruce (*Picea abies*) roots (Reininger & Sieber 2013).

This study found AM and DSE fungal associations in solanaceous plants. Although the effects of AM fungi on growth and nutrient uptake have been demonstrated in solanaceous crops, such benefits are yet to be established for DSE fungi. As many solanaceous crops are propagated in nurseries and *in vitro*, early association of seedlings or plantlets with efficient endorhizal fungal symbionts might enhance their survival and growth in field conditions.

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