

Endorrhizal fungal symbiosis in aroids of the Western Ghats, southern India

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Abstract

Information of dark septate endophyte (DSE), arbuscular mycorrhizal (AM), and fine root endophyte (FRE) fungal symbioses of aroids in the Western Ghats region are very low. Therefore, we assessed the endorrhizal symbiosis in 25 aroid species belonging to 16 genera of Araceae from six different locations of the Western Ghats. The results revealed co-occurrence of the DSE and AM symbiosis in all the examined aroids, and FRE presence in seven aroids (*Alocasia × amazonica*, *Alocasia* sp., *Anthurium andraeanum*, *Epipremnum aureum*, *Spathiphyllum* sp., *Syngonium podophyllum*, and *Zantedeschia aethiopica*). We found variance in root length having AM (inter and intracellular hyphae, arbuscules, vesicles and arbusculate coils) and DSE (melanized septate hyphae, microsclerotia, and moniliform hyphae) fungal structures. Moreover, the AM fungal morphology of *Arum-Paris* type was widespread, and intermediate type morphology reported for the first time in five aroids. AM fungi colonized the roots of *Philodendron xanadu* the most, followed by DSE in *Caladium bicolor*, and FRE in *Spathiphyllum* sp. AM fungal spores were present in all aroid soils examined. The percentage of root length comprising FRE hyphae was significantly and positively correlated root length with FRE arbuscules, AM fungal spore numbers and total colonization. Our study revealed that, the aroids tend to form associations with various endorrhizal fungi.

Keywords: AM fungi; Araceae; colonization; DSE fungi; FRE fungi

Introduction

The members of the family Araceae, commonly recognized as *Arum* family, are colloquially known as aroids. Araceae is the third largest family among monocots with eight subfamilies, and is the oldest group of angiosperms (Croat, 2019; Stevens, 2020). It comprises of herbaceous plants widely distributed in Asia and tropical American regions (Croat *et al.*, 2018). Around 144 genera and 3645 species of aroids are reported worldwide (Cabrera *et al.*, 2008; Boyce and Croat, 2018) and about 60% of the species reported have Neotropical distribution. The genera *Anthurium* (950 species) and *Philodendron* (482 species) present the highest species diversity among aroids (Boyce and Croat, 2011). Most of the aroids in the genera *Alocasia*,

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Anthurium, *Philodendron*, *Dieffenbachia*, *Monstera*, and *Zantedeschia* are valued as ornamentals (Pedralli, 2002). In addition, aroids belonging to *Amorphophallus*, *Colocasia*, *Xanthosoma*, *Typhonium*, and *Monstera* are consumed as food (Zulhazman *et al.*, 2021) and for treating human ailments (Swain *et al.*, 2022).

Endorrhizal fungi are symbiotic and non-pathogenic, which gives habitat fitness to genetically varied host plants and they also provide resistance to plants against several abiotic stresses (Diagne *et al.*, 2020; Begum *et al.*, 2019). Dark septate endophyte (DSE) and arbuscular mycorrhizal (AM) fungal symbiosis are two of the most important forms of endorrhizal fungal symbioses having great ecological importance. The widespread AM fungi are placed under phylum Mucoromycota and subphylum Glomeromycotina (Spatafora *et al.*, 2016). The fungi colonize over 72% of the plants growing in the natural environment (Brundrett and Tedersoo, 2018).

In exchange for carbon, the AM fungi enhances the availability and transfer of various nutrients like potassium (K), nitrogen (N), phosphorus (P), and further minerals to plants from nutrient deficient soil (Smith and Read, 2008; Roupael *et al.*, 2015). AM fungus form different structures within the plant roots, such as hyphae, vesicles, arbuscules, hyphal and arbusculate coils (Muthukumar *et al.*, 2016). The pattern of AM fungal colonization is primarily separated into the *Arum*, *Paris*, and intermediate types based on the distribution of AM fungal structures in plant roots. The *Arum* type, which is prevalent in field crops, has intracellular linear hyphae and forming arbuscules on the short lateral branches (Smith and Read, 2008). Fungal hyphae spread intracellularly among root cells in the *Paris* type, forming arbusculate or hyphal coils (Dickson *et al.*, 2007). Brundrett (2004) refers to the *Arum* and *Paris* types as 'linear' and 'coiling' respectively, based on longitudinal hyphal formation inside the roots. The intermediate type has features of *Paris* type and *Arum* type with the presence of linear hyphae that can be intra- and/or intercellular hyphal and arbuscular coils (Dickson *et al.*, 2004).

The endorrhizal association in Araceae family has not been investigated adequately. For example, AM fungi have been found to colonize Araceae plants including *Alocasia macrorrhizos*, *Amorphophallus bannaensis*, *Rhaphidophora decursiva*, *Arisaema atrovirens*, *Scindapsus aureus*, *Pistia stratiotes*, *Amorphophallus paeniifolius*, *Anthurium affine*, and *Caladium bicolor* (Santos *et al.*, 2000; Muthukumar *et al.*, 2003; Öpik *et al.*, 2013) and some of these species exhibit AM morphology typical to those of *Arum* or intermediate type (Smith and Smith, 1997; Muthukumar *et al.*, 2003). Contrarily, certain aroids such as *Colocasia esculenta*, *P. stratiotes*, *Anthurium consobrinum*, *Anthurium trinerve*, and *Monstera tuberculata* were reported to lack AM fungal structures (Lesica and Antibus, 1990; Seerangan and Thangavelu, 2014).

In addition to typical AM fungi, there are other arbuscule-forming fungi known as fine root endophytes (FRE) that coexist with other fungal endophytes and belong to subphylum Mucoromycotina of Mucoromycota (Orchard *et al.*, 2017). Fine root endophytes are globally distributed and play vital role in both agricultural and semi-natural habitats, across a wide range of host plants (Field *et al.*, 2015; Orchard *et al.*, 2017). Although the benefit of FRE symbiosis to plants is not well resolved like that for AM association, available evidence indicate that FRE fungi like AM fungi can aid plants in their nutrient acquisition (Hoysted *et al.*, 2021; Howard *et al.*, 2022). Nevertheless, the FRE symbiosis in aroids is not well reported as those for AM fungi (Muthukumar and Karthik, 2021).

DSE fungi are distributed worldwide and have functional and ecological level interactions with other soil biota (Mayerhofer *et al.*, 2013). The DSE fungi have melanized regularly septate hyphae (may also be hyaline) that produce clusters of inflated, round, thick-walled cells in the cortical region, known as microsclerotia (Jumpponen and Trappe, 1998). DSE fungi are found in a wide range of environments and host plants, indicating a lack of host and habitat specificity (Mandyam and Jumpponen, 2005). The DSE fungi commonly co-occur with various mycorrhizal fungi and have been noticed in a variety of aromatic plants, field crops, and horticultural plants (Muthukumar *et al.*, 2018; Piszczek *et al.*, 2019). Analogous to AM fungi, the DSE fungi assist plants through enhancing growth, absorption of nutrients, and imparting adaptability to

various environmental circumstances (Vergara *et al.*, 2018; Farias *et al.*, 2020). DSE fungi along with AM fungi reported to co-occur in the roots of *Amorphophallus paenifolius* and *Stenospermatum* species (Rains *et al.*, 2003; Thangavelu and Tamilselvi, 2010).

Endorrhizal fungal symbiosis, as an important component of the soil microbial community in the terrestrial environment, that benefits plants in various ways. AM fungal distribution and activity studies aid in determining the ecological relevance of AM associations (Gianinazzi *et al.*, 2010). As Araceae plants are adapted to different life-forms, investigation on endorrhizal association in these plants growing in various habitats could help in understanding the beneficial role of AM and DSE fungi in these plants. Moreover, the AM and DSE fungal structures of Araceae plants have not been extensively quantified. Therefore, we focused on the following objectives: a) to investigate the occurrence and extent of AM, FRE, and DSE fungal associations, b) to evaluate the colonization patterns and morphology of AM fungi, c) to estimate the diversity of AM fungal spores and d) to determine the relationship of AM, FRE, and DSE fungal structures in selected Araceae taxa belonging to different genera growing in the southern Western Ghats.

Materials and Methods

Site description and aroid species examined

The structural quantification of AM and DSE fungi within the roots of aroids is inadequate. So, the soil samples and root of 25 aroid species belong to 16 genera were collected during January and February 2021 from six different localities of Tamilnadu, India including Bharathiar University campus (Site I; 11°04'N to 76° 93' E), and a Home Garden (Site II; 10° 59' N to 76° 59' E) in Coimbatore. The list of collected aroid species and their locations are presented in Table 1. The average annual maximum and minimum temperatures are 34.7 °C and 21.6 °C respectively and the annual rainfall is 8.7 mm for both site I and II (IMD 2020); Tirupur (Site III; 11° 6' N to 77° 20' E) has an average annual maximum and minimum temperatures of 36.8 °C and 21.6 °C respectively and the annual rainfall is 28.3 mm for site III (IMD 2021); Gobichettipalayam (Site IV; 11° 27' N to 77° 26' E) has an average annual maximum and minimum temperatures are 35.0 °C and 22.1 °C (IMD 2021) for site IV. Ooty (Site V; 11° 24' N to 76° 41' E), and Coonoor (Site VI; 11° 21' N to 76° 47' E) have an average annual maximum and minimum temperatures are 25.5 °C and 13.6 °C respectively and the annual rainfall is 32.6 mm for both site V and VI (IMD 2021). The soils at the sampling sites had a pH of 5.9-10.4, electrical conductivity of 0.13-0.84, 110-190 kg/acre of total nitrogen, 6-24 kg/acre of available phosphorus and 90-270 kg/acre of exchangeable potassium as evaluated according to standard procedures (Jackson, 1971).

Table 1. Status (ST)/economic importance (EI), site of collection, and life-forms of different aroids examined in the study

Aroid species	ST/EI [†]	Site ^{††}	Life form ^{†††}
<i>Alocasia xamazonica</i> Reark	OP	II	TE
<i>Aglaonema commutatum</i> Schott	OP/M	I	TE
<i>Alocasia cuprea</i> K.Koch	OP	II	TE
<i>Alocasia brisbanensis</i> (F.M. Bailey) Domin	OP	VI	TE
<i>Alocasia macrorrhizos</i> (L.) G.Don	OP	V	TE
<i>Alocasia odora</i> (Lindl.) K.Koch	OP/M	III	TE
<i>Alocasia</i> sp.	OP/M	II	TE
<i>Anthurium andraeanum</i> Linden ex André	OP/M	II	TE
<i>Caladium bicolor</i> (Aiton) Vent.	OP/M	I	TE
<i>Colocasia esculenta</i> (L.) Schott	OP/M	VI	TE
<i>Dieffenbachia seguine</i> (Jacq.) Schott	OP/M	I	TE

<i>Epipremnum aureum</i> (Linden & André) G.S.Bunting,	OP/M	I	E
<i>Hamlomena</i> sp.	OP/M	V	TE
<i>Peltandra virginica</i> (L.) Schott	M	IV	TE
<i>Philodendron xanadu</i> Croat, Mayo & J.Boos	OP	I	TE
<i>Philodendron burle-marxii</i> G.M.Barroso	M	I	TE
<i>Philodendron goeldii</i> G.M.Barroso	OP	I	TE
<i>Philodendron hederaceum</i> (Jacq.) Schott	M	I	TE
<i>Pistia stratiotes</i> L.	OP/M	V	A
<i>Remusatia vivipara</i> (Roxb.) Schott	M	II	TE
<i>Spathiphyllum</i> sp.	M	I	TE
<i>Spathiphyllum wallisii</i> Regel	OP/M	I	TE
<i>Syngonium podophyllum</i> Schott	OP	I	TE
<i>Xanthosoma sagittifolium</i> (L.) Schott	M	VI	TE
<i>Zantedeschia aethiopica</i> (L.) Spreng.	OP/M	V	TE

†OP: Ornamental plant; M: Medicinal plant.; ††Site I: Bharathiar University campus (Coimbatore), Site II: Home Garden (Coimbatore), Site III: Tirupur, Site IV: Gobichettipalayam, Site V: Ooty, Site VI: Coonoor. †††TE: Terrestrial; E: Epiphyte; A: Aquatic.

Collection of samples

The root zone soil samples of each aroid (except *P. stratiotes*) were collected using a hand trowel and stored in polybags. The terrestrial roots or substrate of all the plants were collected during soil sampling for the enumeration of AM fungal spores. The roots were then thoroughly washed with tap water and stored in FAA containing plastic vials (Alcohol: Formaldehyde: Acetic acid – 90 ml: 5 ml: 5 ml) for the assessment of endorhizal fungal association. The collected soil samples and roots were then labeled. Within 12 hours of collection, the specimen was sent to the laboratory in an ice box. Then the soil samples were air-dried and kept under 4°C for future analysis.

Enumeration, isolation, and characterization of AM fungal spores

The AM fungal spores were isolated and enumerated from 100 g of each soil sample using a modified wet sieve and decanting technique (Muthukumar *et al.*, 2006). AM fungal spores that lacked contents and/or were parasitized by soil microorganisms were considered as spore cases and spores that were intact with contents and were free from parasitism were categorized as intact or healthy spores.

Processing of root samples for endorhizal fungal colonization

Root samples kept in the FAA were washed with several changes of distilled water, cut into 1 cm long sections, and cleared with 2.5% KOH at 90 °C for 2 hours. The cleared root pieces were then washed with water and immersed in 5 N HCl for 15 minutes. After acidification, the roots were soaked in 0.05% trypan blue staining solution and allowed undisturbed overnight to stain (Koske and Gemma, 1989). Excess stain was removed from 30 randomly selected root pieces by placing them in lacto glycerol and placed on clean microscopic slides, covered with cover glasses, squashed gently, and viewed under an Olympus BX51 microscope. The colonization was determined as AM if the roots contained coarse aseptate linear or coiled hyphae (3-15 µm in diameter) along with or without other AM fungal structures like vesicles, arbuscules or arbusculate coils. The FRE colonization was characterized by aseptate fine hyphae that were <2 µm in diameter, small terminal or intercalary hyphal swellings, fan-like branching, and fine arbuscules. The DSE colonization was recognized by the presence of melanized, regularly septate hyphae that formed linear chains of rounded cells (moniliform hyphae) or tight clusters of cells (microsclerotia) within root cortical cells. The images were taken with a ProgRes 3 digital camera. According to McGonigle *et al.* (1990), the percentage of total root length

colonization by endorhizal fungi was determined at one hundred intersections for each root sample at 400×. The type of AM morphology was determined by the distribution of various AM fungal structures as per Dickson (2004).

Statistical analysis

All the data were evaluated for homogeneity (Levene's test) and examined by one-way analysis of variance (ANOVA). Mean separations were performed using Duncan's Multiple Range Test (DMRT, $P < 0.05$). Pearson's correlation analysis was used to examine the relationship between endorhizal fungal structures and type of AM fungal spores. All statistical analysis was performed using SPSS (Statistical Product and Service Solutions, version 16.0).

Results

AM fungal morphology

The roots of all the aroids were invariably colonized by AM fungi. The majority (52%, 13/25) of the mycorrhizal Araceae plant taxa exhibited *Arum - Paris* type morphology (Table 2). However, one (4%) aroid exhibited typical *Arum*-type morphology characterized by intercellular hyphae and intracellular arbuscules (Figure 1). Four aroid species (16%) had AM morphology of intermediate-type 1. However, *Colocasia esculenta* had intermediate-type 2 AM morphology (4%). The remaining (24%) is non-determined (ND).

The presence of intercellular hyphae, hyphal coils, intracellular hyphae, arbusculate coils, arbuscules, intraradical spores, inter and intracellular vesicles, and appressorium was used to determine AM colonization in aroids (Figure 1, Table 2). Fifty-two percentages of aroids had *Arum - Paris* type morphology characterized by intracellular hyphal/arbusculate coils along with intercellular hyphae with arbuscules (Table 2). Four aroids had intermediate type 1 morphology with intercellular hyphae with arbuscules and intracellular hyphae. *C. esculenta* had intermediate type 2 AM morphology with intracellular linear hyphae containing arbuscules. We could not determine AM morphology in *Alocasia amazonica*, *Alocasia macrorrhizos*, *Anthurium andraeanum*, *Caladium bicolor*, *Peltandra virginica*, and *Pistia stratiotes* due to the absence of arbuscules or arbusculate coils.

AM fungal colonization and spore numbers

The extent of AM fungal colonization and root length with various AM fungal structures differed significantly ($P < 0.001$) among aroid species (Table 3). The percentage of root length with total AM colonization (%TAMC) ranged from 3.20% (*P. stratiotes*) to 95.56% (*Philodendron burle-marxii*) (Table 3). The percentage root length with inter- or intracellular hyphae (%RLH) ranged from 3.20% (*P. stratiotes*) to 63.13% (*Xanthosoma sagittifolium*). Similarly, the percentage of root length with the hyphal coils (%RLHC) ranged from 0.25% (*Spathiphyllum* sp.) to 6.72% (*P. goeldii*), while the percentage root length with arbuscules (%RLAR) ranged from 0.39% (*Syngonium podophyllum*) to 43.95% (*Dieffenbachia seguine*). Besides, the percentage of root length with arbusculate coils (%RLAC) ranged from 0.24% (*S. wallisii*) to 14.79% (*Hamlomena* sp.) and the percentage root length with vesicles (%RLV) varied from 0.20% (*Caladium bicolor* and *Epipremnum aureum*) to 9% (*R. vivipara*) (Table 3). The AM fungal spores were found in the rhizospheres of all Araceae plant species, including those with higher AM colonization rates (Table 5). The intact AM fungal spores ranged from 1.66/100 g (*Peltandra virginica*) to 40.33/100 g (*Spathiphyllum* sp.), while spore cases ranged from 30/100 g (*Alocasia brisbanensis*) to 276/100 g (*Epipremnum aureum*) (Table 4).

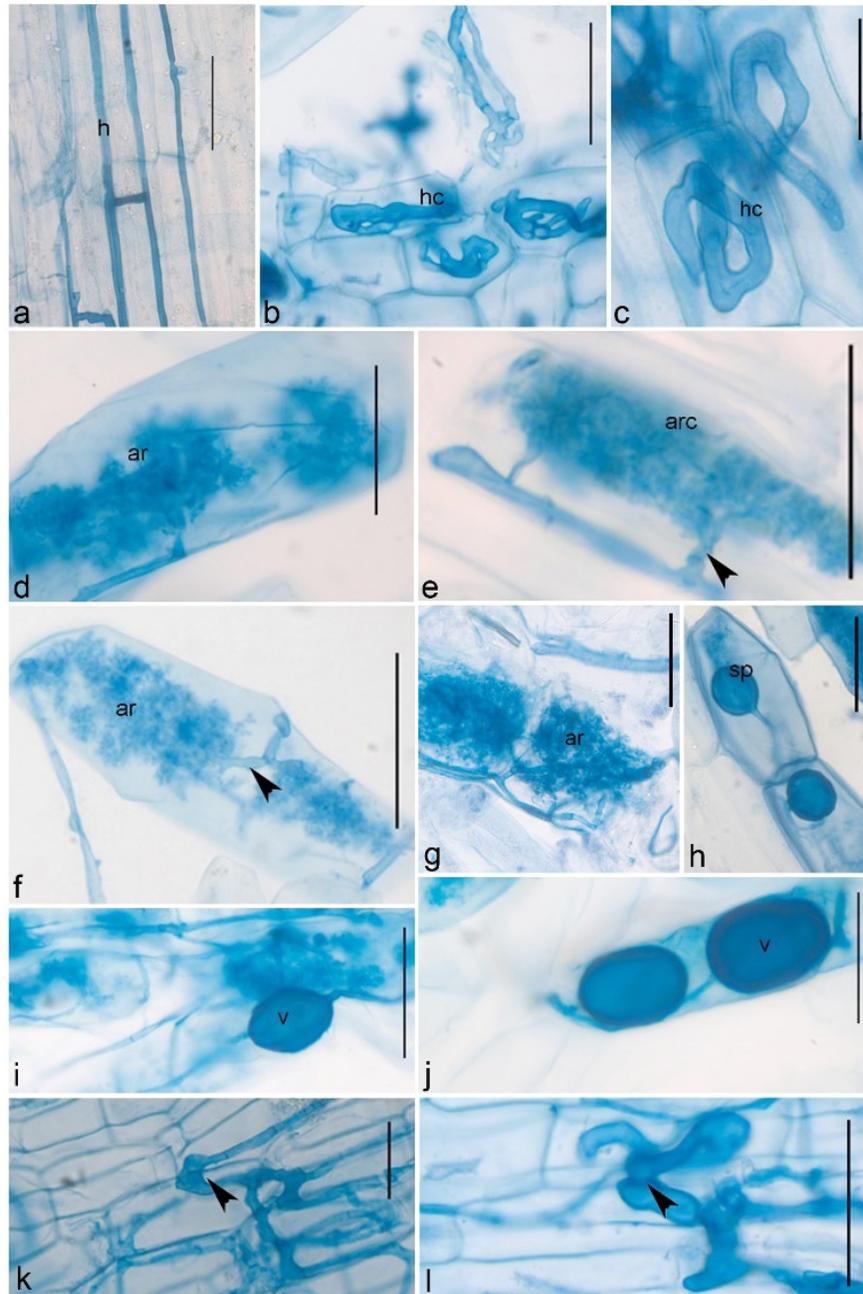


Figure 1. Arbuscular mycorrhizal fungal structures in roots of different aroids. (a) Intercellular hyphae (h) in the roots of *Caladium bicolor*; (b) Hyphal coils (hc) in cells of *Alocasia* sp.; (c) Intracellular hyphal coils (hc) in *Aglaonema commutatum*; (d) Arbuscules (ar) in the roots of *Dieffenbachia seguine*; (e) Arbusculate coils (arc) arising from the arbuscular trunk (black arrow head) in *Spathiphyllum wallisii*; (f) Arbuscule (ar) with hyphal trunk (black arrow head) in cortical cell of *Alocasia* sp.; (g) Arbuscules (ar) in *Epipremnum aureum*; (h) Intraradial spores in the roots of *Philodendron burle-marxii*; (i & j) Intercellular and intracellular vesicles (v) in *Alocasia* sp.; (k & l) Appressorium (black arrow head) on the root surface of *Alocasia* sp. (k) and *Spathiphyllum wallisii* (l). Scale bars = 50 µm.

Table 2. Distribution of various arbuscular mycorrhizal (AM) fungal structures in roots of different Araceae members

Aroid species	AM fungal structures†							AM type††
	IH	IAH	AR	AC	HC	IV	IAV	
<i>Alocasia</i> × <i>amazonica</i> Reark	+	+	-	-	-	-	-	ND
<i>Aglaonema commutatum</i> Schott	+	+	+	+	+	-	-	Arum & Paris type
<i>Alocasia brisbanensis</i> (F.M. Bailey) Domin	-	+	+	+	-	-	+	Arum & Paris type
<i>Alocasia cuprea</i> K.Koch	+	+	+	-	-	-	-	Intermediate type 1
<i>Alocasia macrorrhizos</i> (L.) G.Don	+	+	-	-	-	+	+	ND
<i>Alocasia odora</i> (Lindl.) K.Koch	-	+	+	+	-	-	+	Arum & Paris type
<i>Alocasia</i> sp.	+	+	+	+	-	+	+	Arum & Paris type
<i>Anthurium andraeanum</i> Linden ex André	-	+	-	-	-	-	-	ND
<i>Caladium bicolor</i> (Aiton) Vent.	+	+	-	-	+	-	+	ND
<i>Colocasia esculenta</i> (L.) Schott	-	+	+	-	-	-	-	Inetermediate 2
<i>Dieffenbachia seguine</i> (Jacq.) Schott	+	+	+	-	-	-	-	Intermediate type 1
<i>Epipremnum aureum</i> (Linden & André) G.S.Bunting,	+	+	+	+	-	-	+	Arum & Paris type
<i>Hamlomena</i> sp.	-	+	+	+	-	-	+	Arum & Paris type
<i>Peltandra virginica</i> (L.) Schott	+	+	+	-	-	-	+	ND
<i>Philodendron burle-marxii</i> Croat, Mayo & J.Boos	+	+	+	+	+	+	+	Arum & Paris type
<i>Philodendron goeldii</i> G.M.Barroso	+	+	+	+	+	-	-	Arum & Paris type
<i>Philodendron bederaceum</i> (Jacq.) Schott	+	+	+	-	-	-	+	Intermediate type 1
<i>Philodendron xanadu</i>	-	+	+	+	+	-	+	Arum & Paris type
<i>Pistia stratiotes</i> L.	+	+	-	-	-	-	-	ND
<i>Remusatia vivipara</i> (Roxb.) Schott	+	+	+	+	-	-	+	Arum & Paris type
<i>Spathiphyllum</i> sp.	+	+	+	+	-	-	-	Arum & Paris type
<i>Spathiphyllum wallisii</i> Regel	+	+	+	+	+	+	+	Arum & Paris type
<i>Syngonium podophyllum</i> Schott	+	-	+	-	-	-	-	Arum type
<i>Xanthosoma sagittifolium</i> (L.) Schott	+	+	+	-	-	+	-	Inetermediate 1
<i>Zantedeschia aethiopica</i> (L.) Spreng.	+	+	+	+	-	-	-	Arum & Paris type

†IH - intercellular hyphae; IAH - intracellular hyphae, AR- Arbuscule; AC- Arbuscular coils; HC-Hyphal coils. -: Absent; +: Present; IV - intercellular vesicle; IAV - intracellular vesicle; +, presence; -, absent. †† According to Dickson (2004). ND - not determined.

Table 3. Extent of arbuscular mycorrhizal (AM) fungal colonization in roots of aroids

Plant species	AM colonization					
	%RLH	%RLHC	%RLAR	%RLAC	%RLV	%TAMC
<i>Alocasia</i> × <i>amazonica</i>	55.75±3.70 ^{a,d}	0.00±0.00 ^d	0.00±0.00 ^f	0.00±0.00 ^f	0.00±0.00 ^d	55.75±3.70 ^k
<i>Aglaonema commutatum</i>	44.53±1.94 ^{b,g}	2.91±0.34 ^c	32.08±2.64 ^{b,c}	1.27±0.40 ^f	0.00±0.00 ^d	80.78±1.12 ^{b,f}
<i>Alocasia brisbanensis</i>	55.49±3.36 ^{a,d}	0.00±0.00 ^d	20.16±4.30 ^b	0.86±0.55 ^f	1.05±0.47 ^d	77.57±2.75 ^{a,g}
<i>Alocasia cuprea</i>	59.10±2.02 ^{ab}	0.00±0.00 ^d	3.32±1.31	0.00±0.00 ^f	0.00±0.00 ^d	62.42±2.12 ^j
<i>Alocasia macrorrhizos</i>	55.95±6.59 ^{a,d}	0.00±0.00 ^d	0.00±0.00 ⁱ	0.00±0.00 ^f	4.59±1.27 ^b	60.54±6.22 ^{jk}
<i>Alocasia odora</i>	35.83±1.85 ^{fi}	0.00±0.00 ^d	35.06±1.60 ^{a,d}	12.57±3.25 ^{abc}	0.74±0.74 ^d	84.20±0.97 ^{a,c}
<i>Alocasia</i> sp.	18.09±5.23 ^k	0.00±0.00 ^d	35.59±3.77 ^{a,d}	13.84±2.10 ^f	0.59±0.40 ^d	68.11±3.57 ^{fi}
<i>Anthurium andraeanum</i>	28.63±0.83 ^{i,k}	0.00±0.00 ^d	0.00±0.00 ^j	0.00±0.00 ^f	0.00±0.00 ^d	28.63±0.83 ^l
<i>Caladium bicolor</i>	49.57±5.63 ^{b,f}	4.38±2.15 ^{bc}	0.00±0.00 ^j	0.00±0.00 ^f	0.20±0.20 ^d	54.15±4.01 ^k
<i>Colocasia esculenta</i>	42.74±3.70 ^{c,h}	0.00±0.00 ^d	7.46±2.22 ^{hi}	0.00±0.00 ^f	0.00±0.00 ^d	50.19±2.89 ^k
<i>Dieffenbachia seguine</i>	41.20±5.14 ^{ci}	0.00±0.00 ^d	43.95±6.02 ^a	0.00±0.00 ^f	0.00±0.00 ^d	85.16±1.19 ^{a,c}
<i>Epipremnum aureum</i>	44.13±0.94 ^{c,g}	0.00±0.00 ^d	39.82±1.53 ^{ab}	7.54±1.30 ^{cd}	0.20±0.20 ^d	91.69±2.28 ^{ab}
<i>Hamlomena</i> sp.	43.48±6.29 ^{d,g}	0.00±0.00 ^d	30.51±2.68 ^{b,c}	14.79±6.14 ^{ab}	0.00±0.00 ^d	88.78±2.43 ^{a,d}
<i>Peltandra virginica</i>	38.25±3.66 ^{fi}	0.00±0.00 ^d	24.67±7.36 ^{ef}	0.00±0.00 ^f	0.89±0.66 ^d	63.81±10.94 ^{b,j}
<i>Philodendron burle-marxii</i>	39.60±1.01 ^{ei}	0.69±0.34 ^d	36.10±1.71 ^{a,d}	17.67±1.71 ^a	1.50±0.56 ^d	95.56±2.73 ^a
<i>Philodendron goeldii</i>	40.29±2.76 ^{cf}	6.72±1.78 ^a	32.88±1.73 ^{b,c}	2.85±1.07 ^{ef}	0.00±0.00 ^d	82.75±1.49 ^{b,c}
<i>Philodendron bederaceum</i>	50.44±2.21 ^{a,k}	0.00±0.00 ^d	38.55±4.24 ^{abc}	0.00±0.00 ^f	0.00±0.00 ^d	88.99±2.87 ^{abc}
<i>Philodendron xanadu</i>	50.58±1.16 ^{b,f}	0.65±0.65 ^d	39.63±1.36 ^{ab}	1.68±0.84 ^f	0.22±0.22 ^d	92.77±1.72 ^{ab}
<i>Pistia stratiotes</i>	3.20±1.50 ^l	0.00±0.00 ^d	0.00±0.00 ⁱ	0.00±0.00 ^f	0.00±0.00 ^d	3.20±1.50 ^m
<i>Remusatia vivipara</i>	22.80±1.24 ^{jk}	0.00±0.00 ^d	29.53±0.89 ^c	6.76±2.26 ^{de}	9.20±0.41 ^a	68.15±1.61 ^{fi}
<i>Spathiphyllum</i> sp.	30.29±1.04 ^{b,j}	0.25±0.25 ^d	27.87±1.43 ^{d,f}	0.00±0.00 ^f	0.00±0.00 ^d	58.41±2.30 ^{jk}
<i>Spathiphyllum wallisii</i>	56.39±1.81 ^{a,c}	6.04±1.43 ^{ab}	13.39±2.75 ^{gh}	0.24±0.24 ^f	2.16±0.95 ^{cd}	78.21±3.62 ^{c,g}

<i>Syngonium podophyllum</i>	36.02±7.65 ⁸ⁱ	0.00±0.00 ^d	0.39±0.39 ⁱ	0.00±0.00 ^f	0.00±0.00 ^d	36.41±7.45 ⁱ
<i>Xanthosoma sagittifolium</i>	63.13±5.16 ^a	0.00±0.00 ^d	8.68±4.43 ^{h-i}	0.00±0.00 ^f	3.84±2.37 ^{bc}	75.66±4.65 ^{e-h}
<i>Zantedeschia aethiopica</i>	46.55±4.16 ^{c-g}	0.00±0.00 ^d	12.81±2.13 ^{gh}	10.63±3.17 ^{bcd}	0.00±0.00 ^d	70.00±2.26 ^{e-i}
F (24,124) ^{##}	13.293 ^{***}	9.042 ^{***}	30.025 ^{***}	10.854 ^{***}	10.831 ^{***}	33.067 ^{***}

^aRLH- Root length with hyphae, RLHC - Root length with hyphal coils, RLAR-Root length with arbuscules, RLAC- Root length with arbusculate coils, RLV-Root length with vesicle, TAMC- Total root length with AM colonization ± indicates standard errors. Means in a column followed by a same letter(s) are not significantly (P>0.05) different according to Duncan's Multiple Range Test. ***Significant at 0.1% level. ##- df (degree of freedom) =n-1

FRE colonization

Thirty-two percent of the aroid had FRE colonization in their roots. The percentage of root length colonized by fine root endophyte (%RLTFEC) ranged from 0.21% (*P. hederaceum*) to 25.82% (*Spathiphyllum* sp.). The percentage of root length having FRE hyphae (%RLFEH) ranged from 0.38% (*Syngonium podophyllum*) to 14.48% (*Spathiphyllum* sp.). Likewise, the percentage root length with FRE arbuscules (%RLFEAR) ranged from 0.38% (*S. podophyllum*) to 11.31% (*Spathiphyllum* sp.) (Table 5).

Table 4. Number of arbuscular mycorrhizal (AM) fungal spores present in the root zone soils of different Araceae plant species

Plant species	AM Spore numbers (100 g soil)	
	Intact spores	Spore cases
<i>Alocasia xamazonica</i>	10.33±2.18 ^{c-c}	56.66±24.74 ^{e-g}
<i>Aglaonema commutatum</i>	3.00±0.57 ^{h-k}	225.66±21.45 ^{a-c}
<i>Alocasia brisbanensis</i>	3.66±0.88 ^{g-k}	30.00±2.51 ^g
<i>Alocasia cuprea</i>	4.00±0.57 ^{h-k}	101.66±14.19 ^{d-g}
<i>Alocasia macrorrhizos</i>	5.33±1.4 ^{e-k}	101±17.57 ^{d-g}
<i>Alocasia odora</i>	7.66±2.12 ^{d-i}	119±21.65 ^{e-f}
<i>Alocasia</i> sp.	14.33±3.17 ^c	167.33±29.35 ^{a-d}
<i>Anthurium andraeanum</i>	3.00±0.57 ^{h-k}	75.66±17.91 ^{d-g}
<i>Caladium bicolor</i>	3.33±0.88 ^{h-k}	185±17.57 ^{a-d}
<i>Colocasia esculenta</i>	11.33±2.9 ^{cd}	247.66±54.27 ^a
<i>Dieffenbachia seguine</i>	5.00±0.57 ^{e-k}	73.33±6.64 ^{d-g}
<i>Epipremnum aureum</i>	2.66±1.21 ^{h-k}	276±109.42 ^a
<i>Hamlomena</i> sp.	6.66±2.33 ^{d-j}	125.33±30.60 ^{e-f}
<i>Peltandra virginica</i>	1.66±0.31 ^k	86±18.00 ^{d-g}
<i>Philodendron burle-marxii</i>	5.00±1.15 ^{e-k}	236.33±58.34 ^{ab}
<i>Philodendron goeldii</i>	5.33±1.20 ^{e-k}	72.33±12.81 ^{d-g}
<i>Philodendron hederaceum</i>	9.33±0.88 ^{c-f}	175.66±43.43 ^{a-d}
<i>Philodendron xanadu</i>	5.66±1.45 ^{d-k}	68.66±18.80 ^{d-g}
<i>Remusatia vivipara</i>	20.66±1.4 ^b	86±16.86 ^{d-g}
<i>Spathiphyllum</i> sp.	40.33±3.52 ^a	86±32.65 ^{d-g}
<i>Spathiphyllum wallisii</i>	4.66±0.88 ^{h-k}	72.33±12.81 ^{d-g}
<i>Syngonium podophyllum</i>	8.66±2.0 ^{d-h}	96.66±14.44 ^{d-g}
<i>Xanthosoma sagittifolium</i>	2.33±0.88 ^{h-k}	77.33±17.38 ^{d-g}
<i>Zantedeschia aethiopica</i>	9.00±1.15 ^{d-g}	129.33±37.31 ^{b-f}
F (24,74) ^{##}	24.411 ^{***}	4.287 ^{***}

*Mean ± standard errors (SE) in a column followed by a same letter(s) are not significantly (P>0.05) different according to Duncan's Multiple Range Test. ***significant at 0.1% level. ##- df (degree of freedom) =n-1

Table 5. Fine root endophyte (FRE) and dark septate endophyte (DSE) fungal colonization in roots of aroids (n = 25)

\	FRE colonization [#]			DSE colonization ^{##}			
	%RLFH	%RLFHAR	%RLTFC	%RLDSH	%RLMS	%RLMH	%TDSE
<i>Alocasia xamazonica</i>	5.62±1.55 ^b	2.48±1.29 ^b	8.09±2.49 ^b	1.35±0.95 ^{gij}	4.61±2.98 ^b	0.00±0.00 ^b	5.96±3.81 ^{fk}
<i>Aglaonema commutatum</i>	0.00±0.00 ^d	0.00±0.00 ^c	0.00±0.00 ^c	11.67±0.99 ^{def}	0.00±0.00 ^b	0.61±0.25 ^b	12.72±0.99 ^{ah}
<i>Alocasia brisbanensis</i>	0.00±0.00 ^d	0.00±0.00 ^c	0.00±0.00 ^c	8.18±1.85 ^{c-g}	4.52±2.09 ^b	0.00±0.00 ^b	12.70±2.01 ^k
<i>Alocasia cuprea</i>	0.00±0.00 ^d	0.00±0.00 ^c	0.00±0.00 ^c	3.19±1.32 ^{gij}	14.67±3.40 ^{ab}	0.71±0.71 ^a	18.93±4.03 ^{cd}
<i>Alocasia macrorrhizos</i>	0.00±0.00 ^d	0.00±0.00 ^c	0.00±0.00 ^c	1.52±0.38 ^{gij}	0.00±0.00 ^{ab}	0.73±0.73 ^b	2.25±0.88 ^k
<i>Alocasia odora</i>	0.00±0.00 ^d	0.00±0.00 ^c	0.00±0.00 ^c	12.49±0.64 ^{de}	1.95±0.69 ^b	0.00±0.00 ^b	14.59±0.75 ^{de}
<i>Alocasia</i> sp.	2.39±0.93 ^{3d}	1.19±0.58 ^c	3.58±1.43 ^c	22.55±3.35 ^b	0.99±0.54 ^b	0.00±0.00 ^b	23.54±3.67 ^{bc}
<i>Anthurium andraeanum</i>	1.92±1.26 ^{cd}	0.00±0.00 ^c	1.92±1.26 ^{c-c}	6.03±1.57 ^{fi}	19.53±2.55 ^b	0.00±0.00 ^a	27.01±3.18 ^c
<i>Caladium bicolor</i>	0.00±0.00 ^d	0.00±0.00 ^c	0.00±0.00 ^c	18.43±2.66 ^{bc}	14.52±6.10 ^b	0.00±0.00 ^a	32.95±4.14 ^a
<i>Colocasia esculenta</i>	0.00±0.00 ^d	0.00±0.00 ^c	0.00±0.00 ^c	3.88±0.86 ^{gij}	0.00±0.00 ^b	0.00±0.00 ^b	3.88±0.86 ^k
<i>Dieffenbachia seguine</i>	0.00±0.00 ^d	0.00±0.00 ^c	0.00±0.00 ^c	3.26±0.74 ^{gij}	0.00±0.00 ^b	0.00±0.00 ^b	3.57±0.61 ^k
<i>Epipremnum aureum</i>	1.79±0.55 ^{cd}	1.20±0.37 ^{cd}	2.99±0.85 ^{cd}	0.61±0.25 ^{gij}	0.21±0.21 ^b	0.42±0.42 ^b	1.23±0.77 ^k
<i>Hamomena</i> sp.	0.00±0.00 ^d	0.00±0.00 ^c	0.00±0.00 ^c	6.32±1.74 ^{fi}	0.00±0.00 ^b	0.00±0.00 ^b	6.32±1.74 ^{ck}
<i>Peltandra virginica</i>	0.00±0.00 ^d	0.00±0.00 ^c	0.00±0.00 ^c	14.20±4.34 ^{cd}	0.00±0.00 ^b	0.00±0.00 ^b	14.20±4.34 ^{df}
<i>Philodendron burle-marxii</i>	0.00±0.00 ^d	0.00±0.00 ^c	0.00±0.00 ^c	7.33±1.43 ^{ch}	4.51±1.21 ^b	0.00±0.00 ^b	11.84±2.03 ^{di}
<i>Philodendron goeldii</i>	0.00±0.00 ^d	0.00±0.00 ^c	0.00±0.00 ^c	0.00±0.00 ^{gij}	3.08±2.68 ^b	0.00±0.00 ^b	3.08±2.68 ^k
<i>Philodendron bederaceum</i>	0.00±0.00 ^d	0.00±0.00 ^c	0.21±0.21 ^c	2.08±1.07 ^{gij}	4.93±2.06 ^b	0.00±0.00 ^b	8.55±2.95 ^{ck}
<i>Philodendron xanadu</i>	0.00±0.00 ^d	0.00±0.00 ^c	0.00±0.00 ^c	4.09±1.71 ^{gij}	0.00±0.00 ^b	0.00±0.00 ^b	4.09±1.71 ^{bk}
<i>Pistia stratiotes</i>	0.00±0.00 ^d	0.00±0.00 ^c	0.00±0.00 ^c	2.40±2.40 ^{gij}	0.00±0.00 ^b	0.00±0.00 ^b	2.40±2.40 ^k
<i>Remusatia vivipara</i>	0.00±0.00 ^d	0.00±0.00 ^c	0.00±0.00 ^e	28.66±1.82 ^a	0.49±0.13 ^b	0.00±0.00 ^b	30.59±1.39 ^{ab}
<i>Spathiphyllum</i> sp.	14.48±1.75 ^a	11.31±0.90 ^a	25.82±2.60 ^a	4.54±1.25 ^{gij}	0.00±0.00 ^b	0.00±0.00 ^b	4.89±1.40 ^{fk}
<i>Spathiphyllum wallisii</i>	0.00±0.00 ^d	0.00±0.00 ^c	0.00±0.00 ^c	10.85±1.81 ^{df}	0.00±0.00 ^a	1.35±0.05 ^b	12.20±1.83 ^{di}
<i>Syngonium podophyllum</i>	0.38±0.38 ^d	0.38±0.38 ^c	0.77±0.00 ^{de}	2.59±1.79 ^{gij}	0.36±0.36 ^b	0.00±0.00 ^b	2.95±2.12 ^k
<i>Xanthosoma sagittifolium</i>	0.00±0.00 ^d	0.00±0.00 ^c	0.00±0.00 ^c	4.64±2.83 ^{gij}	0.00±0.00 ^b	0.00±0.00 ^b	4.64±2.83 ^k
<i>Zantedeschia aethiopic</i>	0.81±0.51 ^{cd}	0.00±0.00 ^c	0.81±0.51 ^{de}	7.54±0.81 ^{ch}	2.33±1.78 ^b	0.00±0.00 ^b	9.87±1.99 ^{ef}
F (24,124)	26.930 ^{***}	48.080 ^{***}	38.986 ^{***}	15.674 ^{**}	8.386 ^{***}	2.278 ^{***}	15.674 ^{***}

[#]RLFH-Root length with fine endophyte hyphae, RLFHAR- Root length with fine endophyte arbuscules; RL TFC- Total root length with fine endophyte colonization; ^{##}RLDSH- Root length with dark septate hyphae, RLMH- Root length with moniliform hyphae, RLMS- Root length with microsclerotia, TDSE-Total root length with dark septate endophytic fungi colonization. ± indicates standard errors. Means in a column followed by a same letter(s) are not significantly (P>0.05) different according to Duncan's Multiple Range Test. ***Significant at 0.1% level, ** Significant at 0.5% level

The extent of DSE fungal colonization

DSE fungal symbiosis characterized by melanized dark septate hyphae with moniliform cells and/or microsclerotia were seen in all aroid plant roots (Figure 2). The root length with diverse DSE fungal structures varied significantly (P<0.001) among the aroids. With DSE symbiosis, the extent of total root length colonization (%TDSE) ranged from 5% (*P. stratiotes*) to 98% (*A. odora*, *P. goeldii* and *R. vivipara*) (Table 5). The percentage of root length with DSE fungal hyphae (%RLDH) ranged from 0.61% (*E. aureum*) to 28% (*R. vivipara*). The percentage root length with microsclerotia (% RLMS) ranged from 0.21% (*E. aureum*) to 19% (*Anthurium andraeanum*), while the percentage of root length with moniliform hyphae (%RLMH) ranged between 0.42% (*E. aureum*) and 1% (*S. wallisii*). Moniliform hyphae were infrequent in aroids roots.

Relationship between different endorhizal fungal variables

A significant correlation, either positive or negative was observed in aroids between AM, DSE, and FRE fungal structures and spore numbers of AM fungi (Table 6). The %RLAR of AM fungi exhibited significant negative correlation with %RLMS of DSE fungi. In contrast, %RLMH of DSE fungi showed a significant and positive correlation with the hyphal variables of AM fungi. Additionally, there was a significant positive correlation between intact AM fungal spore numbers and all the FRE variables (Table 6).

Table 6. Pearson's correlation coefficients between arbuscular mycorrhizal (AM), dark septate endophyte (DSE) and fine root endophyte (FRE) fungal structures and spore variables in Araceae family plant species (n=25)

	AM fungi					FRE fungi			DSE fungi				AM spores	
	RLHC	RLAR	RLAC	RLV	RLTC	RLFEB	RLFEB	RLTFC	RLDSH	RLMS	RLMH	RLTDSH	Intact spore	Spore cases
RLH	0.219	-0.110	-0.147	-0.016	0.367	-0.099	-0.104	-0.099	-0.293	0.063	0.400*	-0.179	-0.252	0.151
RLHC		0.016	-0.153	-0.068	0.163	-0.128	-0.107	-0.127	0.209	0.119	0.412*	0.247	-0.164	0.014
RLAR			0.470*	-0.022	0.762**	0.041	0.077	0.041	0.153	-0.422*	-0.177	-0.122	0.174	0.299
RLAC				0.063	0.433*	-0.109	-0.110	-0.109	0.214	-0.191	-0.204	-0.007	0.063	0.389
RLV					0.076	-0.157	-0.137	-0.156	0.475*	-0.219	0.145	0.274	0.168	-0.149
RLTC						-0.092	-0.097	-0.043	0.116	-0.097	0.016	-0.182	-0.093	0.281
RLFEB							0.993**	1.000**	-0.112	-0.078	-0.125	-0.119	0.826**	-0.096
RLFEB								0.993**	-0.105	-0.129	-0.107	-0.146	0.837**	-0.084
RLTFC									-0.112	-0.079	-0.125	-0.119	0.826**	-0.096
RLDSH										0.022	-0.057	0.786**	0.200	-0.005
RLMS											-0.028	0.615**	-0.208	-0.038
RLMH												-0.010	-0.202	0.074
RLTDSH													0.054	-0.004
Intact spore														-0.016

RLH, RLHC, RLAC, RLA, RLV, RLTC, RLFEB, RLFEB, RLTFC, RLDSH, RLMS, RLMH, RLTDSC and RLTC indicates percentage root length with AM fungal, hyphae, hyphal coils, arbusculate coils, arbuscules, vesicles, total colonization, fine endophytic hyphae, fine endophytic arbuscules, total fine colonization, dark septate endophytic, microsclerotia, moniliform hyphae, total dark septate endophytic fungal colonization.

** Correlation is significant at the 0.01 level (2-tailed), * Correlation is significant at the 0.05 level (2-tailed)

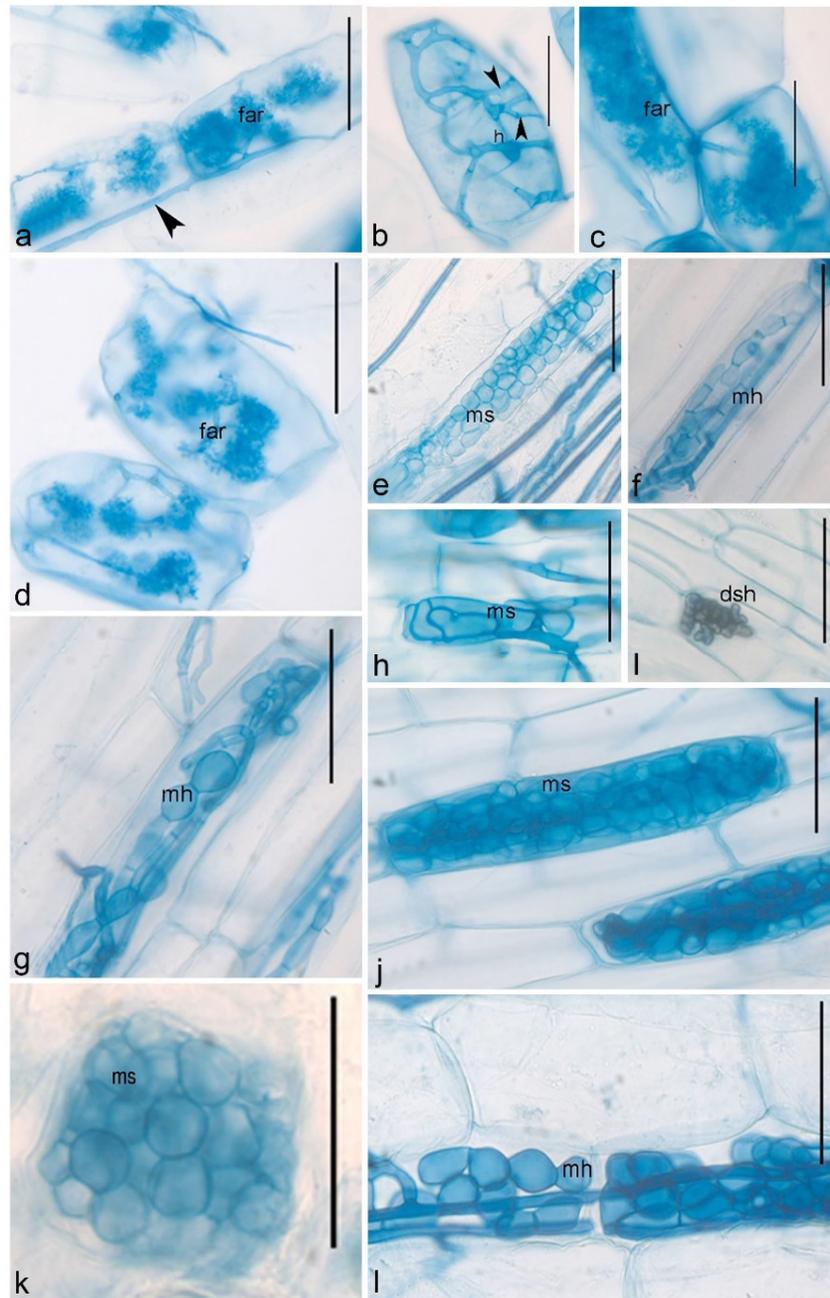


Figure 2. Fine root endophyte (FRE) (a-d) and dark septate endophyte (DSE) (e-l) fungal structures in roots of different aroids. (a,d) Fine intercellular hyphae (black arrow head) and arbuscules (far) in the root cortical cells of *Alocasia* sp.; (b) Hyphae (h) and Hyphal swelling (black arrow head) in *Dieffenbachia suguine*; (c) fine arbuscules (far) in the root cortical cells of *Epipremnum aureum* (e) Microsclerotia (ms) in cortical cells of *Caladium bicolor*; (f & g) Moniliform hyphae (mh) in cells of *Alocasia cuprea*; (h) Microsclerotia (ms) in *Spathiphyllum wallisii*; (i) Dark septate hyphae (dsh) in the root of *Alocasia* sp.; (j & k) Microsclerotia (ms) in the roots of *Alocasia cuprea* and *Anthurium andraeanum*; (l) Moniliform hyphae (mh) in *Caladium bicolor* roots. Scale bars = 50 µm.

Discussion

In the current study we revealed that, all the aroid plant taxa examined had high level of DSE and AM fungal colonization. Further, the number of fungal endophytes co-occurring differed according to species. Because majority of the aroid plant species thrive in natural conditions and the potential confounding factors like climate conditions and soil type that are known to influence AM fungal association were negligible. Previous studies have examined the endorhizal associations in palms at different locations, also reported observations similar to the present study (Balachandar *et al.*, 2022). The incidence of AM morphological characteristics of plants examined was an essential consideration in this investigation. Majority of aroids are presumed to develop typical *Arum*-type morphology which is also named after the aroid *Arum maculatum* (Dickson *et al.*, 2007). However, in a previous study, Thangavelu and Tamilselvi (2010) reported intermediate type of AM morphology in roots of *Amarphophallus paeniifolius*. In another study, Muthukumar *et al.* (2003) reported *Arum*-type AM morphology in three aroids (*Alocasia macrorrhiza*, *Alocasia bannaensis*, *Rhaphidophora decursiva*) examined from the primary and secondary forests of Xishuangbanna in southwest China. Contrarily, *Paris*-type has been described to be more common among wild angiosperms and also in aroids like *Caladium bicolor* (Johnston, 1949; Smith and Smith, 1997). The absence of typical *Paris* or *Arum* type AM morphology examined in the present study clearly suggests that AM morphological types may not be plant family specific. The variation in total root length colonization revealed in the current study and earlier research is reasonable since root colonization by AM fungi is impacted by several characteristics such as host plant, soil, and fungal species (Smith and Read, 2008). Though the presence of arbuscules is considered essential to categorize a plant species as AM, because these structures act as transit points for the exchange of nutrients these structures may be absent in field-collected roots due to their limited life span in actively growing roots (Alexander *et al.*, 1988).

The aroids have complicated mycorrhizal relationships since they include a wide range of life-forms like terrestrial plants, nonmycorrhizal (NM) hydrophytes (*Lemna* and *Pistia* spp.), and NM-AM epiphytes (*Philodendron* spp.) (Maeda, 1954; Santos *et al.*, 2000). The levels of AM colonization seen in the aroids in this study were higher when compared to previously described Araceae members from South India (Muthukumar *et al.*, 2003) and China (Wang and Jiang, 2015). However, Wang and Jiang (2015) showed that AM fungi failed to colonize aquatic *P. stratiotes* (Araceae), which may be due to their aquatic nature. On the other hand, Seerangan and Thangavelu (2014) reported the absence of AM fungal roots of *Colocasia esculenta* and *P. stratiotes* growing in aquatic and wetland habitats of southern India. They also observed AM fungal colonization rate of 8% which is comparable to previous studies involving *Acorus tatarinowii* in Aroid plant taxa (Wang and Jiang, 2015). Although, species of *Philodendron* has been reported to be non-mycorrhizal by Santos *et al.* (2000), roots of all the four *Philodendron* species analyzed in the current study had an intense DSE and AM fungal colonization. Similarly, St. John (1980) also discovered mycorrhizae in the roots of *Philodendron* species growing in Amazon region of south America. Bagyaraj *et al.* (1979) revealed that only three of 12 aquatic specimens they examined had AM and suggested that symbiosis is not favored in aquatic environments. Despite this, *P. stratiotes* analyzed in this study had the presence of inter and intraradical coarse aseptate fungal hyphae resembling those of AM fungi.

Only a few studies in the past have found DSE fungi with AM or FRE fungi (Postma *et al.*, 2007; Giesemann *et al.*, 2020). Also, in this study, *P. stratiotes* had dual colonization of DSE along with AM despite the latter being significantly higher. The occurrence of DSE symbiosis in *Epipremnum aureum* is consistent with the few investigations that have found DSE fungi colonizing roots of this aroid (Rains *et al.*, 2003; Thangavelu and Tamilselvi, 2010; Muthukumar and Karthik, 2021). Lingfei *et al.* (2005) suggested the percentage colonization of AM in plant roots was significantly influenced by the environmental factors, including P concentration in the soil, and precipitation. On the other hand, DSE colonization tends to be more

consistent, with only relative humidity and photoperiod influencing it (Lingfei *et al.*, 2005). Another interesting fact is that non-mycorrhizal plants, such as most Araceae species, have high DSE colonization rates which are thought to have functions similar to mycorrhizal fungi in these situations (Barrow and Aaltonen, 2001; Giesemann *et al.*, 2020).

In the present study, roots of seven aroids belonging to the genus *Alocasia*, *Anthurium*, *Epipremnum*, *Spathiphyllum*, and *Zantedeschia* had FRE fungal association. The morphology of FRE colonization in aroids in this study is comparable to those reported in various other angiosperms in numerous studies (Orchard *et al.*, 2017a; 2017b; Balachandar *et al.*, 2022). It is presumed that the arbuscules formed by FRE are difficult to distinguish from AM fungi, as the hyphal trunk and the arbusculate coils of FRE are much thinner and less prominent than those of AM fungi (Orchard *et al.*, 2017). Although the meta-analysis by Orchard *et al.* (2017) found no significant effect of plant species on the extent of colonization by FRE in plant roots, there was a significant effect on the FRE colonization ratio. The extent of FRE colonization in aroids in the present study ranged from 1-26% with an average colonization of 6.28% which is lower compared to studies where FRE colonization >20% is frequently reported (Orchard *et al.*, 2017). Some of the studies reported high levels of FRE colonization rates originate from agro ecosystems. For example, in a crop rotation experiment performed in Australia, FRE symbiosis was differentiated from AM symbiosis was, colonization by FRE ~40% of the root length for garden pea (*Pisum sativum*) and up to 60% of root length in wheat (*Triticum aestivum*) (Ryan and Kirkegaard, 2012). Likewise, in New Zealand, the colonization levels by FRE in agricultural cereal crops ranged from 9-85% of the root length (Orchard *et al.*, 2017).

Kowal *et al.* (2020) in their study found significant differences in the percentage of FRE colonization in individual roots, plants, site, and growing season. This study also showed that plant roots remained free of FRE during certain seasons, by increasing colonization (>85%) during other seasons (Kowal *et al.*, 2020). Moreover, Kowal *et al.* (2020) also showed that seasonal changes in FRE symbiosis are most likely related to root growth rate responses to climate history. This was supported by the existence of a strong relation between root density and temperature of the sampling site. Therefore, the limitation of the present study appears that the root samples were collected from limited number of plants for each aroid species and during one particular season. This could be one of the reasons behind the low incidence of FRE in the present study as only 32% of the 25 aroids possessed FRE symbiosis. Examining more plants in each aroid taxa coupled with rigorous sampling over different seasons would bring out the actual occurrence of FRE in aroids.

The role of FRE symbiosis in vascular plants is not well resolved like those of AM fungi. Indeed, some recent studies have shown that FRE fungi could benefit plants similar to AM fungi (Balachandar *et al.*, 2022). For instance, Hoystead *et al.* (2019) showed that the exchange of carbon-for-N occurs in *Lycopodiella inundata* colonized by FRE. Moreover, the carbon cost of maintaining FRE by the plants was shown to be in par with that of AM fungi (Hoystead *et al.*, 2019). Furthermore, it is speculated that, as in AM symbiosis, the carbon-for-nutrient exchange among plants and FREs may differ depending on plant and fungal identity, in addition to climatic and soil conditions (Field and Pressel, 2018).

When AM and DSE fungi coexists within the same root system, Wagg *et al.* (2008) theorized that they would occupy different niches within the same root. Trowbridge and Jumpponen (2004) found no antagonistic relationship between DSE and AMF root colonization as observed in the current study. Most research has revealed that DSE and AM fungal interactions coexist among most plant taxa, but in aroids the relationship between different endorhizal fungi colonizing roots and their role in plant growth and health are still unclear.

Conclusions

Our findings demonstrate that endorhizal (AM, DSE, and FRE) fungal associations were found to be widespread in aroids of the Western Ghats, south India. The present study reported the intermediate type morphology of AM for the first time in five aroid species (*Alocasia cuprea*, *Colocasia esculenta*, *Dieffenbachia seguine*, *Philodendron hederaceum*, and *Xanthosoma sagittifolium*). Moreover, our findings reveal that total colonization and root length with distinct fungal structures varied considerably amongst fungal groups. The study also distinguishes the role of root fungal association in the establishment and survival of these plants. Moreover, future experimental studies are needed to ascertain the aroid benefits from these root fungal associations individually and in combinations. This would help in the exploitation of these root fungal symbiosis in the sustainable production of the aroids in horticulture and food industry.

Authors' Contributions

SA and TM wrote the paper. VA carried out the macro and microscopical observations. MB performed statistical analysis and TM designed the study. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

- Alexander T, Meier R, Toth R, Weber HC (1988). Dynamics of arbuscule development and degeneration in mycorrhizas of *Triticum aestivum* L. and *Avena sativa* L. with reference to *Zea mays* L. *New Phytologist* 110(3):363-370.
- Bagyaraj DJ, Manjunath A, Patil RB (1979). Occurrence of vesicular-arbuscular mycorrhizas in some tropical aquatic plants. *Transactions of the British Mycological Society* 72(1):164-167. [https://doi.org/10.1016/S0007-1536\(79\)80023-6](https://doi.org/10.1016/S0007-1536(79)80023-6)
- Balachandar M, Koshila Ravi R, Muthukumar T (2022). Fine root endophyte association in widely cultivated palms of southern India. *Kavaka* 58(3):48-53. <https://doi.org/10.36460/Kavaka/58/3/2022/48-53>
- Barrow JR, Aaltonen RE (2001). Evaluation of the internal colonization of *Atriplex canescens* (Pursh) Nutt. roots by dark septate fungi and the influence of host physiological activity. *Mycorrhiza* 11(4):199-205. <https://doi.org/10.1007/s005720100111>
- Begum N, Qin C, Ahanger MA, Raza S, Khan MI, Ashraf M, ... Zhang L (2019). Role of arbuscular mycorrhizal fungi in plant growth regulation implications in abiotic stress tolerance. *Frontiers in Plant Science* 10:1068. <https://doi.org/10.3389/fpls.2019.01068>
- Boyce PC, Croat TB (2011). The uberlist of araceae totals for published and estimated number of species in aroid genera. Boyce PC, Croat TB (2018) The Überlist of Araceae, totals for published and estimated number of species in aroid genera. *Aroid Society* 7:10.
- Brundrett M (2004). Diversity and classification of mycorrhizal associations. *Biological Reviews* 79(3):473-495. <https://doi.org/10.1017/S1464793103006316>
- Brundrett MC, Tedersoo L (2018). Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytologist* 220(4):1108-1115. <https://doi.org/10.1111/nph.14976>
- Cabrera LI, Salazar GA, Chase MW, Mayo SJ, Bogner J, Dávila P (2008). Phylogenetic relationships of aroids and duckweeds (Araceae) inferred from coding and noncoding plastid DNA. *American Journal of Botany* 95(9):1153-1165. <https://doi.org/10.3732/ajb.0800073>
- Croat TB (2019). Araceae a family with great potential. *Annals of the Missouri Botanical Garden* 104(1):3-9. <https://doi.org/10.3417/2018213>
- Croat TB, Delannay X, Wood R (2018). A revision of *Anthurium* (Araceae) section *Polyneurium* for Carchi province, Ecuador. *Aroideana – Journal of the International Aroid Society* 41(1):4-126.
- Diagne N, Ngom M, Djighaly PI, Fall D, Hocher V, Svistoonoff S (2020). Roles of arbuscular mycorrhizal fungi on plant growth and performance: Importance in biotic and abiotic stressed regulation. *Diversity* 12(10):370. <https://doi.org/10.3390/d12100370>
- Dickson S (2004). The *Arum-Paris* continuum of mycorrhizal symbioses. *New Phytologist* 163:187-200.
- Dickson S, Smith FA, Smith SE (2007). Structural differences in arbuscular mycorrhizal symbioses more than 100 years after Gallaud, where next?. *Mycorrhiza* 17:375-393. <https://doi.org/10.1007/s00572-007-0130-9>
- Farias GC, Nunes KG, Soares MA, de Siqueira KA, Lima WC, Neves ALR, ... Gomes Filho E (2020). Dark septate endophytic fungi mitigate the effects of salt stress on cowpea plants. *Brazilian Journal of Microbiology* 51:243-253. <https://doi.org/10.1007/s42770-019-00173-4>
- Field KJ, Leake JR, Tille S, Allinson KE, Rimington WR, Bidartondo MI, ... Cameron DD (2015). From mycoheterotrophy to mutualism mycorrhizal specificity and functioning in *Ophioglossum vulgatum* sporophytes. *New Phytologist* 205(4):1492-1502. <https://doi.org/10.1111/nph.13263>
- Field KJ, Pressel S (2018). Unity in diversity: structural and functional insights into the ancient partnerships between plants and fungi. *New Phytologist* 220(4):996-1011. <https://doi.org/10.1111/nph.15158>
- Gianinazzi S, Gollotte A, Binet MN, van Tuinen D, Redecker D, Wipf D (2010). Agroecology the key role of arbuscular mycorrhizas in ecosystem services. *Mycorrhiza* 20(8):519-530. <https://doi.org/10.1007/s00572-010-0333-3>

- Giesemann P, Eichenberg D, Stöckel M, Seifert LF, Gomes SI, Merckx VS, ... Gebauer G (2020). Dark septate endophytes and arbuscular mycorrhizal fungi (*Paris*-morphotype) affect the stable isotope composition of 'classically' non-mycorrhizal plants. *Functional Ecology* 34(12):2453-2466. <https://doi.org/10.1111/1365-2435.13673>
- Howard N, Pressel S, Kaye RS, Daniell TJ, Field KJ (2022). The potential role of Mucoromycotina 'fine root endophytes' in plant nitrogen nutrition. *Physiologia Plantarum* 174(3):e13715. <https://doi.org/10.1111/pp.13715>
- Hoysted GA, Kowal J, Pressel S, Duckett JG, Bidartondo MI, Field KJ (2021). Carbon for nutrient exchange between *Lycopodiella inundata* and Mucoromycotina fine root endophytes is unresponsive to high atmospheric CO₂. *Mycorrhiza* 31:431-440. <https://doi.org/10.1007/s00572-021-01033-6>
- Hoysted GA, Jacob AS, Kowal J, Giesemann P, Bidartondo MI, Duckett JG, ... Field KJ (2019) Mucoromycotina fine root endophyte fungi form nutritional mutualisms with vascular plants. *Plant Physiology* 181(2):565-77. <https://doi.org/10.1104/pp.19.00729>
- Jackson ML (1971). *Soil Chemical Analysis*. Prentice Hall, New Delhi, India.
- Jumpponen A, Trappe JM (1998). Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. *New Phytologist* 140(2):295-310. <https://doi.org/10.1046/j.1469-8137.1998.00265.x>
- Johnston A (1949) Vesicular-arbuscular mycorrhiza in Sea Island cotton and other tropical plants. *Tropical Agriculture* 26:118-21.
- Kowal J, Arrigoni E, Serra J, Bidartondo M (2020). Prevalence and phenology of fine root endophyte colonization across populations of *Lycopodiella inundata*. *Mycorrhiza* 30:577-587. <https://doi.org/10.1007/s00572-020-00979-3>
- Koske RE, Gemma JN (1989) A modified procedure for staining roots to detect VA mycorrhizas. *Mycological Research* 92(4):486-488. [https://doi.org/10.1016/S0953-7562\(89\)80195-9](https://doi.org/10.1016/S0953-7562(89)80195-9)
- Lesica P, Antibus RK (1990). The occurrence of mycorrhizae in vascular epiphytes of two Costa Rican rain forests. *Biotropica* 22:250-258. <https://doi.org/10.2307/2388535>
- Lingfei L, Anna Y, Zhiwei Z (2005). Seasonality of arbuscular mycorrhizal symbiosis and dark septate endophytes in a grassland site in southwest China. *FEMS Microbiology Ecology* 54(3):367-373. <https://doi.org/10.1016/j.femsec.2005.04.011>
- Maeda M (1954). The meaning of mycorrhiza in regard to systematic botany. *Kumamoto Journal of Science B* 3:57-84.
- Mandyam K, Jumpponen A (2005). Seeking the elusive function of the root colonising dark septate endophytic fungi. *Studies in Mycology* 53(1):173-189. <https://doi.org/10.3114/sim.53.1.173>
- Mayerhofer MS, Kernaghan G, Harper KA (2013). The effects of fungal root endophytes on plant growth: a meta-analysis. *Mycorrhiza* 23:119-128. <https://doi.org/10.1007/s00572-012-0456-9>
- McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives an objective measure of colonization of roots by vesicular—arbuscular mycorrhizal fungi. *New Phytologist* 115(3):495-501. <https://doi.org/10.1111/j.1469-8137.1990.tb00476.x>
- Muthukumar T, Chinnathambi M, Priyadharsini P (2016). Root fungal associations in some non-orchidaceous vascular lithophytes. *Acta Botanica Brasiliica* 30:407-421. <https://doi.org/10.1590/0102-33062016abb0074>
- Muthukumar T, Karthik S (2021). *Epipremnum aureum* (Araceae) roots associated simultaneously with Glomeromycotina, Mucoromycotina and Ascomycota fungi. *Botanica Complutensis* 45:1-9. <https://doi.org/10.5209/bocm.72399>
- Muthukumar T, Sathiyadash K, Valarmathi V (2018). Arbuscular mycorrhizal and dark septate endophyte fungal associations in plants of different vegetation types in Velliangiri hills of Western Ghats, Southern India. *Acta Botanica Hungarica* 60(1-2):185-222. <https://doi.org/10.1556/034.60.2018.1-2.9>
- Muthukumar T, Senthilkumar M, Rajangam M, Udaiyan K (2006). Arbuscular mycorrhizal morphology and dark septate fungal associations in medicinal and aromatic plants of Western Ghats, Southern India. *Mycorrhiza* 17:11-24. <https://doi.org/10.1007/s00572-006-0077-2>
- Muthukumar T, Sha L, Yang X, Cao M, Tang J, Zheng Z (2003). Mycorrhiza of plants in different vegetation types in tropical ecosystems of Xishuangbanna, southwest China. *Mycorrhiza* 13:289-297. <https://doi.org/10.1007/s00572-003-0234-9>
- Öpik M, Zobel M, Cantero JJ, Davison J, Facelli JM, Hiiesalu I (2013). Global sampling of plant roots expands the described molecular diversity of arbuscular mycorrhizal fungi. *Mycorrhiza* 23:411-430. <https://doi.org/10.1007/s00572-013-0482-2>

- Orchard S, Hilton S, Bending GD, Dickie IA, Standish RJ, Gleeson DB, ...Bass D (2017a). Fine endophytes (*Glomus tenue*) are related to Mucoromycotina, not Glomeromycota. *New Phytologist* 213(2):481-486. <https://doi.org/10.1111/nph.14268>
- Orchard S, Standish RJ, Dickie IA, Renton M, Walker C, Moot D, ... Ryan MH (2017b). Fine root endophytes under scrutiny: a review of the literature on arbuscule- producing fungi recently suggested to belong to the Mucoromycotina. *Mycorrhiza* 27:619-638. <https://doi.org/10.1007/s00572-017-0782-z>
- Orchard S, Standish RJ, Nicol D, Dickie IA, Ryan MH (2017). Sample storage conditions alter colonisation structures of arbuscular mycorrhizal fungi and particularly fine root endophyte. *Plant and Soil* 412:35-42. <https://doi.org/10.1007/s11104-016-2867-4>
- Pedralli G (2002). Distribuição geográfica e taxonomia das famílias Araceae e Dioscoreaceae no Brasil. Inhame e taro: sistemas de produção familiar. Vitória: Incaper, pp 15-26.
- Piszczek P, Kuszewska K, Błaszowski J, Sochacka-Obruśnik A, Stojakowska A, Zubek S (2019). Associations between root-inhabiting fungi and 40 species of medicinal plants with potential applications in the pharmaceutical and biotechnological industries. *Applied Soil Ecology* 137:69-77. <https://doi.org/10.1016/j.apsoil.2019.01.018>
- Postma JW, Olsson PA, Falkengren-Grerup U (2007). Root colonisation by arbuscular mycorrhizal, fine endophytic and dark septate fungi across a pH gradient in acid beech forests. *Soil Biology and Biochemistry* 39(2):400-408. <https://doi.org/10.1016/j.soilbio.2006.08.007>
- Rains KC, Nadkarni NM, Bledsoe CS (2003). Epiphytic and terrestrial mycorrhizas in a lower montane Costa Rican cloud forest. *Mycorrhiza* 13:257-264. <https://doi.org/10.1007/s00572-003-0224-y>
- Rouphael Y, Franken P, Schneider C, Schwarz D, Giovannetti M, Agnolucci M, ... Colla G (2015). Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. *Scientia Horticulturae* 196:91-108. <https://doi.org/10.1016/j.scienta.2015.09.002>
- Ryan MH, Kirkegaard JA (2012). The agronomic relevance of arbuscular mycorrhizas in the fertility of Australian extensive cropping systems. *Agriculture, Ecosystems and Environment* 163:37-53. <https://doi.org/10.1016/j.agee.2012.03.011>
- Santos BA, Silva GA, Maia LC, Alves MV (2000). Mycorrhizae in monocotyledonae of Northeast Brazil: subclasses alismatidae, arecidae and zingiberidae. *Mycorrhiza* 10:151-153. <https://doi.org/10.1007/s005720000068>
- Seerangan K, Thangavelu M (2014). Arbuscular mycorrhizal and dark septate endophyte fungal associations in South Indian aquatic and wetland macrophytes. *Journal of Botany*. <http://dx.doi.org/10.1155/2014/173125>.
- Smith FA, Smith SE (1997). Structural diversity in (vesicular)–arbuscular mycorrhizal symbioses. *New Phytologist* 137(3):373-388. <https://doi.org/10.1046/j.1469-8137.1997.00848.x>
- Smith S, Read D (2008). *Mycorrhiza symbiosis*, 3rd Ed. San Diego, CA: Academic Press.
- Spatafora JW, Chang Y, Benny GL, Lazarus K, Smith ME, Berbee ML, ... Stajich JE (2016). A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. *Mycologia* 108(5):1028-1046. <https://doi.org/10.3852/16-042>
- St. John TV (1980). A survey of mycorrhizal infection in an Amazonian rain forest. *Acta Amazonica* 10:527-533. <https://doi.org/10.1590/1809-43921980103527>
- Stevens PF (2020). Angiosperm phylogeny website. Version 14, 2017.
- Swain J, Devi RS, Kumar S, Antunes LL, Biswal SK, Jena PK (2022). *Amorphophallus paeoniifolius* (Araceae): A nutraceutical for food disorders, novel bacterial & viral infections. *Carpathian Journal of Food Science & Technology* 14(1):118-136.
- Thangavelu M, Tamilselvi V (2010). Occurrence and morphology of endorhizal fungi in crop species. *Tropical and Subtropical Agroecosystems* 12(3):593-604.
- Trowbridge J, Jumpponen A (2004). Fungal colonization of shrub willow roots at the forefront of a receding glacier. *Mycorrhiza* 14:283-293. <https://doi.org/10.1007/s00572-003-0264-3>
- Vergara C, Araujo KEC, Alves LS, de Souza SR, Santos LA, Santa-Catarina C, ... Zilli JÉ (2018). Contribution of dark septate fungi to the nutrient uptake and growth of rice plants. *Brazilian Journal of Microbiology* 49:67-78. <https://doi.org/10.1016/j.bjm.2017.04.010>
- Wagg C, Pautler M, Massicotte HB and Peterson L (2008). The co-occurrence of ectomycorrhizal, arbuscular mycorrhizal, and dark septate fungi in seedlings of four members of the Pinaceae. *Mycorrhiza* 18:103-110. <https://doi.org/10.1007/s00572-007-0157-y>

- Wang M, Jiang P (2015). Colonization and diversity of AM fungi by morphological analysis on medicinal plants in southeast China. *The Scientific World Journal*. <https://doi.org/10.1155/2015/753842>
- Zulhazman H, Fizree MA, Azahar AM, Fadzelly AM, Anis AN (2021). A survey on edible aroids consumed by locals in Kelantan, Peninsular Malaysia. *IOP Conference Series: Earth and Environmental Science* 736(1):012076. <https://doi.org/10.1088/1755-1315/736/1/012076>



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