

Epipremnum aureum (Araceae) roots associated simultaneously with Glomeromycotina, Mucoromycotina and Ascomycota fungi

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Abstract. Only a small number of aroids are examined for their symbiosis with Glomeromycota arbuscular mycorrhizal (AM) fungi and the ascomycetous dark septate endophytic (DSE) fungi. Therefore we examined the aerial and terrestrial adventitious roots of *Epipremnum aureum* (money plant) for different type of fungal association and the soils for AM spores. The aerial roots of *E. aureum* were free from fungal structures, whereas the terrestrial roots were colonized by AM, fine root endophyte (FRE), and DSE fungi. The major portion of the terrestrial roots was colonized by FRE fungi followed by AM and DSE fungi. The colonization pattern was a complex of *Arum-Paris* and intermediate AM types. Spores of *Acaulospora*, *Funneliformis*, *Rhizophagus*, and *Sclerocystis* were isolated from the root zone soils. The results show that *E. aureum* associate with a wide range of endophytic fungi and FRE symbiosis is reported for the first time in aroids.

Keywords: arbuscules; aroids; DSE fungi; endophytic fungi; fine root endophyte; money plant.

[es] Raíces de *Epipremnum aureum* (Araceae) asociadas simultáneamente con los hongos Glomeromycotina, Mucoromycotina y Ascomycota

Resumen. Hay muy pocos estudios de aráceas relacionados con la simbiosis de Glomeromycota o micorrizas arbusculares (AM) y con hongos endófitos del grupo de ascomycetes con filamentos oscuros y septados (DSE). Por esta razón se han estudiado las raíces adventicias y aéreas de *Epipremnum aureum* (poto) para analizar el tipo de asociación fúngica entre ellas y las esporas de AM encontradas en el suelo. Las raíces aéreas de *E. aureum* carecían de estructuras fúngicas, mientras que las raíces subterráneas estaban colonizadas por AM, endófitos de raíces finas (FRE), y hongos DSE. La mayor parte de las raíces subterráneas estaban colonizadas por hongos FRE, seguido por AM y hongos DSE. El patrón de colonización es un complejo de *Arum-Paris* y de tipos intermedios de AM. En el suelo próximo a las raíces subterráneas se han aislado esporas de *Acaulospora*, *Funneliformis*, *Rhizophagus*, y *Sclerocystis*. Los resultados muestran que las raíces subterráneas de *E. aureum* están asociadas con un amplio grupo de hongos endófitos y la simbiosis con estos hongos se reporta por primera vez para las aráceas.

Palabras clave: aráceas; hongos DSE; hongos endófitos; endófitos con raíces finas; micorrizas arbusculares; poto

Introduction

Most terrestrial plant roots are naturally colonized by numerous soil microorganisms of which mycorrhizal fungi are the most prevalent and widespread. Of the various types of mycorrhizal fungi colonizing plant roots, the most prominent and common type are those belonging to subphylum Glomeromycotina and to a lesser extent to Mucoromycotina (Spatafora et al. 2016; Hoysted et al. 2018). The association formed by fungi in these subphyla is distinguished by the formation of specific structures like the arbuscules in the cortical cells of the roots and are therefore termed as arbuscular mycorrhizal (AM) symbiosis. Nevertheless, the structures of Mucoromycotina fungi are much finer and establish functional AM symbiosis and are termed as fine root endophytes or FRE (Orchard et al. 2017a). The fungal hyphae of the glomeromycotan fungi are coarse and are termed as glomeromycotan AM fungi (Orchard et al. 2016). Both AM and FRE fungi acquire carbon from the host plant and

in turn transfer inorganic nutrients like phosphorus (P), nitrogen, and water to their plant hosts. In addition, AM symbiosis also helps plants in ameliorating the effect of various biotic and abiotic stresses thereby helping plants in their establishment, survival and health in natural and anthropogenic ecosystems (Smith and Read 2008).

Plant roots are also colonized by ascomycetous dark septate endophytic (DSE) fungi and this association often co-occurs with the colonization of roots by AM and FRE fungi (Berthelot et al. 2019; Gieseemann et al. 2020). The DSE fungi frequently have dark melanized hyphae that are regularly septate and forms intracellular microsclerotia in root cortex. Like AM fungi, DSE fungi also play a major role in plant growth and development of plants under condition where AM symbiosis cannot function (Shen et al. 2020).

The monocotyledonous plant family Araceae Juss., commonly known as aroids includes around 3750 extant species in 114 genera (Christenhusz and

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Byng 2016). Though many members of this family are tuberous or rhizomatous large herbs, root-climbers are also common in this family. The adventitious aerial roots of Araceae are of two types, namely anchor roots and feeder roots. The anchor roots clasp the plant onto the substrate and the feeder roots reach the soil and absorb nutrients and water for the plant. However in plants like *Epipremnum* the aerial roots perform the function of both holding on the plant to the substrate as well as acquiring water and nutrients from the soil. There is only limited information on the occurrence of mycorrhizal symbiosis in Araceae (Akhmetzhanova et al. 2012; Yaseen et al. 2016). Alismatids, that includes Araceae, is listed as one of the angiospermic plant groups that have high percentage of nonmycorrhizal species (Wang and Qiu 2006). An examination on the occurrence of mycorrhizal association in land plants by Wang and Qiu (2006) indicated the presence of AM in 14 of the 18 species of the aroids examined. Later studies have also noted the occurrence of this association in aroid members (Kumar and Muthukumar 2014; Marins and Carrenho 2017). Brundrett (2009) speculated that around 1600 aroids as variably mycorrhizal (with both mycorrhizal and nonmycorrhizal status). This is also evidenced in a recent global online data base where 88.5% of the 113 genera of aroids had species with variable mycorrhizal status (Soudzilovskaia et al. 2020). Only 54% of the 11 species of aroids occurring in hydric environments were mycorrhizal (Marins and Carrenho 2017). Most of the aroid tuber crops are colonized by AM fungi (e.g., Kumar et al. 2013), however *Colocasia esculenta* (L.) Schott examined from south India was non-mycorrhizal (Kumar and Muthukumar 2014). *Arum*-type characterized by the intercellular hyphae and intracellular arbuscules appears to be the predominant type of AM morphology in Araceae as eight of the nine taxa had this colonization pattern (Dickson et al. 2007). However, *Paris*-type morphology with intracellular hyphal and arbusculate coils was reported only in *Caladium bicolor* (Aiton) Vent. (Johnston 1949). Until 1998 DSE association has been reported in only one species (Jumpponen and Trappe, 1998) and since then this symbiosis has been reported in a few members of aroids (Rains et al. 2003; Muthukumar and Tamilselvi 2010; Kumar and Muthukumar 2015).

The plant genus *Epipremnum* (Araceae) consists of 15 species of which *Epipremnum aureum* (Linden & André) G.S. Bunting, commonly known as money plant, is a popular house plant worldwide including India. This plant is a climbing vine which can reach a height of more than 60 feet. The plant perches to the support by means of the tightly adhering adventitious roots that often grow and reach the soil. *Epipremnum aureum* has also naturalized in forests of sub-tropical and tropical regions and is known to cause certain ecological damage (Moodley et al. 2017). In spite of occurrence in natural ecosystems and under cultivation the fungi associated to the roots

of *E. aureum* have not been examined so far except for a few studies reporting a variable mycorrhizal status (Akhmetzhanova et al. 2012; Yaseen et al. 2016). This prompted us to investigate the fungi associated to the roots of *E. aureum*.

Materials and methods

Aerial (n=5) and terrestrial (n=5) adventitious roots and root zone soil of *E. aureum* perching on to *Roystonea regia* (Kunth) O.F. Cook was collected from the semi-natural area of the Botany Department garden, Bharathiar University, Coimbatore, India during February 2020. The Alfisol soil had a pH of 7.5, electrical conductivity of 0.11 dS/m, 47 mg/kg of total nitrogen, 7.9 mg/kg of available P and 230 mg/kg of exchangeable potassium. The sampled roots were washed thoroughly free of adhering debris, cut into 1-cm long pieces, cleared in 2.5% KOH by heating at 90°C for 120 minutes. The cleared root bits were later washed in several changes of distilled water and acidified for 15 minutes in 5 N HCl. The acidified root bits were stained overnight using 0.05% trypan blue in lactoglycerol. The excess stain from roots was removed using clear lactoglycerol and root squashes were prepared for examination of fungal structures. The proportion of fungal colonization and the percentage root length possessing various fungal structures were determined as per the magnified intersection method of McGonigle et al. (1990). The AM morphology was recorded according to Dickson (2004). The fungal hyphae (n=25) and the vesicle/hyphal swellings of AM and FRE (n=10) were measured from 10 randomly selected 1 cm root pieces using a calibrated ocular micrometer. The intact AM fungal spores in the root zone soils was isolated by modified wet-sieving and decanting technique (Muthukumar et al. 1996) and AM fungal spores were identified by comparing the spore wall and subcellular characters with the original descriptions available in the Schüßler's web page (http://www.amf-phylogeny.com/amphylo_species.html). Microscopic images of fungal colonization were captured using a ProgRes 3 camera attached to a BX51 Olympus trinocular light microscope.

The data are presented as mean and their standard errors. We used student's t-test or one-way Analysis of Variance (ANOVA) to assess the significance ($P < 0.05$) of differences in the total root length colonization and root length containing different fungal structures after checking the data for homogeneity using Kolmogorov-Simrnov test. The values on hyphal width were normalized using square root transformation before analysis. Pearson's correlation was used to analyze the relationship between the various fungal variables (percentage root length with intercellular linear hyphae, intracellular linear hyphae, hyphal coils, arbuscules, arbusculate coils, vesicles, moniliform hyphae, microsclerotia and total colonization). All the statistical procedures were

carried out with Statistical Product and Service Solutions (Version 9.0).

Results

The aerial roots of *E. aureum* were free from fungal structures. However, the same aerial roots on reaching and penetrating the soil hosted different types of associated fungi (Fig. 1A–K).

Glomeromycotan symbiosis

The AM fungi entered the roots through the epidermis after the formation of an appressorium (Fig. 1A, C) and the colonization was characterized by the presence of inter- and intra-cellular linear hyphae, hyphal coils, arbusculate coils, arbuscules and intracellular vesicles (Fig. 1 B–E). The AM fungal hyphae contained several large oil droplets (Fig. 1D). The width of AM fungal hyphae was significantly larger than those of FRE and DSE fungi (Tab. 1). The *Arum*-type arbuscules were either elaborate or much reduced (Fig. 1G, H). Similarly, the vesicles of AM fungi were intracellular, and significantly than the hyphal swellings of FRE fungi (Tab. 1). The AM morphology of *E. aureum* was a combination of *Arum*–*Paris* type and intermediate type 3 (intracellular hyphae bearing arbuscules, Fig. 1E, Dickson 2004).

The AM spore numbers in the root zone soils of *E. aureum* was 25.20 ± 2.65 spores per 100 g soil and spore morphotypes belonging to *Acaulospora scrobiculata* Trappe, *Funneliformis geosporum* (T.H. Nicolson & Gerd.) C.Walker & A.Schüßler, *Rhizophagus aggregatus* (N.C. Schenck & G.S. Sm.) C. Walker, *Rhizophagus intraradices* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüßler and *Sclerocystis sinuosa* Gerd. & B.K. Bakshi was isolated from the root zone soils.

Fine root endophyte

The FRE root colonization was characterized by inter and intracellular fine hyphae (Fig. 1B) that were 0.63–1.88 μm in diameter. The percentage total root length colonization (%RLTC) and root length containing different structures were significantly higher for FRE than AM (Tab. 2). The *Arum*-type arbuscules of FRE had a much finer hyphal trunk and arbuscular branches than those of the coarse AM fungi (Fig. 1G, I). Though vesicles resembling AM fungi were absent in FRE symbiosis, a large number of terminal and intercalary hyphal swellings were observed (Fig. 1F, Tab. 1). Structures of both FRE and AM fungi co-occurred in the same cell (Fig. 1E, G).

Dark septate endophytic fungi

Like AM fungi, the DSE fungi entered the root after the development of an appressorium. The hyphae

of DSE fungi were mostly intracellular, occasionally melanized, regularly septate and microsclerotia were found within the cells (Fig. 1K). The diameter of DSE fungal hyphae ranged between 5.00 and 8.75 μm and was larger than FRE fungi but smaller than AM fungi. The %RLTC for DSE fungi was 3.82 and 6.70 folds lower than the %RLTC of AM and FRE (Tab. 2).

Interaction between fungal endophytes

In the present study, a significant positive correlation existed between the percentage of root length with intercellular linear hyphae (%RLIH) of AM and FRE ($r=0.971$; $P<0.01$). Similarly, a significant positive correlation was also found between %RLIH of FRE and percentage of root length with intracellular linear hyphae (%RLIAH) of AM ($r=0.894$; $P<0.05$). The %RLIAH of AM was significantly and positively correlated to the percentage of root length with hyphal coils (%RLHC) ($r=0.983$; $P<0.01$) and arbuscules (%RLA) ($r=0.891$; $P<0.05$) of FRE. The percentage of root length with arbusculate coils of AM was significantly ($P<0.05$) and positively correlated to vesicles/hyphal swellings (%RLV/HS) of FRE ($r=0.951$). The percentage of root length with DSE fungal microsclerotia was significantly and positively correlated to %RLIH of AM ($r=1.000$; $P<0.001$) and FRE ($r=0.971$; $P<0.01$) and %RLIAH of the later ($r=0.888$; $P<0.05$). Contrarily, in the present study a significant negative correlations existed between FRE %RLIAH and AM %RLA ($r=-0.962$; $P<0.01$) and AM %RLHC and FRE %RLV/HS ($r=-0.929$; $P<0.05$).

Discussion

Unlike in other epiphytes where the aerial roots that are in contact with the phorophyte bear fungal colonization, the aerial roots of *E. aureum* attached to the phorophyte lacked any fungal structures. This observation has to be interpreted with caution as only a limited number of individuals from a single garden were examined. Nevertheless, the lack of fungal structures in aerial roots of *E. aureum* contrasts those of Rains et al. (2003) where the aerial roots of *Anthurium pitieri* Engl. and *Stenospermation* sp., growing in the low montane cloud forests of Costa Rica contained AM structures and the later also possessed DSE fungal hyphae. One reason for the disparity in the occurrence of AM in the aerial roots of *E. aureum* could be the bare surface of the phorophytes on which the plants in the present study was clinging onto. Rains et al. (2003) also found that the roots of epiphytes attached to the bare branches were non-mycorrhizal. The aerial roots of *Syngonium* sp., and *Philodendron aurantiifolium* Schott growing in the Monteverde Cloud Forest of Costa Rica was reported to present AM fungal colonization (Glassman 2007). However,

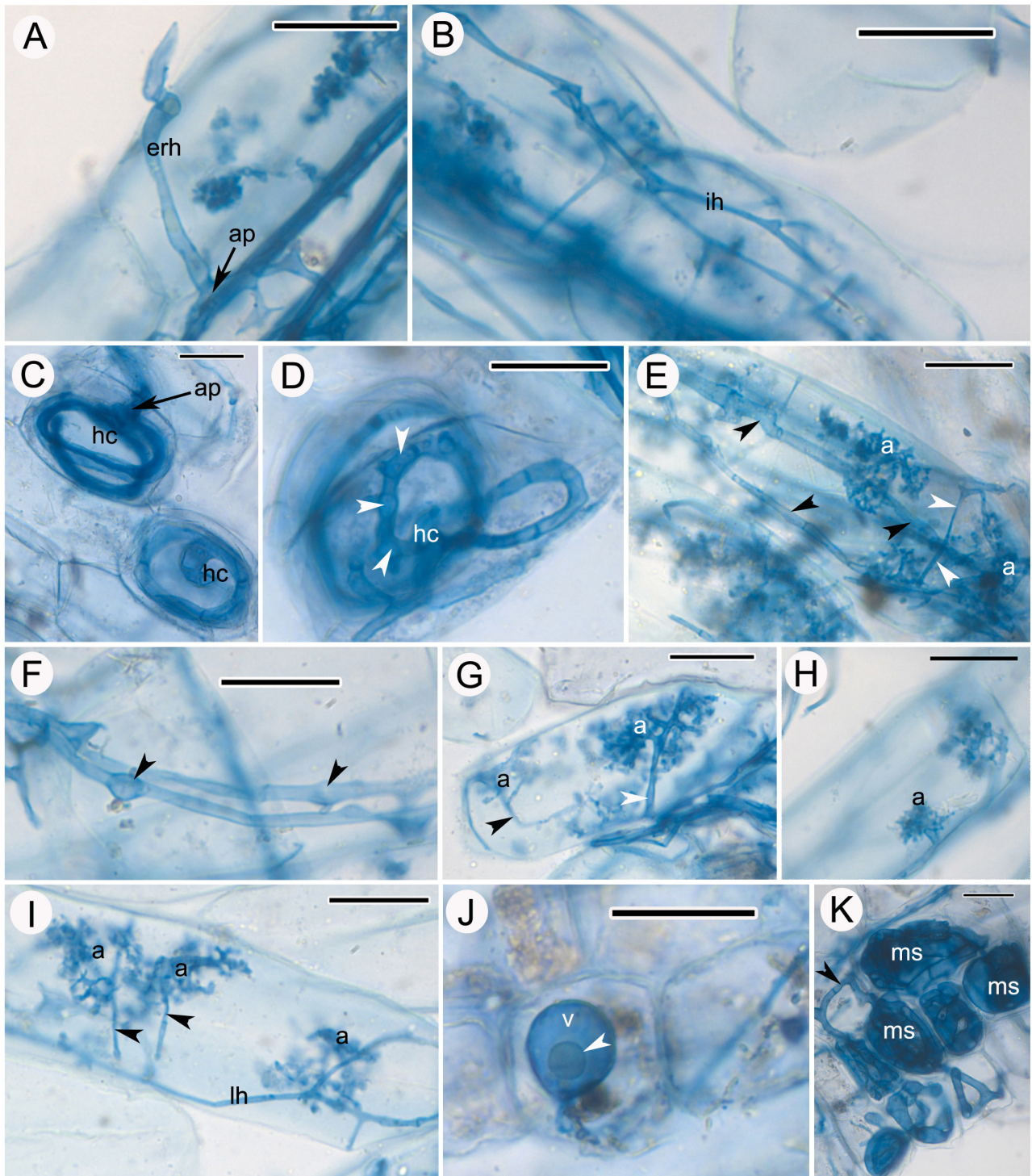


Figure 1. Colonization structures of glomeromycotan arbuscular mycorrhizal (AM), fine root endophyte (FRE) and dark septate endophyte (DSE) fungi in terrestrial adventitious roots of *Epipremnum aureum*. (A) Extraradical hyphae (erh) and appressorium (ap) of AM fungi. (B) Intracellular hyphae (ih) of FRE fungi. (C) Appressorium (ap) and hyphal coils (hc) of AM fungi in the epidermal cells. (D) Paris-type hyphal coil (hc) of AM fungi in the cortex containing oil droplets (white arrow heads). (E) Intracellular hyphae (black arrow heads) and arbuscules (a) of intermediate type AM and FRE (white arrow heads) fungi. (F) Hyphal swellings (black arrow heads) in intercellular hyphae of AM fungi. (G) Cortical cell containing *Arum*-type arbuscule (a) and arbuscular trunk of AM (white arrow head) and FRE (black arrow head) fungi. (H) Miniature *Arum*-type arbuscule (a) of AM fungi. (I) Intracellular hyphae (ih), hyphal trunk (black arrow heads) and arbuscules (a) of FRE fungi. (J) Intracellular vesicle (v) with oil droplet (white arrow head). (K) Septate hyphae (black arrow heads) and microsclerotia (ms) of DSE fungi. Scale bars = 50μm.

a closer look at the images presented in Appendix 3 of that study indicates the structures illustrated are not fungal structures. But the epiphytic roots of *E. aureum* on reaching soil formed a symbiosis with different endophytic fungi. This is in line Nadarajah and Nawawi (1993) who observed AM structures in terrestrial roots of *Scindapsus aureus* Engl., a synonym of *E. aureum*.

Glomeromycotan symbiosis

Although a non-functional AM symbiosis characterized by hyphae and vesicles has been reported in terrestrial roots of *S. aureum* growing in oil palm plantations in Malaysia, this is the first detailed report of functional AM in *E. aureum* (Nadarajah and Nawawi 1993; Luginbuehl and Oldroyd 2017). Formation of an appressorium on the root surface before colonization of roots as observed in *E. aureum* can vary with the associating AM fungi. An appressorium development is mandatory for root penetration by members of Acaulosporaceae and Glomeraceae whereas members of Gigasporaceae can also penetrate the root directly without the formation of an appressorium (Dodd et al. 2000; Gao et al. 2001; Dickson 2004). The presence of large oil droplets in the AM fungal hyphae is consistent with the observations of previous studies where storage lipids are shown occurring in the intraradical mycelium of AM fungi (Bago et al. 2002). Although AM fungi are fatty acid auxotrophs, they obtain fatty acids synthesized by the host plant and store them in their intraradical and extraradical structures (Luginbuehl et al. 2017).

The complex intermediate AM morphology of *E. aureum* is exceptional to the typical *Arum*-type predominantly reported in this family (Dickson et al. 2007). Even though the typical *Arum*-type AM morphology is known after the colonization pattern in *Arum maculatum* L., of Araceae (Gallaud 1905); later studies have shown the occurrence of other types of AM morphology in this family. For example, *Paris*-type colonization was reported in *C. bicolor* and intermediate type AM was reported in *Arisaema tortuosum* (Wall.) Schott, and *Amorphophallus paeoniifolius* var. *campanulatus* (Decne.) Sivad. of this family (Johnston 1949; Muthukumar and Tamilselvi 2010; Muthukumar et al. 2018). Therefore, the observations of the present and other studies clearly show that the AM colonization patterns in aroids are polymorphic. However, the total root length colonized (%RLTC) by AM fungi in *E. aureum* is lower than those reported for other members in this family (Rains et al. 2003; Kumar et al. 2013; Muthukumar et al. 2018). As root colonization by AM fungi is influenced by several factors like the host plant, soil and fungal species the variation in %RLTC observed in the present and other studies are tenable (Smith and Read 2008).

The AM spore numbers reported in the present study is within the range reported for tropical soils and the species of AM fungi recoded are widely re-

ported from tropical soils (Kumar et al. 2016; Gupta et al. 2014; Muthukumar et al. 2018)

Fine root endophytes

Though AM and DSE symbiosis have been reported in aroids, the presence of FRE is reported for the first time in this family (Rains et al. 2003; Muthukumar and Tamilselvi 2010; Orchard et al. 2017a). The morphology of FRE symbiosis observed in the present study is similar to those described previously in the vascular and non-vascular plants (Orchard et al. 2017a; 2017b; Hoysted et al. 2019). The diameter of the FRE hyphae in the present study is $<2 \mu\text{m}$ as reported in previous studies (Orchard et al. 2017b; Kowal et al. 2020). Although aggregation of FRE fungal hyphae in the inter- or intracellular spaces of the cortical cells termed as 'ropes' has been reported in certain studies, we did not observe any such hyphal aggregation in roots of *E. aureum* (Gianinazzi-Pearson et al. 1981; Orchard et al., 2017b). The clear visualization of the arbuscular trunk and arbuscular branches is contrary to the perception that the arbuscular trunks of FRE were hard to perceive in an overlapping FRE and AM colonization (Gianinazzi-Pearson et al., 1981; Orchard et al. 2017b). The size of the FRE hyphal swellings observed in the present study is within the range for these structures reported in previous studies (Orchard et al. 2017b; Kowal et al. 2020). Though the hyphal swellings are termed as vesicles by Thippayarugs et al. (1999) and Kowal et al. (2020), the exact function of these structures in FRE symbiosis is unclear. The dual occurrence of AM and FRE fungi in the same root system or within same cells implies that both the fungal types could benefit the host plant in their acquisition of resources either additively or synergistically (Orchard et al. 2017b). Moreover, inclusion of molecular techniques in addition to morphological methods would be useful in confirming the presence of these fungal types in a root system.

In the present study, the %RLTC of FRE was 73% higher than those of AM and is in line with the studies where %RLTC by FRE either equaled or exceeded AM colonization in certain ecosystems (see Orchard et al. 2017b and references therein). Nevertheless, the %RLTC of *E. aureum* never exceeded 50% of the root length as those reported for plant species growing in pastures, agricultural fields, or native woodlands of Australasia (Cooper 1976; Orchard et al. 2016; Ryan and Kirkegaard 2012). Recent studies have shown that the cost of maintenance of FRE is similar or more than those of AM fungi and FRE fungi could transfer around 3%-9% and 0.6%-1% acquired P and N respectively to their associated host (Hoysted et al. 2019).

Dark septate endophytic fungi

A few studies in the past have recorded the co-occurrence of DSE fungi along with AM or FRE fungi

in different plant species (e.g., Postma et al. 2007; Gieseemann et al. 2020) although the dual occurrence of AM and DSE fungi are quite common (Zubek et al. 2011). The presence of DSE symbiosis in *E. aureum* is in accordance with the limited studies where roots of aroids are reported to be colonized by DSE fungi (Rains et al. 2003; Muthukumar and Tamilselvi 2010). However, the low colonization level of DSE recorded in *E. aureum* is similar to the observations in other studies where DSE colonization levels of <25% are reported for members of this family (Rains et al. 2003; Muthukumar and Tamilselvi 2010). Although the function of DSE symbiosis in plant roots is unclear, it is presumed that DSE symbiosis function similar to AM symbiosis under conditions that are unsuitable for the later (Gieseemann et al. 2020).

Interaction between fungal groups

There is a limited understanding on the coexistence of multiple symbioses in a plant root system. Significant correlations existed between percentage root length containing structures of different fungi despite the lack of significant correlations for the total root length colonized. Our observations on the positive correlation between AM and DSE fungal variables agree with Li et al. (2005) who found positive a correlation between DSE and AM in a grassland site in southwest China. Likewise, a similar relation was also found in grasses occurring in the Colorado Rocky Mountains of US (Ranelli 2015). Significant positive correlations between the root length containing AM and DSE fungal structures are also reported in *Asparagus* (Muthukumar and Muthuraja 2016). However, Mandyam and Jumpponen (2008) found no correlation between AM and DSE fungal variables in plants growing in a tallgrass prairie ecosystem that was influenced by nitrogen supplementation. Although these indicate that AM and DSE fungi do positively interact in the root environment, competition between these fungi can also occur in certain conditions (Pandey et al. 2016; de Mesquita et al. 2018).

One interesting observation made in the present study is the existence of significant negative correlations between certain AM and FRE variables. This suggests that FRE could affect the nutrient exchange sites of AM fungi (arbuscules and hyphal coils) or less the dependence of the host plant on AM fungi. Postma et al. (2007) also observed an inverse correlation between the root colonization of AM and FRE fungi in *Maianthemum bifolium* (L.) F.W.Schmidt growing in nutrient-poor acid soils of beech forests in Sweden. It has been suggested that the abundance and activity of FRE could increase under conditions that are not suitable for AM fungi (Postma et al. 2007). For example, the lycopod *Lycopodiella inundata* growing in semi-natural heathlands of the Netherlands and Britain is predominately colonized by FRE and exclusively benefits from the association (Kowal et al. 2020).

Conclusions

In spite of widespread cultivation as a popular ornamental worldwide, *E. aureum* was examined for FRE and DSE fungal symbiosis and AM morphology for the first time. Moreover, this is also the first report on the presence of FRE symbiosis in aroids. The results of the present study clearly indicate that roots of *E. aureum* can be colonized with different types of fungi. Our results show that the %RLTC and root length with different fungal structures significantly differed between the fungal groups. The predominance of FRE over other fungal groups in *E. aureum* opens up a new avenue for future research in exploring the role of FRE in aroids. Though the interactions between different fungi in *E. aureum* roots are mostly positive, a certain degree of competition tends to exist between AM and FRE fungi.

Author contributions

TM designed the research, analyzed the data and wrote the manuscript. SK performed the research.

Table 1. Hyphal diameter of arbuscular mycorrhizal (AM), fine root endophyte (FRE) and dark septate endophyte (DSE) fungi and vesicle/hyphal swelling dimensions of AM and FRE fungi.

Endophytic fungi	Hyphae (μm)	Vesicles/hyphal swellings (μm)	
		Length	Width
AM fungi	10.65 \pm 0.51a	31.25 \pm 1.76a	27.50 \pm 1.58a
FRE fungi	1.73 \pm 0.22c	10.38 \pm 0.53b	9.25 \pm 0.41b
DSE fungi	6.90 \pm 0.38b	--	--
Statistics	$F_{2,72} = 589.86^{***}$	$t_9 = 12.368^{***}$	$t_9 = 13.303^{***}$

Means \pm standard error in a column followed by the same letter are not significantly ($P > 0.05$) different.

***Significant at $P < 0.001$.

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Table 2. Extent of glomeromycotan arbuscular mycorrhizal (AM), fine root endophyte (FRE) and dark septate endophyte (DSE) fungal colonization in the terrestrial adventitious roots of *Epidendrum aureum*

Endophytic fungi	Fungal structures [#]										
	%RLIH	%RLIAH	%RLHC	%RLA	%RLAC	%RLV	%RLDSH	%RLMS	%RLTC		
AM fungi	2.87 ± 0.49b	4.40 ± 0.54b	5.20 ± 0.57b	4.67 ± 0.47b	8.27 ± 0.86b	0.53 ± 0.25b	--	--	26.00 ± 1.05b		
FRE fungi	7.33 ± 0.60a	6.80 ± 0.49a	11.07 ± 0.72a	9.20 ± 0.44a	11.20 ± 0.65a	2.40 ± 0.27a	--	--	45.60 ± 1.31a		
DSE fungi	--	--	--	--	--	--	2.53 ± 0.44	4.27 ± 0.50	6.80 ± 0.65c		
Statistics	t=-26.92***	t=-2.71*	t=-6.15**	t=-13.95***	t=-3.92*	t=-4.81**	--	--	F _{2,12} =363.43***		

[#]%RLIH, %RLIAH, %RLHC, %RLA, %RLAC, %RLV, %RLDSH, %RLMS, %RLTC, Percentage of root length with intercellular linear hyphae, intracellular linear hyphae, hyphal coils, arbuscules, arbusculate coils, vesicles, moniliform hyphae, microsclerotia and total colonization.

Means ± standard error in a column followed by the same letter are not significantly (P>0.05) different.

*, **, ***Significant at P<0.05, P<0.01 and P<0.001 respectively.