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Fine root endophyte association in widely cultivated palms of southern India

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ABSTRACT

The palm family represents one of the largest plant families of monocotyledons with mycorrhizal symbiosis. However, palms were never examined for the mycorrhizal symbiosis formed by fine root endophyte (FRE) fungi. Therefore, the present study was undertaken to examine the prevalence and intensity of FRE symbiosis in *Borassus flabellifer, Caryota urens, Cocos nucifera, Cyrtostachys renda, Dypsis lutescens,* and *Roystonea regia* cultivated in Tamilnadu. Further, the physicochemical properties of soils of these palm species were analyzed. The results of the present study revealed the presence of FRE colonization in all the examined palm species. There was a significant variation in the soil characteristics and the percentage of root length with different FRE fungal structures except for FRE hyphal coils among the palm species. The percentage of total root length colonization (%TRLC) by FRE fungi ranged from 24.32% (*R. regia*) to 47.18% (*C. nucifera*). Soil pH was significantly and negatively correlated to the percentage root length containing hyphae, arbuscules, and % TRLC of FRE fungi. To the best of our knowledge, this is the first report on the prevalence of FRE fungal colonization in the studied palm species. This FRE fungal association may aid the growth of the cultivated palms as the endophytic fungal symbiosis

Keywords: *Arecaceae*, *Cocos nucifera*, Fine Hyphae, FRE Colonization, *Planticonsortium tenue*

INTRODUCTION

The plant family *Arecaceae* (Palmae) is restricted to tropical regions, including northern and southern hemispheres comprising >2500 species belonging to 217 genera (Temikotan *et al.,* 2021). *Arecaceae* is represented in India by 24 genera and 225 species (Raj *et al.,* 2018). The woody perennial monocotyledonous palms form mutualistic associations with arbuscular mycorrhizal (AM) fungi and dark septate endophytic (DSE) fungi (Fisher and Jayachandran, 2008; Dreyer *et al.,* 2010; Rajeshkumar *et al.,* 2015). Recently, another root colonizing arbuscule-forming fungus *Planticonsortium tenue* (=*Glomus tenue*), often referred to as fine root endophyte (FRE) of the phylum *Mucoromycota* is gaining more interest in the field of mycorrhizal research (Walker *et al.,* 2018; Sinanaj *et al.,* 2021). Like typical AM fungi, FRE also establishes a nutritional symbiotic relationship with the majority of vascular plants (Field *et al.,* 2019) and benefits the host plants through increased nutrient uptake (Sinanaj *et al.,* 2021). Nevertheless, the significance and taxonomical status of FRE fungi are far from complete.

The AM fungi and FRE exhibit distinctive morphological traits within the plant roots. The hyphal diameter of AM fungi is >2µm, whereas FRE is characterized by the occurrence of fine hyphae with <2µm in diameter (Hoysted *et al.,* 2019). A palmate or fan-shaped entry point in the root epidermis and outer region of the cortex is often related to FRE. Consequently, fine hyphae produced by FRE spread both intra- and inter-cellularly inside the root cortical regions. Also, vesicle-like swellings and fine arbuscules are produced by aseptate hyphae in the inner root cortical cells (Orchard *et al.,* 2017a). It is speculated that isolation and observation of FRE spores are difficult due to their smaller dimension (≤20µm diameter) and colourless nature when young; but the spores turn dark brown at maturity (Albornoz *et al.,* 2020). In their review on FRE, Orchard *et al.* (2017b) listed the plant species that are colonized by FRE in both natural and agricultural ecosystems. Nevertheless, none of the plant species forming FRE association is from the palm family (Orchard *et al.,* 2017b). In addition, only limited studies have reported the occurrence of FRE fungi in India (Ganesan *et al.,* 1991; Parmar *et al.,* 2013; Bharathy *et al.,* 2021; Muthukumar and Karthik, 2021). Therefore, an attempt was made to examine the colonization by FRE fungi in widely cultivated palm species, *Borassus flabellifer, Caryota urens, Cocos nucifera, Cyrtostachys renda, Dypsis lutescens,* and *Roystonea regia* of Western Ghats region in South India.

MATERIAL AND METHODS

The roots and rhizosphere soil samples of six palm species were collected from three randomly selected trees growing in the foothills of Maruthamalai (11°2'20.47" N, 76°52'35.10" E), Coimbatore, Tamilnadu, India during April and May 2019. The Maruthamalai hill is located at an elevation of 450 m above sea level with a relative humidity of 70-75% and is mostly characterized by dry deciduous forest. The average annual minimum and maximum temperatures are 18°C and 38°C, respectively. This region receives rainfall from the north-west and south-west monsoons with an annual average of about 450 mm.

The collected roots were washed thoroughly to remove the debris attached to the roots and preserved in FAA (formalin: glacial acetic acid: 70% ethanol, 5:5:90) until processing. The physicochemical characteristics of the soil, such as pH and electrical conductivity (EC), were measured using respective digital meters. The total nitrogen (N), available phosphorus (P), and exchangeable potassium (K) were determined according to Jackson (1971).

The preserved root samples were washed free of FAA and cut into 1-2 cm pieces. These root bits were cleared with 2.5% potassium hydroxide (KOH) solution at 90°C for 3 hours, followed by acidification with 5N HCl, and stained using

0.05% Trypan blue overnight (Koske and Gemma, 1989). The stained roots were destained and mounted with lactoglycerol on clean microscope slides and observed for the presence of FRE structures under Olympus binocular light microscope at ×40 magnifications. The intensity of total FRE colonization and percentage of root length containing different FRE structures was quantified according to McGonigle et al. (1990) with three replicates each.

One-way analysis of variance (ANOVA) was used to access all the data after testing homogeneity using Levene's test. Pearson's correlation was used to determine the relationship between the soil characters and FRE variables. All the statistical analysis was performed using SPSS (version 16.0).

RESULTS

Soil characteristics

The soil characteristics such as pH, EC and macronutrients significantly (P<0.05) differed among the examined palm species (**Table 1**). The soil pH was slightly acidic to slightly alkaline, ranging from 6.65 (*C. renda*) to 7.75 (*R. regia*). Likewise, the EC ranged between 0.17 dSm^{-1} (*D. lutesens* and *R. regia*) and 0.32 dSm^{-1} (*C. renda*). The total N (114 kg ha^{-1}) and available P (127.33 kg ha^{-1}) were at the least in the soils of *C. nucifera* when compared to other studied palm species. Nevertheless, the maximum exchangeable soil K occurred in *B. flabellifer* (327.50 kg ha^{-1}).

Table 1: Soil characteristics of the cultivated palm species

Plant species	pH	Electrical conductivity (dSm^{-1})	Total nitrogen (kg ha^{-1})	Available phosphorus (kg ha^{-1})	Exchangeable potassium (kg ha^{-1})
Borassus flabellifer	#7.54±0.15ab	0.22±0.04b	118.50±1.44b	7.63±0.15b	327.50±7.22a
Caryota urens	7.21±0.02a	0.23±0.03b	118.50±0.87b	7.65±0.14b	300.00±5.77a
Cocos nucifera	6.65±0.20c	0.23±0.03b	114.00±1.15c	7.20±0.12b	314.50±19.92a
Cyrtostachys renda	7.37±0.08ab	0.32±0.03a	116.67±0.67bc	7.50±0.23b	117.67±2.85c
Dypsis lutescens	7.53±0.22ab	0.17±0.00b	127.33±0.33a	7.60±0.17b	325.00±8.66a
Roystonea regia	7.75±0.08a	0.17±0.00b	116.67±0.33bc	9.67±0.12a	240.33±0.33b
$F_{(5,17)}$	7.324**	3.655*	26.051***	31.290***	70.662***

#Means±standard error. Means in a column followed by different letters are significantly (P<0.05) different according to Duncan's Multiple Range Test. *Significance at 5% level, **Significant at 0.5%, ***Significant at 0.1%.

Morphology of FRE fungi

The incidence of FRE fungal colonization in the palm roots was characterized by an appressorium on the root surface (**Fig. 1 A**), fine hyphae that spread intra- and inter-cellularly inside the cortical region of roots (**Fig. 1 E, F**). The hyphae of FRE fungi had terminal and intercalary swellings (**Fig. 1 B, F**). The fine hyphae formed coils within the root cells, and the fine hyphae producing these coils were <2 μm in diameter (**Fig. 1 C, D**). The FRE fungi formed fine arbuscules (**Fig.1 G-J**) on slender hyphal trunks or indistinct branches (**Fig. 1G**).

The extent of FRE fungal colonization

The FRE fungi were characterized by the presence of fine hyphae, hyphal coils, and fine arbuscules in the palm roots. All the FRE fungal variables and total root length colonization (% TRLC) significantly (P<0.001) varied among the examined palm species except for hyphal coils (**Table 2**). The percentage of root length colonization with FRE hyphae (% FRLH) and arbuscules (% FRLAR) was 29.96% **-** 74.89% and 24.40% **-** 58.70% higher in *C. nucifera* than other examined palm species, respectively. Similarly, the maximum % TRLC was also observed in the roots of *C. nucifera* with 47.18%, which was 10.47% - 48.45% greater when compared to other palm species.

Relationship between soil characters and FRE fungal variables

Pearson's correlation (*n*=6) revealed a significant and negative relationship between soil pH and % FRLH (r=-0.990; P<0.01), % FRLAR (r=-0.874; P<0.05), and % TRLC (r=-0.938; P<0.01). However, % FRLH was positively correlated to % FRLAR (r=0.852; P<0.05) and % TRLC (r=0.959; P<0.01). Similarly, a significant positive correlation existed between % FRLAR and %TRLC (r=0.887; P<0.05).

DISCUSSION

Many studies have reported AM fungal colonization levels

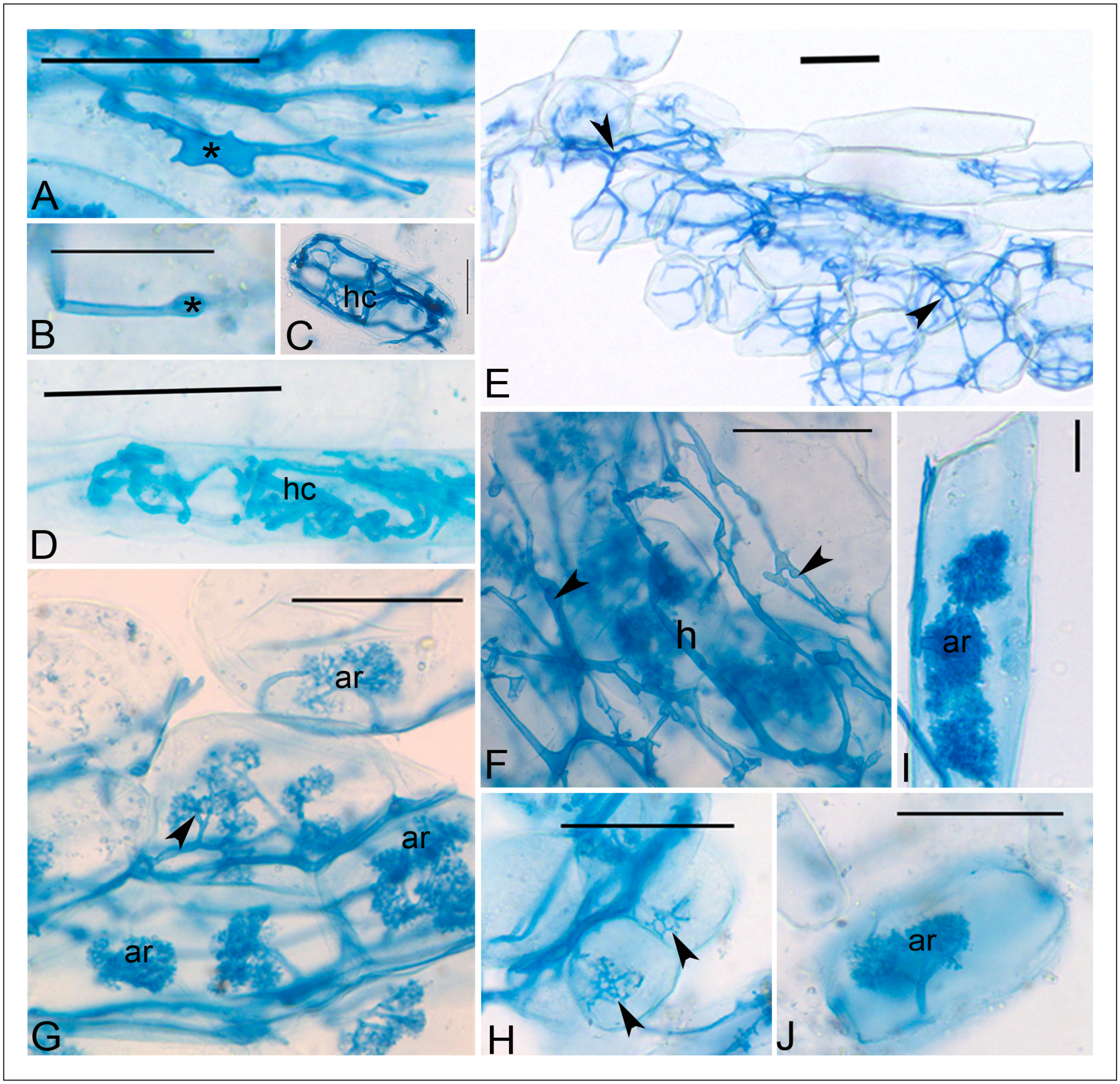

Fig. 1: Morphology of fine root endophyte (FRE) fungi in the cultivated palm species. Appressorium (black astrick) on the root surface of *Roystonea regia* (A); Hyphal swelling (black astrick) of FRE hyphae in *Caryota urens* (B); Fine hyphal coils (hc) in *Cocos nucifera* (C) and *R. regia* (D); Fine hyphae (black arrowheads) in *C. nucifera* (E); Fine hyphae (h) with intercalary hyphal swellings (black arrowheads) in *Dypsis lutesens* (F); Fine arbuscules (ar) on slender abuscular trunks (black arrowhead) in *C. urens* (G); Fine arbuscule (black arrowheads) in *C. nucifera* (H); Fine arbuscules (ar) in *Borassus flabellifer* (I), and *C. renda* (J). Scale bar = 50 µm

and patterns in most plant species, including palms. However, studies about FRE symbiosis in plant species are scarce (Kowal *et al.,* 2020). FRE is reported for the first time in all the examined palm species. Besides FRE, AM and DSE fungal structures were also observed in palm roots. The FRE fungi colonize the plant roots by forming a fan-shaped appressorium on the surface of the root (Gianinazzi-Pearson *et al.,* 1981; Orchard *et al.,* 2017b; Bharathy *et al.,* 2021). Nevertheless, the entry of FRE fungi in the examined palm roots was not preceded by such a structure. In addition, the roots of palm species colonized by prominent structures like fine hyphae and arbuscules, and intercalary and terminal swellings in the present study are similar to those reported in the earlier studies (Nicolson and Schenck, 1979; Gianinazzi-Pearson *et al.,* 1981; Thippayarugs *et al.,* 1999; Orchard *et al.,* 2017b; Bharathy *et al.,* 2021; Muthukumar and Karthik, 2021). In addition, the fine arbuscules on slender arbuscular trunks which are evident in the roots of palm species resemble

Table 2: Fine root endophytic fungal association in the cultivated palm species.

Plant species	Colonization (%)†			
	RLFH	RLFAR	R LFHC	TRLC
Borassus flabellifer	#11.21±1.82b	12.92±0.72b	5.78±1.55a	27.70±0.68b
Caryota urens	19.19±2.31a	14.81±1.70a	2.11±0.53a	42.24±3.23a
Cocos nucifera	27.40±2.23cd	19.59±2.36cd	6.25±2.23a	47.18±4.24bc
Cyrtostachys renda	14.88±1.03bc	9.03±1.28cd	5.4 0±1.35a	2 9.31±2.10bc
Dypsis lutescens	10.11±3.74cd	12.66±0.58bc	6.03±1.45a	28.69±3.29c
Roystonea regia	6.88±1.00d	8.09±0.96d	5.97±1.57a	24.32±0.51bc
$F_{(5,17)}$	11.163***	8.758***	1.047ns	16.366***

†RLFH- Root length colonization with fine hyphae, RLFHC- Root length colonization with fine hyphal coils, RLFAR- Root length colonization with fine arbuscules, TRLC- Total root length colonization.

#Means ± standard error. Means in a column followed by different letters are significantly (P<0.05) different according to Duncan's Multiple Range Test. ***Significant at 0.1% level, ns- non significant.

those observed in the roots of *Plantago lanceolata* (Orchard *et al.,* 2017b) and *Epipremnum aureum* (Muthukumar and Karthik, 2021). These fine arbuscules could help in the uptake of plant nutrients in particular P like arbuscules of AM fungi.

The hyphal swellings formed at intercalary or terminal regions of root cortical cells in the examined palm roots of the present study were referred to as vesicles (Nicolson and Schenck, 1979; Thippayarugs *et al.,* 1999; Orchard *et al.,* 2017b). Nevertheless, the role of these hyphal swellings is yet to be ascertained. On the contrary, a structure called 'ropes' formed by grouping numerous fine hyphae within inter- or intra-cellular spaces in the root cortex have been reported in the previous study (Nicolson and Schenck, 1979; Gianinazzi-Pearson *et al.,* 1981). Nevertheless, such structures were not observed in the roots of any of the examined palm species coinciding with the observations of Muthukumar and Karthik (2021) in the roots of *E. aureum* and roots of medicinal plants by Bharathy *et al.* (2021). These FRE associations in plants are attributed to enhanced nutritional status, including the transfer of P and N in exchange for photosynthetically fixed carbon from the host and assimilation of P in host plants (Sinanaj *et al.,* 2021).

In the present study, the %TRLC in examined palm species ranged between 27% - 47%, which is similar to the observations of other studies reporting <50% of FRE colonization in various plant species growing in different habitats (Orchard *et al.,* 2016; Bueno de Mesquita *et al.,* 2018; Muthukumar and Karthik, 2021). In the present study, %TRLC by FRE was lower in *R. regia* growing in higher soil P levels. Likewise, a higher %TRLC in *C. nucifera* may be due to low P content in the soil. Therefore, the colonization levels may vary depending on the nutrient acquisition over time (Bueno de Mesquita *et al.,* 2018). Besides, the soil characteristics also have a crucial role in root endophytic fungal colonization.

Palm species growing in low pH levels were colonized more by FRE and *vice versa* in the present study. This is supported by a negative correlation between soil pH and most of the FRE variables which are in line with the observations of previous studies (Wang *et al.,* 1985; Thippayarugs *et al.* 1999; Postma *et al.,* 2007). In addition, colonization by FRE fungi has been reported to inversely correlate with AM fungal association (Postma *et al.,* 2007; Orchard *et al.,* 2017a). The reduced FRE colonization at higher pH levels could be because of competition from AM fungi (Postma *et al.,* 2007) that are favoured by increasing soil pH (Frater *et al.,* 2018; Liu *et al.,* 2021). Therefore, the lower colonization by FRE fungi with increasing soil pH in the palms could be attributed to a higher level of AM fungal colonization.

CONCLUSION

The present study reveals the existence of *Mucoromycotina* FRE colonization in all the examined palm species for the first time. In addition to widely reported AM symbiosis in palm species, the prevalence of FRE fungi in the *Arecaceae* family could direct more research on FRE symbiosis in palm species in the future. Moreover, like AM fungi, the FRE association could assist in plant growth promotion of cultivated palm species.

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