KAVAKA 59(1): 16-24 (2023) DOI: 10.36460/Kavaka/59/1/2023/16-24

Diversity of Arbuscular Mycorrhizal Fungi in Association with Mangrove Plants in Tamil Nadu, India

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(Submitted on February 27, 2023; Accepted on February 28, 2023)

ABSTRACT

The present study investigated the diversity of arbuscular mycorrhizal fungi (AMF) associated with four mangrove plant species viz., *Avicennia marina*, *A. officinalis*, *Rhizophora apiculata* and *R. mucronata* in two locations of Cuddalore and Mayiladuthurai districts in Tamil Nadu, India. Soil properties were analysed to determine their potential effects on the distribution of AM fungi. The present study revealed that all the mangroves had AM fungal association with varying amount of root colonization (55-86%) and soil spore density (176 to 350 spores/100g soil). Among them, *R. mucronata* was recorded with the maximum spore density (350 spores/100 g soil) while *A. officinalis* had the minimum spore density (176 spores/100 g soil). Physico chemical analyses showed the soil had slightly acidic pH (6.2-6.5), low level of phosphorus (P) (14.23-17.25 kg/acre), and high level of nitrogen (N) (51.2-54.5 kg/acre). Soil P and salinity appeared to be the important factors influencing AM fungal association in mangrove plants. The AM fungal spores of four different genera viz., *Acaulospora*, *Gigaspora*, *Glomus*, and *Scutellospora* were recorded. The AM fungi were found to be an important component on the landward fringe of mangrove habitats.

Keywords: Arbuscular mycorrhizal fungi, *Acaulospora*, Mangroves, *Avicennia marina*, *A. officinalis*, *Gigaspora*, *Glomus*, *Scutellospora*, *Rhizophora apiculata*, *R. mucronata*

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi are keystone organisms with myriads of ecosystem roles in mangrove habitats. The external hyphal network of AM fungi permeates into the microsites of rocks and soils surrounding the plant roots (Finlay, 2008) increasing the root absorbing surface area by 100 or 1000-folds (Larcher, 1995). Therefore, AM fungi improve soil structure, water relation, nutrient supply, mangrove plant survival, growth, and reproductive success as well as increases tolerance of the plants to biotic and abiotic stressors (Finlay, 2008; Gianinazzi *et al.*, 2010; Karthikeyan and Krishna Kumar, 2012; Manaut *et al.*, 2015). AM fungi are considered to play a pivotal role in plant community assembly and succession (Janes, 1980; Van der Heijden *et al.*, 1998; Kikvidze *et al.*, 2010; Lin *et al.*, 2015).

The AM fungi play an important role in translocation and assimilation of soil nutrients in the wetland (Beena *et al.*, 2001). Earlier studies have reported that AM fungi predominate in soils with high salinity or alkalinity and low nutrients (Carvalho *et al.*, 2004; Evelin *et al.*, 2009). Although AM fungi require oxygen to develop well and it is assumed to be of little relevance in aquatic anaerobic conditions, recent studies have shown that AM fungi survive and colonize many halophytes (Mathur *et al.*, 2007). Flooding is suggested to influence the mycorrhizal association in aquatic plant species of wetlands in less fertile area with controlled water levels (Wang *et al.*, 2010). Miller and Bever (1999) recorded the distribution of nine different AM fungi in two wetlands, dominated by the grass species *Panicum hemitomon*. Sengupta and Chaudhuri (1990) reported AM fungi are associated with four species of pioneer salt marsh plants. Presence of mycorrhizae and dark septate mycelial endophytes in mangrove roots from the Ganges River Estuary have been reported by Sengupta and Chaudhuri (1994). Several studies have reported the occurrence and diversity of AM fungi in mangrove ecosystem (Kumar and Ghose, 2008; Wang *et al.*, 2010; Rodrigues and Rodrigues, 2013). However, only a few reports are available on the diversity of AM fungi in association with different mangrove plant species (Wang *et al.*, 2010; Kumar and Ghose, 2008). Hence, this study was undertaken on the diversity of AM fungi associated with four mangrove plant species in the Vellar and Pazhayar estuaries in Tamil Nadu, India.

MATERIAL AND METHODS

Study location and sample collection

Two study sites viz., Vellar estuary (11°29 N; and 79°46' E) and Pazhayar estuary (40°2' 39.2" N and 66°47'6.0" E) were chosen. Four dominant mangrove plant species *viz.*, *Avicennia marina*, *A. officinalis* (Avicenniaceae), *Rhizophora apiculata* and *R. mucronata* (Rhizophoraceae) were selected for the study. Both the study sites are warm and humid, with marshy soils. Mean temperature is in a range of 28-35°C, and average rainfall is 5037 mm.

Soil samples were randomly collected from the rhizosphere of all the four mangrove plant species during the pre-monsoon season (March-May, 2019) from the two study sites. The soil samples were placed in polyethylene bags, transported to the laboratory, and stored at 4 °C until processed. The soil samples of individual plants were air-dried at room temperature, sieved through mesh size of 150 µm, 250 µm, 300 µm and 500 µm and divided into two parts: one part was used for isolation, enumeration, and identification of AM fungal spores, while the other part was used to prepare trap culture and for soil analysis. The young feeder root samples attached to the plants were collected for estimating the percent colonization.

Soil analysis for physico-chemical properties

Four soil samples (from a depth of 0-25 cm) from each study site were separately collected in polyethylene bags, air-dried in the laboratory before passing through a 2 mm sieve, and mixed thoroughly to obtain a composite sample. Soil pH was measured in soil water (1:2) suspension using pH meter (LI 120 Elico, India). Electrical conductivity (EC) was measured at room temperature in 1:5 soil suspension, using a conductivity meter (CM -180 Elico, India). Standard soil analysis techniques, viz., Walkley and Black rapid titration method (Walkley and Black, 1934) and Bray and Kurtz method (Bray *et al.*, 1945) were employed for determination of organic carbon, available P and N, respectively, and available potassium was estimated by ammonium acetate method (Hanway *et al.*, 1952) using a flame photometer (model Systronic 3292).

Assessment of AM fungal root colonization

Collected root samples were rinsed with tap water and then the root samples were cleared with 10% KOH solution at 90 °C for 0-40 min, then washed with 2% HCl and stained with 0.05% Trypan blue stain (Phillips and Hayman, 1970). The stained root samples were examined under a compound light microscope for the presence of hyphae, arbuscules, vesicles, and spores. Root colonization was estimated using the grid-line intersect method (Newman, 1966; Giovannetti and Mosse, 1980). The total colonization rate (TC%) was calculated by summing the number of occurrence of vesicles, arbuscules or hyphae, while when the structures occurred together, they were counted as a single colony. The colonization rate of hyphae (HC%), colonization rate of vesicles (VC%) and colonization rate of arbuscules (AC%) were all counted using the method as described by McGonigle *et al*. (1990).

Isolation, characterization, and identification of AM fungal spores

As density of AM fungal spores in mangrove rhizosphere soil samples is low due to anaerobic condition, the soil samples collected from the root zone of the four mangrove plant species in two study locations were mixed with sterilized sand, and this soil mixture was kept in separate pots and sown with *Zea mays* (maize) seeds as host plant in order to multiply the number of existing AM fungal spores (Leal *et al.*, 2009; Stürmer and Siqueira, 2011). After 90 days, the soil samples were taken and analyzed to isolate AM fungal spores by using the wet sieving and decanting technique (Gerdemann and Nicolson, 1963). About 100 g of representative soil samples of each study location (in triplicate) was suspended in sufficient quantity of water and stirred thoroughly. The resulting soil suspension was sieved through the mesh sizes viz., 500 µm, 250 µm, 100 µm and 50 µm, arranged in a descending order. The sievates after sieving were collected carefully and filtered through Whatman No.1 filter paper and examined under a stereo zoom dissection microscope. Diagnostic slides with spores were prepared and examined for their morphological characteristics such as shape, size, attachment, and wall layers. The intact and the crushed spores were examined under a compound microscope and identified at genus and species level (Trappe, 1982; Schenck and Perez, 1987). Spores were identified based on spore morphology and sub-cellular characters and compared with original descriptions of Schenck and Perez (1987). Spore morphology was also compared with the culture data base established by INVAM (http://invam. cag.wvu.edu).

Diversity studies and statistical analysis:

Diversity studies were conducted for each site separately by calculating Simpson's Index of diversity 1-D (Simpson, 1949). D = 1 $-1-\sum(pi)2,$ where $p_{i=n_{i/N}}$ the relative abundance (RA) of the species was calculated as the proportion of individuals of a given species to the total number of individuals in a community. Shannon diversity index (H') is commonly used to characterize species diversity in a community, accounting for both abundance and evenness of the species present $H' = -\Sigma(pi \ln pi)$ (Shannon and Weaver, 1949). Species richness (SR) is the number of species present. Species evenness (E), which indicates the distribution of individuals within species, was calculated by using following formula $\sum(H') = H'/H'$max' where H'max' = 1n (SR). Pearson's correlation co efficient was calculated to assess the relationship between spore density and species richness at each site using SPSS 6.0 Windows student ver 3.5. Relative abundance of AM fungal species common to all seasons was correlated with soil pH, P and EC ($p \leq 0.05$).

RESULTS

Soil analysis

Physio-chemical properties of soils are given in **Table** 1. The soil pH was acidic, 6.6 in Pazhayar and 6.8 in Vellar estuaries. The EC was recorded as 7.39 dSm^{-1} in Vellar estuary and 10.15 dSm^{-1} in Pazhayar estuary. Both the study sites had high organic carbon and low level of P. Levels of N and K varied at the two study sites.

Table 1: Soil properties of the study sites.

Soil characteristics	Pazhayar estuary	Vellar estuary
pH	6.6 ± 0.39	6.8 ± 0.05
EC (dSm^{-1})	10.15 ± 2.06	7.39 ± 0.96
OC	4.45 ± 1.41	4.01 ± 1.37
P (Kg/acre)	17.25 ± 4.19	14.23 ± 3.12
K (Kg/acre)	418.75± 65.6	432.23 ± 85.82
N (Kg/acre)	52.5± 4.63	54.5 ± 6.55

Values are means of four readings at each study site; ± indicates SD

AM fungal spore density

Data on AM fungal spore density recorded from the soil samples of four mangrove plant species by using the test plant maize is presented in **Table** 2. AM fungal spore density significantly varied between mangroves plants at two different study sites. Mean spore density was significantly higher in Vellar estuary than in Pazhayar estuary. Maximum AM fungal spore density was recorded in *R. mucronata* (356 spores 100 g^{-1}), while minimum (172 spores 100 g^{-1}) was recorded in *A. officinalis* at the Vellar estuary site. While in Pazhayar estuary, the maximum AM fungal spore density was recorded in *R. mucronata* (246 spores 100 g^{-1}) and minimum (131 spores 100 g^{-1}) in *A. officinalis*.

Table 2: Spore density of AM fungal in four mangrove plant species at the study sites.

S. No.	Plant species	Vellar estuary	Pazhayar estuary
1	*Rhizophora mucronata*	356.75 ± 13.25	246.05 ± 8.85
2	*Avicennia marina*	240.00 ± 7.07	234.00 ± 10.48
3	*Rhizophora apiculata*	176. 05 ± 0.06	142.75 ± 4.75
4	*Avicennia officinalis*	172.25 ± 10.01	131.25 ± 2.98

AM fungal root colonization

Data on percent AM fungal root colonization in the mangrove plant species undertaken for the study is presented in **Table** 3. Spore density of AM fungi significantly varied between the mangroves plant species in the two study sites. The mean spore density was significantly higher in Vellar estuary than in Pazhayar estuary. Maximum AM fungal spore density was recorded from the rhizosphere samples of *R. mucronata* (356 spores 100 g^{-1}) and minimum (172 spores 100 g^{-1}) from the rhizosphere samples of *A. officinalis* at the Vellar estuary site, while maximum AM fungal spore density was recorded from the rhizosphere samples of *R. mucronata* (246 spores 100 g^{-1}) and minimum (131 spores 100 g^{-1}) from the rhizosphere samples of *A. officinalis* at Pazhayar estuary site.

Table 3: Percent root colonization of AM fungi in association with different mangrove plants at two study sites.

S. No.	Plant species	Vellar estuary	Pazhayar estuary
1	*Rhizophora mucronata*	84%	78%
2	*Avicennia marina*	82%	70%
3	*Rhizophora apiculata*	75%	68%
4	*Avicennia officinalis*	72%	62%

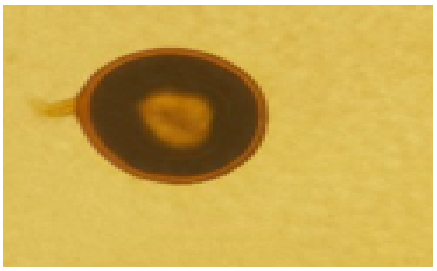
Frequency of occurrence and Relative abundance of AM fungal species

A total of 23 AM fungal species belonging to four genera *viz*., *Acaulospora*, *Gigaspora*, *Glomus*, and *Scutellospora* were recovered from the rhizosphere soils of four mangrove plant species collected from the two study sites (**Table 4**; **Plate 1**). The genus *Glomus* was dominant, followed by *Scutellospora*, *Acaulospora*, and *Gigaspora*. One sporocarpic species, viz., *Glomus aggregatum* was recovered. Within AM fungal species, the highest RA was recorded for *Glomus fasciculatum*, followed by *Glomus* sp. 3, *Glomus dimorphicum*, *Acaulospora laevis*, and *Gigaspora gigantea* whereas the lowest RA was recorded for *Acaulospora denticulata*. *G. fasciculatum*, *G. intraradices*, *A. denticulata*, *Glomus microaggregatum*, *G. aggregatum*, *G. gigantea*, *Acaulospora taiwania*, *Scutellospora verucosa*, *Glomus mosseae*, *G. palidum*, and *Scutellospora nigra* at the two study sites. The root samples of all the four mangrove plant species showed AM fungal colonization as evident by the presence of vesicles, arbuscules and hyphae (**Plate 1**).

Table 4: Relative abundance of AM fungi in the selected study sites.

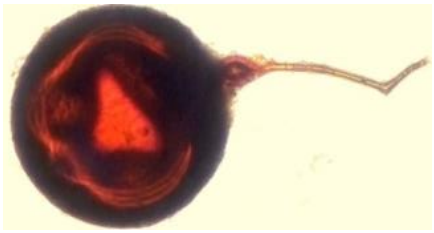
S.No.	AMF species	Vellar estuary	Pazhayar estuary
1	*Glomus dimorphicum* (Boyetchko & Tewari)	6.54	-
2	*Glomus* sp.1	6.07	-
3	*Scutellospora nigra* (Red Head) Walker & Sanders.	4.20	0.50
4	*Glomus* sp. 3	7.00	-
5	*Acaulospora taiwania*	2.80	0.36
6	*Glomus aggregatum* (Schenck & Smith)	3.73	0.82
7	*Acaulospora* sp.1	3.73	-
8	*Gigaspora gigantea* (Nicolson, Gerd. & Trappe)	1.86	0.50
9	*Glomus microaggregatum* (Koske, Gemma & Olexia)	2.80	0.82
10	*Glomus fasciculatum* (Thaxt) Gerd & Trappe	7.47	1.05
11	*Scutellospora* sp. 2	2.33	-
12	*Glomus intraradices*	4.20	0.73
13	*Glomus mosseae*	2.33	0.78
14	*Glomus austral* (Beek) S.M. Berch	2.80	0.73
15	*Glomus palidum* (L.R. Hall)	2.33	0.87
16	*Glomus* sp. 2	4.20	-
17	*Gigaspora* sp. 1	2.80	-
18	*Acaulospora denticulata* (Sieverd and Toro)	1.86	0.45
19	*Glomus* sp. 4	5.14	-
20	*Glomus constrictum*	-	1.03
21	*Scutellospora verrucosa* (Koskee,Walker, Read & Sander	4.20	-
22	*Scutellospora* sp. 3	1.86	-
23	*Scutellospora heterogenus* (Nicolson & Gerd.)	2.80	-

All the values are means of the composite sample of four plant species for each study site.

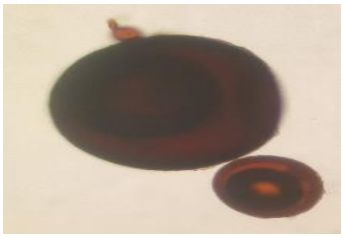


Glomus fasciculatum

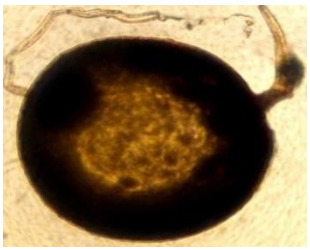
Acaulospora sp.



Gigaspora sp. (x 200)

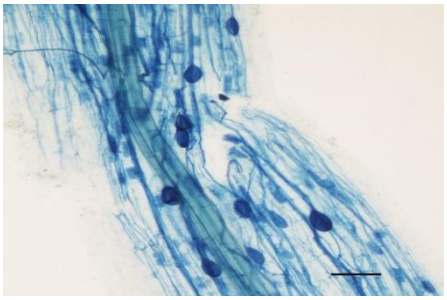


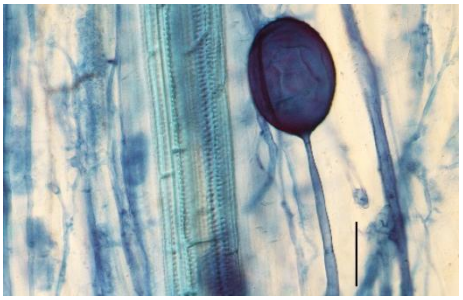
Scutellospora sp. (x 400)



Glomus intraradices (x 400)

Arbuscular mycorrhizal fungal spores isolated from the rhizosphere soil samples of different mangrove plants





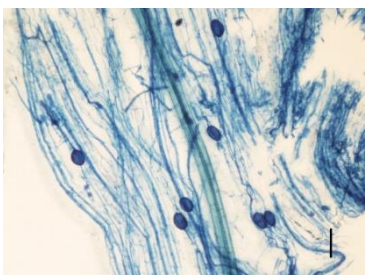


Plate 1: Root segments showing vesicles, arbuscules, and hyphae in root of different mangrove plants (x 200 to x 400).

Species Richness and Species Evenness

Species richness was maximum (22 spp.) in Vellar estuary and minimum (12 spp.) in Pazhayar estuary. In Vellar estuary, the Shannon's diversity index was highest in *R. mucronata* (4.221) and was least in *A. officinalis* (1.730). In Pazhayar estuary, Shannon's diversity index was highest in *A. marina* (3.464) and was least in *A. officinalis* (3.198). In both the study sites, *A. officinalis* showed that lowest Shannon diversity index.

DISCUSSION

Root-associated mycorrhizas are known to significantly make a contribution to plant community nutrient balances (Read, 1996). Mycorrhizas are known to improve plant species' tolerance to a variety of physical and chemical stresses (Marschner *et al.,* 1995). Because saline soils cover more than 7% of the earth's surface, the AM fungal interaction with vegetation in saline habitats is becoming increasingly important in understanding and rehabilitating the ecosystem to maximize benefits. AM fungi have been shown to play an important role in community development in nitrogen mineralizing, organic carbon, and phosphate deficient mangrove soils.

This study reports the occurrence and distribution of AM fungi in association with four mangrove plant species from the two study sites. The present study revealed that 94% of mangrove species were mycorrhizal. Similar observations have been reported earlier (Sengupta and Chaudhuri, 2002; Kumar and Ghose, 2008; Wang *et al.,* 2010). However, the present study contradicts with the earlier observation of Mohankumar and Mahadevan (1987) who reported absence of AM fungi in mangroves of Pichavaram, Tamil Nadu in India. Sengupta and Chaudhuri (2002) studied AM fungal colonization in a mangrove plant community with four successional stages and found that decreasing soil-water salinity increased the intensity of colonisation, as seen in our study. Glomalean fungi spores (7 species) were found in all four stages of mangrove succession in Sundarban mangroves, with *Glomus mosseae*, *G. fasciculatum*, and *Gigaspora margarita* dominating. They believe that AM fungal spores and root fragments containing AM fungi were transported upstream and exhibited adaptive tolerance to salinity and inundation in mangroves. Kumar and Ghose (2008), on the other hand, concluded that inundation has no effect on the spore density of AM fungi in Sundarban mangroves.

The present study analyzed AM fungi in relation to soil properties. EC was significantly correlated to AM fungal root colonization with *R. mucronata* in Pazhayar estuary ($r = -0.56$, $p = 0.088$) while other soil parameters did not show any correlation with AM fungal colonization for both the study sites. Analyses of the data for all the species together revealed that the soil P had significant negative correlation with AM root colonization and spore density. This finding is corroborated with Kumar and Ghose (2008). Earlier reports have shown that abiotic factors affect AM root colonization and their hydrological conditions (Miller and Sharitz, 2000; Wang *et al.,* 2010), and levels of P (Chen *et al.,* 2008), N (Eom *et al.,* 1999), and organic matter content (Albertsen *et al.,* 2006) in soil. Soil P content was very low in this study, and this might be the restricting factor for AMF root colonization and soil spore density. However, soil N content did not influence AMF root colonization and spore density. Salinity showed negative correlation with spore density, which is in conformity with earlier studies in mangrove (Sengupta and Chaudhuri, 2002) and coastal ecosystem (Guo and Gong, 2014). The root hairs of mangrove plant species were small and poorly developed. This feature is known to create potential plant mycotrophic, enhancing nutrient acquisition in stressed environments (Baylis, 1975). Many host factors (e.g., host species, phenology, mycorrhizal dependency, and changes in soil environment) have been predicted to influence AM fungal colonization and spore density in the rhizosphere (Eom *et al.,* 2000). In our study, there appears to be a direct impact of host on AM fungal colonization, spore density, and frequency of occurrence, as seen by Kumar and Ghose (2008) in Sundarban mangroves.

In present study, hyphae were the dominant AM fungal structures as observed in an earlier study (Sengupta and Chaudhuri, 2002). This can possibly be explained by the high moisture levels in mangrove habitat. The mechanism by which AM fungi survive in hypoxic conditions is known (Miller and Bever, 1999). Since AM fungi are aerobic microorganisms, their occurrence is related to the well-developed aerenchyma, and when flooded, they may survive by relying on oxygen provided by the aerenchyma. The AM fungi have potential to alter at least some aspects of plant morphology under stressful conditions. However, further research is needed to understand the adaptive mechanism of AM fungi in mangrove environment. Other studies have found that the AM fungal spore richness does not decrease significantly with increasing soil salinity, as demonstrated by Kumar and Ghose (2008). Salt stress appears to promote sporulation in AM fungi (Tresner and Hayes, 1971). The extent of salinity most likely determines the function of AM fungi (vegetative vs. reproductive phase) in mangrove habitats, which benefits the host plant species. As demonstrated by Kumar and Ghose (2008) in the Sundarbans, AM colonization in *Canavalia cathartica* in Nethravathi was negatively correlated with rhizosphere salinity. However, the

fact that spore richness in *Spinefix littoreus* was positively correlated with rhizosphere salinity supports the notion that AM spore richness is more dependent on host plant species than soil.

CONCLUSION

Mangrove plant communities interact with rhizosphere soil and can modify edaphic properties. Similarly, edaphic factors interact with plant communities and modify the rhizosphere composition. In this study, there was no clear observation on the seasonal variations of AM fungal sporulation. Ecological studies of AM fungi help to predict the abiotic factors affecting their development. Further, targeted seasonal studies are needed to consider the combined effect on the occurrence and distribution of AM fungi in different phenological stages of mangroves to provide an accurate picture of AM fungal development and functions prevailing in the mangrove ecosystem. Until now, our understanding of root AM fungal colonization and spore density in mangroves is still in its infancy. Studying the characterization of AM fungal root colonization and spore density in mangroves will help in understanding the factors that influence their distribution, as well as their application in bioremediation of mangroves.

ACKNOWLEDGEMENTS

The authors thankfully acknowledge the Director, Institute of Forest Genetics and Tree Breeding, Coimbatore, Tamil Nadu, India for providing the necessary facilities.

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