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Seasonal variations in diversity and distribution of arbuscular mycorrhizal fungi in mangrove species of Indian East and the West coast

Sankrita Gaonkar[1*] and B.F. Rodrigues[2]

[1]*Department of Botany, Govt. College of Arts, Science and Commerce, Quepem, Goa - 403 705*

[2]*School of Biological Sciences and Biotechnology, Goa University, Taleigao Plateau, Panjim, Goa - 403 206*

[*]*Corresponding author Email: sankrita002@gmail.com*

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ABSTRACT

The impact of arbuscular mycorrhizal (AM) fungi on the diversity and successional pattern of the plant population have stimulated the necessity to identify the factors maintaining their diversity and abundance. The present study investigated AM fungal communities colonizing the roots of three plant species, *Avicennia marina* (Forssk.) Vierh., *Bruguiera cylindrica* (L.) Blume and *Excoecaria agallocha* L. in two different Indian coasts, Chorao Island, Goa, and Pichavaram Forest, Tamil Nadu, to varying seasons. The results of the present study revealed that root colonization, spore density, and relative abundance varied among the three plant species in all seasons. The highest root colonization and spore density were detected in pre-monsoon and monsoon, respectively. The two coastal habitats hosted different AM fungal communities. Chorao Island presented the dominance of AM fungal species belonging to the family *Acaulosporaceae*, while there was the dominance of *Glomeraceae* at Pichavaram Forest. The study revealed that the season, host plant, and soil properties influence AM fungal symbiosis. The CCA indicated that the soil attributes such as OC, N, Mn, Zn, and Fe significantly influenced the abundance of *Acaulospora, Funneliformis, Gigaspora, and Sclerocystis. In contrast, EC affected the Rhizophagus, Glomus,* and *Entrophospora* species.

Keywords: Canonical correspondence analysis, Dominant species, Relative abundance, Root colonization, Spore density

INTRODUCTION

Mangroves are hydrohalophytes inhabiting tropical and sub-tropical estuaries with regular tidal inundation and fluctuating salinity (Gopinathan *et al.,* 2017). They are endowed with high productivity levels, fulfilling vital economic functions (Kathiresan*,* 2000). The coastal plains of India display vast and diverse mangrove ecosystems, of which 56.7% (2, 75,800 ha) are situated on the east coast, 23.5% (1, 14,700 ha) on the west coast, and 19.8% (96,600 ha) in Andaman and Nicobar Islands (Selvam *et al.,* 2010). Chorao Island on the west coast is the largest Island in Goa, supporting rich mangrove forests of about 178 ha, and its major mangrove area is declared a Reserved Forest under the Indian Forest Act, 1927. Besides, it was declared a Bird Sanctuary in 1988 (Nagi *et al.,* 2014). Creeks and backwaters divide Chorao Island having tidal variations formed by Mandovi and Mapusa Rivers. During monsoon season, the efflux of freshwater makes the estuary limnetic from head to mouth (D'Souza *et al.,* 2015). Pichavaram Forest on the East coast is among the best-studied mangrove ecosystems in India (Kathiresan, 2000), with a total mangrove cover of 1,350 ha separated by small Islands. It is located in the extreme North of the Cauvery river near the mouth of the Coleroon estuary (Selvam *et al.,* 2010).

Phosphorus (P), the major seawater element, is a limiting nutrient as it is either bound to or adsorbed to silt and clay particles (Dastager and Damare, 2013). thereby restricting the growth of mangrove plants (Reef *et al.,* 2010). Microbes inhabiting the rhizosphere may promote solubilization and mobilization of the bound P into available form (Bhattacharyya and Jha, 2012; Gaonkar and Rodrigues, 2020). Arbuscular mycorrhizal fungal symbiosis is a promiscuous association that colonizes the plant roots and assists in better nutrient uptake, especially P, thereby improving plant growth, salinity tolerance, and reduced biotic stress (Neelakandan, 2015; Yinan *et al.,* 2017; Ramírez-Viga, 2018). Studies have shown that AM fungi can tackle salinity either by exclusion or tolerance of salts in their cells, thus reducing the host's salinity stress (Xie *et al.,* 2014). Since AM fungi need oxygen to thrive, flooding (forming hypoxic conditions) has been defined as one of the chief abiotic factors affecting root colonization by these fungi in the mangrove ecosystem (Wang *et al.,* 2011). It is recognized that AM fungi rely upon oxygen provided by aerenchyma during flooded conditions (Wang *et al.,* 2010).

Hydrological conditions, which fluctuate with seasons, affect the abundance and colonization of AM fungi in the soil, which may successively influence the performance of the host plant (Escudero and Mendoza, 2005). Throughout the growing season, plants experience variations in soil moisture with the incidence of flooding and dry periods. Reduction in soil moisture leads to depletion of nutrients, favouring the AM fungal association as a response to host nutrient demands (Muthukumar and Udaiyan, 2002).

Earlier studies suggest that AM fungi are significant drivers of the diversity and distribution of the plant community (Wang *et al.,* 2010; Sridhar *et al.,* 2011). However, until now, no investigations have been conducted to analyze the comparative seasonal variations in the composition of AM fungal communities on the East and West coasts of India. Hence, the study proposes to determine the effect of season, host, and soil parameters on the diversity of AM fungi in three common mangrove plant species from two selected estuarine regions, i.e., Chorao Island on the West coast and Pichavaram on the East coast of India.

MATERIAL AND METHODS

Study area and sample collection

Chorao Island is situated on the southwest coast of India, surrounded by the Mandovi and Mapusa rivers of Goa (**Fig. 1**). In the Western region, the island is roofed by a thick stretch of about 1.78 km^2 of conserved mangrove forest. The geographic location of the site is 15°32′50.7″N and 73°52′45.8″E. Both diurnal and semidiurnal tidal inundations influence the estuary. The average annual rainfall on the island is nearly 3000 mm, inhabited by diverse mangrove species, with *Rhizophora, Avicennia,* and *Sonneratia* being the dominant plant genera in that order (Sappal *et al.,* 2014).

Pichavaram mangrove forest is situated at 11^{O} 29' N and 79^{0} 46' E on the southeast coast of India (**Fig. 1**). The forest comprises 51 islets divided by intricate waterways connecting Vellar and Coleroon estuaries. It experiences micro and diurnal tides. It receives an average annual rainfall of 1310 mm, having most of the rainfall occurring during the northeast monsoon season (Selvam *et al.,* 2003). The dominant mangrove genera at Pichavaram Forest are *Avicennia, Excoecaria,* and *Rhizophora* (Kathiresan, 2000).

The years arranged into three seasons are different for the East and West coast. The seasonal months for the East coast are June to September (Pre-monsoon), October to December (Monsoon), and January to May (Post-monsoon). Whereas for the West coast, it is February to May (Pre-monsoon), June to October (Monsoon), and November to January (Post-monsoon).

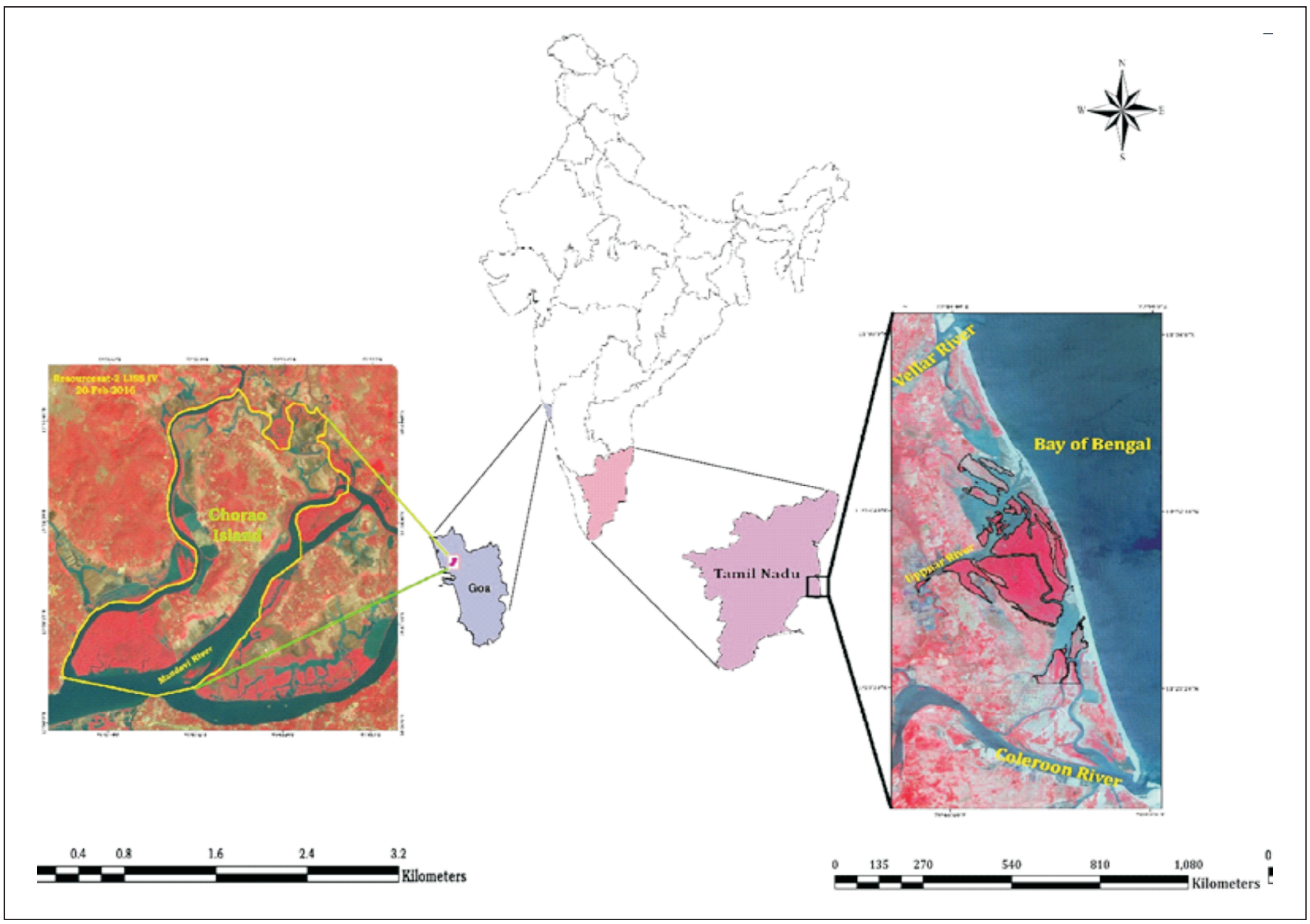

Fig. 1: Map of India showing the study sites of East and West coastal mangroves.

Rhizosphere samples of three common plants *viz., Avicennia marina* (Forssk.) Vierh., *Bruguiera cylindrica* (L.) Blume and *Excoecaria agallocha* L. from both the sites were collected during all three seasons. Rhizosphere and root samples for each of the three plants were collected from three representative individuals. The soil samples were carried in polyethylene bags and air-dried in the laboratory. One part of the soil sample was used as an inoculum for preparing trap cultures, and the other was used for the isolation and identification of AM fungi.

Soil analyses

The soil samples from 0-15 cm depth were collected separately in triplicates from the two mangrove sites during all three seasons. All the soil samples were air-dried before analysis. Soil pH and electrical conductivity (EC) were measured with pH meter (LI 120 Elico, India) and Conductivity meter (CM-180 Elico, India), respectively, in soil water suspension (1:2 ratio). Organic carbon (OC) was estimated by Walkley and Black's (1934) method by oxidizing it using potassium dichromate in an acidic medium and titrating the residual dichromate against ferrous ammonium sulphate (FAS). Available nitrogen (N) was estimated by oxidative hydrolysis of liberated ammonia using $KMnO_4$, absorbing it on boric acid, and titrating against standard acid (Subbiah and Asija, 1956). Available P was extracted with 1.5% Dickman and Bray's reagent and determined by colorimetry (Bray and Kurtz, 1945). The available potassium was extracted with 1N ammonium acetate and estimated by flame photometry (Hanway and Heidel, 1952). Available zinc (Zn), copper (Cu), manganese (Mn), and iron (Fe) were extracted using DTPA (diethylene triamine penta acetic acid) extractant with soil: reagent ratio of 1:2 (Lindsay and Norvell, 1978) and quantified using atomic absorption spectrophotometer (AAS) (nova 400P, Analytik Jena, Germany).

Assessment of AM root colonization

Root bits (1 cm) were rinsed in water, hydrolyzed in 10% KOH solution at 90°C for 1.5 hours, cleared in 5N HCl for 10 minutes, and stained in 0.05% Trypan blue stain overnight (Phillips and Hayman, 1970). After staining, root bits were placed on glass slides observed under a bright field Olympus BX41 research microscope.

The following formula was used for the estimation of percent AM root colonization:

$$\text{Relative abundance (\%)} = \frac{\text{Number of spores of a species/genus}}{\text{Total number of spores in all soil samples}} \times 100$$

Preparation of trap cultures

Trap cultures were prepared to propagate healthy viable spores to identify AM species. Pots were filled with the rhizosphere sample and sterilized sand (1:1) (w/w) using *Plectranthus scutellarioides* (coleus) as the host plant and were kept in the polyhouse at 27°C for six months.

Spore extraction and identification

AM fungal spores were extracted from field samples and trap cultures following the Wet sieving and decanting technique (Gerdemann and Nicolson, 1963). Intact and healthy spores were identified according to the description given by Blaszkowski (2012), Rodrigues and Muthukumar (2009), and the International Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM). The names were confirmed with the recommendations of Schüßler and Walker (2010) and Redecker *et al.* (2013).

Ecological studies and statistical analysis

$$\text{Percent colonization} = \frac{\text{Number of root segments colonized}}{\text{Total number of root segments observed}} \times 100$$

Canonical correspondence analysis (CCA) was used to test the association of AM fungal relative abundance (RA) with soil parameters at two different sites during three different seasons. Multivariate Statistical Package (MVSP) v3.1 was used to perform CCA.

RESULTS

Soil properties

Results of soil analysis are depicted in **table 1**. The results revealed that the soils of Chorao Island are acidic, whereas Pichavaram soils are almost neutral. Both the sites exhibited high levels of EC during the pre-monsoon season and were least during monsoon. Phosphorus levels were low at both locations. Iron content in Chorao soils was found to be higher

Table 1: Soil characteristics at the two sites during different seasons

Parameters	Pre-monsoon		Monsoon		Post-monsoon	
	Chorao	Pichavaram	Chorao	Pichavaram	Chorao	Pichavaram
pH	5.3 ± 0.5^{bc}	6.8 ± 0.7^{a}	4.9 ± 0.5^{d}	6.9 ± 0.7^{a}	5.1 ± 0.5^{bc}	6.2 ± 0.6^{ab}
EC (mS/cm)	15.6 ± 1.7^{a}	16.0 ± 1.8^{a}	1.9 ± 0.2^{d}	8.7 ± 0.9^{c}	14.1 ± 1.6^{ab}	9.6 ± 1.2^{c}
OC (%)	1.3 ± 0.2^{ab}	0.2 ± 0.0^{c}	2.4 ± 0.3^{a}	1.0 ± 0.1^{ab}	2.0 ± 0.2^{a}	1.0 ± 0.1^{ab}
N (g/kg)	0.1 ± 0.0^{a}	0.03 ± 0.0^{ab}	0.1 ± 0.0^{a}	0.05 ± 0.0^{ab}	0.1 ± 0.0^{a}	0.07 ± 0.0^{a}
P (g/kg)	0.05 ± 0.0^{a}	0.04 ± 0.0^{a}	0.05 ± 0.0^{a}	0.07 ± 0.0^{a}	0.05 ± 0.0^{a}	0.04 ± 0.0^{a}
K (g/kg)	1.4 ± 0.4^{a}	0.8 ± 0.3^{ab}	1.2 ± 0.4^{a}	0.9 ± 0.2^{ab}	1.5 ± 0.2^{a}	0.7 ± 0.2^{ab}
Fe (ppm)	112.5 ± 4.5^{c}	15.9 ± 8.7^{f}	218.1 ± 9.5^{ab}	63.5 ± 0.6^{d}	236.3 ± 2.5^{a}	41.1 ± 1.6^{de}
Mn (ppm)	43.6 ± 4.4^{a}	19.3 ± 3.9^{d}	39.6 ± 4.2^{ab}	24.5 ± 1.9^{c}	42.0 ± 2.5^{a}	25.3 ± 2.5^{c}
Zn (ppm)	22.1 ± 2.5^{a}	3.4 ± 0.2^{bc}	2.2 ± 0.7^{cd}	3.4 ± 0.4^{bc}	5.9 ± 0.4^{b}	4.5 ± 0.5^{bc}
Cu (ppm)	0.7 ± 0.2^{a}	0.3 ± 0.1^{a}	0.4 ± 0.2^{a}	0.3 ± 0.1^{a}	0.7 ± 0.1^{a}	0.3 ± 0.1^{a}

Note: All values are the mean of three readings; ± = Standard error; EC= Electrical conductivity; OC= Organic carbon. Values in the same row not sharing the same letters are significantly different ($P \leq 0.05$).

than in Pichavaram soils.

Root colonization and spore density

The AM root colonization was observed in all the three plants investigated during all the seasons (**Fig. 2. a, b**). This suggests the dependency of the host plant on AM fungi throughout the year. At Chorao, root colonization intensity was high during pre-and post-monsoon seasons. Whereas, at Pichavaram, all the plant species studied showed differential patterns of colonization rates in different seasons. The percent root colonization was highest in *E. agallocha* at Chorao Island, while the lowest was in *A. marina* at Pichavaram in the post-monsoon season (**Fig. 3**). Spore density during pre-monsoon ranged from 22-83 spores, 53-124 spores in monsoon, and 39-

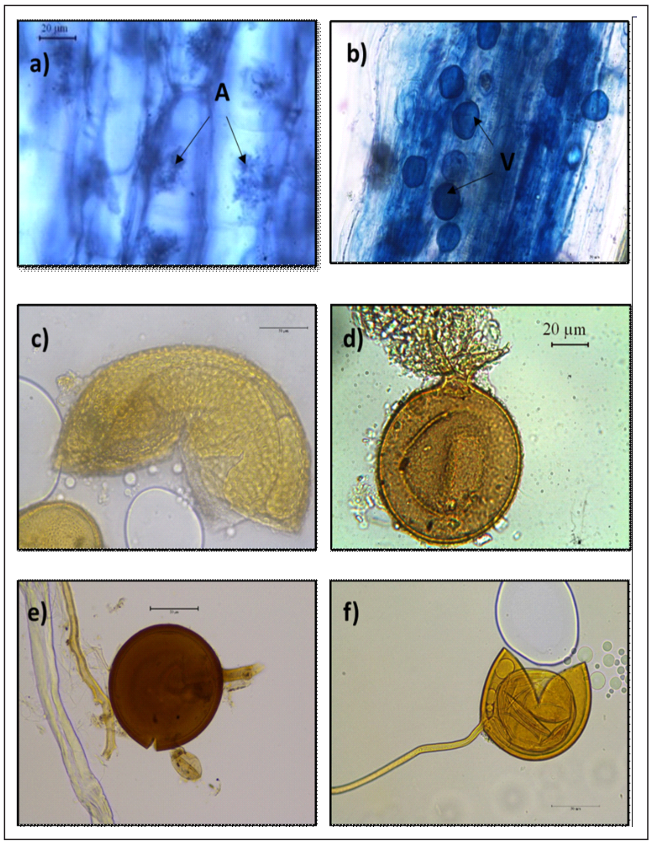

Fig. 2: AM fungal species and their structures in host roots. a) A stands for arbuscule b) V stands for vesicles c) *Acaulospora undulata* d) *Entrophospora* sp. e) *Funneliformis geosporus* f) *Rhizophagus fasciculatus*

162 spores/100g of soil in the post-monsoon (**Fig. 4**).

AM species diversity and relative abundance (RA)

In total, 19 AM species of eight genera belonging to five families were recovered from different sites, seasons, and host plants. Out of 19 AM species, four species viz., *A. undulata, Entrophospora* sp., *F. geosporus,* and *R. fasciculatus* were found in almost all the seasons at both the sites (**Fig. 2. c-f**). Both sites recorded high AM diversity and species evenness during pre-monsoon. Species of *Acaulosporaceae* were most abundant at Chorao, whereas at

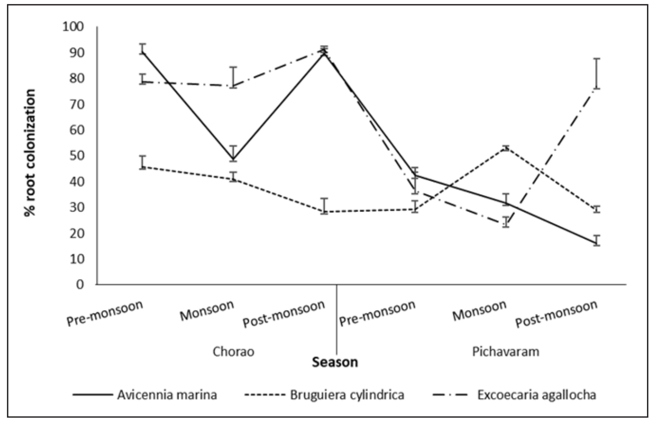

Fig. 3: Seasonal variations in arbuscular mycorrhizal root colonization

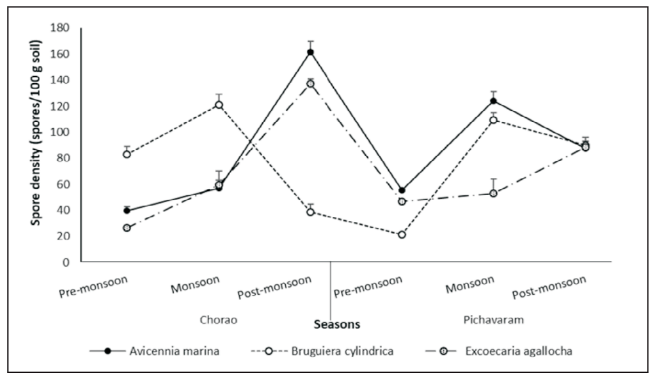

Fig. 4: Seasonal variations in arbuscular mycorrhizal spore density.

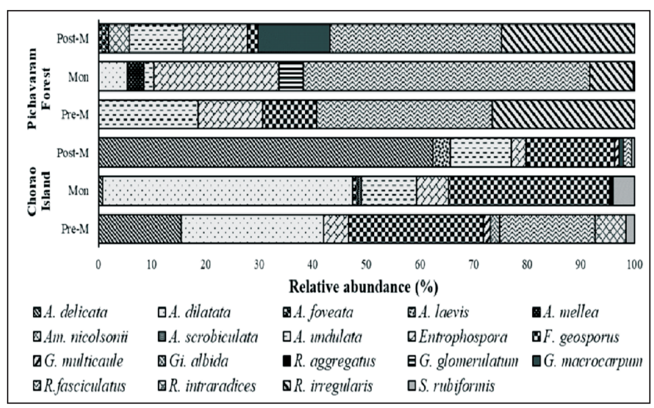

Fig. 5: Seasonal variation in relative abundance (%). Am=*Ambispora,* A=*Acaulospora,* F=*Funneliformis,* G=*Glomus,* Gi=*Gigaspora,* R=*Rhizophagus,* S=*Sclerocystis*.

Pichavaram, *Glomeraceae* showed high abundance. Season-wise results of RA at the different study sites are represented in **fig. 5**.

Relationship between AM fungal species and soil

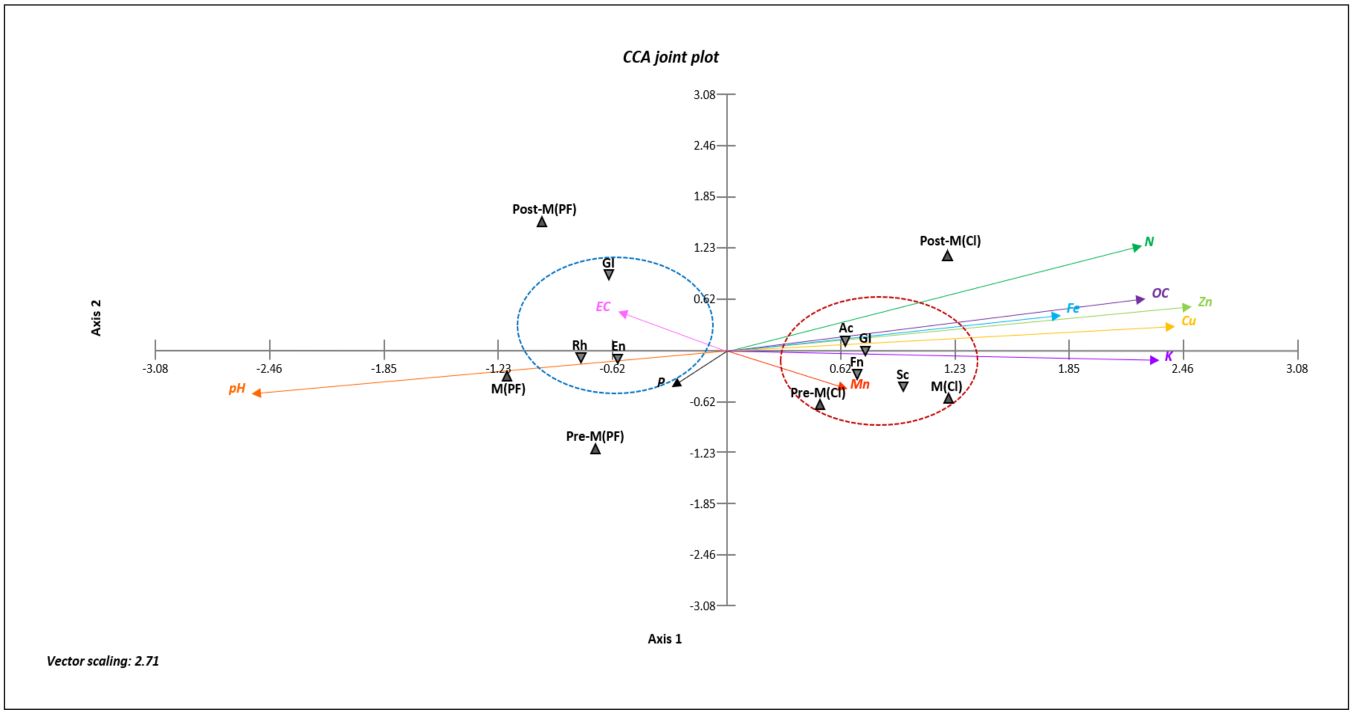

Fig. 6: Canonical correspondence analysis (CCA) of the relationship between AMF genera and soil variables during three seasons (Pre-M Pre-monsoon, M Monsoon, Post-M Post-monsoon) in two mangrove sites (CI Chorao Island, PF Pichavaram forest).

variables

CCA was performed to determine the probable correlation between AM fungal RA and soil variables in different seasons (**Fig. 6**). The Eigenvalues of the first and second axes were 0.496 and 0.054, respectively. The variance of AM genera was 82.98% and 8.98% on the first and second axes, respectively. The variables of soil parameters such as OC, N, Mn, Zn, Cu, and Fe significantly affected the abundance of *Acaulospora, Funneliformis, Gigaspora,* and *Sclerocystis*. Whereas, *Rhizophagus, Glomus,* and *Entrophospora* were governed mainly by EC with lesser effects of pH.

DISCUSSION

In the present study, the occurrence of AM symbiosis during all seasons indicates their perpetual importance in mangrove habitats of India's East and West coasts. However, it appears that colonization, spore density, diversity, and abundance of AM fungi in the mangrove rhizosphere exhibit seasonal shifts under natural conditions. Moreover, AM fungal species revealed a remarkable correlation with the soil parameters. A seasonal change in values of soil factors was significant. Higher EC values during the pre-monsoon could be due to higher evaporation rates, while the rainfall and ingression of landward water are known to cause a decrease in salinity (Prabu *et al.,* 2008). At Pichavaram, freshwater inflow from Vellar and Coleroon rivers reduces salinity in the monsoon (Kathiresan, 2000). A higher concentration of Fe at Chorao could be due to the mining activities in the Mandovi basin (Nayak, 1998).

In the present study, *R. fasciculatus* was found to be better adapted in all the seasons of the Pichavaram Forest. Still, the same cannot thrive in the wet seasons of Chorao Island. Whereas, *Acaulospora* spp. (*A. dilatata* and *A. delicata*) were ill-adapted to damp and dry conditions of Pichavaram Forest but succeeded better even in seasonal changes at Chorao Island. The relative abundance of AM fungal taxa changes with a shift in environmental conditions (Oehl *et al.,* 2009). It could be attributed to alterations in gene number of developing AM fungal mycelium (Yang *et al.,* 2010). This phenomenon represents seasonal niche differentiation in sporulation patterns between AM species (Escudero and Mendoza, 2005).

In the present study, variation in root colonization and spore density was detected both in plant species and seasons. The study also revealed low AM root colonization levels in monsoon, whereas earlier studies have shown humidity favouring AM spore germination, resulting in increased root colonization during the rainy season (Mirdhe and Laxshman, 2011; Nandi *et al.,* 2014). However, it has been well demonstrated that seasonality, host plant, and soil factors influence AM colonization and sporulation (Sigüenza *et al.,* 1996; D'Souza and Rodrigues, 2013). Moreover, a fungus can colonize at different levels in different plant species (Smith and Read, 2008). Variation in AM colonization at the two sites could also be attributed to different phenological patterns of the plant species studied. Enhanced plant growth during vegetative and fruiting stages leads to high metabolic activity and, in turn, to greater nutrient demand. The hyphae, arbuscules, and vesicles are the storage and nutrient uptake sites (Su *et al.,* 2011), controlling the colonization rates in their host plants during the different growing seasons. Furthermore, some AM species have a seasonal framework of sporulation, elucidating temporal variation in their sporulation pattern. Nonetheless, several other AM fungi sporulate contemporarily with the growing season of the host (Lugo *et al.,* 2002; Oehl *et al.,* 2009).

The other factors, such as soil microbes, host species, and host preference (Klironomos, 2003; Lugo *et al.,* 2003; Dauber *et al.,* 2008), may also affect colonization. Some earlier studies suggested that flooding reduces sporulation (Aziz and Sylvia, 1995), and spore number negatively correlates with soil moisture (Anderson *et al.,* 1984). In another study, it was reported that the spore density was observed to be higher in wet than in dry areas (Rickerl *et al.,* 1994). They also proposed that high spore production is a response to hostile environments. Hence, lower spore density in the present study during pre-monsoon could be due to less moisture content during this season.

The prevalence of *Acaulospora* species at Chorao Island and *Glomus* at Pichavaram Forest reflects the significance of soil parameters on the AM fungal populations. Both the sites differed in vegetation and soil type. Hence these factors could be the reason for the diverse composition of AM fungal community. Further, spores of *Acaulospora* are more greatly affected by seasonal variations than *Glomus* species (Oehl *et al.,* 2009). Besides, soil pH could cause the prevalence of *Acaulosporaceae* at Chorao and *Glomeraceae* at Pichavaram, with the soils being acidic and neutral, respectively. *Acaulosporaceae* species are often abundant in acidic soils, whereas those of *Glomeraceae* are known to be in neutral soils (Abbott and Robson, 1991).

CONCLUSION

The study showed variation in AM fungal symbiosis on the East and West coasts of India. It was apparent that these mangrove habitats supported varied AM communities, and the constitution of these communities is highly influenced by edaphic factors having OC, EC, pH, N, Mn, Zn, Cu, and Fe as key drivers. The colonization rates were much lower at Pichavaram in the pre-monsoon season compared to Chorao. Our study also indicated differential seasonal patterns, with a higher number of spores in the monsoon season and higher root colonization in the dry seasons. The predominance of *Acaulospora* at Chorao and *Rhizophagus* at Pichavaram indicates their adaptation to two different ecological conditions of the mangrove forest. In light of the ecological services of AM fungi for the conservation of mangrove habitats, the observations in this study emphasize the need for the application of AM fungi in the restoration of the mangrove ecosystem. The present study provides scope for further research on AM fungal status during different phenological stages of mangrove plants and periodically flooded conditions.

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