Status of arbuscular mycorrhizal fungi (AMF) in the Sundarbans of India in relation to tidal inundation and chemical properties of soil

Tanumi Kumar · Monoranjan Ghose

Abstract The arbuscular mycorrhizal status of fifteen mangroves and one mangrove associate was investigated from 27 sites of three inundation types namely, diurnal, usual springtide and summer springtide. Roots and rhizospheric soil samples were analysed for spore density, frequency of mycorrhizal colonization and some chemical characteristics of soil. Relative abundance, frequency and spore richness of AMF were assessed at each inundation type. All the plant species except *Avicennia alba* exhibited mycorrhizal colonization. The study demonstrated that mycorrhizal colonization and spore density were more influenced by host plant species than tidal inundation. Forty four AMF species belonging to six genera, namely *Acaulospora*, *Entrophospora*, *Gigaspora*, *Glomus*, *Sclerocystis* and *Scutellospora*, were recorded. *Glomus mosseae* exhibited highest frequency at all the inundation types; *Glomus fistulosum*, *Sclerocystis coremioides* and *Glomus mosseae* showed highest relative abundance at sites inundated by usual springtides, summer springtides and diurnal tides, respectively. Spore richness of AMF was of the order usual springtide > diurnal > summer springtide inundated sites. The mean spore richness was 3.27. Diurnally inundated sites had the lowest concentrations of salinity, available phosphorus, exchangeable potassium, sodium and magnesium. Statistical analyses indicated that mycorrhizal frequency and AMF spore richness were significantly negatively correlated to soil salinity. Spore richness was also significantly negatively correlated to available phosphorus. The soil parameters of the usual springtide inundated sites appeared to be favourable for the existence of maximum number of AMF. Glomus mosseae was the predominant species in terms of frequency in the soils of the Sundarbans.

Keywords Indian Sundarbans · Mangroves · Arbuscular mycorrhizal fungi · Mycorrhizal colonization · Tidal inundation · Chemical soil parameters · Relationships

Introduction

Arbuscular mycorrhizal fungi (AMF) occur in a wide variety of ecosystems, such as farmland and forestland, as well as many stressful environments. The distribution of AMF in different ecological regions and their relations to soil characteristics and native plants have been explored by several researchers (Khan 1974; Walker et al. 1982; Kim and Weber 1985; Rozema et al. 1986; Koske 1987; McGee 1989; Stahl and Christensen 1991; Cooke et al. 1993; Hoefnagels et al. 1993; Udaiyan et al. 1996; Barrow et al. 1997; Bhardwaj et al. 1997; Hildebrandt et al. 2001). AMF populations in saline soils are variable and affected by

many factors. For example, Hildebrandt et al. (2001) reported mycorrhizal colonization as well as high spore density in Central European salt marshes. Landwehr et al. (2002) found the number of spores in European saline, sodic and gypsum soils to be rather variable but high on the average when compared with the reports of Hildebrandt et al. (2001), with *Glomus geosporum* as the dominant species. Aliasgharzadeh et al. (2001) reported that the spore number of AMF in saline soil of the Tabriz plain of Iran was not correlated with soil salinity but suffered adverse effects of the accumulation of some anions and cations. Wang et al. (2004) suggested that the poor plant diversity and low vegetation cover severely restricted colonization and diversity of AMF in the saline-alkaline soils of the Yellow River delta of China. Variation in the populations of AMF and their symbiosis with plant roots is related to both soil properties and host plants (Hayman 1982). In addition, species and isolates of AMF differ in their tolerance to adverse physical and chemical conditions in soil (Sengupta and Chaudhuri 1990; Juniper and Abbott 1993; Mankarios and Abdel-Fattah 1994). Recent investigations showed that salt tolerance of some plants increases under saline conditions when they are mycorrhizal with certain AMF (Pond et al. 1984; Poss et al. 1985; Jindal et al. 1993; Aboulkhair and El-Sokkary 1994).

Worldwide various researchers (Schenck and Perez 1990; Walker 1992) have used morphological approaches for the specific identification of AMF spores, but it is also true that sometimes spores are difficult to identify by morphology (Reddy et al. 2005). It is also an established fact that molecular techniques to species identification can reveal results that spore morphology may fail to recognize (Reddy et al. 2005). However, it may not be realistic for every researcher to employ molecular approaches.

Though molecular investigations on AMF have started yielding promising results in some cases yet several obstacles are encountered in this way (Reddy et al. 2005). The lack of clear species concept and polymorphism of currently used marker genes make it difficult to clearly define AMF species by molecular methods (Reddy et al. 2005). It has also been felt that a single genetic locus (e.g. r DNA) does not provide clear differentiation of genetic variation in intra-species and inter-species (Redecker et al. 2003; Reddy et al. 2005). Due to these constraints of molecular methods, identification of AMF, based on the morphological features of the asexually produced propagules i.e. chlamydospores, still remains the most popular and feasible approach for AMF taxonomy and classification.

Saline soils occupy over 7% of the earth's land surface. Solutions to saline toxicity in plants should include both plant breeding for salt tolerance and use of biological factors such as mycorrhiza. In this respect, it is very important to study the diversity and effectiveness of AMF in saline soils and their effects on host plants.

Mangrove is a type of coastal woody vegetation that fringes muddy saline shores and estuaries in tropical and subtropical regions (Naskar and Guha Bakshi 1987). On the Gangetic and Brahmaputra deltas of West Bengal and Bangladesh lies the world's second largest mangrove forests, the Sundarbans (Ghose 2001). According to satellite imagery of the Forest Survey of India (1999) total area of the Indian Sundarbans was 2125 square kilometer, excluding the anastomosing network of creeks and backwaters. The creeks and backwaters of the Sundarbans are subjected to tidal influence and are therefore saline. Sites can be broadly divided into three types/classes (Watson 1928)—diurnal, usual springtide and summer springtide.

The only studies on the occurrence of arbuscular mycorrhiza (AM) in mangroves of Sundarbans were reported by Sengupta and Chaudhuri (1990, 1991, 1994, 2002). However, they did not quantify the mycorrhizal colonization of the plants in relation to the properties of soil and tidal inundation. Studies on AMF populations of the Sundarbans are meagre. Study of relative abundance and frequency of species, their large scale propagation and their application in the field might facilitate reforestation programmes in the future.

The objectives of the present study were to investigate the distribution of AMF in the rhizospheres of mangroves and mangrove associates of the Sundarbans, and to evaluate mycorrhizal frequency in the roots in relation to tidal inundation and chemical properties of soil.

Materials and methods

Study sites

Root and soil samples were collected between September 2004 and February 2007 from 27 sites of

six different areas (including two islands) in the Sundarbans of West Bengal (Fig. 1). Each site is equivalent to a quadrate of size 4 m × 16 m. The latitude-longitude values of the six areas are presented in Table 1. Sites were broadly divided into three inundation types (Watson 1928) viz. diurnal inundation (Diurnal)—10 sites, usual springtide inundation (Usp)—nine sites and summer springtide inundation (Ssp)—eight sites.

Sampling

Root and soil samples for all available species (Table 2) at a particular site were collected from three to five individuals at different stages of growth (vegetative and reproductive). The distribution of species significantly varied with inundation type (Table 2). Care was taken during collection of an individual plant that roots were positively identified as belonging to the same plant. Young seedlings were uprooted together with some soil adhering to the roots. Fine roots of mature trees were traced by digging, and taken out with adhering soil. In both the cases samples were brought to the laboratory, roots were separated from the adhering soil, washed gently under tap water and fixed in FAA (formalin-acetic acid-alcohol) for estimation of AM colonization. Root adhering or rhizospheric soil of each individual was air dried at room temperature, sieved, and divided into two portions. One portion was used for AMF spore isolation, enumeration and identification, and the other portion for determination of chemical characteristics of soil.

Estimation of AMF colonization

Roots were taken out from FAA, rinsed with distilled water, cleared in 10% KOH at 90°C, bleached in alkaline H_2O_2, acidified with 1% HCl (Kormanik and McGraw 1982), and stained with 0.05% trypan blue lacto-glycerol stain at 80–90°C (Phillips and Hayman 1970). The material was then destained in lacto-glycerol.

For estimation of AM colonization in the root systems, 30 pieces (in case of seedlings) to 60 pieces (for mature trees) of 1-cm fragments of roots with diameter <2 mm were mounted on slides (10 per slide) in polyvinyl alcohol-lactoglycerol (PVLG).

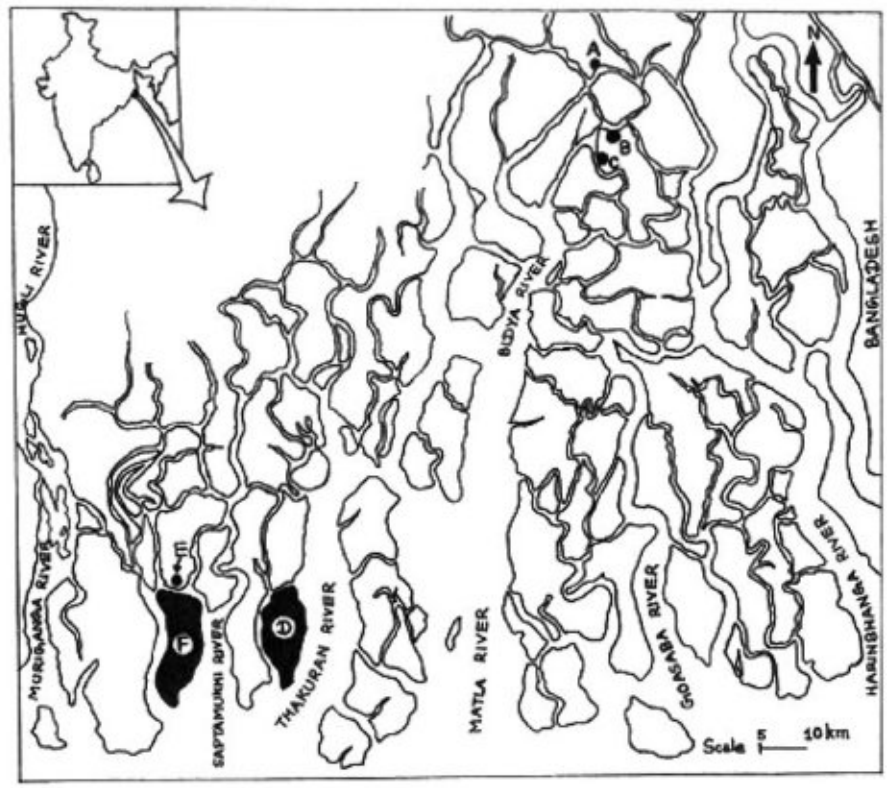

Fig. 1 Map of the Sundarbans showing study areas. A—Manmathanagar, B—Sajnekhali, C—Sudhanyakhali, D—Dhanchi island, E—Bhagwatpur and F—Lothian island

Table 1 Study areas, their geographic locations and number of sites studied at each area

Areas	Location	Number of sites studied
Bhagwatpur	21°44′33″ N & 88°18′29″ E	1
Dhanchi (island)	21°36′54″ N to 21°42′33″ N & 88°24′54″ E to 88°28′30″ E	16
Lothian (island)	21°32′50″ N to 21°42′30″ N & 88°18′10″ E to 88°21′30″ E	6
Manmathanagar	22°11′2″ N & 88°48′44″ E	1
Sajnekhali	22°7′27″ N & 88°49′48″ E	1
Sudhanyakhali	22°6′42″ N & 88°46′46″ E	2

Table 2 Plants associated with mycorrhizae, their habit and habitat at three tidal inundation types

Family	Species	Abbreviation	Habit	Habitat sites
Acanthaceae	*Acanthus ilicifolius* L.	A.i	MWH	I, II, III
Arecaceae	*Phoenix paludosa* Roxb.	P.p	MT	II
Avicenniaceae	*Avicennia alba* Bl.	A.a	MT	I, II, III
	Avicennia marina (For.) Vierh.	A.m	MT	I, II, III
	Avicennia officinalis L.	A.o	MT	I, II, III
Euphorbiaceae	*Excoecaria agallocha* L.	E.a	MT	II
Meliaceae	*Xylocarpus granatum* Koenig.	X.g	MT	II
	Xylocarpus mekongensis Pierre.	X.m	MT	II
Myrsinaceae	*Aegiceras corniculatum* (L.) Blanco	A.c	MT	I, II
Plumbaginaceae	*Aegialitis rotundifolia* Roxb.	A.r	MS	II, III
Poaceae	*Porteresia coarctata* (Roxb.) Tak.	P.c	MAH	I
Rhizophoraceae	*Bruguiera gymnorrhiza* (L.) Lam.	B.g	MT	I, II
	Bruguiera parviflora W. & A.	B.p	MT	I, II
	Ceriops decandra (Griff.) Ding Hou.	C.d	MT	II, III
	Ceriops tagal (Pers.) Robins	C.t	MT	II, III
Sterculiaceae	*Heritiera fomes* Buch. Ham.	H.f	MT	II

MWH, Mangrove woody herb; MT, Mangrove tree; MAH, Mangrove associate herb; I, sites inundated diurnally; II, sites inundated by usual springtides and III, sites inundated by only summer springtides. Species habitat varies significantly with inundation types ($P < 0.001$)

Each root fragment was ranked under a compound microscope for quantification of infection (colonization). Frequency of mycorrhizal colonization in the root system of an individual plant (F%) was estimated by using the method of Trouvelot et al. (1986).

Isolation, enumeration and identification of AMF spores

Spores were extracted from 10 g of rhizospheric soil in triplicate for individual plant by wet-sieving and decanting method of Gerdemann and Nicolson (1963), followed by sucrose centrifugation method of Daniels and Skipper (1982). The finest sieve used was 37 μm. The spores were collected on grid pattern filter paper and washed with distilled water to spread the spores evenly over the entire grid. The spores were counted using a dissecting microscope with objectives of ×40 magnification and ×10 eye-piece. Only intact and healthy spores were counted, and each sporocarp was considered as one unit.

Spores were mounted on glass slides in PVLG and PVLG+Melzers reagent. Spore morphology and subcellular characters were compared to the type descriptions of the species (Schenck and Perez 1990) and also to the culture database established by INVAM (http://invam.cag.wvu.edu) for identification.

Numbers and distribution of AMF spores

Spore density, frequency, relative abundance and spore richness of AMF at the three specified inundation types were expressed as follows: spore density (Sp) = number of AMF spores in 10 g of rhizospheric soil in triplicate for individual plant; frequency (Fr) = (number of the samples in which the genus or the species was observed/ total samples) × 100%; relative abundance (RA) = (number of spores of a genus or a species/ total spores) × 100%; Spore richness = number of AMF species (based on spore morphology) in 10 g of rhizospheric soil for individual plant.

Determination of soil characters

Soil pH, salinity (Sal, calculated from electrical conductivity), available nitrogen (N), available phosphorus (Olsen P), organic carbon (OC), available potassium (K), sodium (Na), calcium (Ca) and magnesium (Mg) were measured according to Jackson (1973).

Statistical analyses

Chi-square test was performed after cross tabulation of the mangrove species versus inundation type to check whether the species composition varied significantly with inundation or not. Two-way Analysis of Variance (ANOVA) was used to test whether there were significant differences in spore density and mycorrhizal frequency between species and inundation types. The data on soil parameters were subjected to one-way ANOVA to judge whether the variation in the parameters between the inundation types were significant or not. Pearson's correlation coefficients (r) were calculated between spore density and spore richness and the soil parameters. χ^2 test was also performed after cross tabulation of the AMF species (based on spore morphology) versus inundation type to check whether the species composition varied significantly with inundation type or not. Means and standard errors were estimated for the replicate values. For all the above statistical analyses SPSS software version 11.0 was used.

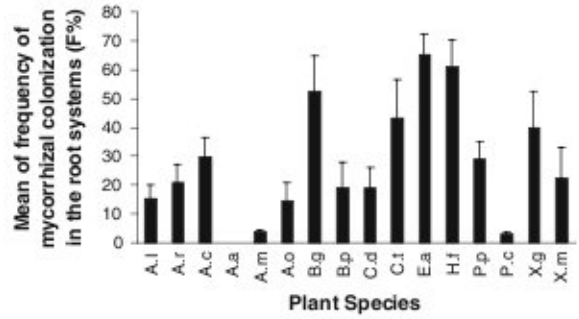

Fig. 2 Mean of frequency of mycorrhizal colonization in the root systems (F%) of different plant species. Bars represent standard errors

Results

Mycorrhizal colonization

Figure 2 represents the AMF status of 15 mangroves and one mangrove associate. All species except *Avicennia alba* were colonized by AMF. The mean frequency of mycorrhizal colonization (F%) ranged from 0.83% to 65.12%. The highest F%s occur in *Excoecaria agallocha* (65.12%), *Heritiera fomes* (61.16%) and *Bruguiera gymnorrhiza* (52.63%) and lowest in *Porteresia coarctata* (0.83%) and *Avicennia marina* (1.19%). Significant differences in mycorrhizal frequency occur between species, but not between inundation levels; however, significant interaction was observed in mycorrhizal frequency between species and inundation types (Table 3).

AMF spore density

The highest mean spore density (60.09) occurs in the rhizosphere of *Phoenix paludosa*, followed by *Heritiera fomes* (48.89) and *Ceriops decandra* (41.24), whereas the lowest spore density (2.92) occurs in *Avicennia marina* (Fig. 3). Table 3 shows statistically significant differences in spore density among rhizospheres of different plant species. However, inundation has no significant effect on spore density. The lowest spore density occurs in diurnally inundated sites. Interaction between host plant and inundation was significant (Table 3).

Table 3 Two-way ANOVA of mean spore density and mean frequency of mycorrhizal colonization at the three inundation types with species, inundation and species * inundation as factors. All effects entered simultaneously

Parameter	Inundation			Factors	F
	Diurnal	Usp	Ssp		
Sp	8.85 (1.26)	26.98 (5.55)	20.12 (2.32)	Species	2.197**
				Inundation	1.248 ns
				Species * Inundation	2.264**
F%	17.73 (3.21)	31.37 (3.56)	10.83 (0.82)	Species	6.029**
				Inundation	0.099 ns
				Species * Inundation	6.547**

Values in parenthesis represent standard errors. Sp, mean spore density in 10 g of rhizospheric soil; and F%, mean frequency of mycorrhizal colonization in the root systems in percentage. Diurnal, diurnally inundated sites; Usp, sites inundated by usual springtides; and Ssp, sites inundated by only summer springtides. ** $P < 0.01$, ns = not significant

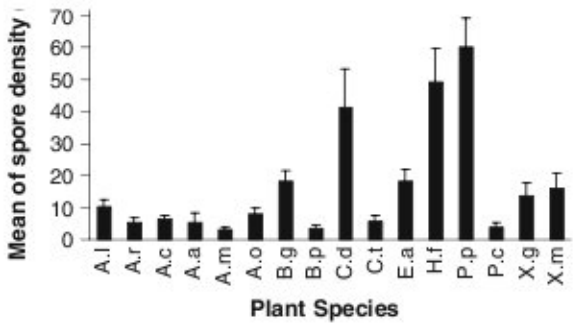

Fig. 3 Mean of spore density (Sp) of different plant species. Bars represent standard errors

Genera and species of AMF

A total of 44 species representing 6 genera, including 10 species of *Acaulospora*, 2 of *Entrophospora*, 3 of *Gigaspora*, 21 of *Glomus*, 2 of *Sclerocystis* and 6 of *Scutellospora*, were isolated from the rhizosphere of the plants from 27 sites of the Sundarbans. Twelve forms of the 44 species isolated do not fit into the known descriptions for AMF (Table 4). Out of the 44 species, 43 species occur in usually springtide inundated sites, 21 in diurnally inundated areas and only 7 in summer springtides sites (Table 4). *Acaulospora* sp. (1), *Glomus coronatum*, *G. etunicatum* and *G. mosseae* occurred at all the inundation classes (Table 4).

Relative abundance and frequency of AMF

Considering all the study sites together

The highest frequency (Fr) and relative abundance (RA) of spores occur in the genus *Glomus*, and second highest in *Acaulospora*, whereas the lowest Fr and RA occur in *Entrophospora* (Fig. 4). Species wise, the highest RA occurs in *Glomus etunicatum* and *G. fistulosum* and lowest in *Entrophospora* sp. (1), *E.* sp. (2), *Gigaspora decipiens*, *Glomus fasciculatum*, *G. viscosum* and *Scutellospora dipapillosa*. The highest frequency of spores occurs in *G. mosseae* and second highest in *G. etunicatum* (Fig. 5). *A. delicata*, *A. lacunosa*, *A.* sp. (3), *G. etunicatum*, *Scutellospora* sp. (1) and *Scu.* sp. (2) register intermediate frequency of spores.

Considering the inundation types separately

G. mosseae spores had the highest Fr in all of the three inundation sites but the highest RA of *G. mosseae* occured only in diurnally inundated sites (Figs. 6–8). On the other hand the highest RA of *G. fistulosum* and *Sclerocystis coremioides* occurs in usual springtide (Fig. 7) and summer springtides (Fig. 8) respectively.

AMF spore richness

The highest spore richness (5.877) occurs in usual springtide inundated sites, the lowest (0.666) in summer springtide sites and intermediate (3.28) in diurnally inundated sites. The mean spore richness is 3.27.

Soil parameters at the three inundation types

Table 5 illustrates some chemical parameters of soil at three inundation classes. Usual springtide

Table 4 Distribution of AMF species (based on spore morphology) at the three inundation types. Abbreviations as in Table 3

AM fungal species	Inundation		
	Diurnal	Usp	Ssp
A. bireticulata Rothwell &Trappe	+	+	−
A. delicata Walker, Pfeiffer & Bloss	+	+	+
A. foveata Trappe & Janos	−	+	+
A. lacunosa Morton	+	+	−
A. mellea Spain & Schenck	+	+	−
A. scrobiculata Trappe	−	+	−
A. spinosa Walker	+	−	−
A. sp. (1)	+	+	+
A. sp. (2)	+	+	−
A. sp. (3)	+	+	−
E. sp. (1)	−	+	−
E. sp. (2)	−	+	−
Gi. decipiens Hall & Abbott	−	+	−
Gi. margarita Becker & Hall	−	+	−
Gi. sp.	+	+	−
Gl. aggregatum Schenck & Smith emend. Koske	−	+	−
Gl. albidum Walker & Rhodes	−	+	−
Gl. clarum Nicolson & Schenck	−	+	−
Gl. coronatum Giovannetti	+	+	+
Gl. eburneum Kenn., Stutz & Morton	−	+	−
Gl. etunicatum Becker & Gerdemann	+	+	+
Gl. fasciculatum (Thaxter) Gerd. & Trappe emend. Koske & Walker	−	+	−
Gl. fistulosum Skou. & Jakob.	+	+	−
Gl. geosporum (Nicol. & Gerd.) Walker	+	+	−
Gl. intraradices Schenck & Smith	−	+	−
Gl. lacteum Rose & Trappe	−	+	−
Gl. lamellosum Dalpé, Koske & Tews	−	+	−
Gl. mosseae (Nicol. & Gerd.) Gerd. & Trappe	+	+	+
Gl. multicaule Gerdemann & Bakshi	+	+	−
Gl. occultum Walker	−	+	−
Gl. verruculosum Blaszk.	−	+	−
Gl. viscosum Nicolson	−	+	−
Gl. sp. (1)	+	+	−
Gl. sp. (2)	−	+	−
Gl. sp. (3)	+	+	−
Gl. sp. (4)	−	+	−
Scl. coremioides Berk. & Broome	−	+	+
Scl. sinuosa Gerd. & Bakshi	+	+	−

Table 4 continued

AM fungal species	Inundation		
	Diurnal	Usp	Ssp
Scu. calospora (Nicol. & Gerd.) Walker & Sanders	+	+	−
Scu. dipapillosa (Walker & Koske) Walker & Sanders	−	+	−
Scu. pellucida (Nicol. & Gerd.) Walker & Sanders	−	+	−
Scu. persica (Koske & Walker) Walker & Sanders	−	+	−
Scu. sp. (1)	+	+	−
Scu. sp. (2)	+	+	−
Total species	21	43	7

Species distribution varies significantly with inundation types ($P < 0.001$)

inundated sites had the lowest concentrations of Sal, P, K, Na and Mg; summer springtide inundated sites exhibited highest concentrations of Sal, OC, N, Na, K and Mg. P and N did not differ significantly between the inundation types. P was highest and N lowest in the soils of the diurnally inundated sites (Table 5). The cationic analysis of the soil indicated that Na, Ca, Mg and K ions dominated as soil salinity increased.

Correlation analysis

Table 6 represents the correlation of spore density, mycorrhizal frequency and spore richness with soil

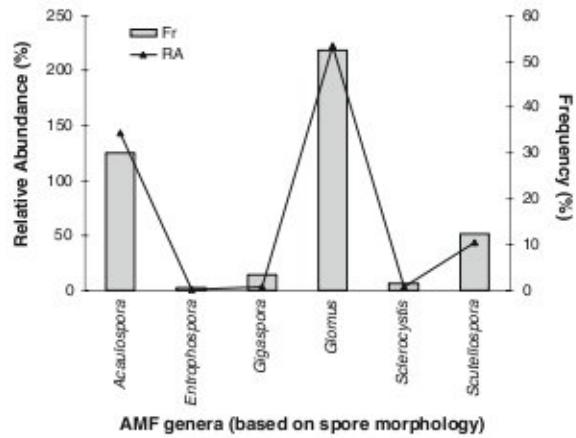

Fig. 4 Relative abundance and frequency of AMF genera

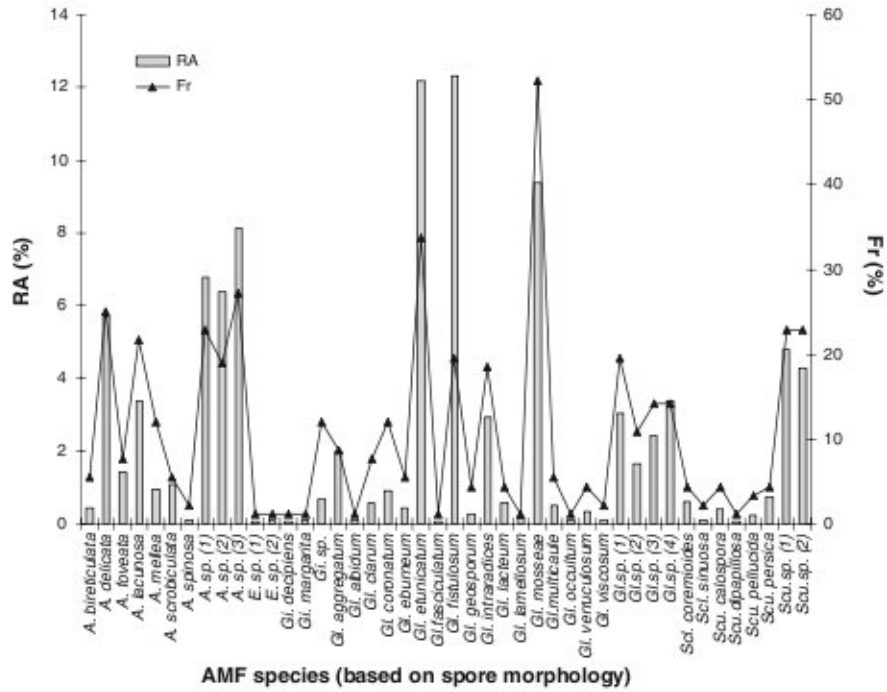

Fig. 5 Relative abundance and frequency of AMF species

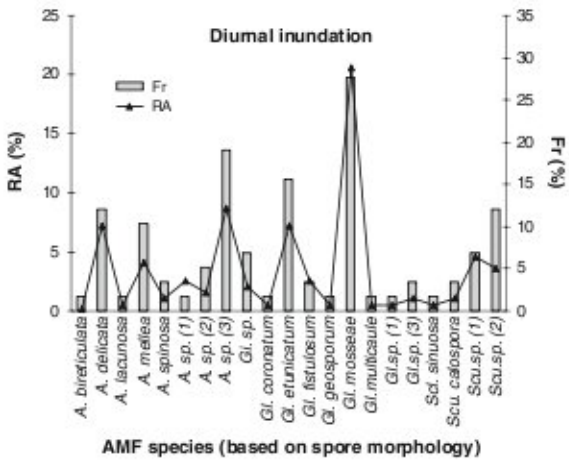

Fig. 6 Relative abundance and frequency of AMF species at the diurnally inundated sites

parameters. Soil salinity is negatively correlated ($P < 0.01$) with mycorrhizal frequency and spore richness, and available phosphorus is negatively correlated ($P < 0.01$) with spore richness.

Discussion

Root hairs are generally absent or are small and poorly developed on mangrove roots (Tomlinson 1986). These features of roots would make mangroves potentially mycotrophic (Baylis 1975) for nutrient acquisition from stressed environments.

Variation in spore density and colonization of AMF associated with different host plant species may be generated by a variety of mechanisms, including variation in host species and their phenology, mycorrhizal dependency, host-mediated alterations of the soil microenvironment, or other unknown host-plant traits (Eom et al. 2000; Lorgio et al. 1999). The present study indicates that, the host plants apparently had direct effects on spore density and frequency of mycorrhizal colonization in the roots. For example, these were both high in case of *Heritiera fomes* and quite low in the rhizospheres of *Avicennia marina* and *Porteresia coarctata*.

This study also indicates that the number of spores did not decrease significantly with increasing soil salinity. These findings are in conformity with Aliasgharzadeh et al. (2001), where they found the same trend between some crop plants (non-saline) compared with two halophytes. Hirrel (1981), and Tressner and Hayes (1971) are of the opinion that sporulation of AMF is stimulated under salt-stress conditions. This may again cause accumulation of spores in saline soil. The occurrence of relatively low spore numbers (mean of 19 per 10 g soil) in our study

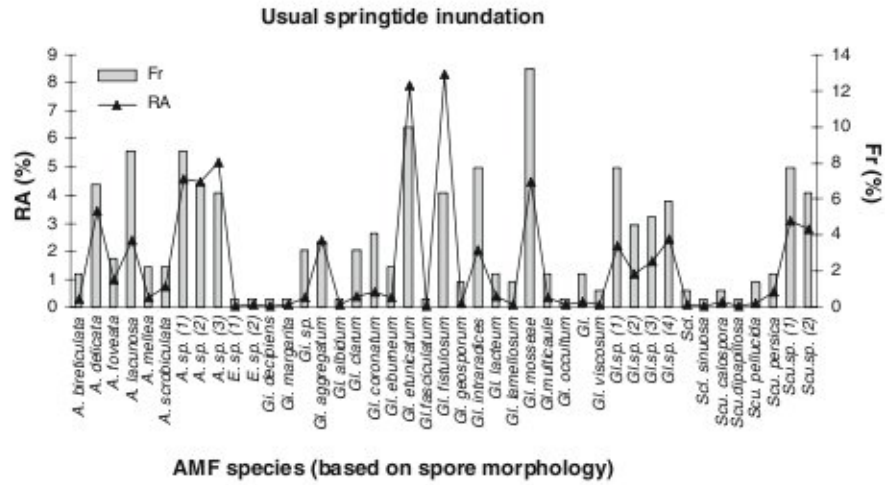

Fig. 7 Relative abundance and frequency of AMF species at the usual springtide inundated sites

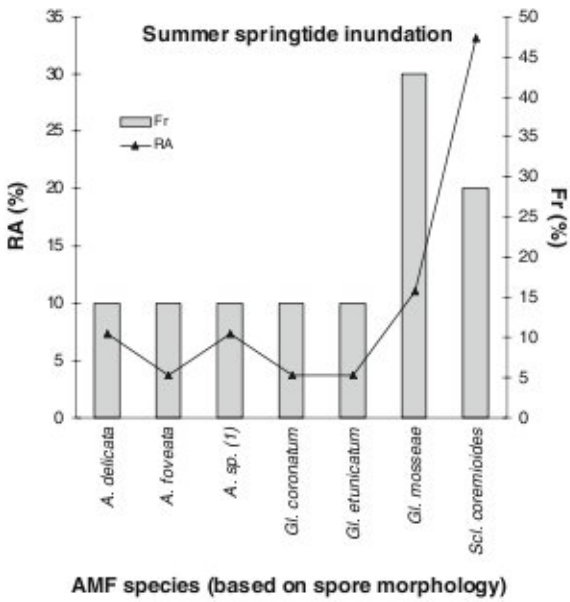

Fig. 8 Relative abundance and frequency of AMF species at the summer springtide inundated sites

supports some studies of saline soils where low spore numbers were reported (Barrow et al. 1997; Hirrel et al. 1978; Kim and Weber 1985), but our results contrast with the reports of spore density of soils of Tabriz Plain where relatively high spore numbers (mean of 100 per 10 g soil) were recorded.

Although *Avicennia alba* showed no mycorrhizal colonization, we cannot designate it as a non-mycorrhizal species because the mean salinity of

Table 5 One-way ANOVA of mean values of soil parameters at three specified tidal inundation types

Soil parameters	Inundation			F
	Diurnal	Usp	Ssp	
pH	8.04 b	8.03 b	7.57 a	15.26**
	(0.04)	(0.03)	(0.09)	
Sal	15.62 a	9.92 b	21.67 c	87.16**
	(0.40)	(0.40)	(1.39)	
OC	0.68 b	0.74 b	1.08 a	6.69*
	(0.05)	(0.06)	(0.08)	
N	292.19	310.86	337.03	1.54 ns
	(18.97)	(28.34)	(33.01)	
P	33.92	18.67	27.36	0.75 ns
	(11.42)	(1.46)	(2.78)	
K	2.66 a	1.95 b	3.38 c	20.03**
	(0.11)	(0.09)	(0.37)	
Na	27.26 a	21.97 b	40.53 c	20.87**
	(0.99)	(0.94)	(5.38)	
Ca	19.58 a	13.79 b	12.38 b	15.09**
	(1.02)	(0.73)	(0.99)	
Mg	10.58 b	9.99 b	14.99 a	8.68*
	(0.51)	(0.55)	(1.11)	

Sal-salinity (ppt, parts per thousand); OC, Organic carbon (%); N, available nitrogen (mg/kg); P-available phosphorus (mg/kg); K, Na, Ca and Mg, exchangeable potassium, sodium, calcium and magnesium (meq./100g) respectively. Values in parentheses represent standard errors. The digits followed by same letter are not significantly different according to LSD. * $P < 0.05$, ** $P \leq 0.01$, ns = not significant. Other abbreviations as in Table 3

Table 6 Pearson's correlation coefficient (r) values between frequency of mycorrhizal colonization, AMF spore density and spore richness with different soil parameters

Soil properties	Frequency of mycorrhizal colonization	Spore density	Spore richness
pH	0.000	−0.057	−0.092
Sal	−0.209*	−0.134	−0.392*
OC	0.003	0.097	−0.026
N	0.134	0.093	0.147
P	−0.051	−0.040	−0.308*
K	−0.008	0.018	−0.130
Na	−0.083	−0.055	−0.197
Ca	−0.074	−0.110	−0.147
Mg	−0.006	0.114	−0.002

Abbreviations as in Table 5. * $P < 0.01$

our study sites was 17.29 ppt and a previous report stated that it is mycorrhizal at sites having salinity less than 15 ppt (Sengupta and Chaudhuri 2002). This may serve as an evidence to show that, at high salt concentrations, mycorrhiza formation of even the mycotrophic plants may be adversely affected (as opined by Juniper and Abbott 1993). This study shows that mycorrhizal colonization in the root systems and spore density in the rhizospheric soils are more dependent on host plant species than on tidal inundation.

The mycorrhizal frequency and spore density decreased with increase in available soil phosphorus. This supports the general conclusion of Anderson (1992) that more is the amount of available P in the soil more is the inhibition of root colonization and spore number in the rhizosphere. Spore richness was not only influenced by soil salinity, but also by available phosphorus.

The mangrove species distribution significantly varied with tidal inundation type, again inundation levels had significant effect on most of the measured soil parameters. This is in conformity with the view that local patterns of tidal inundation influence soil characteristics that control plant species distribution in mangrove wetlands (Tomlinson 1986).

In the mangrove soils tidal inundation causes submergence, thereby influencing soil saturation and hence redox potential (Tomlinson 1986). The present study shows that spore density is least in diurnally inundated sites; this could be due to more anoxic microenvironment and more negative redox potentials ranging from −100 mV to −400 mV (Mitsch and Grosslink 1993). Both these factors are known to decrease AM spore density (Khan 1993). Underground water table of the Sundarbans is shallow and enriched with high salt contents (Naskar and Guha Bakshi 1987). During the dry season, salt molecules move along with water to the surface soil. As the water in the soil evaporates the salt molecules are left on the surface soil (Yadav et al. 1981). Since the summer springtide inundated sites receive the least number and lowest duration of tides, mean salinity as well as the concentrations of Na, K and Mg show highest values. Quite high salinity and high concentrations of the estimated cations might suggest that though in the diurnally inundated sites water runs in opposite directions twice a day (Naskar and Guha Bakshi 1987), yet the leaching of the salt molecules are not much for these sites. Soil parameters of sites inundated by usual springtides apparently seem to be most favourable for AMF spore density, richness and mycorrhizal colonization.

In contrast to the assumption that the genera *Acaulospora* and *Scutellospora* are particularly diverse in the tropics (Walker 1992; Allen et al. 1995), the present study indicates a predominance of *Glomus* over other genera. Similar observations have been made in the tropics by Ragupathy and Mahadevan (1993), Thapar and Khan (1985) and Muthukumar and Udaiyan (2000). Two possible reasons for the predominance of *Glomus* are, firstly, that *Acaulospora* species are often associated with acidic soils (Morton 1986; Abbott and Robson 1991), whereas the soils examined at the Sundarbans were neutral to sightly alkaline. *Glomus* species are known to be more common in neutral and slightly alkaline soils (Mukerji et al. 2002). Secondly, *Gigaspora* species predominate in soils with high sand content, especially dunes (Day et al. 1987; Lee and Koske 1994); *Scutellospora* is ancestral to *Gigaspora* (Walker 1992) and probably prefers similar sandy soils. The soil of the Sundarbans of West Bengal was less sandy (Naskar and Guha Bakshi 1987) than those examined by most of the authors cited above.

Sites inundated by usual springtides support the existence of 43 AMF species out of the 44 species so far recorded in our study. One major reason could be that the soil microenvironment is more favourable for the growth of AMF than those of the diurnally and

summer springtide inundated areas. The mean spore richness obtained in our results is higher than those of some saline tracts and salt marshes, explored by some researchers (Barrow et al. 1997; Carvalho et al. 2001; Wang et al. 2004). Mycorrhizal colonization is reduced with increase in salinity, but plant growth is still improved by the symbiosis as compared with non-mycorrhizal plants growing under similar conditions (Gupta and Krishnamurthy 1996). This study indicates that spore richness and composition are influenced by tidal inundation.

Though *Glomus mosseae* and *G. etunicatum* occurred as the two most frequent species [as observed by Aliasgharzadeh et al. (2001) in the soils of the Tabriz Plain of Iran] and *G. fistulosum* the most abundant species of AMF in the Sundarban soils, yet these species might not be the main root colonizers of the mangroves. Moreover, the possibility that these species of *Glomus* can improve tolerance of mangroves to salt stress should be tested to select useful isolates.

It appears that spore richness and composition of AMF is more influenced by tidal inundation than AM colonization in the roots or spore density in the rhizospheres.

Much of the data presented in our work elucidates patterns of spore distribution and their identification based on morphological parameters. Despite the constraints of molecular approaches, we hope to employ such techniques for AMF identification in the near future (at least for the spores whose specific identification could not be established on the grounds of spore morphology and their subcellular characteristics).

Acknowledgement We are obliged to Dr. D. Roy of the Indian Statistical Institute, Kolkata for his statistical assistance. We are also thankful to the Conservator and Joint Director, Sundarbans Biosphere Reserve, and DFO, South 24-Parganas, West Bengal for the necessary permission they provided for the fieldwork. Last, but not the least, we thank the field assistants for their sincere help during root and soil collection.

References

Abbott LK, Robson AD (1991) Factors influencing the occurrence of vesicular-arbuscular mycorrhizas. Agric Ecosyst Environ 35:121–150

Aboulkhair KS, El-Sokkary IH (1994) Effects of salinity, boron and sodium on the growth and root infection of VAM, *Rhizobium* and *Frankia* of seedlings of three tree species. J Agric Sci Egypt 19:2969–2980

Aliasgharzadeh N, Rastin NS, Towfighi H, Alizadeh A (2001) Occurrence of arbuscular mycorrhizal fungi in saline soils of the Tabriz Plain of Iran in relation to some physical and chemical properties of soil. Mycorrhiza 11:119–122

Allen EB, Allen MF, Helm DJ, Trappe JM, Molina R, Rincon E (1995) Patterns and regulation of mycorrhizal plant and fungal diversity. Plant Soil 170:47–62

Anderson AJ (1992) The influence of the plant root in mycorrhizal formation: an integrative plant fungal process. Chapman and Hall, New York, pp 37–64

Barrow JR, Havstad KM, McCaslin BD (1997) Fungal root endophytes in four-wing saltbush, *Altiplex canescens*, on arid rangeland of southwestern USA. Arid Soil Res Rehabil 11:177–185

Baylis GTS (1975) The magnolioid mycorrhiza and mycotrophy in root systems derived from it. In: Sanders FE, Mosse B, Tinker PB (eds) Endomycorrhizas. Academic Press, London, pp 373–389

Bhardwaj S, Dudeja SS, Khurana AL (1997) Distribution of VAM fungi in the natural ecosystem. Folia Microbiol 42:589–594

Carvalho LM, Caçador I, Martins-Loução MA (2001) Temporal and spatial variation of arbuscular mycorrhizas in salt marsh plants of the Tagus estuary (Portugal). Mycorrhiza 11:303–309

Cooke JC, Butler RH, Madole G (1993) Some observations on the vertical distribution of vesicular-arbuscular mycorrhizae in roots of salt marsh grasses growing in saturated soils. Mycologia 85:547–550

Daniels BA, Skipper HD (1982) Methods for the recovery and quantitative estimation of propagules from soil. In: Schenck NC (ed) Methods and principles of mycorrhizal research. American Phytopathological Society, St. Paul, pp 20–45

Day LD, Sylvia DM, Collins ME (1987) Interaction among vesicular-arbuscular mycorrhizae, soil, and landscape position. Soil Sci Soc Am J 51:635–639

Eom AH, David C, Hartnett A, Gail WT, Wilson C (2000) Host plant species effects on arbuscular mycorrhizal fungal communities in tallgrass prairie. Oecologia 122:435–444

Forest Survey of India (1999) The state of forest report. Forest survey of India. Ministry of environment and Forests, Dehra Dun

Gerdemann JW, Nicolson TH (1963) Spores of mycorrhizal fungi isolated from soil by wet sieving and decanting. Trans Br Mycol Soc 46:235–244

Ghose M (2001) Status of mangroves in Sundarbans of West Bengal, India. In: Bhat NR, Taha FK, Al-Nasser AY (eds) Mangrove ecosystems: natural distribution, biology and management. Proceedings of International Symposium on Mangrove Ecology and Biology. Kuwait Institute for Scientific Research, Kuwait, pp 7–16

Gupta R, Krishnamurthy KV (1996) Response of mycorrhizal and non-mycorrhizal *Arachis hypogaea* to NaCl and acid stress. Mycorrhiza 6:145–149

Hayman DS (1982) Influence of soils and fertility on activity and survival of VAM fungi. Phytopathology 72: 1119–1125

Hildebrandt U, Janetta K, Ouzaid F, Renne B, Nawrath K, Bothe H (2001) Arbuscular mycorrhizal colonization of halophytes in central European salt marshes. Mycorrhiza 10:175–183

Hirrel MC (1981) The effect of sodium and chloride salts on the germination of *Gigaspora margarita*. Mycologia 73:610–617

Hirrel MC, Mehranaran H, Gerdemann JW (1978) Vesicular-arbuscular mycorrhiza in the Chenopodiaceae and Cruciferae: do they occur? Can J Bot 56:2813–2817

Hoefnagels MH, Broome SW, Shafer SR (1993) Vesicular-arbuscular myorrhizae in salt marshes in North Carolina. Estuaries 16:851–858

Jackson ML (1973) Soil chemical analysis. Prentice Hall of India Private Limited, New Delhi, India

Jindal V, Atval A, Sekhon BS, Singh R (1993) Effect of VAM on metabolism of mung plants under NaCl salinity. Plant Physiol Biochem 31:475–481

Juniper S, Abbott L (1993) Vesicular arbuscular mycorrhizas and soil salinity. Mycorrhiza 4:45–57

Khan AG (1974) The occurrence of mycorrhizas in halophytes, hydrophytes and xerophytes, and *Endogone* spores in adjacent soils. J Gen Microbiol 81:7–14

Khan AG (1993) Occurrence and importance of mycorrhizae in aquatic trees of New South Wales, Australia. Mycorrhiza 3:31–38

Kim CK, Weber DJ (1985) Distribution of VA mycorrhiza on halophytes on inland salt playas. Plant Soil 83:207–214

Kormanik PP, McGraw AC (1982) Quantification of vesicular-arbuscular mycorrhizae in plant roots. In: Schenck NC (ed) Methods and principles of mycorrhizal research. American Phytopathological Society, St. Paul, pp 20–45

Koske RE (1987) Distribution of VAM fungi along a latitudinal temperature gradient. Mycologia 79:55–68

Landwehr M, Hildebrandt U, Wilde P, Nawrath K, Toth T, Biro B, Bothe H (2002) The arbuscular mycorrhizal fungus *Glomus geosporum* in European saline, sodic and gypsum soils. Mycorrhiza 12:199–211

Lee PJ, Koske RE (1994) *Gigaspora gigantia*: Seasonal, abundance and ageing of spores in a sand dune. Mycol Res 98:453–457

Lorgio EA, Julio RG, Peter LM (1999) Variation in soil microorganisms and nutrients underneath and outside the canopy of *Adesmia bedwellii* (Papilionaceae) shrubs in arid coastal Chile following drought and above average rainfall. J Arid Environ 42:61–70

Mankarios AT, Abdel-Fattah GM (1994) Ecology of VA mycorrhiza in some Egyptian soils. Egypt J Bot 34: 135–152

McGee PA (1989) Variation in propagule numbers of VAM fungi in a semi-arid soil. Mycol Res 92:28–33

Mitsch WJ, Gooselink JG (1993) Mangrove wetlands. In: Mitsch WJ, Gooselink JG (eds) Wetlands. J.G. Van Nostrand Reinfold Publication, New York, pp 293–328

Morton JB (1986) Three new species of *Acaulospora* (Endogonaceae) from high-aluminium, low pH soils in West Virginia. Mycologia 78:641–648

Mukerji KG, Manoharachary C, Chamola BP (eds) (2002) Techniques in mycorrhizal studies. Kluwer Academic Publishers, Dordrecht, Boston, London

Muthukumar T, Udaiyan K (2000) Arbuscular mycorrhizas of plants growing in the Western Ghats region, Southern India. Mycorrhiza 9:297–313

Naskar KR, Guha Bakshi DN (1987) Mangrove swamps of the Sundarbans: an ecological perspective. Naya Prokash, Kolkata, India

Phillips JM, Hayman DS (1970) Improved procedure for clearing root and staining parasitic and VA-mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc 55:158–161

Pond EC, Menge JA, Jarrell WM (1984) Improved growth of tomato in salinized soil by VAM fungi collected from saline soils. Mycologia 76:74–84

Poss JA, Pond E, Menge JA, Jarrell WM (1985) Effect of salinity on mycorrhizal onion and tomato in soil with and without additional phosphorus. Plant Soil 88:307–319

Ragupathy S, Mahadevan A (1993) Distribution of vesicular-arbuscular mycorrhizae in plants and rhizosphere soils of the tropical plains, Tamil Nadu, India. Mycorrhiza 3:123–136

Reddy SR, Pindi PK, Reddy SM (2005) Molecular methods for research on arbuscular mycorrhizal fungi in India: problems and prospects. Curr Sci 89:1699–1709

Redecker D, Hijri I, Wiemken A (2003) Molecular identification of arbuscular mycorrhizal fungi in root: Perspectives and problems. Folia Geobot 38:113–124

Rozema J, Arp W, Diggelen JV, Esbroek MV, Broekman R, Punte H (1986) Occurrence and ecological significance of vesicular-arbuscular mycorrhiza in the salt marsh environment. Acta Bot Neerl 35:457–467

Schenck NC, Perez Y (eds) (1990) Manual for the identification of VA-mycorrhizal fungi. Synergistic, Gainesville, Fla

Sengupta A, Chaudhuri S (1990) Vesicular arbuscular mycorrhiza (VAM) in pioneer salt marsh plants of the Ganges river delta in West Bengal (India). Plant Soil 122:111–113

Sengupta A, Chaudhuri S (1991) Ecology of heterotrophic dinitrogen fixation in the rhizosphere of mangrove plant community at the Ganges river estuary in India. Oecologia 87:560–564

Sengupta A, Chaudhuri S (1994) Atypical root endophytic fungi of mangrove plant community of Sundarban and their possible significance as mycorrhiza. J Mycopathol Res 32:29–39

Sengupta A, Chaudhuri S (2002) Arbuscular mycorrhizal relations of mangrove plant community at the Ganges river estuary in India. Mycorrhiza 12:169–174

Stahl PD, Christensen M (1991) Population variation in the mycorrhizal fungus *Glomus mosseae*: breadth of environmental tolerance. Mycol Res 95:300–307

Thapar HS, Khan SN (1985) Distribution of VA mycorrhizal fungi in forest soils of India. Indian J For 8:5–7

Tomlinson PB (1986) The Botany of Mangroves. Paperback edn. Cambridge University Press, New York

Tressner HD, Hayes JA (1971) Sodium chloride tolerance of terrestrial fungi. Appl Microbiol 22:210–213

Trouvelot A, Kough JL, Gianinazzi-Pearson V (1986) Mesure du taux de mycorhization VA d'un systéme radiculaire. Recherche de méthodes d'estimation ayant une signification fonctionnelle. In: Gianinazzi-Pearson V, Gianinazzi S

(eds) Physiological and genetical aspects of mycorrhizae. INRA Press, Paris, pp 217–221

Udaiyan K, Karthikeyan A, Muthukumar T (1996) Influence of edaphic and climatic factors on dynamics of root colonization and spore density of vesicular-arbuscular mycorrhizal fungi in *Acacia farnesiana* Willd. and *A. planifrons* W. et. A. Mycorrhiza 11:65–71

Walker C (1992) Systematics and taxonomy of the arbuscular endomycorrhizal fungi (Glomales)—a possible way forward. Agronomie 12:887–897

Walker C, Mize CW, McNabb HS (1982) Populations of endogonacae fungi at two locations in central Iowa. Can J Bot 60:2518–2529

Wang FY, Liu RJ, Lin XG, Zhou JM (2004) Arbuscular mycorrhizal status of wild plants in saline-alkaline soils of the Yellow River Delta. Mycorrhiza 14:133–137

Watson J (1928) Mangrove forests of the Malay Peninsula. Malayan Forest Records 6. Fraser & Neave Limited, Singapore

Yadav JSP, Bandopadhyay AK, Rao KVGK, Sinha TS, Biswas CR, Bandopadhyay BK, Dutta SK (1981) Management of coastal saline soils of Sundarbans. CSSRI-ICAR Bull 7: 1–32