Mycorrhizal status (VAM) of some mangroves growing in saline and non-saline soils

TANUMI KUMAR AND MONORANJAN GHOSE

Agricultural and Ecological Research Unit, Indian Statistical Institute, 203 Barrackpore Trunk Road, Kolkata 700108, West Bengal

The present study describes the mycorrhizal associations in five mangrove species namely, Bruguiera gymnorrhiza (L.) Lam., Excoecaria agallocha L., Heritiera fomes Buch. Ham., Phoenix paludosa Roxb., and Xylocarpus mekongensis Pierre. growing in two distinct ecological sites. One group is growing in non-saline garden soil of the Indian Statistical Institute, Kolkata and the other group in the ridge saline zone of the Sundarbans forest. The vesicular arbuscular mycorrhizal (VAM) status of these plants were evaluated in terms of VA fungal spore number (Sp) per 10 g of rhizospheric soil, frequency of mycorrhiza (F%), intensity of mycorrhizal colonization (M%), arbuscule (A%), vesicle (V%) and hyphal abundance (H%) in the root systems. The soil salinity levels of the garden ranged from 0.32 to 0.96 ppt and were more nutrient poor [in terms of available nitrogen (N)], whereas salinity levels of forest soils ranged from 5.33 to 10.67 ppt with high concentrations of exchangeable sodium (Na) and potassium (K). The extent of mycotrophy was more in fresh water condition of the garden than that of the saline Sundarbans forest. The garden mangroves showed the mean F% and Sp as 94.91 and 16.73, whereas the forest mangroves possessed only 28.67 and 14.8 respectively, Excoecaria agallocha showed high F%, M%, A% and highest Sp in the rhizospheric soils at both the sites. In the saline tracts of the forest, Heritiera fomes lacked mycorrhizal colonization in the roots. Both 'Arum' and 'Paris' types of mycorrhizal colonization were common in Bruguiera gymnorrhiza, Excoecaria agallocha, Xylocarpus mekongensis at both garden and forest and in Heritiera fomes at the garden. Phoenix paludosa exhibited 'Arum' type at the garden, but 'Paris' type at the forest. Spore numbers showed significant positive correlation with F%, whereas negative correlation with available phosphorus (P) content of the soil. The F%, M%, and H% were highly positively correlated to each other, but were highly negatively correlated to soil salinity, exchangeable Na, K and available P content. The A% was positively correlated to F% and M% but not significantly correlated to any of the soil characteristics. Glomus and Acaulospora were frequent in both garden and forest soils, but Glomus versiforme was predominant in the garden soils and G. mosseae and G. etunicatum in the soils of the Sundarbans.

Key words: Mangroves, vesicular arbuscular mycorrhiza, garden vs Sundarbans forest, soil parameters, correlation

INTRODUCTION

Mangrove is a type of coastal woody vegetation that fringes muddy saline shores and estuaries in tropical and subtropical regions (Blasco *et al.*, 1975). Tomlinson (1994) defined mangroves as tropical trees restricted to intertidal and adjacent communities. On the Gangetic and Brahmaputra deltas of West Bengal and Bangladesh, lies the world's one of the largest mangrove forests, the Sundarbans. The term 'Sundarbans' came into

existence either from the local Bengali name 'Sundri' of the once prevalent mangrove species *Heritiera fomes* Buch. Ham. or from the words 'Sundar' (meaning beautiful) and 'bans' (meaning forests) i.e. beautiful forests (Naskar and Guha Bakshi, 1987). The creeks and back waters of the Sundarbans are subjected to tidal influence and are therefore saline. The tidal amplitude ranges from 3 m to 8 m high from mean sea level depending upon the solar phases and position of rivers (Naskar and Guha Bakshi, 1987).

A good deal of work has been published on the occurrence of vesicular arbuscular mycorrhizal (VAM) fungi in halophytes of salt marshes from different parts of the world (Brown and Bledsoe, 1996; Carvalho *et al.*, 2001, 2003, 2004; Cooke and Lefor, 1990; Cooke *et al.*, 1993; Juniper and Abbott, 1993; Hildebrandt *et al.*, 2001; Hoefnagels *et al.*, 1993; Kim and Weber, 1985; Mason, 1928; Mohankumar and Mahadevan, 1986; Rozema *et al.*, 1986; Van Duin *et al.*, 1989). The only studies on the occurrence of VAM in mangroves of Sundarbans were reported by Sengupta and Chaudhuri (1990, 1991, 1994 and 2002). However, they did not quantify the internal mycorrhizal structures in the mangroves.

More than ten years back seedlings and fruits of some important mangroves were collected from the Sundarbans and planted in the non-saline garden soil of the Indian Statistical Institute, Kolkata. These mangroves are surviving and growing well under fresh water terrestrial conditions.

In order to explore the mycorrhizal status, we selected five species of mangroves from the terrestrial garden soil and the same species from the saline Sundarbans region. Hence the objectives of the present study were (i) to compare and contrast the possible differences of mycorrhizal status of five species of mangroves growing in two distinct ecological habitats (i.e. non-saline and saline), and (ii) to quantitatively evaluate mycorrhizal colonization, internal mycorrhizal structures and fungal spores in relation to some physico-chemical properties of the soil.

MATERIALS AND METHODS

Study sites

The first site constituted the garden of the Indian Statistical Institute (ISI), Kolkata (22.65N and 88.38E) at an elevation of 9 m above mean sea level, where some mangroves were grown under fresh water terrestrial condition. The second site was selected on the saline ridge forest of the Sundarbans (22.11N-22.12N and 88.77E-88.83E). During winter, the temperature of both the study sites is cool, but not cold, hardly goes down to

10°C, and in summer it is warm to hot, sometimes rising above 40°C in Kolkata, but the temperature never exceeds 38°C in the Sundarbans due to prevalence of vast amount of water surface (Naskar and Guha Bakshi, 1987). The average rainfall and humidity during monsoon in both the sites are more or less the same, being 170 cm and 96% respectively.

The first site was designated as garden and the second site as forest.

Sample collection

Root and soil samples of five mangrove species (see Table 1) were collected from each site during the month of September 2004. Three to six individuals of each species of different stages of growth (vegetative and reproductive) formed the samples. Care was taken during collection of an individual plant that roots were positively identified as belonging to the particular 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 VAM colonization. Root adhering soil of each individual was air dried at room temperature, sieved, and divided into two portions. One portion was used for VAM fungal spore isolation, enumeration and identification, and the other portion for determination of physical and chemical properties of soil.

Estimation of VAM fungal colonization

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

For estimation of VAM fungal colonization, 30 pieces of 1-cm fragments of roots with diameter <

2 mm were mounted on slides (10 per slide) in polyvinyl alcohol-lactoglycerol (PVLG). Each root fragment was ranked under a compound microscope for the presence and quantification of infection (colonization) and extent of development of arbuscules, vesicles, intercellular and intracellular hyphae including hyphal coils. The mycorrhizal percentages such as, F% (frequency of mycorrhiza in the root system), M% (intensity of mycorrhizal colonization), A% (arbuscule abundance), V% (vesicle abundance), and H% (fungal hyphae in the root system) for individual plant were calculated according to the method of Trouvelot et al., (1986) with slight modifications and using the computer programme 'Mycocalc' (http://www.dijon.inra.fr/ Mychintec/Protocole/Workshop_Procedures.html).

The mycorrhizal colonization was designated as either 'Arum' type (A) or 'Paris' type (P) according to the descriptions given by Smith and Smith (1997). Plants exhibiting both the types were designated as (A+P). Plant species were classified as consistently mycorrhizal (all individuals of a species having mycorrhizal colonization) or inconsistently mycorrhizal (only some individuals colonized) according to Koske *et al.* (1992) and Muthukumar *et al.* (2003).

Isolation, enumeration and identification of VAM spores

Spores were extracted from 10 g of soil in triplicate for each sample by wet-sieving and decantation 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 patterned filter paper and washed with distilled water to spread the spores evenly over the entire grid. The spores were counted using a dissecting microscope at ×40 magnification. 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. Photomicrographs of VAM structures and spores were taken.

Determination of soil characters

Soil pH, salinity (Sal, calculated from electrical conductivity), available nitrogen (N), available phosphorus (Olsen P), exchangeable potassium (K) and sodium (Na) were measured according to Jackson (1973).

Statistical analysis

Two-way Analysis of Variance (ANOVA) was used to test whether there were significant differences in spore number, different mycorrhizal percentages (F%, M%, A%, V% & H%) and soil parameters between species and site. Pearson's correlation coefficient analysis was employed for evaluation of the relationships between spore number, mycorrhizal percentages and soil parameters. SPSS software version 7.5.1. 1996 was used and the values for individual plant were entered for the statistical analyses.

RESULTS

Mycorrhizal colonization

Table 1 shows the VAM status of the five mangroves along with soil characteristics in two different sites. VAM fungi (Fig. 1) occurred at both the sites in Bruguiera gymnorrhiza, Excoecaria agallocha, Phoenix paludosa, and Xylocarpus mekongensis. Heritiera fomes showed VAM colonization only at the garden. The highest F% (100%), M% (66.11%) and H% (40.43%) occurred in Xylocarpus mekongensis at the garden soil, whereas they were very low (22.22%, 12.11% and 3.52% respectively) in the saline forest soil. Excoecaria agallocha (Fig. 1 l, m) showed the highest A% and V% (31.79% and 20.95% respectively) in the non-saline garden soil. At the forest soil E. agallocha possessed the highest F% (78.89%), M% (33.52%), A% (22.45%) and H% (8.74%), but Phoenix paludosa (Fig. 1a, b, c) showed the highest V% (8.74%). P. paludosa exhibited 'Arum' type hyphae with arbuscules and

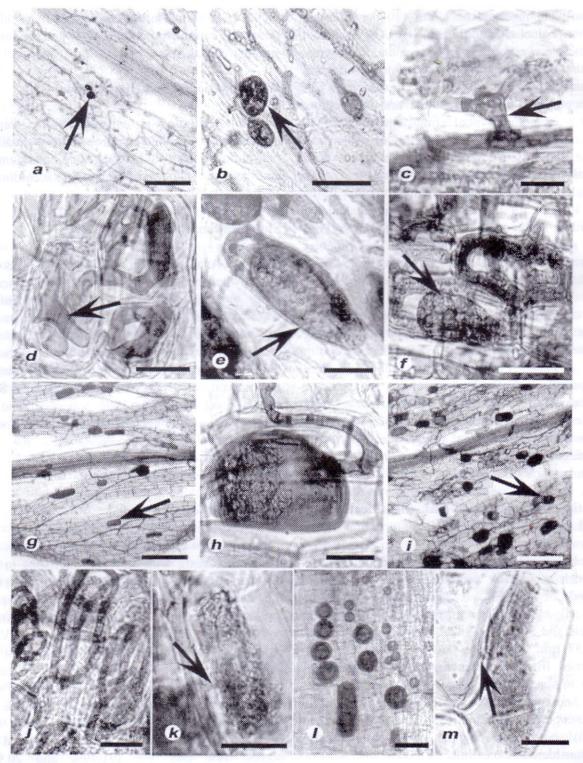


Fig. 1: Vesicular arbuscular mycorrhiza (VAM) in five mangrove species. a, b, c, d, e VAM structures in *Phoenix paludosa*. a Vesicles (one indicated by an arrow) and hyphae; bar 200 μm. b Vesicles (arrow indicating a single vesicle) and hyphae; bar 50 μm. c Arbuscule (trunk indicated by an arrow) in the cortical root cell; bar 10 μm. d Intracellular hyphal coils in cortical cells; bar 30 μm. e Intracellular hyphal coil and vesicle (arrow); bar 30 μm. f Hyphal coils and vesicle (arrow) in *Bruguiera gymnorrhiza*; bar 30 μm. g, h VAM structures in *Heritiera fomes*. g Hyphae and vesicles (one indicated by an arrow); bar 200 μm. h Single vesicle; bar 30 μm. i, j, k VAM structures in *Xylocarpus mekongensis*. i Hyphae and vesicles (one indicated by an arrow); bar 200 μm. j Intracellular hyphal coils; bar 30 μm. Arbuscule (trunk indicated by an arrow); bar 20 μm. l, m VAM structures in *Excoecaria agallocha*. I Vesicles and spores, bar 50 μm. m Single root cortical cell with arbuscule (trunk of which indicated by an arrow); bar 20 μm. a, b, g exhibit 'Arum' type and d, e, f, j 'Paris' type VAM structures.

vesicles at the garden soil but 'Paris' type colonization with characteristic intracellular hyphae, hyphal coils (Fig. 1 d), intracellular vesicles (Fig. 1 e) and arbuscules (with occasional absence of typical arbuscules in some individuals) at the forest. The remaining four species showed both types of colonization (Fig. 1 f-m), with occasional absence of vesicles in some individuals of the species. *P. paludosa* and *E. agallocha* were consistently mycorrhizal, whereas the rest three species were inconsistently mycorrhizal. It was interesting to note that mycorrhizal colonization was absent in *Heritiera fomes* in the saline forest, although a few spores occurred in the rhizospheric soils.

Table 2 shows that there were significant differences in F% between the species, between sites, and between species and sites. Fungal spores (Sp) in the rhizospheric soils differed significantly

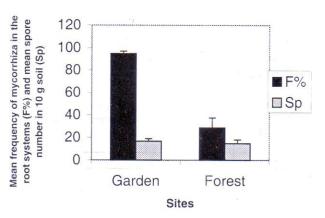


Fig. 2: Mean frequency of mycorrhizae (F%) in the root systems and mean spore numbers (Sp) in 10 g of rhizospheric soil at the two sites viz. garden and forest. Bars represent standard errors.

among the species, and M% between sites. The H% also differed significantly between sites. A% and V% did not differ significantly between species, between sites and between species and sites.

Table 1: Mean spore number in 10 g dry weight soil (Sp), frequency of mycorrhiza (F%), intensity of mycorrhizal colonization (M%), arbuscule abundance (A), vesicle abundance (V), fungal hyphae (H%) in the root system, and rhizospheric soil characteristics of the five species at the two sites viz. garden (g) and forest (f). The scientific names of the plants have been given together with their families in parentheses. Values in parentheses represent standard deviations of means. Other abbreviations are as follows: s-shrub, t-tree, M structures, Values in parentheses represent standard deviations of means. Other abbreviations are as follows: s-shrub, t-tree, M structures, values in parentheses represent standard deviations of means. Other abbreviations are as follows: s-shrub, t-tree, M structures, values in parentheses represent standard deviations of means. Other abbreviations are as follows: s-shrub, t-tree, M structures, values in parentheses represent standard deviations of means. Other abbreviations are as follows: s-shrub, t-tree, M structures, values in parentheses represent standard deviations of means. Other abbreviations are as follows: s-shrub, t-tree, M structures, values in parentheses represent standard deviations of means. Other abbreviations are as follows: s-shrub, t-tree, M structures, values in parentheses. Values in parentheses represent standard deviations of means. Other abbreviations are as follows: s-shrub, t-tree, M structures, values in parentheses. Values in parentheses represent standard deviations of means. Other abbreviations are as follows: s-shrub, t-tree, M structures, values in parentheses.

Parameter	Bruguiera gymnorrhiza¹ (L.) Lam. (Rhizophoraceae)		Excoecaria agallocha ^t L. (Euphorbiaceae)		Heritiera fomes ¹ Buch. Ham (Sterculiaceae)		Phoenix paludosa ^s Roxb. (Arecaceae)		Xylocarpus mekongensis' Pierre. (Meliaceae)	
	g	f	g	f	g	f	g	f	g	f
M struc.	VA(A+P)	VA(A+P)	VA(A+P)	VA(A+P)	VA(A+P)	e r %	VA(A)	VA(P)	VA(A+P)	VA(A+P)
Sp	14.33	16.67	29	29.33	5	2.67	17.33	9.67	18	15.67
	(± 3.51)	(± 9.07)	(± 1.73)	(± 18.01)	(± 1.73)	(± 1.53)	(± 6.51)	(± 4.51)	(±7)	(± 10.50)
F%	88.33	7.78	98.33	78.89	97.66	0	90.22	34.45	100	22.22
	(± 12.58)	(± 13.47)	(± 2.89)	(± 2.39)	(± 2.04)		(± 9.68)	(± 26.73)	(0)	(± 38.49)
M%	47.24	1.9	65.18	33.52	61.26	0	42.30	22.62	66.11	12.11
	(±29.91)	(± 3.29)	(± 5.67)	(± 19.19)	(± 3.83)		(± 22.05)	(± 27.76)	(± 34.55)	(± 20.98)
A%	12.92	1.33	31.79	22.45	16.38	0	23.46	12.32	23.68	8.34
	(± 19.81)	(± 2.31)	(± 26.92)	(± 16.89)	(± 8.42)		(± 21.63)	(± 12.05)	(± 22.44)	(± 14.45)
V%	2.42	0.51	20.95	2.31	17.74	0	1.22	8.74	1.99	0.45
	(± 2.79)	(± 0.88)	(± 26.73)	(± 2.20)	(± 15.64)		(± 1.52)	(± 14.69)	(± 2.67)	(± 0.78)
H%	31.91	0.06	12.43	8.77	33.14	0	17.63	1.57	40.43	3.32
	(±33.26)	(± 0.09)	(± 5.57)	(± 5.17)	(± 21.26)		(± 5.14)	(± 2.01)	(± 33.49)	(± 5.74)
pH	8.11	7.8	8	8.83	8.05	7.68	8	7.3	8.11	8.23
	(± 0.09)	(± 0.79)	(0)	(± 0.05)	(± 0.04)	(± 0.25)	(0)	(± 1.4)	(± 0.09)	(± 0.64)
Sal	0.64	10.67	0.64	5.33	0.32	6.08	0.96	6.05	0.64	6.08
	(0)	(± 0.58)	(0)	(± 0.67)	(0)	(± 1.99)	(0)	(± 3.29)	(0)	(±2.88)
N	289.33	281.4	231.47	498.4	204.47	374.27	220.33	901.6	214.2	372.71
	(± 129.42)	(± 134.89)	(± 344.43)	(± 344.43)	(± 13.30)	(± 60.21)	(± 3.00)	(± 421.79)	(± 32.74)	(± 8.42)
P	4.07	9.11	6.43	8.79	3.04	16.71	3.81	8.09	6.32	15.09
	(± 3.79)	(± 1.45)	(± 5.69)	(± 8.23)	(± 4.20)	(± 21.84)	(± 2.86)	(± 6.37)	(± 5.69)	(± 14.34)
K	0.41	1.89	0.67	1.22	0.37	1.23	0.39	1.28	0.39	1.1
	(± 0.12)	(± 0.56)	(± 0.23)	(0)	(± 0.14)	(± 0.47)	(± 0.03)	(± 0.05)	(± 0.02)	(± 0.22)
Na	2.01	16.45	2.14	14.93	2.11	17.53	2.32	6.36	2.09	17.52
	(± 1.04)	(± 4.68)	(± 1.39)	(± 4.89)	(± 0.57)	(± 4.18)	(± 1.13)	(± 4.91)	(± 0.27)	(± 5.87)

Fig. 2 illustrates that the average frequency of mycorrhizae in the root systems of the garden mangroves (94.91%) was much higher than that of the forest mangroves (28.67%).

Table 2: Two-way ANOVA of different parameters by species, site and species*site. All effects entered simultaneously. Abbreviations as in Table 1.

Parameter	Factors	F				
Sp	Species Site Species * Site	7.584** 0.432 ns 0.326 ns				
F%	Species - Site Species * Site	6.100** 124.279*** 5.133**				
M%	Species Site Species * Site	1.270 ns 31.975*** 1.006 ns				
A%	Species Site Species * Site	1.680 ns 3.574 ns 0.028 ns				
V%	Species Site Species * Site	1.048 ns 2.613 ns 1.615 ns				
Н%	Species Site Species * Site	0.531 ns 15.896*** 1.062 ns				
рН	Species Site Species * Site	1.690 ns 0.175 ns 1.704 ns				
Sal	Species Site Species * Site	2.958* 120.500*** 2.968*				
N	Species Site Species * Site	2.458 ns 14.281** 2.968*				
P	Species Site Species * Site	0.284 ns 3.914 ns 0.338 ns				
K	Species Site Species * Site	2.209 ns 88.140*** 2.767 ns				
Na	Species Site Species * Site	2.509 ns 91.516*** 2.745 ns				

^{*}P<0.05, **P<0.01, ***P<0.001, ns=not significant

Spore analysis

The highest spore number (29) occurred in the rhizospheric soils of E. agallocha at both the sites (Table 1). The lowest VAM spores (2.67) occurred in the rhizospheric soils of H. fomes at the forest. Table 2 shows that there were significant differences in the spore number between the species (F=7.584, P<0.01). Neither sample site nor the interaction between species and site showed any significant difference on the spore number. Fig. 2 also shows that the mean number of spores in the rhizosperic soils of garden (16.73) and forest (14.8) did not vary much. The VAM fungal spores belonging to four genera, namely Glomus, Acaulospora, Gigaspora and Scutellospora occurred at the garden, while only the first three genera occurred at the forest. The predominating spores of Glomus versiforme (Karsten) Berch occurred at the garden, whereas Glomus mosseae (Nicol. & Gerd.) Gerdemann & Trappe and Glomus etunicatum Becker & Gerdemann occurred at the forest.

Soil analysis

There was no significant difference in pH and available P content of the two sites (Tables 1, 2). However, significant differences occurred in salinity of the rhizospheric soils between species, between sites, and between species and sites (Table 2). The mean salinity of the garden soils ranged from 0.32 to 0.96 ppt, whereas in forest soils it ranged from 5.33 to 10.67 ppt (Table 1). The amounts of exchangeable K and Na, and available N occurred much more in the forest soils than those of the garden (Table 1). These parameters differred significantly (P< 0.01 & P< 0.001) mainly between sites (Table 2). Soils of both garden and forest were

Table 3: Pearson's correlation coefficients (r) between Sp, different mycorrhizal percentages and soil characters including the two sites viz. garden and forest. Abbreviations as in Table 1.

0.10	Sp	F %	M %	A %	V %	H %	pH	Sal	N	P	K	Na
Sp	1.000	.421*	.293	.351	.059	.100	.197	046	.067	337*	057	023
7%	.421*	1.000	.852**	.532**	.332	.613**	.154	791**	246	495**	769**	762**
M %	.293	.852**	1.000	.645**	.336	.732**	.114	678**	241	448*	665**	720**
1%	.351	.532**	.645**	1.000	042	.127	.067	360	016	238	309	350
1%	.059	.332	.336	042	1.000	049	.004	238	.047	132	210	389*
H %	.100	.613**	.732**	.127	049	1.000	.109	556**	368*	382*	596**	539**

^{*} Correlation is significant at the 0.05 level (P< 0.05)

^{**} Correlation is significant at the 0.01 level (P< 0.01)

poor in major plant nutrients, particularly available N and P (Table 1).

Correlation analysis

Table 3 illustrates the correlation between different soil parameters and the mycorrhizal characteristics. The Sp showed significant positive correlation (P<0.05) with F% and negative correlation with P (P< 0.05). The F% also showed significant positive correlation with M%. A% and H%, whereas negative correlation with Sal, P, K and Na (P< 0.01). The M% also registered significant positive correlation with A% and H% (P< 0.01) and negative correlation with Sal, Na, K (P< 0.01), and P (P< 0.05). The A% showed significant positive correlation with F% and M% (P< 0.01), but not correlated to any of the soil characteristics. The V% showed only significant negative correlation with Na (P< 0.05). The H% indicated significant negative correlation with Sal (P< 0.01), N (P< 0.05), P (P<0.05), K (P< 0.01) and Na (P< 0.01) in addition to positive correlation with F% and M%.

DISCUSSION

The five mangrove species of Sundarbans growing in fresh water conditions in the garden support the view of Waisel (1972), that halophytes grow in saline habitats not because they are salt loving, but because they can tolerate high concentrations of salt better than other plants of non-saline habitats.

The present study indicates that the extent of mycotrophy was much greater in fresh water condition than in saline water condition. However, 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) were of the opinion that sporulation of arbuscular mycorrhizal (AM) fungi is stimulated under salt-stress conditions.

The mangrove Heritiera fomes of the saline Sundarbans forest soils did not show any VAM structure except a few spores, although its counterpart in non-saline garden soil possessed quite high amount of VAM. This indicated that salinity might plays a big role in this species which inhibited VAM infection, and might be designated as a facultative mycotrophic species. This fact could be one of the reasons for its gradual depletion (Naskar and Guha Bakshi, 1987) from the highly saline substrate of the western Sundarbans. The AM fungal spores present in the rhizospheric soils of H. fomes might have come from the interwoven roots of adjacent AM fungi colonizing plants as suggested by Muthukumar and Udaiyan (2000) and Muthukumar et al. (2003). Juniper and Abbott (1993) also postulated that in high salt concentrations, mycorrhiza formation of even the mycotrophic plants may be adversely affected. McMillen et al. (1998) reported that spore germination and hyphal growth were inhibited by high concentration of NaCl. However, Bowen (1987) opined that salinity induced by NaCl might differ from natural salinity which contained different salts. Among the five mangroves, Exceocaria agallocha showed almost equal spore numbers and high VAM infections in both garden and forest soils, which indicated its wide ecological amplitude in regards to mycorrhizal infection.

Root characters are known to significantly affect the mycorrhizal status of plant species (Hetrick, 1991). Absence of root hairs make the mangroves more dependent on mycorrhizae for nutrient acquisition from the soil (Baylis, 1975).

According to Smith and Smith (1997) most of the experimental studies had involved cultivated plants that form 'Arum' type VAM, rather than 'Paris' type in wild plants. In our study this was found to be true in case of Phoenix paludosa, but not in Bruguiera gymnorrhiza, Excoecaria agallocha, and Xylocarpus mekongensis, as they exhibited both 'Arum' and 'Paris' types of mycorrhizae in both garden plants as well as in wild forest plants. Occurrence of both types of mycorrhizae in the forest plants of these latter three species were in accordance with the findings of Sengupta and Chaudhuri (2002).

Higher percentages of arbuscular abundance in the root systems of the studied mangroves was an indication of symbiosis between plants and VAM fungi (Smith and Smith, 1989). However, arbuscule

abundance might be related to sampling season, since the present study was carried out during the wet period (September), when arbuscules were found to be more abundant than vesicles (Sigüenza et al., 1996).

The study revealed that spore numbers (Sp) varied significantly among the species but was positively correlated to F% and negatively correlated to soil available P. On the other hand, F% varied significantly between species, between sites, and between species and sites, but correlated to various factors such as, Sp, M%, A%, H%, Sal, P, K, and Na. These results supported the general conclusion that variation in spore numbers and frequency of mycorrhizae in the root systems might be due to several factors, including mycorrhizal dependency, variation in host species and their phenology, host plant-mediated alteration of the soil microenvironment, or other unknown host-plant traits (Eom et al., 2000; Lorgio et al., 1999; Wang et al., 2004). The high spore number in the rhizospheric soils of Excoecaria agallocha at both the sites indicated and supported the conclusion of Wang et al., (2004) in that the host species apparently had direct effects on spore density and colonization of AM fungi.

The spore density in the saline soils of the Sundarbans (present study) ranged between 2.67 to 29.33 per 10 g soil which, was higher compared to 3 to 30 per 50 ml soil of the Yellow River Delta reported by Wang et al. (2004). However, Sengupta and Chaudhuri (2002) reported spore number ranged between 170-793 per 10 g soil of the Sundarbans, which was much higher and contradictory. Low density of AM fungal spores was also reported in humid tropical forests (Janos, 1980; Fischer et al., 1994) and in primary tropical forests of Xishuangbanna (Muthukumar et al., 2003). According to Brundrett (1991) AM fungal spore numbers decreased during root growth and increased during root senescence. This fact might be the reason for the low spore numbers which we obtained in our study. Significant positive correlation between Sp and F% in the present study contradicts reports of Brundrett (1991) and Brundrett et al. (1996). However, the observation is consistent with the studies of Muthukumar and Udaiyan (2000), Aliasgharzadeh et al. (2001) and Muthukumar et al. (2003).

The significant positive correlation of Sp with F% and negative correlation with available P, and the highly significant negative correlation of F% with Sal, P and Na were in accordance with the findings of Aliasgharzadeh *et al.* (2001). Contrary to the results of Trouvelot *et al.* (1986), F%, M% and H% were found to be more sensitive to P content in the soil than A%. Since F%, M% and H% were highly correlated to each other, they showed more or less similar behaviour to the soil characteristics. There was a significant negative correlation between V% and Na, and between Sp and P. This finding remains unexplained and requires further investigation.

Glomus and Acaulospora were frequent in the soils of both garden and the Sundarbans. This is in accordance with the observations that species of Glomus and Acaulospora were more abundant in the tropical soils (Muthukumar and Udaiyan, 2000; Muthukumar et al., 2003). Glomus mosseae occurred as one of the most predominant species of AM fungi in the saline soils (as in the observations of Aliasgharzadeh et al., 2001 and Sengupta and Chowdhuri, 2002). Some of the spores have been identified to the species level, the rest are yet to be identified. Further studies are in progress to characterize the different AM fungal species in the garden and at the forest. In due course of time we intend to apply molecular approaches to identify AM fungi within colonized roots.

ACKNOWLEDGEMENT

We thank Professor D. Roy of the Indian Statistical Institute, Kolkata for his help in 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 and help 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

Aliasgharzadeh, N.; Rastin, N. S.; Towfighi, H. and 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.

- Baylis, G. T. S. 1975. The magnolioid mycorrhiza and mycotrophy in root systems derived from it. In: Sanders, F. E.; Mosse, B. and Tinker, P. B. (eds) Endomycorrhizas. Academic Press, London, pp 373-
- Blasco, F. 1975. The Mangroves in India (translated by Mrs.Thanikaimoni from Les mangroves de l'inde. Institute Français de Pondicherry, Inde). Shri Aurobinda Ashram, Pondicherry, India.
- Bowen, G. D. 1987. The biology and physiology of infection and its development. In: Safir, G. R. (ed) Ecophysiology of VA Mycorrhizal Plants. CRC Press, Boca Ratan, Florida, pp 27-57.
- Brown, A. M. and Bledsoe, C. 1996. Spatial and temporal dynamics of mycorrhizas in Jaumea carnosa, a tidal saltmarsh halophyte. J Ecol 84: 703-715.
- Brundrett, M. C. 1991. Mycorrhizas in natural ecosystems. In: Begon, M.; Fitter, A. H. and Macfadyen, A. (eds) Advances in Ecological Research. Vol. 21. Academic Press, London, pp 171-313.
- Brundrett, M. C.; Ashwath, N. and Jasper, D. A. 1996. Mycorrhizas in the Kakadu region of tropical Australia. I. Propagules of mycorrhizal fungi and soil properties in natural habitats. Plant Soil 84: 159-171.
- Carvalho, L. M.; Caçador, I. and Martins-Loução, M. A. 2001. Temporal and spatial variation of arbuscular mycorrhizas in salt marsh plants of the Tagus estuary (Portugal). Mycorrhiza 11: 303-309.
- Carvalho, L. M.; Correia, P. M. and Martins-Loução, M. A. 2004. Arbuscular mycorrhizal fungal propagules in a salt marsh. Mycorrhiza 14: 165-170.
- Carvalho, L. M.; Correia, P. M.; Caçador I. and Martins-Loução, M. A. (2003) Effects of salinity and flooding on the infectivity of salt marsh arbuscular mycorrhizal fungi in Aster trifolium L. Biol Fertil Soils 38: 137-
- Cooke, J. C.; Butler, R. H. and Madole, G. 1993. Some observations on the vertical distribution of vesiculararbuscular mycorrhizae in roots of salt marsh grasses growing in saturated soils. Mycologia 85: 547-550.
- Cooke, J. C. and Lefor, M. W. 1990. Comparison of vesiculararbuscular mycorrhizae in plants from disturbed and adjacent undisturbed regions of a coastal salt marsh in Clinton, Connecticut, USA. Envir Mgmt 14: 131-137.
- Daniels, B. A. and Skipper, H. D. 1982. Methods for the recovery and quantitative estimation of propagules from soil. In: Schenck, N. C. (ed) Methods and principles of mycorrhizal research. American Phytopathological Society, St. Paul, pp 20-45.
- Eom. A. H.; David, C.; Hartnett, A.; Gail, W. T. and Wilson, C. 2000. Host plant species effects on arbuscular mycorrhizal fungal communities in tallgrass prairie. Oecologia 122: 435-444.
- Fischer, C. R.; Janos, D. P.; Perry, D. A.; Linderman, R. G. and Sollins, P. 1994. Mycorrhiza inoculum potentials in tropical secondary succession. Biotropica 26: 369-377.
- Gerdemann, J. W. and Nicolson, T. H. 1963. Spores of mycorrhizal fungi isolated from soil by wet sieving and decanting. Trans Br Mycol Soc 46: 235-244.

- Hildebrandt, U.; Janetta, K.; Ouzaid, F.; Renne, B.; Nawrath, K. and Bothe, H. 2001. Arbuscular mycorrhizal colonization of halophytes in central European salt marshes. Mycorrhiza 10: 175-183.
- Hetrick, B. A. D. 1991. Mycorrhizas and root architecture. Experientia 47: 355-362.
- Hirrel, M. C. 1981. The effect of sodium and chloride salts on the germination of Gigaspora margarita. Mycologia 73 : 610-617.
- Hoefnagels, M. H.; Broome, S. W. and Shafer, S. R. 1993, Vesicular-arbuscular myorrhizae in salt marshes in North Carolina. Estuaries 16: 851-858.
- Jackson, M. L. 1973. Soil chemical analysis. Prentice Hall of India Pvt Ltd, New Delhi, India.
- Janos, D. P. 1980. Vesicular-arbuscular mycorrhizae affect lowland tropical rain forest plant growth. Ecology 61:
- Juniper, S. and Abbott, L. 1993. Vesicular arbuscular mycorrhizas and soil salinity. Mycorrhiza 4: 45-57.
- Kim, C. K. and Weber, D. J. 1985. Distribution of VA mycorrhiza on halophytes on inland salt playas. Plant Soil 83: 207-214.
- Koske, R. E.; Gemma, J. N. and Flynn, T. 1992. Mycorrhizae in Hawaiian angiosperms: a survey with implications for the origin of the native flora. Am J Bot 79: 853-862.
- Kormanik, P. P. and McGraw, A. C. 1982. Quantification of vesicular-arbuscular mycorrhizae in plant roots. In: Schenck, N. C. (ed) Methods and principles of mycorrhizal research. American Phytopathological Society, St. Paul, Minn, pp 37-45.
- Lorgio, E. A.; Julio, R. G. and Peter, L. M. 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.
- Mason, E. 1928. Note on the presence of mycorrhiza in the roots of salt marsh plants. New Phytol 2: 193-195.
- McMillen, B. G.; Juniper, S. and Abbott, L. K. 1998. Inhibition of hyphal growth of a VA mycorrhizal fungus in soil containing sodium chloride limits the spread of infection from spores. Soil Biol Biochem 30: 1639-1646.
- Mohankumar, V. and Mahadevan, A. 1986. Survey of vesicular-arbuscular mycorrhizae in vegetation. Curr Sci 55: 936.
- Muthukumar, T.; Sha, L.; Yang, X.; Cao, M.; Tang, J. and Zheng, Z. 2003. Mycorrhiza of plants in different vegetation types in tropical ecosystems of Xishuangbanna, southwest China. Mycorrhiza 13: 289-
- Muthukumar, T. and Udaiyan, K. 2000. Arbuscular mycorrhizas of plants growing in the Western Ghats region, Southern India. Mycorrhiza 9: 297-313.
- Naskar, K. R. and Guha Bakshi, D. N. 1987. Mangrove Swamps of the Sundarbans: An Ecological Perspective. Naya Prokash, Kolkata, India.

- Phillips, J. M. and Hayman, D. S. 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.
- Rozema, J.; Arp, W.; Diggelen, J. V.; Esbroek, M. V.; Broekman, R. and Punte, H. 1986. Occurrence and ecological significance of vesicular-arbuscular mycorrhiza in the salt marsh environment. Acta Bot Neerl 35: 457-467.
- Schenck, N. C. and Perez, Y. 1990. Manual for the identification of VA-mycorrhizal fungi. Synergistic, Gainesville, Fla.
- Sengupta, A. and Chowdhuri, 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. and Chowdhuri, 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. and Chowdhuri, 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. and Chowdhuri, S. 2002. Arbuscular mycorrhizal relations of mangrove plant community at the Ganges river estuary in India. *Mycorrhiza* 12: 169-174.
- Sigüenza, C.; Espejel, I. and Allen, E. B. 1996. Seasonality of mycorrhizae in coastal sand dunes of Baja California.

- Mycorrhiza 6: 151-157.
- Smith, F. A. and Smith, S. E. 1989. Membrane tranport at the biotrophic interface: an overview. Aust J Plant Physiol 16: 33-43.
- Smith, F. A. and Smith, S. E. 1997. Structural diversity in (vesicular)-arbuscular mycorrhizal symbioses. New Phytol 137: 373-388.
- Tomlinson, P. B. 1994. The Botany of Mangroves. Paperback ed, Cambridge University Press, New York.
- Tressner, H. D. and Hayes, J. A. 1971. Sodium chloride tolerance of terrestrial fungi. Appl Microbiol 22: 210-213.
- Trouvelot, A.; Kough, J.L. and 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. and Gianinazzi, S. (eds) *Physiological and Genetical Aspects of Mycorrhizae*. INRA Press, Paris, pp 217-221.
- Van Duin, W.E.; Rozema, J. and Ernst, W.H.O. 1989. Seasonal and spatial variation in the occurrence of vesicular-arbuscular (VA) mycorrhiza in salt marsh plants. *Agric Ecosyst Environ* **29**: 107-110.
- Waisel, Y. 1972. Biology of halophytes. Academic Press, New York.
- Wang, F. Y.; Liu, R. J.; Lin, X. G. and Zhou, J. M. 2004. Arbuscular mycorrhizal status of wild plants in saline-alkaline soils of the Yellow River Delta. *Mycorrhiza* 14: 133-137.