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Arbuscular Mycorrhizal Fungi of Tropical Sand Dunes of West Coast of India

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ABSTRACT

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Root colonization and spore density of arbuscular mycorrhizal fungi in the rhizosphere of 12 plant species growing on sand dunes in the west coast of India were studied during post-monsoon season. *Borreria articularis, Ipomoea pes-caprae* and *Launaea sarmentosa* were dominant plant species on the dunes. The level of AM fungal root colonization ranged from 34 to 80 percent. Highest number of vesicles and arbuscules were found in *I. pes-caprae* and *Emelia sonchifolia* respectively. Except for *I. pes-caprae,* AM fungal spores were found in the rhizosphere of all plants. The mean spore density was 0.75 g $^{-1}$. Out of a total of 16 species of AM fungi, nine species belonged to genus *Glomus.* Among the spore communities, *Gigaspora ramisporophora, Glomus albidum, Glomus clarum* and *Scutellispora gregaria* were dominant. *Glomus albidum* colonized all plant species except *I. pes-caprae.* Five species of AM fungal spores were recorded from non-vegetated dune adjacent to vegetated dune, of them *Glomus albidum* and *Glomus lacteum* were abundant. A strong positive correlation was found between spore density and number of species; relative abundance and frequency of occurrence of AM fungal spores in rhizophere.

ADDITIONAL INDEX WORDS: *AM fungi, colonization, dune vegetation, rhizosphere, edaphic features.*

INTRODUCTION

Arbuscular mycorrhizas (AM) are obligate symbiotic associations between plant roots and zygomycetes. They are also known to colonize plant species of maritime sand dunes (GIOVANNETTI and NICOLSON, 1983; KOSKE, 1975, 1988). Studies have shown that AM fungi aid in the establishment of plants in coastal sand dunes by improving the uptake of water and nutrients especially phosphorus (FORSTER and NICOLSON, 1981; KOSKE *et al.,* 1975; NICOLSON, 1960). They also enhance dune stability by increasing the aggregation of sand (KOSKE and POLSON, 1984; SUTTON and SHEPPARD, 1976). Although investigations on AM fungi of coastal sand dunes of India are scarce (MOHANKUMAR *et al.,* 1988) several studies have been conducted in other tropical and subtropical regions *e.g.* Australia (KOSKE, 1975; LOGAN *et al.,* 1989), Brazil (STURMER and BELLEI, 1994), Hawaii (KOSKE, 1988; KOSKE and GEMMA, 1990), Pakistan (KHAN, 1971, 1974) and Singapore (LOUIS, 1990).

The objectives of the current study were to examine the colonization rate of AM fungi with vegetation of a tropical coastal sand dune system on the west coast of India. Qualitative and quantitative estimates were also made of AM fungal spores in vegetated and non-vegetated sand dunes; and the physicochemical characteristics of dune sand.

MATERIALS AND METHODS

Study Site

This study was conducted in the coastal sand dunes located at Someshwara, Mangalore Coast of Karnataka (Figure 1) during September 1994 (post-monsoon season). Stable dunes are located at a distance of 25 m from the first dune ridge. Vegetation exists as patches in a stretch on sand dunes. The area of the vegetated dune selected for study is about 75 m^2. It supports several herbaceous plant species. Cultivated *Acacia* and *Casuarina* forests exist parallel to the stretch of dune vegetation. Occasionally the oceanic waves reach the dune vegetation and deposit detritus. The climate is tropical, air temperature ranges between 23–33°C. The average annual rainfall is 390 cm, of which 87% rainfall occurs during June–September due to southwest monsoon. Relative humidity is high throughout the year especially during the southwest monsoon months (JAYAPPA, 1987).

Edaphic Features

Particle size distribution of the samples was determined by sieving the soil samples through sieves (see Table 2). Immediately after the collection soil moisture percentage was estimated by drying known amount of samples at 85°C to a constant weight. Sand pH was detected after dilution with distilled water (1:1 v/v) using glass electrode.

Organic carbon was determined by Walkley and Black's rapid titration method, where organic carbon was oxidised to carbon dioxide by potassium dichromate. Excess potassium dichromate leftover after oxidation was titrated against ferrous ammonium sulphate with diphenylamine indicator (JACKSON, 1973).

Sand samples were dissolved in neutral ammonium acetate and calcium, sodium and potassium were determined by

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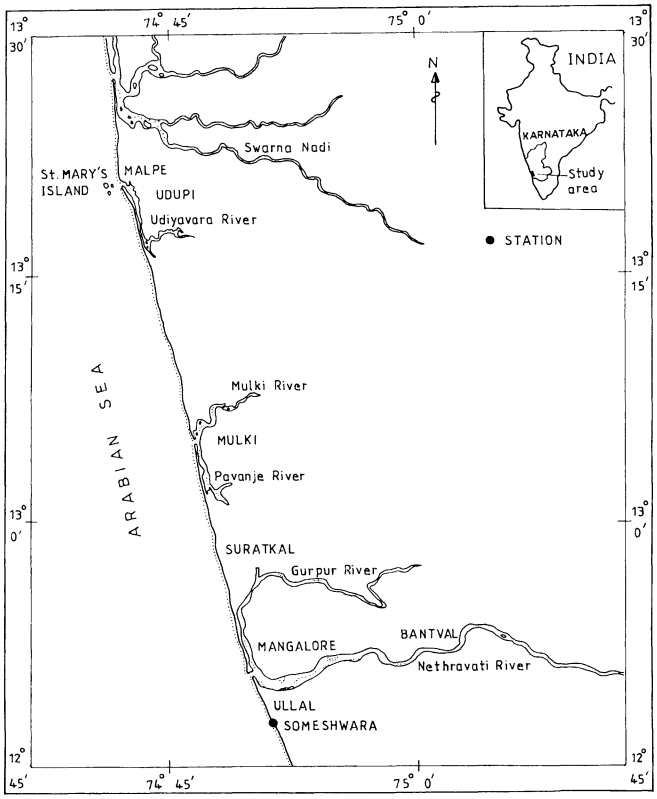

Figure 1. Map of the sampling site Someshwara of Mangalore Coast investigated for AM fungi.

flame photometry. Known concentrations of calcium carbonate, sodium chloride and potassium chloride were used as standards (JACKSON, 1973).

Sand samples were mixed with distilled water by shaking and the water extract was prepared. Later the extract was titrated against standard silver nitrate with potassium chromate as indicator to detect chloride content (JACKSON, 1973).

To detect the available phosphate of sand, Olsen's method was employed. Sand samples were mixed with bicarbonate extractant, shaken after the addition of activated charcoal. Aliquots of the filtrate were mixed with bicarbonate solution and molybdate reagent, shaken again and diluted. Optical density was determined at 660 nm between 10–20 min interval after dilution. Potassium dihydrogen phosphate was used to prepare standards (JACKSON, 1973).

Available nitrogen was estimated titrimetrically. Sand samples were mixed with alkaline potassium permonganate and distilled. The generated ammonia was absorbed by 2% boric acid, excess of which was titrated against 2.5% sodium hydroxide with methylred indicator (JACKSON, 1973).

Colonization of AM Fungi

Twelve plant species growing on the dune system were selected for the study (see list in Table 1). Eight plants each of 12 plant species were excavated carefully (nodal roots for *Ipomoea*) and eight 2–3 mm diameter feeder roots were collected in sterile polythene bags and transported to the laboratory. From each root of each species one piece of one cm length was cut and then pooled in 2.5% aqueous KOH solution. The composited sample of each species was heated at 90°C for 20 min, rinsed in distilled water, bleached in 3% alkaline hydrogen peroxide for 20 min, rinsed in distilled water, soaked in 1% HCl overnight and then stained with acidic glycerol containing 0.05% trypan blue for 20 min at 90°C. Later the roots were destained and stored in acidic glycerol (KOSKE and GEMMA, 1989). Later these root pieces were mounted on glass slides with coverglass and scanned (125–500 ×) for arbuscules/vesicles. The presence or absence of arbuscules or vesicles out of 64 root pieces were considered to estimate the percent colonization. The arbuscules/vesicles or both were enumerated to assess their number per cm in each piece of root.

Table 1. *Rhizosphere and roots of coastal dune plants assessed for AM fungal association from Someshwara, Mangalore coast of India.*

Plant Species	Relative Cover (%)[a]	Status of Plants[b]
Asteraceae:		
Ageratum conyzoides L.	+	F, S
Emelia sonchifolia (L.) DC.	+	F, S
Launaea sarmentosa (Willd.) Alston	+++	F
Tridax procumbens L.	+	F
Caryophyllaceae:		
Polycarpaea corymbosa (L.) Lam.	++	F, S
Convolvulaceae:		
Ipomoea pes-caprae (L.) R. Br.	++++	PF
Lamiaceae:		
Leucas aspera (Willd.) Link	+	F
Papilionaceae:		
Alysicarpus rugosus (Willd.) DC.	+	PF, N
Canavalia rosea (Swartz) DC.	+	PF, N
Rubiaceae:		
Borreria articularis (L.F.) F.N. Will.	+++	PF
B. pusilla (wall.) DC.	+	F, S
Oldenlandia aspera (Roth.) DC.	++	F, S

[a]+, <5%; ++, 5–10%; +++, 10–20%; ++++, 65% (during September 1994)

[b]F, flowering; PF, preflowering; S, seeds present; N, roots nodulated (during September 1994)

Spore Density of AM Fungi

About 2 kg of sand sample at 10–20 cm depth of rhizosphere of each plant was collected in sterile polythene bags and stored at 5°C until they were processed. VAM spores were recovered by modified floatation-adhesion technique (SUTTON and BARRON, 1972). Air dried 20 g of each sand sample was placed in 100 ml of distilled water in a cylinder, shaken vigorously for 30 sec and then allowed to stand for 10 sec. The supernatant was collected in a separating funnel. This procedure was repeated four times and all the supernatant in separating funnel was filtered through Whatman No. 4 filter paper. Settled material on filter paper was scanned under a dissecting microscope (20 ×). Individual

Table 2. *Soil texture of sand dunes of Someshwara, Mangalore coast of India (n = 5; ± SD; range in parentheses).*

Size Class	Grain Size (mm)	Vegetated Dune (%)	Non-vegetated Dune (%)
Gravel	>2.0	1.07 ± 0.20 (0.73–1.28)	0.56 ± 0.21 (0.32–0.83)
Very coarse sand	1.0–2.0	5.96 ± 0.39 (5.54–6.58)	1.11 ± 0.09 (0.95–1.20)
Coarse sand	0.5–1.0	31.38 ± 5.36 (25.85–40.37)	35.96 ± 1.49 (34.52–38.35)
Medium sand	0.25–0.5	53.72 ± 2.86 (48.32–56.48)	52.03 ± 5.58 (44.02–57.19)
Fine sand	0.13–0.25	6.48 ± 2.77 (2.03–9.72)	8.04 ± 1.26 (6.51–10.20)
Very fine sand	0.06–0.13	0.34 ± 0.04 (0.28–0.40)	0.37 ± 0.30 (0.10–0.74)

spores and clumps of spores were transferred from filter paper to microscope slides and mounted with polyvinyl alcohol-lacto-glycerol (PVLG) mountant (polyvinyl alcohol, 8.33 g; distilled water, 50 ml; lactic acid, 50 ml; glycerine, 5 ml) (KOSKE and TESSIER, 1983). Identifications were made according to MORTON (1988), MORTON and BENNY (1990) and SCHENCK and PEREZ (1988). The analyses of rhizosphere sand samples (60 samples: five subsamples per host plant species) yielded a total of 902 AM fungal spores.

The relative abundance was calculated as follows:

$$\frac{\text{Number of sand samples possessing AM fungal spores of a particular species}}{\text{Total number of sand samples analysed}} \times 100$$

The frequency of occurrence was calculated as below:

$$\frac{\text{Number of AM fungal spores of a particular species recorded out of 60 sand samples}}{\text{Total number of all AM fungal spores recorded out of 60 sand samples}} \times 100$$

Similarly sand samples from a non-vegetated dune adjacent to vegetated dune were also analysed.

RESULTS

Edaphic Features

The soil was predominently composed of sand of coarse to medium texture (~85% of the mass had particles 0.25–1.0 mm in diameter) (Table 2). Analyses of physicochemical characteristics of sand samples of vegetated and non-vegetated dunes using paired 't' test reveals that there is significant difference in the levels of phosphate ($p = 0.05$), moisture ($p < 0.01$); pH, sodium, potassium and nitrogen ($p < 0.001$); but no significant difference was observed for organic carbon, calcium and chloride ($p > 0.05$) (Table 3).

Colonization of AM Fungi

All 12 plant species analysed for AM fungal colonization contained either arbuscules or vesicles or both (Figure 2). The level of colonization ranged from 34–80% with an overall average of 60%. Colonization was above 70% in *Ageratum conyzoides, Borreria articularis, Emelia sonchifolia* and *Leucas aspera,* and below 50% in *Alysicarpus rugosus, Canavalia rosea* and *Launaea sarmentosa.* Of the 12 plant species six did not possess vesicles. The maximum number of vesicles were found in roots of *Ipomoea pes-caprae* (18 cm^{-1}), but the average was about 4 cm^{-1}. All plant species tested had arbuscules and the mean of arbuscules in roots was 22 cm^{-1}. The highest number of arbuscules was found in *E. sonchifolia* (42 cm^{-1}) and the lowest in *Canavalia rosea* (6 cm^{-1}). A positive correlation ($r = 0.51$; $p < 0.05$) was found between percent colonization and number of arbuscules.

Table 3. *Edaphic characteristics of the sand dunes of Someshwara, Mangalore coast of India (n = 5; ± SD; range in parentheses).*

Characteristics	Vegetated Dune	Non-vegetated Dune
Moisture, %**	1.70 ± 0.14 (1.50–1.90)	1.34 ± 0.15 (1.17–1.57)
pH***	6.67 ± 0.02 (6.65–6.67)	7.09 ± 0.01 (7.07–7.10)
Organic carbon, %	0.03 ± 0.01 (0.02–0.04)	0.02 ± 0.01 (0.01–0.03)
Calcium, μg g^{-1}	14.69 ± 1.53 (12.24–16.32)	15.50 ± 1.00 (14.28–16.32)
Chlorides, μg g^{-1}	849.80 ± 36.66 (791–889)	862.44 ± 39.30 (795–894)
Sodium, μg g^{-1}***	77.27 ± 5.29 (67.26–82.91)	59.44 ± 3.56 (54.75–64.13)
Potassium, μg g^{-1}***	27.15 ± 0.21 (27.00–27.45)	8.85 ± 0.42 (8.55–9.45)
Phosphate, μg g^{-1}*	58 ± 7.48 (50–70)	70 ± 8.94 (60–80)
Nitrogen, μg g^{-1}***	6.55 ± 0.12 (6.44–6.72)	1.47 ± 0.21 (1.26–1.82)

*p = 0.05; **p < 0.01; ***p < 0.001

Spore Density of AM Fungi

With exception of *I. pes-caprae,* rhizosphere of all other plant species contained AM fungal spores (Figure 3). Spore abundance ranged from 0–1.6 g^{-1} sand with an overall average of 0.75 g^{-1}. Maximum number of spores were encountered in *Borreria articularis* (1.60 g^{-1}), followed by *Leucas aspera* (1.21 g^{-1}) and *Ageratum conyzoides* (1 g^{-1}). The spore load was least in *Launaea sarmentosa* (0.10 g^{-1}). Spores of 16 species of AM fungi were found in association with plants in the sand dune (Table 4). Members of the family Glomaceae were dominated (62%), followed by the Gigasporaceae (38%). The genus *Glomus* was recorded most frequently (47%), followed by *Scutellispora* (33%) and *Gigaspora* (20%). Spores of *Gigaspora ramisporophora, Glomus albidum, G. clarum* and *Scutellispora gregaria* were dominant among the spore communities. Spore densities and frequencies were very low in *Glomus reticulatum* and *Sclerocystis pachycaulis* (0.1 vs 1.7%).

Though spores of 10 species of Glomaceae were found in the rhizosphere, their spore density in the rhizosphere was 47.3%, whereas six species of Gigasporaceae produced 52.7% of spores in the rhizosphere. A strong positive correlation ($p < 0.0005$) was found between AM fungal spore density and number of species ($r = 0.86$); relative abundance and frequency of occurrence of AM fungal species ($r = 0.90$); and

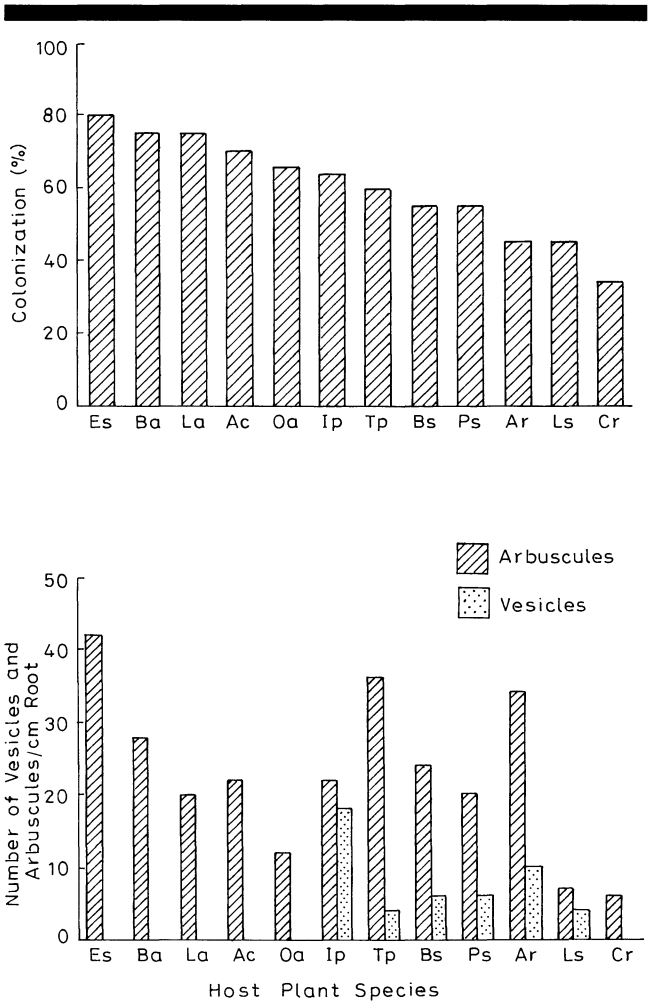

Figure 2. Percent colonization of root by AM fungi; arbuscules and vesicles per cm root length of 12 host plant species: Es, *Emelia sonchifolia*; Ba, *Borreria articularis*; La, *Leucas aspera*; Ac, *Ageratum conyzoides*; Oa, *Oldenlandia aspera*; Ip, *Ipomoea pes-caprae*; Tp, *Tridax procumbens*; Bs, *Borreria stricta*; Ps, *Polycarpaea corymbosa*; Ar, *Alysicarpus rugosus*; Ls, *Launaea sarmentosa*; Cr, *Canavalia rosea*.

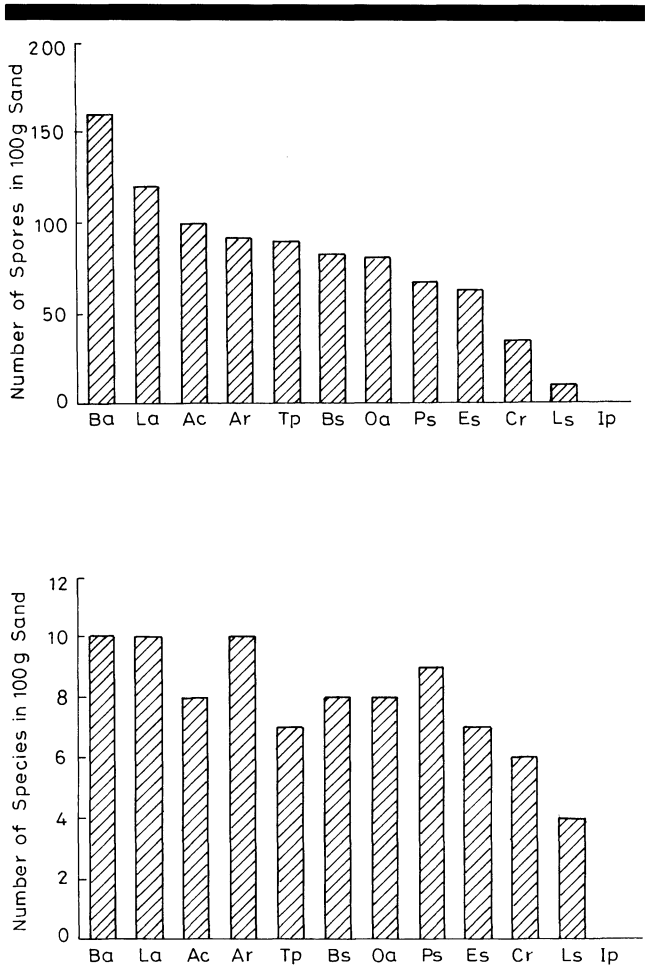

Figure 3. Number of AM fungal spores and species in 100 g dry weight of rhizosphere sand of 12 host plant species (see legend to Figure 2).

also relative abundance and frequency of occurrence of spores among the members of Asteraceae (r = 0.83), Caryophillaceae (r = 0.82), Lamiaceae (r = 0.95) and Rubiaceae (r = 0.87). Weak positive correlation ($p < 0.05$) was found between root colonization and spore density (r = 0.49); relative abundance and frequency of occurrence among the members of Papilionaceae (r = 0.50).

Spores of five species were recorded from sand samples of non-vegetated dune adjacent to vegetated dune (Table 5). Of these, *Glomus albidum* and *G. lacteum* were dominant.

DISCUSSION

Species richness of AM fungi found in the present study is higher than that of sand dunes of other tropical regions in Australia (KOSKE, 1975), Hawaii (KOSKE, 1988) and east coast of India (MOHANKUMAR *et al.*, 1988). In a preliminary study MOHANKUMAR *et al.*, (1988) reported that out of 53 plant species surveyed over a stretch of 36 km along south east coast (Madras) of India, 35 species had AM fungal colonization. All plant species had AM fungal spores in the rhizosphere and spores of nine species of AM fungi were recovered. Of these *Glomus clarum* and *G. pustulatum* were found in the present study. Despite of repeated examination, *Oldenlandia aspera* (Roth.) DC. (Rubiaceae) have not shown AM fungal root colonization (MOHANKUMAR *et al.*, 1988), however in the present study 65% of the roots of *Oldenlandia aspera* had been colonized. Normally the members of Caryophyllaceae are not colonized by AM fungi (GERDEMANN, 1968; LINDROTH *et al.*, 1973, cf. LOUIS, 1990), but in the present study *Polycarpaea corymbosa* (Caryophyllaceae) had an AM fungal root colonization of 55% and a spore density in rhizosphere of 0.67 g^{-1}.

Generally, production of AM fungi is maximum at the end of growing season in many plants (KOSKE and HALVORSON, 1981). Though roots of *I. pes-caprae* had 65% of colonization by AM fungi, rhizosphere did not contain any AM fungal spores. This plant had maximum relative cover (65%) at pre-

Table 4. *AM fungal spore population associated with coastal sand dune vegetation at Someshwara, Mangalore coast.*

	Relative Abundance (%)[a]	Frequency (%)[b]
Gigasporaceae		
Gigaspora decipens Hull & Abbott	1.6	13.3
G. gigantea (Nicolson & Gerdemann) Gerdemann & Trappe	1.2	20.0
G. ramisporophora Spain, Sieverding & Schenck	17.5	75.0
Scutellispora erythropa (Koske & Walker) Walker & Sanders	4.2	31.7
S. gregaria (Schenck & Nicolson) Walker & Sanders	27.7	65.0
S. nigra (Redhead) Walker & Sanders	0.4	5.0
Glomaceae		
Glomus albidum Walker & Rhodes	16.5	76.7
G. clarum Nicolson & Schenck	11.1	48.3
G. convolutum Gerdemann & Trappe	0.4	3.3
G. dimorphicum Boyetchko & Tewari	4.3	11.7
G. fasciculatum (Thaxter) Gerdemann & Trappe emend. Walker & Koske	4.3	16.7
G. lacteum Rose & Trappe	5.5	21.7
G. mosseae (Nicolson & Gerdemann) Gerdemann & Trappe	2.1	11.7
G. pustulatum Koske, Friese, Walker & Dalpe	2.8	26.7
G. reticulatum Bhattacharjee & Mukerji	0.1	1.7
Sclerocystis pachycaulis Wu & Chen	0.1	1.7

[a]Number of spores of AM fungal species as percent of total number of all AM spores encountered
[b]Number of sand samples possess AM spores of a particular fungal species divided by total number of soil samples screened × 100

Table 5. *AM fungal spores recorded from non-vegetated sand dune at Someshwara, Mangalore coast.*

	Relative Abundance (%)[a]	Frequency (%)[b]
Gigasporaceae		
Gigaspora ramisporophora Spain, Sieverding & Schenck	11.1	6.7
Glomaceae		
Glomus albidum Walker & Rhodes	44.4	26.7
G. clarum Nicolson & Schenck	11.1	6.7
G. lacteum Rose & Trappe	22.2	13.3
G. pustulatum Koske, Friese, Walker & Dalpe	11.1	6.7

[a,b]See details in Table 4

flowering stage in September 1994. During subsequent months in a seasonal study the plant cover decreased drastically with seed setting and several AM fungal spores were recovered (K. R. SRIDHAR *et al., unpublished observation*). The number of arbuscules and vesicles in *I. pes-caprae* in September 1994 was almost equal (18–22 cm^{-1} root). The vesicles were maximum compared to other plant species on the dune. It is interesting to study the extent of arbuscules and vesicles when *I. pes-caprae* produce AM fungal spores in its rhizosphere.

Presence of AM fungal spores in non-vegetated dune suggests that they are dispersed by biotic or abiotic factors. Many plants possess seeds in September along with flowers, their dispersal usually take place by wind and waves. The dispersal of AM fungal spores along with seeds may have definite advantage in establishment of plants as well as AM fungi. Besides these factors, dispersal of spores might be dependent on distribution of roots. *Ipomoea pes-caprae* produce roots at nodal regions, *Launaea sarmentosa,* a runner has deep roots in addition to horizontal spreading. Members of Poaceae were also found on different dunes of study site, they possess horizontal rhizomes and smaller biomass of fibrous roots. Such plants might function as spreading zones of AM fungi in these sand dunes.

STURMER and BELLEI (1994) found no correlation between frequency of occurrence and relative abundance of AM fungal spores on coastal sand dunes of Brazil. They concluded that the observed difference in frequency of occurrence and spore abundance of AM fungal species was due to unequal spatial distribution. In this study a strong correlation ($r = 0.90$; $p < 0.005$) was found between overall frequency of occurrence and relative abundance of AM fungal spores suggesting possible uniform distribution of spores in vegetated dune.

KOSKE (1987) studied the distribution of AM fungi along the sand dunes of the Atlantic Coast and did not find significant correlation between soil nutrients (N, P or K) or other soil properties and abundance of AM spores. In the present study significant difference of physicochemical features of vegetated and non-vegetated dune might be due to the influence of plants as well as AM fungi. Arbuscular mycorrhizal fungi are known to enhance the uptake of phosphorus by plants (ALLEN *et al.,* 1980). The lower available phosphorus status in the current study in vegetated dune ($p = 0.05$) might had been influenced by increased plant uptake. Similarly significantly high level of sodium and potassium ($p < 0.001$) in the vegetated dune might be influenced by accumulation by plants and fungi in the rhizosphere. The higher nitrogen level ($p < 0.001$) in vegetated dune is attributed to increased synthesis of nitrogenous compounds and decomposition of detritus in the rhizosphere. Sand pH was slightly acidic (vegetated dune) to neutral (non-vegetated dune) ($p < 0.001$).

The diversity of glomalean populations has mainly been studied in coastal sand dunes (GIOVANNETTI and GIANINAZZI-PEARSON, 1994). The activity of glomalean AM fungi under experimental conditions is affected by many edaphic factors, of which pH plays a very important role (HETRICK, 1984; SIEVERDING, 1991). The relationship between AM fungi, host plants and edaphic factors in coastal sand dunes needs further study.

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