

## **Effect of disturbance on Arbuscular Mycorrhizal (AM) Fungal population in Coastal Sand Dune Vegetation of Goa**

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### **Abstract**

A survey was conducted to study the occurrence and distribution of AM fungi in disturbed and un-disturbed coastal sand dunes of Goa. Eight plant species were found dominant at the un-disturbed site while seven were dominant at the disturbed site. Of these, only three plant species viz., *Ipomoea pes-caprae*, *Cocos nucifera* and *Borassus flabellifer* were common to both the sites.

The study compares the spore density and diversity of AM fungi in disturbed and un-disturbed sand dunes. The avg. spore density and species richness was higher at un-disturbed site than at the disturbed site. In all, 27 AM fungal species were recorded from both the sites. Ten of the identified AM fungal species were commonly occurring at both the sites but their abundance drastically varied at the two sites.

**Keywords:** Dune vegetation, rhizosphere, AM spore density, AM species richness

### **Introduction**

Arbuscular mycorrhizal (AM) fungi are obligate symbionts that are of vital importance to the establishment, growth and survival of the dominant plant species that colonizes dunes

(Koske and Polson 1984; Puppi and Riess 1987). Arbuscular mycorrhizal fungi appear to benefit by improving the uptake of phosphate and other nutrients from the soil, conferring increased drought and disease tolerance to the host plant and contributing to the soil structure and stability (Koske et al 1975; Sutton and Sheppard 1976; Nelson 1987; Newsham et al 1995). They also have a role in plant community composition (Reeves et al 1979) and secondary succession (Janos 1980).

The beach dune system protects coastal environments by absorbing energy from wind, tide and wave action (McHarg 1972). Coastal dune system may be damaged by erosion, which results from natural processes, such as the gradual rise in sea level, or human activity, predominantly building construction and recreational use (Dean 1976). Any perturbation to an existing ecosystem that includes physical removal of plants and changes in the physico-chemical characteristics of soil will have a major impact on symbiotic association (Sylvia 1986; Sylvia and Will 1988; Beena et al 2000). Consequently, as species of plant differ in their response to AM fungi in the soil, the presence or absence of AM fungi has been linked to the composition of plant communities that developed in dune sites (Read 1989; Koske and Gemma 1992; Francis and Read 1995). Several surveys on AM fungi of coastal sand dunes have been conducted in temperate, subtropical and other tropical regions (Koske and Halvorson 1981; Koske 1975; Sturmer and Bellei 1994; Louis, 1990). Investigations on AM fungi of coastal sand dunes of India are scarce (Kumar et al 1988, Kulkarni et al 1997).

Goa is one of the smallest State of India situated along the Central West Coast lying in between latitudes  $15^{\circ}48'$  and  $14^{\circ}43'54''$  N and long  $74^{\circ}20'13''$  to  $73^{\circ}40'33''$  E. The coast of Goa, which extends approximately 120 km in length, has beautiful stretches

of sandy shores and beaches, which attract a large number of tourists from home and abroad. The dimension of tourism and hotel industry on the beaches is surely affecting the dune system. Since AM fungi are obligate symbionts, the population of viable propagules of AM fungi declines steadily with the destruction of the vegetation (Janos 1980; Miller 1979), until it is too low to contribute to the successful establishment of plant species that can benefit from the symbiosis. Hence it could be critical to introduce such propagules to improve the recovery rate of the system. An understanding of occurrence and distribution of AM fungi in sand dunes of Goa is essential in any attempt to use them in environmental conservation.

This study compares the occurrence, distribution, spore density and species richness of AM fungi in disturbed and un-disturbed sand dunes of Goa. The rhizosphere edaphic features at both the sites were also studied to determine the chemical characteristics of soils.

## **Materials and Methods**

Two Coastal areas viz., Varca and Miramar beaches were selected for the study. Varca beach located in south Goa is an un-disturbed beach and has rich sand dune vegetation. The total length of the beach is 4 km and width is 600 m. Here one can clearly see three different types of dunes namely the fore dunes, mid dunes and hind dunes. Miramar beach in north Goa in contrast, is a disturbed site. It is about 90-100 m in width. The dune system is disturbed by tourism activities. There is a little natural vegetation dominated by *Ipomoea pes-caprae* in the fore dunes. There is a total absence of back dunes and this can be attributed to urbanization.

Climatic factors are almost uniform at both the sites. The climate is tropical with three main seasons viz., monsoon (Jun to Oct), winter (Nov to Jan) and summer (Feb to May). The avg. annual rainfall recorded is 3250 mm with max humidity 96%. The mean max and min temp recorded are 37.2°C and 15.9°C respectively.

The dominating plant species at each site were selected for the study. Rhizosphere soil samples were collected randomly from three individuals of each plant species. Later, a composite sample was made for each species, and analyzed. Rhizosphere soil samples were excavated from a depth of 0-25 cm, placed in plastic bags, labeled and stored at 4°C and processed within a month. While collecting soil samples, soil was dug at three places for each plant so as to cover the entire rhizosphere.

Soil pH was measured in 1:2 soil water suspension using pH meter. Electrical conductivity was measured at room temp 1:5 soil suspension, Standard soil analysis techniques viz., Walkely and Black's rapid titration (Walkley and Black 1934), micro-Kjeldahl (Jackson 1971) and Bray and Kurtz (Bray and Kurts 1945) were employed for determination of organic carbon, total nitrogen (N) and available phosphorus (P) respectively. Available potassium (K) was estimated by ammonium acetate method (Hanway and Heidal 1952) using flame photometer (Systronic 3292).

For quantification of spore density, 100 g of air dried composite sample was assayed for spore count using wet sieving and decanting procedure (Gerdemann and Nicolson 1963) and quantification of spore density of AM fungi was carried out using the method of Gaur and Adholeya (1994). Each composite sample was processed three times. Spores were observed under Leica Stereo-microscope.

Baiting of native AM fungi were carried out using open pot cultures (Gilmore 1968). *Eleusine coracana* (L.) Gartner and *Coleus* sp. were used as host plants for baiting the native AM fungi. The plants were maintained under glass house conditions and watered adequately. Five pot cultures were maintained per plant species for each of the sites.

The roots of host species were checked for AM colonization after 45 d. Pots showing successful mycorrhization were maintained for a period of 6 mo. and application of water was reduced at final 3 wk to maximize spore production (Menge 1982). At the end of 6 mo. the plants were cut near the base, the cultures were air-dried and checked for the presence of spores. Spores isolated from pot cultures were used for identification for AM fungi.

Diagnostic slides containing intact and crushed spores and sporocarps of AM fungi were prepared in polyvinyl alcohol lactoglycerol (Koske and Tessier 1983). Spore morphology and wall characteristics were considered for the identification of AM fungi and these characteristics were ascertained using compound microscope (Leica WILD MP 3 and Nikon E 800).

Arbuscular mycorrhizal fungal spores were identified to species level using various bibliographies (Almeida and Schenck 1990; Morton and Benny 1990; Schenck and Perez 1990; Bentivenga and Morton 1995; Redecker et al 2000; Morton and Redecker 2001). Taxonomic identifications of spores were also carried out by matching the descriptions provided by International Collection of Vesicular Arbuscular Mycorrhizal Fungi (<http://invam.caf.wvu.edu>). Names and epithets of AM fungi were followed according to the earlier given recommendations (Walker and Trappe 1993).

Relative abundance (RA) and frequency of occurrence (FO) was calculated as  
 $RA (\%) = \text{AM fungal spores of a particular species} / \text{Total AM fungal spores of all species} \times 100$ ,  
and  $FO (\%) = \text{Soil samples with spores of a particular AM fungal species} / \text{Total soil samples screened} \times 100$ .

## Results and Discussion

The study of soil analysis revealed differences in mean values for available P between both the sites (Table 1). Among the edaphic features, available P is one of the major limiting factors in the severely disturbed dunes (Sturmer and Bellei 1994). Arbuscular mycorrhizal association increases the capacity of the plants to accumulate phosphates as it is released by other microorganisms (Gupta and Rorison 1975).

A total of three plant species viz., *Ipomoea pes-caprae*, *Cocos nucifera* and *Borassus flabellifer* were common to both the sites. The avg. spore density observed at Varca was 1041 spores + 9 sporocarps/100 g soil. Spore count was max. in *C. nucifera* (3404 spores + 32 sporocarps) followed by *Anacardium occidentale* (2076 spores + 20 sporocarps) and *B. flabellifer* (1832 spores + 16 sporocarps) and min. in *Vitex trifolia* (28 spores/100 g soil). The average spore density observed at Miramar was 485 spores + 7 sporocarps. Spore count was max. in *C. nucifera* (996 spores + 20 sporocarps) followed by *Acacia auriculiformis* (756 spores) and *Lantana camara* (640 spores) and min. in *Casuarina equisetifolia* (92 spores + 16 sporocarps). The species recovered represented four genera of AM fungi i.e. *Acaulospora*, *Gigaspora*, *Glomus* and *Scutellospora*. At Varca site, 21 AM fungal species were identified. Most AM fungal species were associated with roots of *B. flabellifer* and *A. occidentale* but were the least with those of *I. pes-caprae* (Table 2). The fungal species most frequently occurring at Varca were

*Gigaspora margarita* (with a frequency of occurrence 62.5%). *Acaulospora spinosa*, *Scutellospora calospora* and *S. coralloidea* ranked second (50%). At Varca site, nine of the AM fungal species identified occurred in more than 25% of the samples examined with *Glomus fasciculatum* as the most abundantly sporulating species (Table 3). Spores of this fungus constituted 25% of all the spores recovered. Other AM fungi producing numerous spores were *Glomus claroideum*, *A. spinosa* and *S. calospora*. At Miramar site, 14 AM fungal species were identified. The highest number of AM fungal species co-occurred with *L. camara*, *A. auriculiformis* and *C. nucifera* while the lowest numbers of AM fungal species were recorded in *I. pes-capre* and *C. equisetifolia* (Table 2).

It was observed that the spore density showed a drastic reduction (by 50%) in disturbed site compared to un-disturbed site. Also, the species richness was lower in disturbed site (14 AM fungal species) and higher at un-disturbed site (21 AM fungal species). The dominance of AM fungal species from un-disturbed dunes was different than those from the disturbed dunes. According to Beena et al (2000) the disturbance severely affects the reproduction of AM fungi on the dune. The impact of disturbance seems to be higher on spore production than on species richness. Changes in the AM fungal community between un-disturbed and disturbed dunes reflect its response to disturbance.

The results of this study support the findings of Louis (1990) that reduced activity of AM fungi in a disturbed site also affects its plant community structure and ecosystem stability. Giovannetti and Nicolson (1983) reported greater spore numbers in a well fixed stable dune system and low spore numbers in the dunes that were considerably eroded by anthropogenic factors in their study of AM fungi in Italian sand dunes. According to

Reeves et al (1979) disturbance of soil leads to elimination or reduction in number of viable propagules of AM fungi. Mayr (1965) concluded that reduced number of AM fungal propagules in disturbed sites results from lowered number of plant hosts of AM fungi. Soil disturbance can reduce the colonization potential of AM fungi in several ways; Propagules may be physically damaged i.e. spores may be crushed and/or the soil hyphal network and colonized root fragments may be disrupted (Evans and Miller 1990). Disturbance may alter the physical, chemical, or biological environment of the soil, which in turn prevents the colonization by or germination of AM propagules (Stahl et al 1988). Disturbance may also eliminate host plants, leading to changes in the carbon supply available to the fungus (Abbott and Robson 1981). Brundrett (1991) commented that the relative importance of these mechanisms has not yet been fully established. Additionally, individual fungal species may exhibit different responses to both the direct and indirect impacts of soil disturbance, depending upon their own specific host and environmental requirements.

The AM fungal species most frequently found at Miramar site were *A. spinosa*, *Glomus macrocarpum*, *G. deserticola*, *S. coralloidea* and *S. pellucida*. Ten of the identified AM fungal species occurred in more than 25% of samples examined (Table 3). The Miramar soils were dominated by the spores of *G. macrocarpum*, *A. spinosa*, *G. deserticola*, *Scutellospora pellucida* and *Glomus heterosporum*. Among the identified AM fungal species, 10 species viz., *S. coralloidea*, *S. pellucida*, *S. verrucosa*, *S. weresubiae*, *Glomus heterosporum*, *G. sinosum*, *G. microaggregatum*, *Gi. margarita*, *Acaulospora foveata* and *A. spinosa* were common to both the sites but their abundance drastically varied at the two sites (Table 3) (Plate 1).



The survey of dune soils of coastal vegetation in Goa confirms the universal occurrence of AM species. The spores of *A. spinosa*, *Gi. margarita*, *S. calospora*, *S. coralloidea* and *S. pellucida* were numerous and widely distributed in coastal sand dune vegetation of Goa. Rose (1988) recovered *Glomus fasciculatum*, *Scutellospora coralloidea* and *Scutellospora calospora* from sand dunes of Northern California and found that distribution of spores and species paralleled plant succession and vascular plant diversity. Koske and Tews (1987) recovered *Glomus macrocarpum* as commonly occurring AM fungi in Wisconsin (USA) sandy soils. Giovannetti and Nicolson (1983) reported wide distribution of *Glomus fasciculatum* and *Scutellospora calospora* in Italian sand dunes. Koske (1975) recovered *Scutellospora calospora* from Australian sand dunes and found that spore diversity was greater in fixed dunes than from younger dunes. The frequency of occurrence of spores and their abundance increased with increasing stabilization of the dunes. Kulkarni et al (1997) reported the presence of *Scutellospora gregaria* as dominant species from Mangalore coast of Karnataka.

Nicolson (1967) suggested that AM fungi might be a significant factor to colonize bare areas such as sand or industrial waste. The absence of AM fungal propagules in the coastal reclaimed land in Singapore resulted in the failure of colonization of mycorrhizal plant species even after five years of reclamation (Louis 1990). Stabilization of disturbed ecosystems like coastal dunes is dependent upon successful establishment of the most effective plant community. There is a great potential for land reclamation programmes in manipulating symbiotic association to accelerate the success of more desirable plants.

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**Table 1. Chemical characteristics of coastal sand dune soils.**

Site	pH	E. C. M mhos/cm	Total N (%)	Available P kg/ha	Organic Carbon (%)	Available K (kg/ha)
Undisturbed site (Varca)	7.5 (0.05)	0.17 (0.05)	0.07 (0.00)	14.2 (1.08)	0.14 (0.01)	200.00 (5.01)
Disturbed site (Miramar)	7.6 (0.09)	0.20 (0.00)	0.06 (0.00)	10.2 (1.20)	0.10 (0.01)	180.00 (3.91)

Std. values in parenthesis

**Table 2. Spore density and species richness of AM fungi in un-disturbed (Varca) and disturbed (Miramar) sites.**

Site	Plant species	Spore density/100g	Species richness/ plant species
Undisturbed site:	<i>Anacardium occidentale</i> L.	2076 (479) spores + 20 (14) sporocarps	11
	<i>Borassus flabellifer</i> L.	1832 (300) spores + 16 (6) sporocarps	13
	<i>Cocos nucifera</i> L.	3404 (598) spores +32 (22) sporocarps	7
	<i>Ipomoea pes-caprae</i> (L.) Sweet	116 (26) spores	3
	<i>Ixora coccinea</i> L.	324 (36.17) spores + 4 (2) sporocarps	4
	<i>Spinifex littoreus</i> Merrill.	100 (19.97) spores	4
	<i>Urginea indica</i> Kunth	444 (70) spores+8 (3) sporocarps	8
	<i>Vitex trifolia</i> L.	28 (11.14) spores	4
Average value		1041 spores + 9 sporocarps	7
Disturbed site:	<i>Acacia auriculiformis</i> Benth.	756 (147.09) spores	8
	<i>Borassus flabellifer</i> L.	452 (60) spores + 12 (2) sporocarps	6
	<i>Casuarina equisetifolia</i> J. R. Forster & G. Forster	92 (20) spores + 16 (6) sporocarps	4
	<i>Cocos nucifera</i> L.	996 (234.08) spores + 20 (4) sporocarps	8
	<i>Ipomoea pes-caprae</i> (L.) Sweet	224 (58.18) spores	4
	<i>Lantana camara</i> L.	640 (140) spores	9
	<i>Zizyphus rugosa</i> Lam.	232 (43) spores + 4 (3) sporocarps	5
Average value		485 spores + 7 sporocarps	6

**Table 3. Frequency of occurrence and relative abundance of AM fungi in disturbed (Miramar) and undisturbed (Varca) sites.**

AM species	Frequency of occurrence (%)		Relative abundance (%)	
	Un-disturbed site	Disturbed site	Un-disturbed site	Disturbed site
<i>Acaulospora delicata</i> Walker, Pfeiff. & Bloss	NR	10.0	NR	00.28
<i>Acaulospora elegans</i> Trappe & Gerd.	25.0	NR	01.71	NR
<i>Acaulospora foveata</i> Trappe & Janos	25.0	10.0	04.14	00.19
<i>Acaulospora nicolsonii</i> Walker, Reed & Sanders	12.5	NR	00.81	NR
<i>Acaulospora scrobiculata</i> Trappe	37.5	NR	02.28	NR
<i>Acaulospora spinosa</i> Walker & Trappe	50.0	80.0	07.71	21.95
<i>Acaulospora</i> sp.	12.5	30.0	00.05	06.42
<i>Gigaspora margarita</i> Becker & Hall	62.5	30.0	01.38	02.60
<i>Glomus constrictum</i> Trappe	12.5	NR	00.43	NR
<i>Glomus claroideum</i> Schenck & Sm. Emend Walker & Vestberg	37.5	NR	08.62	NR
<i>Glomus coremioides</i> (Berk. & Broome) Redecker & Morton	NR	30.0	NR	00.47
<i>Glomus deserticola</i> Trappe, Bloss & Menge	NR	60.0	NR	19.63
<i>Glomus fasciculatum</i> (Thaxt.) Gerdemann & Trappe emend. Walker & Koske	37.5	NR	25.13	NR
<i>Glomus formosanum</i> Wu & Chen	25.0	NR	01.67	NR
<i>Glomus heterosporum</i> Sm. & Schenck	25.0	40.0	03.05	03.72
<i>Glomus macrocarpum</i> Tul. & Tul.	NR	70.0	NR	23.16
<i>Glomus microaggregatum</i> Koske, Gemma & Olexia	37.5	20.0	00.62	00.19
<i>Glomus sinusum</i> (Gerd. & Bakshi) Almeida & Schenck	12.5	30.0	00.05	00.65
<i>Glomus</i> sp. 1	37.5	NR	00.29	NR
<i>Glomus</i> sp. 2	25.0	30.0	26.56	01.40
<i>Scutellospora calospora</i> (Nicolson & Gerd.) Walker & Sanders	50.0	NR	05.14	NR
<i>Scutellospora coralloidea</i> (Trappe, Gerd. & Ho) Walker & Sanders	50.0	70.0	0.19	03.26
<i>Scutellospora gregaria</i> (Schenck & Nicolson) Walker & Sanders	25.0	NR	00.57	NR
<i>Scutellospora pellucida</i> (Nicolson & Schenck) Walker & Sanders	12.5	60.0	00.43	13.40
<i>Scutellospora verrucosa</i> (Koske & Walker) Walker & Sanders	12.5	40.0	00.66	02.42
<i>Scutellospora weresubiae</i> Koske & Walker	37.5	10.0	00.33	00.09
<i>Scutellospora</i> sp.	NR	10.0	NR	00.19

NR = Not reported.

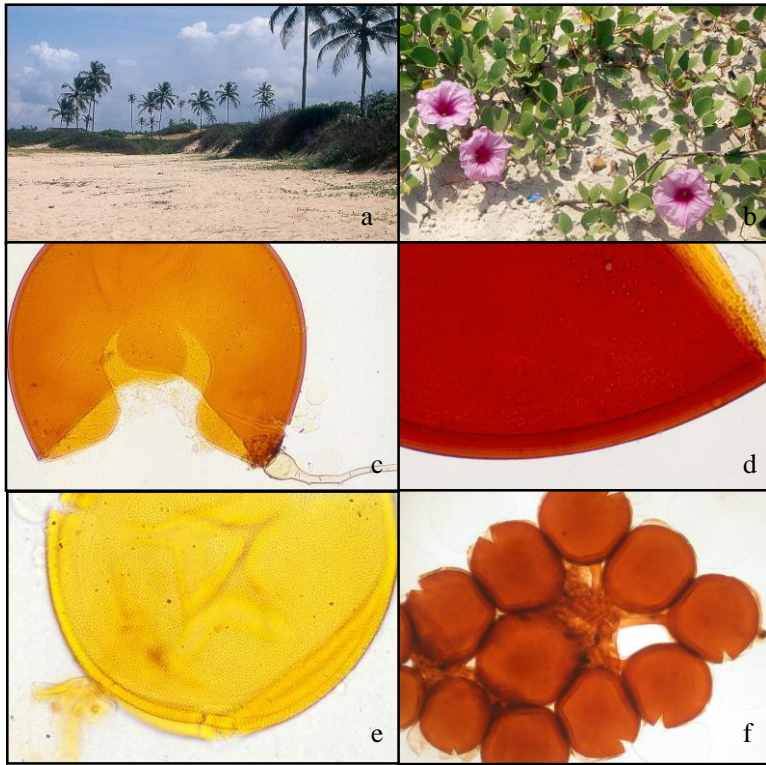


Plate 1: a. Coastal sand dunes with vegetation. b. *Ipomoea pes-capre* – a natural sand binder. c. Spore of *Gigaspora margarita* (100X) d. Spore wall laminations in *Gigaspora margarita* (400X) e. Spore of *Acaulospora spinosa* (100X) f. Broken sporocarp of *Glomus macrocarpum* (200X).