STUDIES ON ARBUSCULAR MYCORRHIZAL FUNGI IN COASTAL SAND DUNE VEGETATION OF GOA.

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By

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Dedicated to my Parents

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Varsha Jaiswal

CERTIFICATE

I certify that the thesis entitled "STUDIES ON ARBUSCULAR MYCORRHIZAL FUNGI IN COASTAL SAND DUNE VEGETATION OF GOA" submitted by Ms. VARSHA JAISWAL, is a record of research work done by her during the period from 1997-2001 when she worked under my supervision. The thesis has not formed the basis for award of any Degree, Diploma, Associate-ship or Fellowship to Ms. VARSHA JAISWAL.

I affirm that the thesis submitted by Ms. VARSHA JAISWAL incorporates the independent research work carried out by her under my supervision.

(B. F. RODRIGUES)
(Signature of Guide)

DECLARATION

I hereby declare that the Ph.D. thesis entitled "STUDIES ON ARBUSCULAR MYCORRHIZAL FUNGI IN COASTAL SAND DUNE VEGETATION OF GOA" submitted to Goa University, forms an independent work carried out by me in the Department of Botany, Goa University, under the supervision of Dr. B. F. Rodrigues, Reader, Department of Botany, Goa University and the thesis has not formed previously the basis for the award of any Degree, Diploma, Associate-ship or other similar titles.

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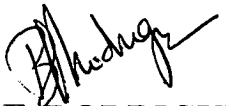

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INTRODUCTION

Coastal sand dunes, result from the stabilization of transported sediment by vegetation. Krumbein and Slack (1956) divide the beach zone into two *viz.*, the foreshore where there is transport by water currents, by waves and occasionally by wind; and the backshore where there is transport primarily by wind with breaking waves having only a minor influence. The two requisites for dune formation are adequate supply of sand and sufficient wind. Stabilization of the mobile sand by vegetation is the second phase in the process.

Coastal sand dunes are found in many parts of the world in arid, semi-arid and temperate climates, though on semi-tropical and tropical coasts luxuriant vegetation, low wind velocities and damp soil make them less frequent (Packham and Willis, 1997). Most coastal dunes are essentially phytogenic, evolving with a partial cover of vegetation which both helps to fix the ground and modifies its surface properties with respect to air flow. At a later stage in their development and seldom permanently dunes often become inactive or fixed, held by the roots of the plants covering them. The great arid deserts of the world are generally devoid of permanent vegetation and their dunes are active or live constantly changing under wind currents and fed from blowouts.

Barbour *et al.*, (1985) and Chapman (1976) have extensively reviewed the abiotic component of coastal sand dunes. According to them high temperature, wind speed, light intensity and potential evapo-transpiration are of considerable significance. The sand is coarse with low level of inorganic content. In most

PLATE-1

VEGETATION COVER OF COASTAL
SAND DUNE OF GOA (SITE: VARCA).

dunes, the primary source of water is rain, but once it has percolated through the dune to the underlying water table it is unavailable to the dune ridge plants. Three main strategies have evolved in response to this problem. (1) Dune annuals undergo their vegetative cycle in autumn and spring or other wet seasons and survive the dry periods as seeds. Perennials either (2) produce a deep rooting system and are thus able to draw water from a vertical zone or (3) produce an extensive shallow shower exploiting root system. Water availability is enhanced by diurnal temperature change, which leads to dew formation, the dune acting as large water condensers.

Coastal dune systems are dynamic natural features of vital economic and ecological importance. They function as flexible barriers to storm, tides and waves (Woodhouse, 1982). Fore dunes protect areas behind them from wave damage and salt water intrusion during storms and also are a source of sand for the beach during periods of erosion. Vegetated fore dunes restrict wind, sand and salt spray intrusion into hind dune areas. The protective action of the fore dunes allow the development of a more complex plant community on the hind dunes. Land ward dune parallel to fore dunes serve as a second line of defense against water and wind erosion.

Vegetation plays an important role in the formation and stabilization of coastal sand dunes. Pioneer plants trap and hold wind blown sand in the frontal dune and help to create condition, which encourage the establishment and growth

of other plant communities such as woodland, scrub heath and forest. The above ground parts of the dune plants act as obstruction, increase surface roughness and cause reduction in the surface speed of sand carrying wind. The reduction in wind movement results in the deposition of sand around the plants. Thus dune vegetation helps in keeping the coastal land free from erosion and also prevents internal desertification.

Pioneer plants make up the initial dune vegetation. They are found on the dune nearest to the sea where their survival depends on their ability to establish, grow and reproduce in order to colonize newly formed dunes.

Mycorrhiza, are critical components of the root soil interface. These fungi are present quite consistently on the root surfaces or in the tissues or cells of the roots of many species so that dual organs of consistent morphological and histological patterns are formed. They conform to a number of common kinds the world over, and in them the fungus and the host co-exist in a physiologically, ecologically and reproductively active state for long periods, a state called mutualistic symbiosis. **Table-1** summarizes the characteristics of the common kinds of mycorrhizae. These symbiotic associations that participate in the uptake of phosphorus are involved in pioneer colonization of nutrient deficient sites (Harley, 1970). The arbuscular mycorrhizal symbiosis is a wide- spread phenomenon which occurs in 80% of the plant species including Angiosperms, Gymnosperms and Pteridophytes showing a little host specificity (Azon, 1994;

Baylis, 1975). It is now well known that these organism are ubiquitous in distribution. Grass-lands, muckfarms, rainforests, sand dunes and arid regions support variable levels of arbuscular mycorrhizae. Disturbed habitats may support relatively few natural infections by mycorrhizae (Hayman, 1982).

Table1: Characteristics of important kinds of mycorrhizas in their mature state (Harley, 1989).

Character	**Kinds of mycorrhiza**						
	Arbuscular	Ecto	Ectendo	Arbutoid	Monotropoid	Ericoid	Orchid
Fungi							
Septate	-	+	+	+	+	+	+
Aseptate	+	(+)	-	-	-	-	-
Hyphae enter cells	+	-	+	+	+	+	+
Fungal sheath present	-	+	+ or -	+	+	-	-
Hartig net formed	-	+	+	+	+	-	-
Hyphal coils in cells	+	-	+	+	-	+	+
Haustoria							
Dichotomous	+	-	-	-	-	-	-
Not dichotomous	-	-	-	-	+	-	+ or -
Vesicles in cells/tissues	+ or -	-	-	-	-	-	-
Host Taxon	Bryo, Pterido, Gymno, Angio	Gymno, Angio, (Pterido)	Gymno, Angio.	Ericales	Monotrop-aceae	Ericales	Orchid-aceae

Legend:

+ = Present - = Absent ( ) = rare

Arbuscular mycorrhizae belonging to Zygomycetes can be identified by vesicles and arbuscules formation in roots (Abbott and Robson, 1979), dimorphic branching of extramatrical hyphae (Mosse, 1963; Nicolson, 1959) and production of large numbers of chlamydospores and azygospores in the soil. Arbuscular mycorrhiza develop when a hypha from a spore or an already infected root contacts a suitable host root (Powell, 1976). The development of AM fungi in root can be divided into four stages (Tommerup and Briggs, 1988).

a) Spore germination and hyphal growth from infective propagules of AM fungi.

b) Growth of hyphae through soil to host roots. The mycelial system surrounding the roots are dimorphic (Mosse, 1959b; Nicolson, 1967).

c) Penetration and successful initiation of infection in roots. Hyphae penetrates mechanically and enzymatically into cortical cells (Kinden and Brown, 1975). At the point of penetration, hypha may or may not form appressoria (Abbott, 1982).

d) Spread of infection and development of internal hyphal system, arbuscules, which bifurcate inside a cell and bring about nutritional transfer between two symbionts and vesicles which, develop as terminal or intercalary swellings in inter- or intra-cellular hyphae. They are responsible for storage and vegetative reproduction.

The role of AM fungi in phosphorus acquisition of plants has been well documented for more than two decades. In general, most large growth enhancement effects of root infection with mycorrhizal fungi are caused by increases in phosphorus absorption; particularly from sparingly soluble phosphorus sources (Bolan *et al.,* 1987). When root exploration is restricted up to 80% of the plant phosphorus can be delivered by the external Am fungal hyphae to the host plant over a distance of more than 10 cm from the root surface (Li *et al.,* 1991).

Besides this increase in spatial availability of phosphorus, effective phosphorus acquisition by the external hyphae is related to a) formation of polyphosphates in the hyphae, and thus, maintaining low internal phosphate (Pi) concentration (b) the small hyphal diameter leading to a relatively larger soil volume delivering phosphorus per unit surface area compared to the root surface (Jungk and Claassen, 1989) and a correspondingly 2-6 times higher phosphorus influx rate per unit length of hyphae (Jakobsen *et al.*, 1992).

With respect to phosphorus nutrition, the growth response to AM infection depends on soil and plant factors which determine the phosphorus acquisition of the host plant, extent of phosphorus deficiency-induced root response and the phosphorus status of the soil. With increasing soil phosphorus, the growth enhancement effect of AM fungi declines and may either be abolished or lead to growth depressions.

When AM fungi improve phosphorus nutrition of the host plant there may be a corresponding increase in nodulation, nitrogen fixation and growth (Robson *et al.*, 1981). There are numerous reports on the enhancement of K (Bethlenfalvay *et al.*, 1989), Ca (Rhodes and Gerdemann, 1978a) and SO_4 –S (Cooper and Tinker, 1978) uptake by AM infection. It also depresses root penetration and larval development of nematode (Sikora, 1978).

Goa is one of the smallest state of India situated along the Central West Coast lying in between Latitudes 15 ^{0} 48' 00" and 14^0 43' 54" North and Longitude 74^0 20'13" to 73^0 40' 33" East (Anonymous, 1979). 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 tourist from home and abroad. Demands for land, for development put increasing pressure on all land areas particularly those of relatively low agricultural values such as dunes. Many dunes have been used as residential sites – often in connection with the tourism industry. A well planned development of beaches is essential not only for sand dune vegetation but also for an ecofriendly development of tourism and other industries which will indeed be a contribution to the economy of the State.

Recent scientific results provide data which support the hypothesis that arbuscular mycorrhizal plants are effective colonizers of disturbed habitats and the lack of AM fungi exert profound influences on species composition (Tommerup and Abbott, 1981). The understanding of mycorrhizal association in sand dunes

and their distribution in soil is necessary for wise management of disturbed sand dunes.

Arbuscular mycorrhizal fungi directly mediate interaction between plants in at- least four ways:

a) They allow trees to compete successfully with grasses and herbs for resources and they detoxify allelochemicals produced by these plants as well.

b) They may decrease competitive interactions between the plants and increase the productivity of species mixture, particularly in soil where phosphorus is limiting.

c) The hyphae that link the same and different species act as a route of material transfer among plants.

d) Arbuscular mycorrhizal fungi and other microbes affect soil formation and structural characteristics by producing humic compounds, accelerating decomposition of primary minerals and producing organic glues that bind soil particles into water stable aggregates (Rose, 1988; Varma *et al.*, 1990).

Severing the close links between plant and soil microorganisms has contributed to degradation of many ecosystems. The re-establishment of functional ecosystems presumes knowledge of micro-elements of the system and therefore

they must be studied to understand the ecosystem changes. No effort seems to be directed to isolate and identify the native AM fungi in the sand dune soils of Goa.

The present study is taken up to determine the status of AM fungi in the sand dune vegetation of Goa. The main objectives of the present study are as follows:

1. Survey of coastal sand dune vegetation at different sites in Goa.
2. Determination of colonization of AM fungi in roots and spore density in rhizosphere of dune plants.
3. To study the diversity of the AM fungi in natural and disturbed sites of sand dunes.
4. To find AM fungal diversity in rhizosphere of each host plant species.
5. To determine the seasonal fluctuations in AM fungal colonization, spore density and diversity.
6. Preparation of pure inoculum of AM fungi in association with dune plants.
7. To assess the response of dune plants to arbuscular mycorrhization.

REVIEW OF LITERATURE

Arbuscular mycorrhiza, a type of mutualistic symbiosis formed between plants and zygomycetous fungi in the Glomales, is certainly the most ancient and widespread mycorrhizal symbiosis. Klironomos and Kendrick (1993) estimated that from 1951- 1990, there were about 8900 papers on mycorrhizas published but almost 3500 from 1991-1995 alone.

Frank (1885) hypothesized that a mycorrhiza is a fungus in the root that functions to provide soil resources to the host in exchange for energy. Frank (1887) differentiated endomycorrhiza and ectomycorriza. He described endomycorrhizas as the fungi that do not cover the roots and are within the roots. Schlicht (1888) developed a list of plants forming endomycorrhiza. Gallaud (1905) described arbuscules and vesicles. Thus by the early 1900s, a mycorrhiza was inferred if a particular plant- fungal interaction did not result in necrosis. Further, the type of mycorrhiza was generally inferred from the types of structures present. If a root had a mantle and hartig net, it was an ectomycorrhiza. If the fungus penetrated the cell wall, it was an endomycorrhiza. Since arbuscules and vesicles were within the cortical cells, AM were endomycorrhizal. The term AM remains primarily used to define a structure despite attributing a function to it (Allen, 1991).

The AM type of mycorrhiza has undergone several name changes from endomycorrhiza to Vesicular Arbuscular Mycorrhiza (VAM) to Arbuscular Mycorrhiza (AM). The shift to VAM from endomycorrhiza followed the

recognition that evolutionarily and functionally VAM did not resemble other types of endomycorrhiza that penetrated the root cells. The VAM were not *Rhizoctonia*, such as were mycorrhizal with orchids, nor were they Ascomycetes that formed ectendomycorrhizae. The fungi forming VAM were all Zygomycetes in the Glomales, that appears to have diverged simultaneously with the ancestral group leading to the Ascomycetes and Basidiomycetes (Berbee and Taylor, 1993). They form easily distinguishable structures including arbuscules, vesicles, coils and distinctive hyphae. More recently, the letter 'V' in VAM was dropped because members of the Gigasporaceae do not form vesicles (Morton and Benny, 1990).

Arbuscular mycorrhiza were present in the earliest land plants (Taylor *et al.*, 1995). Hyphae, arbuscules and vesicles can be clearly seen in material from early Devonian Rhynie chert (Kidston and Lang, 1921; Taylor *et al.*, 1995). The molecular clock evidence suggests that AM fungi appeared over 300 million years ago (Simon *et al.*, 1993).

Research on AM associations went through a long gestation period. From the first comprehensive description of an AM (Gallaud, 1905) until workers in the 1950s demonstrated convincingly that AM could enhance plant growth (Nicolson, 1967), research was confined to the range of plants forming these associations and the taxonomic position of the symbiotic fungi. However, this was a crucial period because these observations established that these mycorrhizas were widespread (Janse 1896; Lohman, 1927) and ancient (Kidston and Lang, 1921).

Janse (1896) undertook the first broad scale survey in Java, showing that the great majority of tropical plants formed mycorrhizas. Stahl (1900) categorized plants into obligatory mycotrophic, facultatively mycotrophic and non- mycotrophic families.

The first description of fungi in the Endogonaceae (*Glomus*) appeared in the mid -1800s (Tulasne and Tulasne, 1845). Butler (1939) linked members of the Endogonaceae with the AM type of mycorrhiza.

FUNCTION OF AM AND MODES OF ACTION

Following the determination that a distinct group of fungi formed AM, rapid evaluation of the mechanisms whereby AM affected plant growth was undertaken. Mosse (1957) described the role of AM in improved nutrient uptake and, Gerdemann (1964) demonstrated that in the low nutrient (especially phosphorus) soils, AM enhanced the growth of Maize. Nicolson (1960) linked grasses with AM and placed the AM activity in a successional context by demonstrating that early seral land plant were non mycorrhizal and that the later seral grasses and forbs were mycorrhizal.

Several studies demonstrated that AM fungal hypha could expand beyond the root depletion zone up to several cm and transport ions such as P, Ca, S (Rhodes and Gerdemann, 1975, 1978a,b). While most research in the 1970s concentrated on phosphorus uptake, a few studies began to look at other limiting resources. Safir *et al.*, 1972, first demonstrated enhanced water uptake in AM plants, but attributed the increase to elevated phosphorus transport. Arbuscular mycorrhiza-enhanced waterthrough flow occurred during the dry down phase (Allen and Allen, 1986) when the shrinkage of the roots would have occurred and air pockets between soil and root developed.

There are several consistent structure/function relationships associated with all AM symbiosis which are critical to understanding their role in ecosystem dynamics. The arbuscule is an important point of contact for exchange of resources between plant and fungus. Both organisms retain a membrane to separate their adjacent cells (Cox and Tinker, 1976). Enzymes such as phosphatases are concentrated along the interface, presumably facilitating nutrient movement from fungus to plant (Gianinazzi *et al.*, 1979). Intraradical structures, because they exist, create an altered physiology of the host. Several research laboratories are presently studying the mechanisms whereby signals are exchanged between host and fungus which regulate their development (Giovannetti *et al.*, 1994).

Associated with the hyphae that penetrate a root is an external matrix that radiates out into the soil. There are important architectural features associated with these extramatrical hyphae. These include runner, or arterial hyphae that are thick walled and often traverse long distances to invade uncolonized roots (Mosse, 1959a; Friese and Allen, 1991). These hyphae may be major carbon sinks in storing and transporting a large amount of energy necessary for colonizing new roots. These hyphae may also serve as bridges between plant, transporting resources between individuals (Read, 1992).

When a hypha penetrate a root, the runner hypha produces finer branched hypha that radiate out into the soil matrix. Friese and Allen (1991) suggested that this pattern was a distinct architectural feature of the symbiosis that is critical to AM functioning.

Associated with this mycelium appears to be a highly regulated development of other organisms. Klironomos and Kendrick (1996) found that soil arthropods, when presented with a choice, would preferentially graze on saprotrophic rather than AM fungal hyphae. While soil animals appear readily to clip hyphal connections between the extramatrical and internal hypha (Fitter and Sanders, 1992), they do not appear to feed preferentially upon the entire hypha as they do saprotrophic fungi.

TAXONOMY OF AM FUNGI

A. Morphological Characters

Morphological features, macro- and micro- anatomy, form the basis for the identification and taxonomy of Glomalean fungi. Identification is a fundamental requirement to understanding biodiversity and essential for monitoring changes in natural, managed or disturbed ecosystems. Diversity in AM fungi can be explored at this level by studying spore characteristics, ultrastructural features and infection patterns.

a. Spores

Arbuscular mycorrhizal fungi are obligate biotrophs (Lewis, 1973) that can only be maintained pure in pot cultures with host plants. Many attempts to grow these organisms *in vitro* in association with genetically transformed roots were implemented in the last decades (Becard and Piche, 1992; St. Arnaud *et al.,* 1996; Declerck *et al.,* 1998). However, most of them form relatively large asexual spores in soil and their identification has therefore traditionally been based almost exclusively on morphological descriptions of the different spore types, giving rise progressively to more accurate classifications of the different taxa (Gerdemann and Trappe, 1974; Morton and Benny, 1990). Many new species of AM fungi have been reported since the historical revision of the Endogonaceae by Thaxter (1922). Recently, a new order, the Glomales consisting of two new suborders, the Glomineae {Families: Glomaceae (Genera- *Glomus, Sclerocystis*),

Acaulosporaceae (Genera- *Acaulospora, Entrophospora)*} and the Gigasporineae {Family: Gigasporaceae (Genera- *Gigaspora, Scutellospora*)}, has founded on the basis of morphological criteria (Morton and Benny, 1990).

Walker, 1992 discussed the problems involved in Taxonomy of AM fungi. Morton, (1988) and Schenck and Perez, (1990) gave taxonomical keys to identify species. The most important taxonomical parameters can be summarized as follows:

1) Sporocarp occurrence, shape, colour and size.
2) Peridium occurrence and characteristics.
3) Spore colour, size and shape.
4) Spore walls number, colour, thickness and ornamentation.
5) Hyphal attachment, shape and type of occlusions.

b. Wall structure and cytochemistry

Few Glomalean species have been examined ultrastructurally to decide which wall features might contribute most to significant taxonomic variability. Scanning electron microscope (SEM) studies have confirmed light microscope observations that different ornamentations can be observed on spore walls (Koske and Walker, 1985; Maia and Kimbrough, 1993). Comparative transmission electron microscope (TEM) investigations have revealed variations in the fine architecture of wall components of both spores and hyphae.

c. Infection patterns

Some authors have proposed considering intraradical mycelium morphology as a criterion for taxonomic differentiation between AM fungi and for their identification *in planta* (Abbott and Robson 1979; Abbott, 1982). In fact some fungi in the Glomales can differ in their pattern of colonization and the structures they form. After appressorium formation, infection can begin by the formation of either simple unbranched, inter- or intra- cellular hyphae or large hyphal loops in the epidermal cells. The only infection structure common to all genera is the intracellular haustorium called the arbuscule. The Glomineae also form vesicles and some times spores inside roots, distinguishing them from the Gigasporineae which never form intraradical vesicles or spores, but develop characteristic soil-borne structures called auxiliary cells (Morton and Benny,1990). There is only one AM fungi (probably a group of fungi), currently called *Glomus tenue* (Greenhall) Hall, which can be easily identified within root tissues because of its very different infection morphology, with extremely small hyphae, the formation of thickened appressoria and fan- shaped hyphal structures within roots (Hall,1977).

Otherwise, such criteria based on mycorrhiza morphology have not been widely applied to fungal identification since variations are small and changes often depend on the host plant (Daniels Hetrick *et al.*, 1985). *Glomus mosseae* has a distinct infection pattern within *Gentiana* roots where it forms only large intracellular coils, no typical arbuscules and no intercellular hyphae (Jacquelinet-

Jeanmougin and Gianinazzi-Pearson, 1983). For this reason, it has been claimed that infection patterns by Glomalean fungi depend more on the genome of the host plant than on that of the fungal species involved (Bonfante and Fontana, 1985; Gianinazzi-Pearson *et al.*, 1991).

B. Biological characters

Information concerning the life cycle and developmental sequences of these biotrophic fungi, with that relative to their symbiotic performance, must take into account factors relating to spore ontogeny and germination, infectivity and efficiency, biogeography, edaphic requirements.

a. Ontogeny of spores

Increasing attention is given to the taxonomic character of spore and sporocarp ontogeny in descriptions of new species or studies of the established ones (Morton and Benny, 1990; Walker, 1992). Ontogenic diversity has been considered diagnostic in the differentiation of two genera, *Acaulospora* and *Entrophospora* (Ames and Schneider, 1979). In *Acaulospora*, the spores are borne laterally on the stalk of a large vesicle while in *Entrophospora* they are borne within the stalk of the mother vesicle. Sporocarp development occurs in some species of the Glomaceae but not in the same manner. Recently Almeida and Schenck (1990) transferred most of the species of *Sclerocystis* (except *Sclerocystis coremioides*) to *Glomus* based on spore ontogeny. However, Wu (1993a) argued that distribution

of shared characters justified to group all species with highly organized sporocarps into *Sclerocystis.*

b. Spore germination

Germination is an integral part of the life cycle of AM fungi and germination characters have importance for their taxonomy since they are used to distinguish between the genera *Gigaspora* and *Scutellospora,* in the Gigasporineae. In the former, germination takes place directly through the spore wall whilst in the latter, it occurs from a germination shield formed upon or within the inner wall layer (Walker and Sanders, 1986).

Although AM fungi are unculturable obligate biotrophs, isolated spores will germinate on nutrient or water agar (Mosse, 1962; Hepper, 1981). The germinating ability, the pattern of germination and the quantity of mycelium produced are characters that can all show a high degree of variation (Hepper and Smith, 1976; Pons and Gianinazzi-Pearson, 1984; Giovannetti *et al.,* 1991). Spores of *Gigaspora* species germinate very quickly in water agar and produce extensive hyphal growth, whilst *Acaulospora* species are generally slow, and *Glomus species* vary in their rates and levels of germination (Tommerup, 1983; Pons and Gianinazzi-Pearson, 1984).

C. Molecular characters

Analysis of molecular features offers an alternative approach for more accurate identification, for establishment of more precise phylogenetic relationships and for detection *in planta*.

Attempts have been made to characterize AM fungi by analysis of total or enzymically-active proteins extracted from spores and separated by electrophoresis. The first fungal enzyme to be identified electrophoretically, alkaline phosphatase, does not show a high degree of polymorphism between different Glomalean species (Gianinazzi *et al.*, 1992). In contrast, other isozyme activities detected in Glomalean spores display clear variations between species and amongst geographically different isolates (Hepper *et al.*, 1988b; Rosendahl and Sen, 1992). Diagnostic isozyme bands showing stability under variable growth conditions have been used to distinguish between fungi in individual and combined, morphologically similar, infections (Hepper *et al.*, 1986; Rosendahl *et al.*, 1989), and to monitor variability in infection during competitivity experiments between fungal species in a microcosm experiment (Hepper *et al.*, 1988a).

Amount of DNA per nucleus has been found to differ considerably among species. It ranges from 0.25 in *Glomus versiforme* to 0.77 pg in *Gigaspora margarita* (Bianciotto and Bonfante, 1992). Amplification of genomic DNA with short arbritary primers (RAPD) and electrophoretic analysis of amplification products derived from different fungi has shown that, as could be expected,

similarity in banding patterns is maximum in spores of different lines of the same isolate, decreases between isolates and is minimum in spores of different species (Wyss and Bonfante, 1993). Recently Redecker *et al.*, (2000) transferred *Sclerocystis coremioides*, the only retained species in genus *Sclerocystis* to *Glomus*. According to him molecular divergence of these species was not sufficiently great to separate them from *Glomus*. However many questions about the formation of sporocarps in *Sclerocystis* still remains unanswered. Progress is occurring in this direction.

Molecular techniques will, in the future, provide powerful tools to better appreciate the extent of genetic variability in the Glomales *in situ* and lead to a more precise evaluation of the consequences of evolutionary processes for the magnitude of biodiversity in these symbiotic soil microorganisms.

BIOGEOGRAPHY, EDAPHIC REQUIREMENTS & E NVIRONMENTAL TOLERANCE

Whilst AM fungi can be found in most terrestrial ecosystems, they dominate in Temperate and Tropical grassland, scrub and desert ecosystems, as well as Tropical forests (Read, 1991). Some species have been reported to occur in geographically different regions and at least one species, *Glomus intraradices*, has been found on almost every continent (Morton, 1990). Arbuscular mycorrhiza can also be found in unexpected plant groups and locations *viz.*, Khan (1993) found AM in aquatic trees. Stenlund and Charvat (1994) reported AM in floating *Typha*

mats. Palacios-Mayorga and Perez- Silva (1993) found AM in parasitic plants. Bellgard *et al.*, (1994) reported AM in Proteaceae. Several workers have noted AM in arboreal habitats (Janos, 1993); to depths of 4m in desert soils (Virginia *et al.*, 1986); to depths of 12-20m in Amazonian forest soils (Nepstad *et al.*, 1994) and in extreme deserts such as the Namib (Jacobson *et al.*, 1993) and the Anaconda (Dhillion *et al.*, 1995).

ARBUSCULAR MYCORRHIZAE IN SAND DUNE VEGETATION

Koske and Halvorson (1981) reported species of *Gigaspora* as the dominant genus of AM fungi in sand dunes of the Atlantic coast of U.S. Bergen and Koske (1984) investigated the occurrence of AM fungi from sand dunes of Capecod-Massachusetts. They obtained five species of *Gigaspora* in association with roots of *Ammophila breviligulata*. *Gigaspora gigantea* was the dominating species occurring in 66% of the samples. Koske (1987) reported fourteen species of AM fungi from the sandy soils of Wisconsin. *Glomus etunicatum* was the most frequently isolated AM fungi. Sylvia (1986) gave the spatial and temporal distribution of AM fungi associated with *Uniola paniculata* in Florida fore dunes. Spore densities in non-vegetated areas adjacent to vegetated dunes averaged less than 6% of the spore densities found in the root zone of sea oats. Giovanneti and Nicolson (1983) reported presence of *Glomus mosseae* and *G. fasiculatum* in Italian sand dunes. Plant species of cosmopolitan families were found to be heavily infected with AM fungi. Koske (1975) worked on the AM spores in

Australian sand dunes. He found that the density of spores in sand was greater in older, more stabilized dunes than younger fore dunes and mobile dunes. El-Giahmi *et al.*, (1976) reported AM in coastal sandy soils of Libya, wherein most of the host plant species showed infection levels of between 40-60%. Khan (1971) reported the AM spores in West Pakistan soils.

Mohankumar *et al.*, (1988) studied the distribution of AM in the sandy beach soils of Madras coast. They reported the presence of *Entrophospora* and *Glomus* species. According to them, soil temperature and moisture status of the soil influenced the infection of AM fungi in the coastal soils. Kulkarni *et al.*, (1997) recorded the presence of sixteen species of AM fungi in Mangalore coast of Karnataka. *Gigaspora ramisporophora, Glomus albidum*, *Glomus clarum* and *Scutellospora gregaria* were dominant.

Several edaphic factors have been shown to affect spore germination, root colonization and efficiency of AM fungi under experimental conditions (Daniels Hetrick, 1984) and Sieverding (1991) has proposed that a general distinction can be made between fungi occurring under broad or narrow ranges of soil conditions. One of the most decisive soil factors appears to be pH, and many fungi show a wide diversity in tolerance to distinct pH ranges, which is reflected in the occurrence of species, rather than genera.

Whilst species from different genera can be found in soils covering a broad pH range, others like *Glomus mosseae* have only been reported from soils with pH value greater than 5.5 (Sieverding, 1991). Although variations in the behaviour of AM fungal species are known to exist with respect to other soil factors (heavy metals, texture, moisture, temperature, nutrient levels, salinity *etc.*), the significance of these for fungal diversity in native habitats is still poorly understood. Temperature appeared to be the main abiotic factor determining the structure of the AM fungal community in the study of distribution of AM fungi along a latitudinal temperature gradient (Koske, 1987). There is a negative correlation between mycorrhizal formation and increase in initial soil depth, moisture and pH (Al-Agely and Reeves, 1995). Interaction between AM fungi is of less importance in determining species presence and spore density than are the species of host plant and other environmental factors (Koske, 1981).

Gemma *et al.*, (1989) examined the seasonal dynamics of five AM fungi occurring in Massachusetts sand dune to determine the period of maximum sporulation. They observed high levels of spore abundance of one species in a sample were associated with significantly lower levels of sporulation by the other species in the same sample.

Sturmer and Bellei (1994) studied the composition and seasonal variation of spore populations of AM fungi in dune soils on the Island of Santa Catarina, Brazil. The number of spores of *Glomus constrictum*, *G. etunicatum* and

Acaulospora species was maximum in winter, whereas that of *Gigaspora albida* peaked in spring. Beena *et al.*, (1997) studied the seasonal fluctuations in AM fungal populations, their colonization and the rhizosphere edaphic features of *Launea sermentosa* on the coastal sand dunes of West coast of India. The colonization of roots by AM fungi revealed a peak during post-monsoon, while the AM fungal species richness and spore diversity in the rhizosphere were highest during monsoon.

As far as natural ecosystems are concerned, sand dunes probably represent those where diversity of Glomalean populations has been most studied. Louis (1990) surveyed the vegetation of coastal reclaimed land in Singapore. He observed the absence of AM and predominance of plants generally regarded as non-mycorrhizal species. Sylvia and Will (1988) studied the changes in the populations of AM fungi and other soil microorganisms in replenished sand planted with *Uniola paniculata* and *Panicum* species. There was a shift in the dominant AM fungi found in the planted zone with respect to those in established dunes. Beena *et al.*, (2000) reported that the vegetation cover, AM fungal colonization, species richness and diversity were greater in moderately disturbed dunes than in severely disturbed dunes of West coast of India. In general, the variety of fungal species appears to be greater in more stabilized dunes than in younger or disturbed ones (Koske, 1975; Giovannetti and Nicolson, 1983). Mycorrhizal associations are potential factors determining diversity in ecosystems. They can probably modify the structure and functioning of a plant community in a

complex and unpredictable way (Grime *et al.*, 1987; Read, 1990). Any shift in the mycorrhizal fungal population could have consequences for the composition of plant communities (survival, competition and floristic diversity), causing changes in biology of natural ecosystems (Miller and Allen, 1992 ; Molina *et al.*, 1992). Therefore, knowledge of the different factors influencing the population biology of AM fungi is essential in any attempt to use them in environment conservation (Allen, 1991), biotechnology (Mulongoy *et al.*, 1992) or in sustainable agriculture (Bethlenfalvay and Linderman, 1992).

CHAPTER I

SURVEY OF COASTAL SAND DUNE VEGETATION OF GOA.

INTRODUCTION

Coastal sand dunes are common in different parts of the world. These are natural structures which protect the coastal environment by absorbing energy from wind, tide and wave action (McHarg, 1972). Despite geographical differences, sand dunes have been considered as a specific ecosystem due to several common environmental features. Coastal sand dunes constitute a variety of microenvironments due to substrate mobility and physical processes. Plants establishing on coastal sand dunes are subjected to several environmental fluctuations which affect their growth, survival and community structure. The most important factors include high soil surface temperature, desiccation, low moisture retention, soil erosion, sand accretion, soil salinity, salt spray and nutrient deficiency (Martinez and Moreno-Casasola, 1993). Survival of plants under such harsh conditions depends on symbiosis with mycorrhizal fungi, rhizobia and other endophytes.

Studies on coastal sand dune vegetation has been reported from various parts of the world. Cooper (1958, 1967) and Terrell (1979) have contributed significantly to the studies of sand dune vegetation of the United States. A characteristic vegetation zonation occurs where successive dune vegetation has been formed parallel to the shoreline. The zonation reflects the succession of the vegetation types from the pioneer colonizing species (typically grasses) on the fore dune, through shrub species, to a dune scrub with wood line or even heath on the

older ridges. Extensive rearrangement of older parallel dune system has resulted in succession. The parabolic dunes carry younger soil and dune scrub vegetation, whereas the undisturbed parallel ridges are covered with heath or woodland. The older parabolic dunes with acid soils are heath covered and more recent parallel dunes support a dense scrub (Parsons, 1966).

Kutiel *et al* ., (1980) studied the vegetation along the coastal plain of Israel which extends 1 to 6 Km east of the Mediterranean Coast.

Hesp *et al.*, (1989) reviewed the dune system on African coast. They distinguished four principal coastal dunes *viz.*, fore dunes, relict fore dune plains, parabolic dunes and transgressive dune fields. The evolution and morphology of each complex is briefly studied by them. Transgressive dune fields are the dominant dune complexes. They are found on the high energy beaches experiencing high littoral drift and form two main types, tubular fields and buttress fields. Both tend to be dominated by transverse dunes.

Maarel *et al* ., (1985) analyzed the vegetation on the coastal dunes in South West Netherlands, while Simpson and Marson (1984) reported the dune flora of Canterbury. Classification and ordination of coastal sand dune vegetation along the gulf of Caribbean Sea of Mexico was reported by Moreno-Casasola and Espejel, (1986).

Gehu and Uslu (1989) studied the halophilous and psamophilous coastal vegetation of the Turkish straits area.

In the flora of Japan, Ohwi (1953) reported the Tropical grasses of South East Asia. According to him, plants like *Spinifex* surely indicate the presence of coastal sand dunes.

Doing (1985) has studied and tabulated the important species of fore dunes from various parts in the world. According to him, most extensive and complicated dune area occurs in the regions where disturbances of the wind and fixation of dune by plants are equally strong.

Rao and Agarwal, (1964, 1971); Rao and Sastry, (1972, 1974) and, Rao *et al.*, (1975) under the Botanical Survey of India have worked extensively on the ecological studies of coastal sand dune vegetation along the coasts of Saurastra and the neighbouring islands, Tamil Nadu, Orissa and West Bengal.

Untawale (1980) gave a report of sandy coasts of India. Sandy coasts have been reported in Gujarat (Kutch), Saurastra and South Gujarat which is limited between muddy and rocky shores. Sandy strips along rocky cliffs are also observed in Maharashtra. The sandy shores of Karnataka are of limited width, while Kerala has extensive sandy beaches intercepted with coastal lagoons. They are often laterite or rock bound. Tamil Nadu has sand strips along the deltic shores

and rock bound beaches. In Andhra Pradesh the sandy beaches are of limited width intercepted by river Godavari, Krishna and their tributaries. Konark Puri in Orissa have extensive sand strips. The West Bengal coast is also limited. The coast of Andaman Islands has sand strips intercepted by rocks or shingle along the coast line. Lakshadweep atolls have long stretches of coralline sandy beaches with unique vegetation.

Goa is situated on the West coast of India with 120 km coastline. The coastline of Goa is segmented as a result of seven rivers like Mandovi, Zuari, Chapora, Sal and others. Besides, there are cliffs of Western Ghats, bays and creeks which intercept the coast (Ahmed, 1972). The lengths of the sandy shores in Goa have been indicated in **Table-2**.

Desai (1995) surveyed the beaches in Goa from North at Keri to South at Pollem. The best of the dunes in Goa were found on the stretch of Majorda, Colva, Benaulim, Varca, Betul and Cavelossim. At North, Keri and Mandrem had vegetated foreshores and backshores. Shores from Chapora to Baga, near Dona Paula, Ushal, Kankara and Navshi, and down South in Canacona, are intercepted by river basins, cliffs and rocks. Morjim, Harmal, stretches between Calangute to Sinquerim, Campal, Miramar and shores between Marmugao and Velsao are severely disturbed beaches.

Table-2: Length of sandy shores of Goa (Dhargalkar, 1981).

Name of the sandy coast	Length in Km
Terekhol	2.04
Keri	1.32
Harmal	4.32
Mandrem to Morjim	7.38
Chapora	1.50
Vagator	0.85
Anjuna	2.55
Baga	1.30
Calangute to Sinquerim	5.75
Campal	0.45
Miramar to Caranzalem	4.15
Dona Paula	0.65
Ushal, Kankire and Navshi	2.20
Marmugao head	0.15
Baina	1.70
Binkade	0.45
Bogmola	0.70
Velsao	0.80
Colva, Benaulim, Varca, Betul	26.25
Betul point	0.60
Cabo de Rama	1.85
Talpona	1.24
Kankon Island	1.55
Galgibhag	0.69
Loliem point	0.13
Pollem	0.25
St. Georges Island	0.55
Total length in Km	**71.37**
% Sandy shores	**59.47**

The present chapter deals with the survey studies of coastal sand dune vegetation of eight different and prominent sites in Goa. The objective of this work was to study the diversity of vegetation and to find out the dominant plant species existing on the dunes.

MATERIALS AND METHODS

The sand dune vegetation survey was carried out in eight different sites *viz.*, Varca, Mobor, Benaulim, Colva and Majorda (in the South) and Bambolim, Miramar and Junes (Mandrem) beach (in the North) (Fig 1). All the plant species thus collected were identified using local floras.

RESULTS AND DISCUSSION

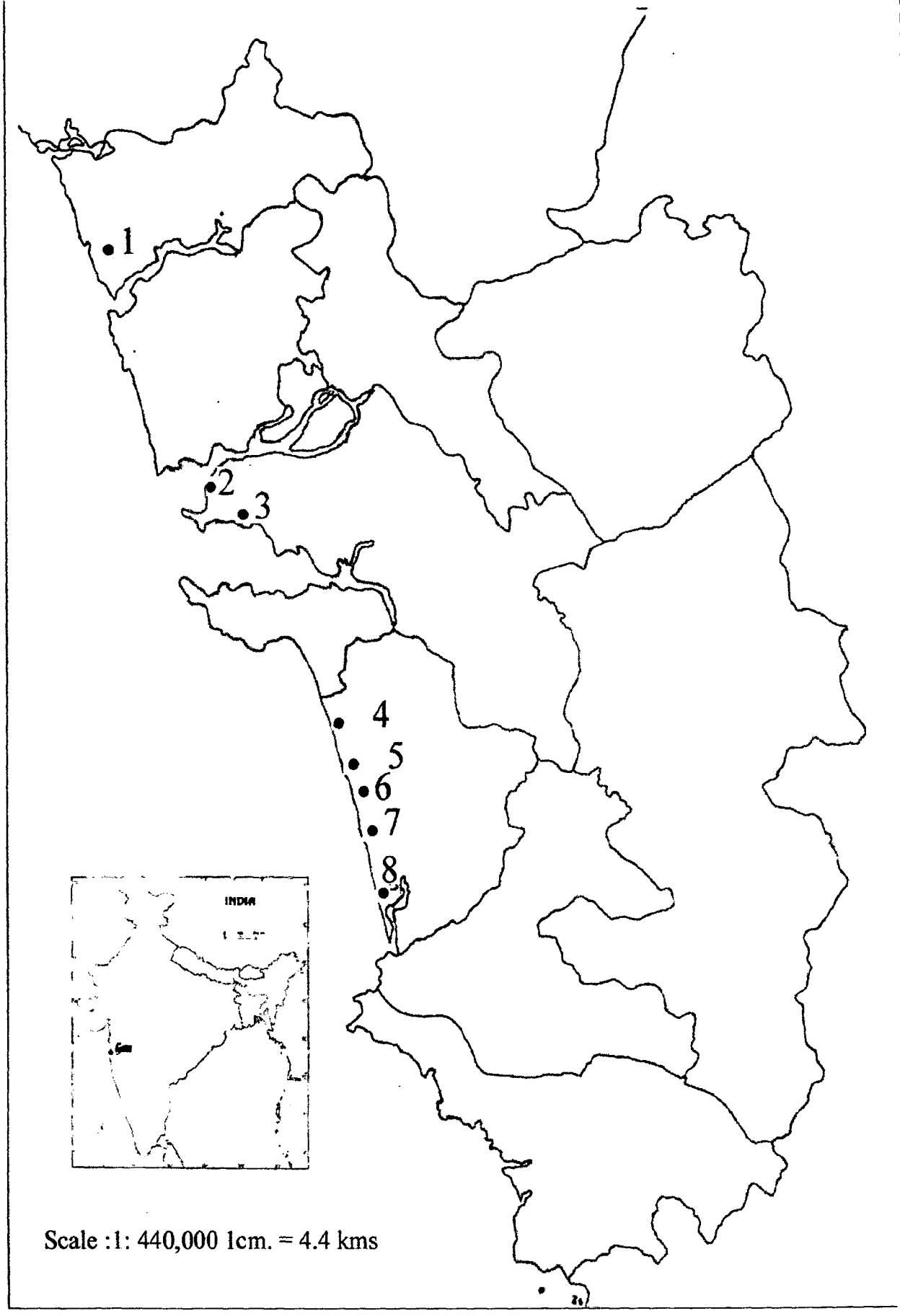
It was observed that all the beaches in South Goa *viz.*, Varca, Mobor, Benaulim, Colva and Majorda had well developed sand dunes, which could be well differentiated into fore dunes, mid dunes and hind dunes, while the sand dunes in Miramar and Bambolim beaches comprised of only fore dunes. However Junes (Mandrem) beach had well developed fore dunes and mid dunes. Depending upon the magnitude of disturbance all the sites studied could be grouped into three categories *viz.*,

FIG. 1

COASTAL SAND DUNE SITES IN GOA
SELECTED FOR THE STUDY.

1. Junes (Mandrem)
2. Miramar
3. Bambolim
4. Majorda
5. Colva
6. Benaulim
7. Varca
8. Mobor

Fig. 1



1. **Least disturbed:** This includes Varca and Mobor.
2. **Moderately disturbed:** This includes Colva, Benaulim, Junes and Majorda.
3. **Severely disturbed:** This includes Miramar and Bambolim.

Least disturbed dunes possess dense vegetation cover and high plant diversity. Frequency of occurrence of the plant species are depicted in **Table-3.** In all, a total of 55 plant species belonging to 33 families have been recorded.

It was observed that a total of five plant species *viz., Ipomoea pes-caprae, Spinifex littoreus, Vitex trifolia, Cocos nucifera* and *Casuarina equisetifolia* were the most dominant and common at all the eight study sites.

Ipomoea pes-caprae and *Spinifex littoreus* were the most dominating in the pioneer zone. *Ipomoea pes-caprae* is a dominant and widely distributed stoloniferous creeping sand binder in tropical sand dunes (Beena *et al.*, 2000). Rao and Agarwal (1971) recognized communities of *Hydrophylax maritima* and *Ipomoea pes-caprae* growing on the Saurastra Coast. Along the Orissa Coast, Rao and Sastry (1972) reported the growth of *Canavalia maritima, Cyperus arenarius, Ipomoea pes-caprae, Launea sarmentosa* and *Hydrophylax maritima.* In the same paper they reported the occurrence of *Ipomoea pes-caprae, Cyperus arenarius, Canavalia maritima, Launea sarmentosa, Sporobolus virginicus* and *Zoysia matrella* on the Andhra Coast. On the West Bengal coast, the open

Table-3: Frequency of occurrence of plant species on coastal sand dunes of Goa.

Family and Plant species (In order of species dominance)	Frequency of occurrence (%)	Location
ASTERACEAE		
*Acanthospermum sp.***	12.5	7
Ageratum conyzoides Linn.**	12.5	7
Epaltes divaricata Cass**.	12.5	7
Launea fallax (Jaub. & Spach.) Kuntze**	37.5	1,6,8
*Vernonia sp.***	12.5	7
Chromolaena odorata (L.) King & Robinson	37.5	4,6,8
VERBENACEAE		
Vitex trifolia Linn. ***	87.5	1,3-8
Lantana camara Linn.	87.5	1-7
Premna integrifolia Linn.	25	4,7
Clerodendrum inerme (Linn.) Gaertn.**	25	1,4
CONVOLVULACEAE		
Ipomoea pes-caprae (Linn.) Sweet***	100	1-8
Evolvulus alsinoides Linn.	12.5	7
Cuscuta sp.	37.5	1,4,5
FABACEAE		
Crotolaria sp.	25	2,3
Erythrina variegata Linn.	12.5	4
Abrus precatorius Linn.	12.5	7
EUPHORBIACEAE		
Fluggea sp.	25	1,5
Bridelia retusa (Linn.) Spreng.	12.5	1
Crignella sp.	12.5	7

Contd.

PANDANACEAE		
Pandanus tectorius Soland**.	62.5	1,3,4, 5,7
ASCLEPIADACEAE		
Calotropis gigantea (Linn.) R.Br.	75	2-6
AMARANTHACEAE		
Achyranthes aspera Linn.	12.5	5
RHAMNACEAE		
Ziziphus rugosa Lamk.	25	2,4
LILIACEAE		
Urginea indica (Roxb.) Kunth	25	1,5
TILIACEAE		
Microcos paniculata Linn.	12.5	6
MIMOSACEAE		
Acacia auriculiformis A. Cunn**	37.5	1,2,7
CLUSIACEAE		
Calophyllum inophyllum Linn.	37.5	1,4,5
NYCTAGINACEAE		
Bougainvillea spectabilis Willd.	12.5	4
CACTACEAE		
Opuntia sp.	25	6,8
TACCACEAE		
Tacca sp.	12.5	7

Contd.

POACEAE		
Spinifex litttoreus (Burm.f.) Merr.***	75	1,4, 5-8
Ischaemum sp.	12.5	4
Panicum sp.	12.5	2
ARECACEAE		
Cocos nucifera Linn.***	100	1-8
Borassus flabellifer Linn.	25	1,2
LAMIACEAE		
Leucas aspera Spreng.**	37.5	5,6,8
Hyptis suaveolens (Linn.)Poit.	12.5	5
RUBIACEAE		
Ixora coccinia Linn.	37.5	1,4,7
Neanotis sp.	12.5	7
APOCYNACEAE		
Ichnocarpus frutescens (Linn.) R.Br.	12.5	4
Carissa carandas Graham	12.5	1
MYRTACEAE		
Syzygium caryophyllatum (Linn.) Alston	12.5	1
Syzygium cumini (Linn.) Skeels	12.5	1
ANACARDIACEAE		
Anacardium occidentale Linn.**	37.5	1,5,7
Mangifera indica Linn.	12.5	4
CASUARINACEAE		
Casuarina equisetifolia Forst.**	75	1,2,5-8

Contd.

LYTHRACEAE		
Ammania sp.	12.5	7
CYPERACEAE		
*Cyperus sp.***	25	1,7
COMMELINACEAE		
Cyanotis sp.	12.5	7
ONAGRACEAE		
Ludwigia parviflora Roxb.	12.5	7
SOLANACEAE		
Datura metel Linn.	12.5	4
MALVACEAE		
Abutilon sp.	12.5	6
ERIOCAULACEAE		
Eriocaulon sp.	12.5	7
MORACEAE		
Ficus religiosa Linn.	12.5	4
VITACEAE		
Leea indica (Burm.f.) Merril	12.5	1

Legend:

*** = Abundant
** = Frequent
No asterisk = Less Frequent

1 = Varca	2 = Miramar	3 = Bambolim	4 = Majorda
5 = Colva	6 = Benaulim	7 = Mobor	8 = Junes

pioneer zone is being covered with *Ipomoea pes-caprae, Launea sarmentosa, Dactyloctenium aegypticum* and *Cyperus arenarius* (Rao *et al.*, 1974).

Ipomoea pes-caprae, Vitex trifolia, Cocos nucifera, Casuarina equisetifolia, Pandanus tectorius, Clerodendrum inerme, Launea fallax and *Leucas aspera* were the dominant plants growing on the Goa Coast in the mid dune region. Rao and Sastry (1972) reported the occurrence of *Halopyrum mucronatum, Lotus garcinii, Borreria* articularis, *Asparagus dumosus, Enicostema hyssopiflorum, Peplidium maritimum* and *Cassytha filiformis* in the mid dune of the Western Coast. Along the Orissa Coast, the growth of *Euphorbia rosea, Geniospermum tenuiflorum* and *Phyllanthus rotundifolius* was reported, While on the Andhra Pradesh Coast, *Spinifex littoreus, Coniogyne hirta, Perotis indica, Trachys muricata* and *Fimbristylis polytrichoides* were common (Rao *et al.*, 1974). Along the West Bengal Coast, Rao *et al.*, (1974) reported the presence of *Synostemon bacciforme, Borreria articularis, Brachiaria reptens, Eleusine indica, Euphorbia thymifolia, Leucas lavandulaetolia, Narenga porphyrochoma, Rothia indica* and *Trianthema pentandra.*

"Migratory dune system" present in the other parts of the world and reported by Cooper (1958) and Wiedmann (1984), was not observed in most of the sites in Goa as this area has been highly urbanized. Back dunes is either planted with *Cocos nucifera, Anacardium occidentale* and *Casuarina equisetifolia* or covered

with naturally growing plant species like *Calophyllum inophyllum, Ziziphus rugosa*, and *Ficus religiosa.*

The dune vegetation is essential in shaping the coastal landscape. It either actively interacts with the wind blown sand or by its mere presence it passively interacts in protecting the shore from wind and water erosion. Moreover, if proper vegetation is maintained on the moving dunes, it would check the sand erosion. Hence, it is essential to study the various aspects of dune vegetation.

CHAPTER II

PRELIMINARY SURVEY OF ARBUSCULAR MYCORRHIZAL ASSOCIATION IN COASTAL SAND DUNE VEGETATION OF GOA.

INTRODUCTION

Sand dunes on the coasts are the natural defense structures against soil erosion by the action of wind and waves. Wind erosion of the beach and unvegetated frontal dunes results in coastline recession (Anonymous, 1981). Dune vegetation play a major role in stabilizing the sand dunes (Woodhouse, 1982). For rapid growth of plants in the dunes, beneficial micro-organisms especially Arbuscular mycorrhizal (AM) fungi play an important role (Koske and Polson, 1984).

On the coastal dunes of Goa, *Ipomoea pes-caprae* and *Spinifex littoreus* are the most dominating plants in the pioneer zone. *Ipomoea pes-caprae, Vitex trifolia, Cocos nucifera, Casuarina equisetifolia, Pandanus tectorius, Clerodendrum inerme, Launea fallax* and *Leucas aspera* are the dominant plants growing on the Goa Coast in the mid dune region. Back dunes is either planted with *Cocos nucifera, Anacardium occidentale* and *Casuarina equisetifolia* or covered with naturally growing plants species like *Calophyllum inophyllum, Ziziphus rugosa*, and *Ficus religiosa*. Back dunes are not observed in most of the sites in Goa as this area has been highly urbanized.

Arbuscular mycorrhizal (AM) fungi are obligate symbionts which enhance the plant growth by helping in uptake of phosphorus, water and other nutrients. They help in translocation of nutrients between hosts through hyphal connections (Heap and Newman, 1980). They also have a role in seedling establishment (Read *et al.*, 1976), plant competition (Hall, 1978), community composition (Reeves *et*

al., 1979) and secondary succession (Janos, 1980). The role of mycorrhizae in building and improving soil characters is well recognised (Clough and Sutton, 1978; Koske *et al.*, 1975).

In the present chapter, a preliminary survey of Arbuscular Mycorrhizal status in the Coastal sand dune vegetation of Goa has been carried out. The available literature on the subject has been reviewed under the title " Review of Literature" at the beginning of the thesis.

MATERIALS AND METHODS

Coastal sand dunes from three sites in South Goa *viz.*, Mobor, Colva and Majorda were selected for the study. Mobor is one of the least disturbed beaches in Goa. Colva and Majorda are the moderately disturbed beaches. Fifteen plant species belonging to 10 different families were selected from Mobor beach for the study. Six plant species were selected from each of the two sites *viz.*, Colva and Majorda, out of which four plant species were common to all the three sites. From the sites Colva and Majorda the dominating plants and the plants common to the site Mobor were chosen for the study. In all, 18 plants belonging to 12 families were worked upon. For each plant species, soil and root samples were collected randomly from 3 individuals of the species. Later, a composite sample was made for each species and worked out. The samples were collected in post-monsoon season (October-November). Rhizosphere soil samples were excavated from a depth of 0-25cm, placed in plastic bags, labeled and stored at 10^0

C until processed. While collecting soil samples, soil was dug at three places for each plant so as to cover the entire rhizosphere. While collecting root samples, herbs were dug out entirely. For shrubs and trees, the roots were dug and traced back to the plant which ensured that the roots belonged to the intended species.

Soil analysis

For soil analysis, rhizosphere soils of all the host plant species along with the sub samples were mixed to form a composite sample for each site and was analysed for pH, Total N, Available P and Total K.

Recovery of spores

Hundred grams of air dried composite sample was assayed for spore count using modified Wet Sieving and Decanting procedure (Gerdemann and Nicolson, 1963), the details of which are as follows:

1. 100g of soil was added to 1 litre of tap water in a beaker.
2. Large soil clumps were broken by hand and the mixture was stirred for 10-15 seconds.
3. The course particles were allowed to settle in the water for 5-10 seconds.
4. The soil water mixture was decanted through stacked sieves with the course sieve on top and the fine sieve on the bottom; the range of course sieve openings was 300-250 μm, 105-90 μm f or sieves with medium size openings and 63-45 μm for the sieves with fine pore openings.

5. Steps 1-4 was repeated twice to increase the likelihood that a majority of the spores in the soil had been removed.
6. The spores captured on each sieve were collected in a beaker and were filtered separately through Whatman No.1 filter paper. The filter paper was transferred to a petriplate.
7. Spores were observed under Leica Stereo-microscope. Estimation of Spore density was carried out as per the procedure given by Gaur and Adholeya (1994). Only healthy spores were enumerated; broken and parasitised spores were discarded.
9. For each sample steps 1-7 were repeated 3 times.

Estimation of root colonization

Root samples were washed thoroughly under tap water. Feeder roots were cut into 1cm fragments. The composite sample of each species was cleared in 10% KOH in an autoclave at 121^0C for 15-25 minutes except for softer roots of some herbs which were cleared only for period of 10 minutes. Cleared roots were captured on the sieves, rinsed with water and acidified with 1% HCl. Later the roots were stained with 0.05% Trypan blue (Phillips and Hayman, 1970). Stained roots were mounted in glycerol and observed under Leica Compound Microscope. Percent root colonization was estimated by using Slide technique (Giovannetti and Mosse, 1980). For a sample to be regarded as possessing AM association, it had to be seen to have vesicles, arbuscules or both.

RESULTS AND DISCUSSION

Soil analysis results revealed that soils were slightly alkaline with pH ranging from 7.0-7.7. Electrical Conductivity (E.C.) ranged from 0.148-0.202 M mhos/cm. Available Phosphorus content ranged from 45 µg/g-75 µg/g. Potassium content varied from 0.012-0.035%, while Nitrogen content ranged from 0.068- 0.080% **(Table-4).**

The survey for the prevalence of AM fungi in coastal sand dune vegetation revealed that the symbionts were found to have association with varied types of herbs, shrubs and trees growing at the three sites. It was also observed that all the plants were mycorrhizal but the extent of colonization varied **(Table 5, 6 & 7)**. The mycorrhizal colonization consisted of hypha, vesicles and arbuscules **(Plate-2, Fig. a, b & d)**. Auxillary cells of *Gigaspora sp.* were observed in some root samples indicating the association of *Gigaspora sp.* with the plants **(Plate-2, Fig. c)**. Arbuscular mycorrhizal colonization in plants ranged from 52-100%, lowest being in *Cyperus* sp. All the families comprised plants at least some plants with root colonization greater than 90% except Cyperaceae. Spore number in rhizosphere soil exhibited great variation. It ranged from 12 spores + 2 sporocarps /100g soil in *Vitex trifolia* to 984 spores + 20 sporocarps /100g soil in *Ipomoea pes-caprae* **(Table 5, 6 & 7)**.

At Mobor site, total AM colonization recorded was 100% in 9 plants belonging to 8 different families. Minimum root colonization was 52% in *Cyperus* sp. Root

COASTAL SAND DUNE VEGETATION OF GOA.

a) Arbuscules (x 400)
b) Arbuscules (x 1000)
c) Auxiliary cells of *Gigaspora sp.* (x 400)
d) Vesicles (x 400)

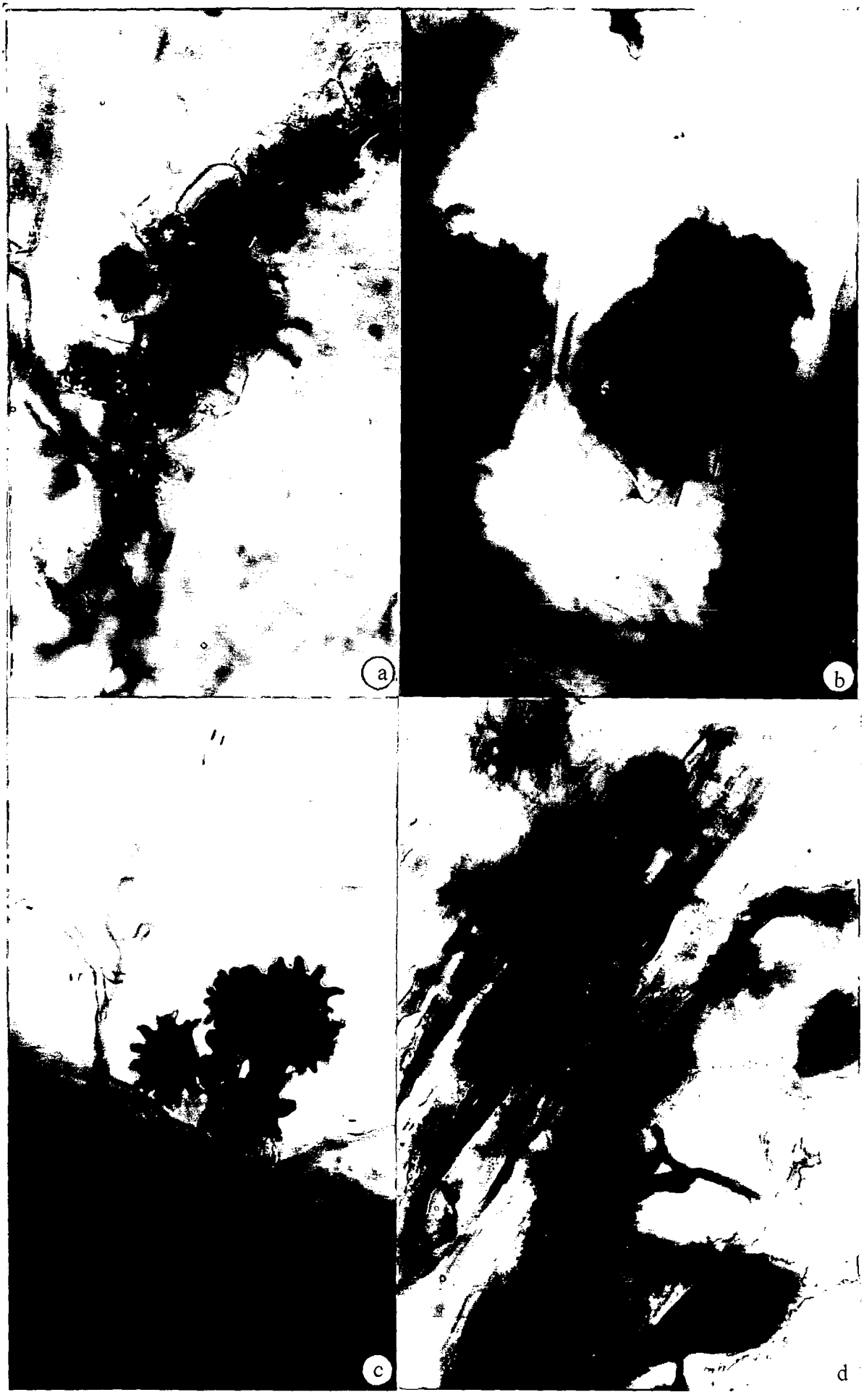

a
b
c
d

Table- 4: Chemical characteristics of sand dune soils.

Site	pH	E. C. M mhos/cm	Total N_2 (%)	Available P (μg/g)	Total K_2O (%)
Mobor	7.0 (0.00)	0.148 (0.001)	0.079 (0.004)	71 (2.00)	0.012 (0.001)
Colva	7.1 (0.01)	0.202 (0.002)	0.080 (0.005)	75 (3.50)	0.035 (0.002)
Majorda	7.7 (0.10)	0.168 (0.001)	0.068 (0.005)	45 (2.50)	0.030 (0.000)

Values are mean of 3 readings.
Values in the parenthesis indicates Standard deviation.

Family and Plant species	Total Colonization (%)	Vesicles	Arbuscules	Spore density /100g soil
VERBENACEAE				
.Premna integrifolia Linn.	100 (0)	+	+	140 (26.46) spores+128 (16) sporocarps
Vitex trifolia Linn.	80 (13.23)	+	+	48 (13.87) spores
Lantana camara Linn.	100 (0)	+	+	64 (18.33) spores
CONVOLVULACEAE				
Ipomoea pes-caprae (Linn.) Sweet	100 (0)	+	+	60 (15) spores
ARECACEAE				
Cocos nucifera Linn.	60 (9.17)	+	-	628 (159.85) spores+8 (3) sporocarps
CASUARINACEAE				
Casuarina equisetifolia Forst.	100 (0)	+	+	60 (17.56) spores+4 (2) sporocarps
Average value	**88.32**			**233.07 spores+17.6 sporocarps**

+ = Present; - = Absent

Values are mean of 3 readings.

Values in the parenthesis indicates Standard deviation.

colonization varied from 80-100% in all other plants except *Ixora coccinea* and *Cocos nucifera* where it was 61.8% and 60% respectively. No Arbuscular colonization was recorded in *Ixora coccinea*, unidentified grass sp., *Tacca sp.*, *Anacardium occidentale* and *Cocos nucifera.* Spore density was maximum in *Ixora coccinea* (848 spores /100g soil), followed by *Cocos nucifera* (628 spores + 8 sporocarps/100g soil) and *Cyperus* sp (536 spores + 4 sporocarps/100g soil). Minimum spore density was observed in *Spinifex littoreus* (44 spores). Average root colonization recorded was 88.32%. Average spore density recorded was 233.07 spores and +17.6 sporocarps/100g soil **(Table-5; Fig. 2)**.

At Colva site, total AM colonization varied from 90-100% in all the 6 plants selected for the study. Arbuscular colonization was not recorded in *Vitex trifolia, Casuarina equisetifolia* and *Calotropis gigantea.* Spore density was maximum in *Ipomoea pes-caprae* (984 spores + 20 sporocarps/100g soil) and minimum in *Casuarina equisetifolia* (76 spores/100g soil). Average root colonization recorded was 95.79%. Average spore density recorded was 488 spores +8 sporocarps/100g soil **(Table-6; Fig. 2)**.

At site Majorda, total AM colonization varied from 82-100% in all the 6 plants selected for the study. Spore density was maximum in *Cocos nucifera* (396 spores + 6 sporocarps/100g soil) and minimum in *Vitex trifolia* (12 spores + 2 sporocarps /100g soil). Average root colonization recorded was 94.92%. Average spore density recorded was 146.67 spores + 4.33 sporocarps/100g soil **(Table-7; Fig. 2)**.

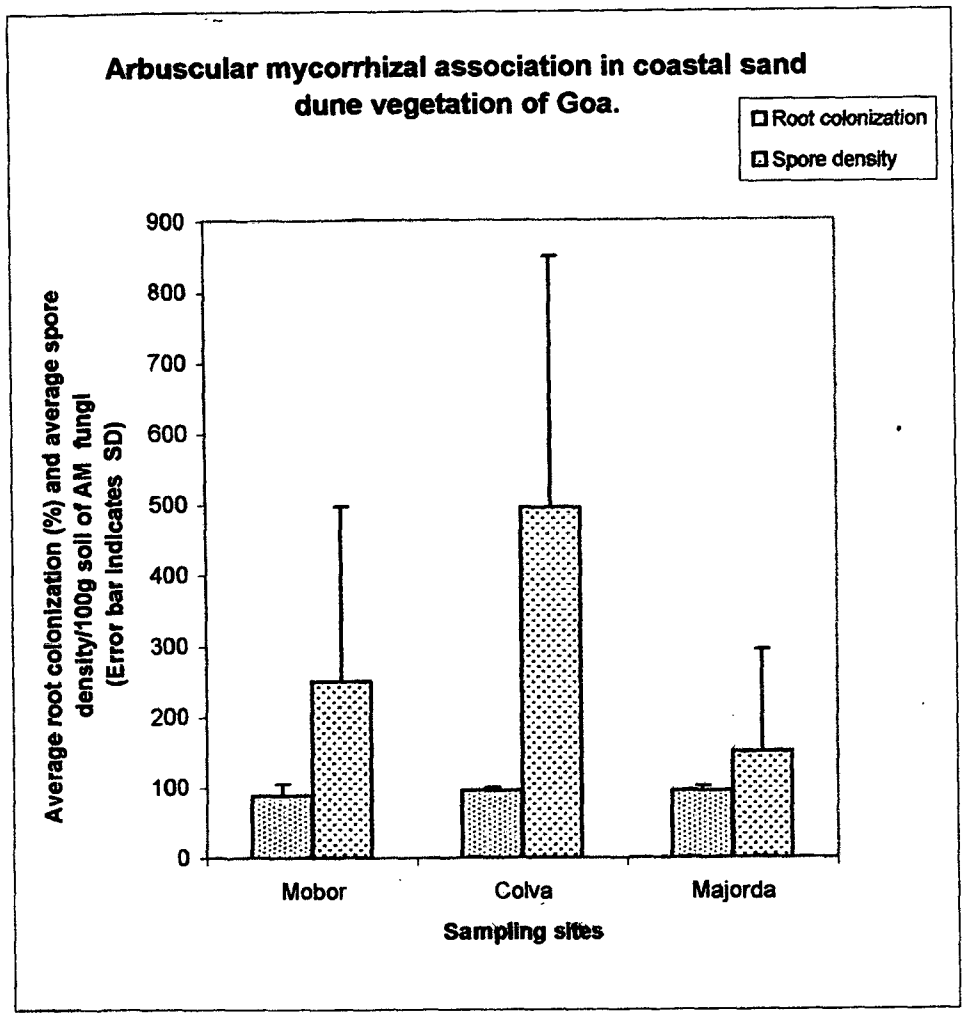

Arbuscular mycorrhizal association in coastal sand dune vegetation of Goa.
Root colonization
Spore density
Average root colonization (%) and average spore density/100g soil of AM fungi (Error bar indicates SD)
900
800
700
600
500
400
300
200
100
0
Mobor
Colva
Majorda
Sampling sites

Fig.2

Table-6: Arbuscular Mycorrhizal association in sand dune vegetation at Colva.

Family and Plant species	Total colonization (%)	Vesicles	Arbuscules	Spore density /100g soil
VERBENACEAE				
Vitex trifolia Linn.	93.33 (15.49)	+	-	156 (22.61) spores
Lantana camara Linn.	100 (0)	+	+	720 (160.93) spores+4 (1) sporocarps
CONVOLVULACEAE				
Ipomoea pes-caprae (Linn.) Sweet	97.51 (3.27)	+	+	984 (72) spores+20 (8) sporocarps
ARECACEAE				
Cocos nucifera Linn.	93 (7)	+	+	372 (49) spores+16 (3) sporocarps
CASUARINACEAE				
Casuarina equisetifolia Forst.	90.91(11.4)	+	-	76 (17.78)spores
ASCLEPIADACEAE				
Calotropis gigantea (Linn.) R.Br.	100 (0)	+	-	620 (135) spores+8 (2) sporocarps
Average value	**95.79**			**488 spores+8 sporocarps**

+ = Present; - = Absent
Values are mean of 3 readings.
Values in the parenthesis indicates Standard deviation.

Table-7: Arbuscular Mycorrhizal association in sand dune vegetation at Majorda.

Family and Plant species	Total colonization (%)	Vesicles	Arbuscules	Spore density /100g soil
VERBENACEAE				
Clerodendrum inerme (Linn.) Gaertn.	100 (0)	+	+	28 (9.16) spores+2 (1) sporocarps
Vitex trifolia Linn.	82 (6)	+	+	12 (4) spores+2 (2) sporocarps
Lantana camara Linn.	97.78 (2.22)	+	+	144 (16) spores+4 (1)sporocarps
CONVOLVULACEAE				
Ipomoea pes-caprae (Linn.) Sweet	100 (0)	+	+	90 (70) spores+2 (2) sporocarps
ARECACEAE				
Cocos nucifera Linn.	92.3 (3.08)	+	+	396 (91.15) spores+6 (3) sporocarps
PANDANACEAE				
Pandanus tectorius Soland.	97 (3)	+	+	210 (70) spores+10 (4) sporocarps
Average value	**94.92**			**146.67 spores+4.33 sporocarps**

+ = Present; - = Absent

Values are mean of 3 readings.

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Logan *et al.*, (1989) assessed root samples of 41 sand dune plant species belonging to 28 families from sites along the coasts of New South Wales for AM colonization. They recorded AM colonization in as many as 36 plant species, out of which 21 plant species showed arbuscules, while 16 plant species showed hyphal coils. Mohan Kumar *et al.*, (1988) observed that most plants growing along the Madras sea coast harboured AM fungi.

Kulkarni *et al.*, (1997) studied the root colonization and spore density of AM in the rhizosphere of 12 plant species growing on sand dunes in the West Coast of India. The level of AM fungal root colonization ranged from 34-80%, while the mean spore density was 0.75 spores/g.

In a particular soil, differences in the population of spores or range of colonization in different plants may be attributed to the specificity between host and endophytes (Fox and Spasoff, 1971). According to Logan *et al.*, (1989) there seem to be no trends by plant family, and contrasting AM status was found even in species within the same genus. He found extensive AM with arbuscules, vesicles and coils in *Isolepis nodosa* a member of Cyperaceae which was presumed to be non-mycorrhizal (Tester *et al.*, 1987). Results of the present study are in accordance with them.

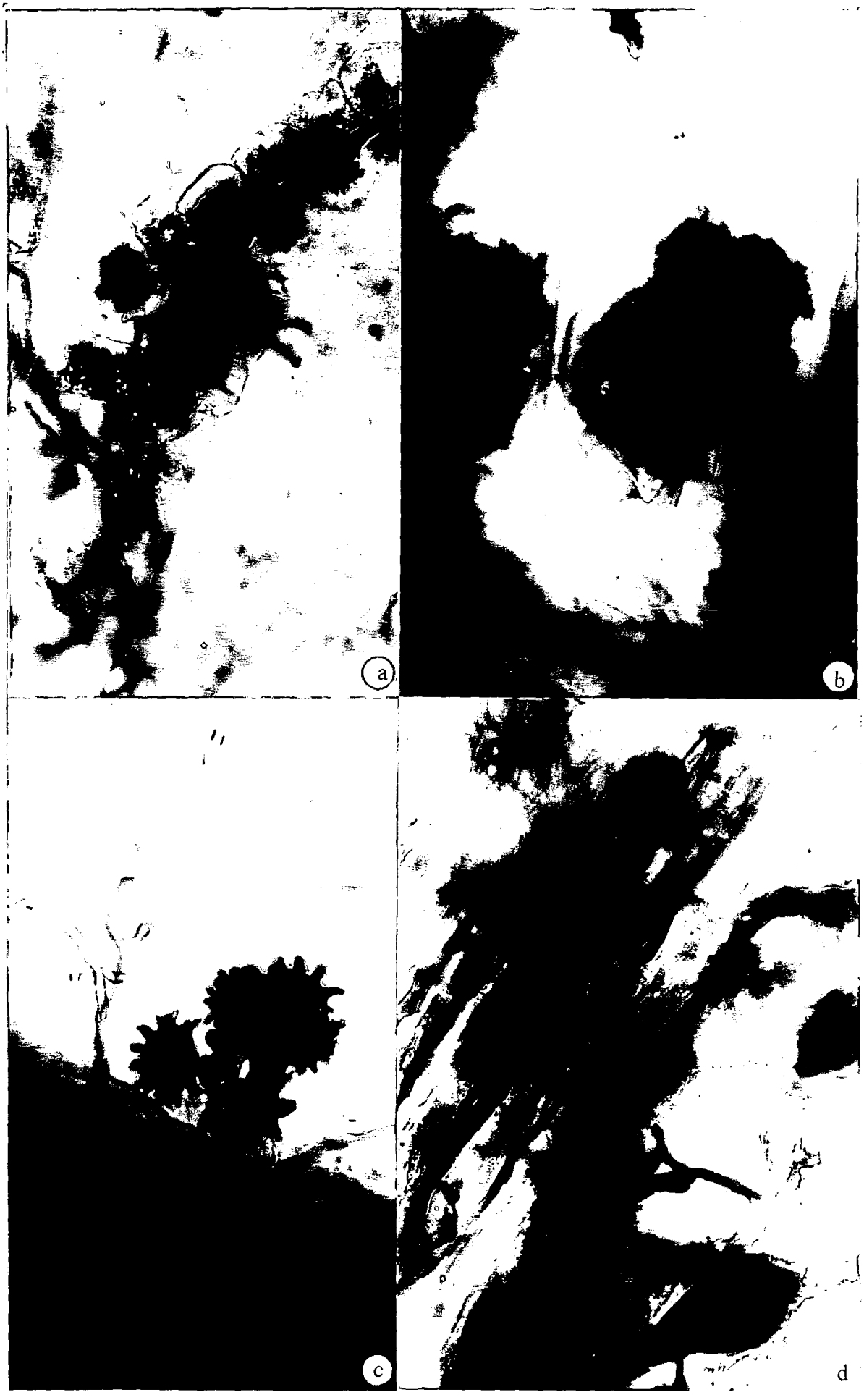
The plants *viz., Ipomoea pes-caprae, Vitex trifolia, Lantana camara* and *Cocos nucifera* were common at all the three sites. The average values of root colonization % and spore density/ 100g soil of these common plants at each site is given in **(Table-8)**. Average total root colonization was maximum in Colva (95.96%) followed by Majorda (93%), while it was minimum in Mobor (85%). Average spore density was maximum in Colva (558 spores+10 sporocarps/100g soil) followed by Mobor (200 spores+2 sporocarps/100g soil), while average spore density was minimum in Majorda (160.5 spores+3.5 sporocarps/100g soil).

Table-8: Arbuscular Mycorrhizal association in four common plants in sand dunes at three sites.

Site	Average colonization (%)	Average spore density /100g soil
Mobor	85	200spores+2 sporocarps
Colva	95.96	558spores+10 sporocarps
Majorda	93	160.5spores+3.5 sporocarps

COASTAL SAND DUNE VEGETATION OF GOA.

a) Arbuscules (x 400)
b) Arbuscules (x 1000)
c) Auxiliary cells of *Gigaspora sp.* (x 400)
d) Vesicles (x 400)


a
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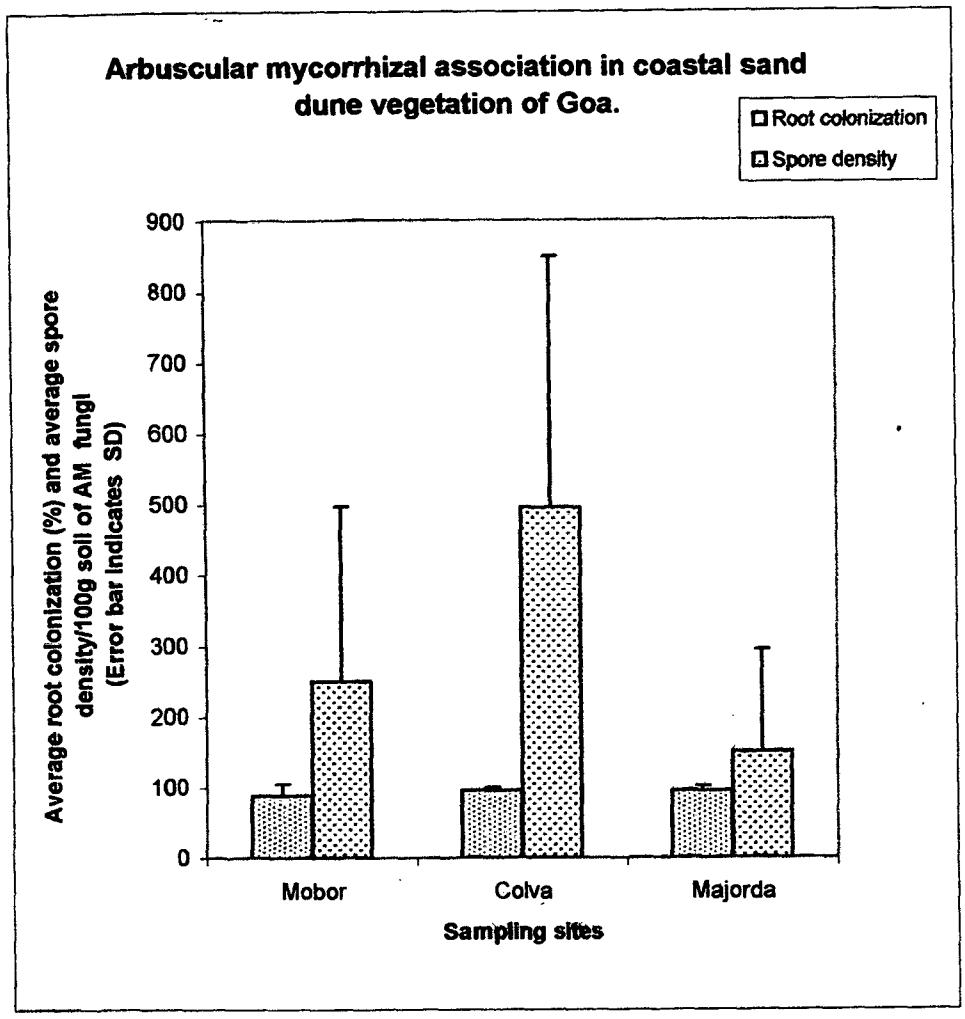
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Physical as well as chemical characteristics of the respective dunes may contribute to the differences in spore density (Rose, 1988).

The results though preliminary, may reflect a high AM status in coastal sand dune vegetation of Goa. This would be likely to enhance plant nutrition and sand binding, and to have implications for sand dune management.

CHAPTER III

OCCURRENCE AND DISTRIBUTION OF ARBUSCULAR MYCORRHIZAL FUNGI IN NATURAL AND DISTURBED SAND DUNE ECOSYSTEM OF GOA.

INTRODUCTION

An understanding of the role of Arbuscular Mycorrhizae (AM) in natural and disturbed ecosystems is one of the more evasive, yet challenging areas in biology today. The influence of disturbance on symbiosis and secondary plant succession has been studied in variety of ecosystems including arid communities (Reeves *et al.,* 1979), Coal wastes (Daft and Nicolson, 1974; Daft *et al.,* 1975; Daft and Hacskaylo 1976; Khan, 1978), Mine spoils (Marx, 1975; Miller, 1979; Allen and Allen, 1980) and Sand dunes (Nicolson, 1960; Nicolson and Johnston, 1979; Koske and Halvorson, 1981; Sylvia, 1986; Gemma and Koske,1988). It is clear that under stress, the benefits of AM association with plants quite outweigh the disadvantages (Aldon, 1975; Daft *et al.,* 1975; Daft and Nicolson 1974., Lindsey *et al.,* 1977 and William *et al.,* 1974).

The stresses associated with these soils are normally related to nutrient deficiencies as well as low precipitation and high evapo-transpiration regime. The growth advantages attributed to AM are believed to be associated with an increase in nutritional status of the plant brought about by increased phosphorus uptake (Daft and Nicolson, 1969) and water transport (Safir *et al.,* 1972). Any perturbation to an existing ecosystem that includes physical removal of plants and changes in the soils physical and chemical characteristics has a major impact on the symbiotic association. When the communities are disturbed, non mycorrhizal plant species predominate (Reeves *et al.,* 1979). The intensity of disturbance and

the type of ecosystem create influence on species composition of the mycorrhizal community, their rates of turnover and their infection potential. Consequently, the mycorrhizal status of early plant colonizers and the pattern of plant community succession after disturbances are also affected.

The beach dune system protects coastal environments by absorbing energy from wind, tide and wave action (McHarg, 1972). This 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). The re- establishment of functional ecosystems presumes a knowledge of both the important macro- and micro-elements of the system *i. e.*, both the above and below ground elements constituting the system. Arbuscular mycorrhizae are among the ubiquitous components in below ground ecosystems (Wilhelm, 1966; Gerdemann, 1975; Smith, 1974; Read *et al.*, 1976). These fungal symbionts appear to be essential in most ecosystems (Hacskaylo, 1972; Mosse, 1973; Gerdemann, 1975). Hence, these fungi must be studied to understand the ecosystem changes.

The present chapter deals with the occurrence and distribution of AM fungi in native and disturbed habitats. This study compares the spore density and diversity of AM fungi in less disturbed verses severely disturbed sand dune vegetation.

MATERIALS AND METHODS

Two beaches *viz.,* Varca beach and Miramar beach were selected for the study. Varca beach located in South Goa is a less disturbed beach. It has rich sand dune vegetation. The total length of the beach is 4 Km and width is 600m. Here one can clearly see three different types of dunes *viz.,* the fore dunes, mid dunes and back dunes. Miramar beach in North Goa in contrast, is a severely disturbed site. It is broad and about 90-100m in length. The dune system is disturbed by tourism activities. There is a little natural vegetation dominated by *Ipomoea pes-caprae* and *Panicum sp.* in the fore dunes. There is total absence of back dunes and this can be attributed to urbanization **(Plate-3).**

Rhizosphere soil samples were collected in the month of March from the selected host plants at each site. For each plant species, rhizosphere soil samples were collected randomly from 3 individuals of the species. Later, a composite sample was made for each species and worked out. Rhizosphere soil samples were excavated from a depth of 0-25cm, placed in plastic bags, labeled and stored at 10^0 C until processed. While collecting soil samples, soil was dug at three places for each plant so as to cover the entire rhizosphere. Hundred grams of air dried composite sample was assayed for spore count using Wet Sieving and Decanting procedure (Gerdemann and Nicolson,1963). Spores were separated using sieves having mesh sizes ranging from 45µm to 300µm. Each composite sample was processed three times. Spores were observed under Leica Stereo-microscope. Estimation of spore density was carried out as per the procedure

PLATE-3

COASTAL SAND DUNE VEGETATION

a) At less disturbed site (Varca).
b) At severely disturbed site (Miramar).


a


b

given by Gaur and Adholeya (1994). Only healthy spores were enumerated, whereas, broken and parasitised spores were discarded. Spores were mounted in Polyvinyl Lacto Glycerol (PVGL) (Koske and Tessier, 1983), examined for their various characteristics using compound microscope (40x-1000x) and identified using the standard keys (Schenck and Perez, 1990; Morton and Benny, 1990; Wu, 1993). Spores in the rhizosphere soils were multiplied using *Eleusine coracana* (L.) Gaertn. as a host plant. The spores isolated from the pot cultures were later used for confirming the identification of the spores recovered from the dunes.

A composite sample was prepared of all the samples collected for each beach for soil nutrient analysis. Available phosphate was detected by Olsen's method.

Relative abundance and Frequency of occurrence was calculated for each site using the formulas given below:

$$\textbf{Relative Abundance (\%)} = \frac{\textbf{Number of AM fungal spores of particular species}}{\textbf{Total number of AM fungal spores of all species}} \times 100$$

$$\textbf{Frequency of Occurrence (\%)} = \frac{\textbf{Number of soil samples that possess spores of particular AM species}}{\textbf{Total number of soil samples screened.}} \times 100$$

RESULTS AND DISCUSSION

Soil analysis indicates that at the less disturbed site (Varca), the soil was alkaline having pH 7.5 with Electrical conductivity (E.C) 0.156 M mhos/cm, Total Nitrogen (0.055%), Total Potassium (0.03%), Organic Carbon (0.14%) and Available Phosphorus (97μg/g), while, at severely disturbed site (Miramar), the soil was alkaline having pH 7.6 with Electrical conductivity (E.C) 0.203 M mhos/cm, Total Nitrogen (0.069%), Total Potassium (0.07%), Organic Carbon (0.115%) and Available Phosphorus (73μg/g) **(Table-9)**.

The average spore density observed at Varca was 1040.5 spores + 10 sporocarps/ 100g soil. Maximum spore count was recorded in *Cocos nucifera* (3404 spores + 32 sporocarps/100g soil) followed by *Anacardium occidentale* (2076 spores + 20 sporocarps/100g soil) and *Borassus flabellifer* (1832 spores + 16 sporocarps/100g soil), while minimum spore count was recorded in *Vitex trifolia* (28 spores/100g soil) **(Table-10)**.

The average spore density observed at Miramar was 424.4 spores + 5.6 sporocarps/ 100g soil. Maximum spore count was recorded in *Cocos nucifera* (996 spores + 20 sporocarps/100g soil) followed by *Acacia auriculiformis* (756 spores /100g soil) and *Lantana camara* (640 spores /100g soil), while minimum spore count was recorded in *Panicum sp.* (12 spores/100g soil) **(Table-11)**.

Table- 9: Chemical characteristics of coastal sand dune soils.

Site	pH	E. C. M mhos/cm	Total N_2 (%)	Available P µg/g	Organic Carbon (%)	Total K_2O (%)
Varca	7.5 (0.1)	0.156 (0.002)	0.055 (0.001)	97.0 (2.00)	0.140 (0.01)	0.03 (0.005)
Miramar	7.6 (0.09)	0.203 (0.001)	0.069 (0.002)	73.0 (1.32)	0.115 (0.01)	0.07 (0.002)

Values are the mean of three readings.
Values in parenthesis indicate standard deviation.

Table-10: Spore density and Species richness of AM fungi in less disturbed site.

Plant species	Spore density/100g	Species richness/ Plant species
***Ipomoea pes-caprae* (Linn.) Sweet**	116 (26) spores	3
***Spinifex litttoreus* (Burm.f.) Merr.**	100 (19.97) spores	4
***Urginea indica* (Roxb.) Kunth**	444 (70) spores+8 (3) sporocarps	8
***Ixora coccinia* Linn.**	324 (36.17) spores + 4 (2) sporocarps	4
***Vitex trifolia* Linn.**	28 (11.14) spores	4
***Cocos nucifera* Linn.**	3404 (598) spores +32 (22) sporocarps	7
***Borassus flabellifer* Linn.**	1832 (300) spores + 16 (6) sporocarps	13
***Anacardium occidentale* Linn.**	2076 (479) spores + 20 (14) sporocarps	11
Average value	**1040.5 spores + 10 sporocarps**	**6.75**

Values are the mean of 3 readings.
Values in the parenthesis indicate Standard deviation.

Table-11: Spore density and Species richness of AM fungi in severely disturbed site.

Plant species	Spore density/100g	Species richness/ Plant species
***Ipomoea pes-caprae* (Linn.) Sweet**	224 (58.18) spores	4
Panicum sp.	12 (8) spores	2
Crotolaria sp.	532 (24.1) spores + 4 (3.51) sporocarps	6
***Lantana camara* Linn.**	640 (140) spores	9
Derris sp.	308 (62.39) spores	4
***Ziziphus rugosa* Lamk.**	232 (43) spores + 4 (3) sporocarps	5
***Cocos nucifera* Linn**	996 (234.08) spores + 20 (4) sporocarps	8
***Borassus flabellifer* Linn.**	452 (60) spores + 12 (2) sporocarps	6
***Casuarina equisetifolia* Forst**	92 (20) spores + 16 (6) sporocarps	4
***Acacia auriculiformis* A. Cunn**	756 (147.09) spores	8
Average value	**424.4 spores + 5.6 sporocarps**	**5.6**

Values are the mean of 3 readings; Values in the parenthesis indicate Standard deviation.

Except for the genera *Entrophospora,* rest of the five genera *viz., Scutellospora, Sclerocystis, Glomus, Gigaspora* and *Acaulospora* were recovered in the survey. At Varca site, 21 AM fungal species were identified. The identified species include *Scutellospora calospora* Walker and Sanders, *Scutellospora coralloidea* Walker and Sanders, *Scutellospora gregaria* Walker and Sanders, *Scutellospora pellucida* Walker and Sanders, *Scutellospora verrucosa* Walker and Sanders, *Scutellospora weresubiae* Koske and Walker, *Sclerocystis sinuosa* Gerdemann and Bakshi, *Glomus constrictum* Trappe, *Glomus convolutum* Gerdemann and Trappe, *Glomus fasciculatum,* Gerdemann and Trappe emend. Walker and Koske, *Glomus formosanum* Wu and Chen, *Glomus heterosporum* Smith and Schenck ,*Glomus macrocarpum* Tul. and Tul., *Glomus maculosum* Miller and Walker, *Glomus microaggregatum* Koske, Gemma and Olexia, *Gigaspora margarita* Becker and Hall, *Acaulospora elegans* Trappe and Gerdemann, *Acaulospora foveata* Trappe and Janos, *Acaulospora nicolsonii* Walker, Reed and Sanders, *Acaulospora scrobiculata* Trappe and *Acaulospora spinosa* Walker and Trappe. Maximum AM fungal species were recorded in *Borassus flabellifer* (13) followed by *Anacardium occidentale* (11) while minimum AM fungal species were recorded in *Ipomoea pes-caprae* (3) **(Table-10)**. *Glomus macrocarpum* and *Gigaspora margarita* were the most frequently occurring species at the site with frequency of occurrence 62.5% each, followed by *Scutellospora calospora, Scutellospora coralloidea* and *Acaulospora spinosa* each having frequency of occurrence 50%. Frequency of occurrence was observed to be above 25% in 15 of the identified AM fungal species **(Table-12)**.

Table-12: Frequency of Occurrence and Relative Abundance of AM fungi in less disturbed site (Varca).

AM species	Frequency of Occurrence (%)	Relative Abundance (%)
Scutellospora calospora	50.0	05.14
Scutellospora coralloidea	50.0	00.19
Scutellospora gregaria	25.0	00.57
Scutellospora pellucida	12.5	00.43
Scutellospora verrucosa	12.5	00.66
Scutellospora weresubiae	37.5	00.33
Sclerocystis sinuosa	12.5	00.05
Sclerocystis sp.	37.5	00.29
Glomus constrictum	12.5	00.43
Glomus convolutum	25.0	01.86
Glomus fasciculatum	37.5	25.13
Glomus formosanum	25.0	01.67
Glomus heterosporum	25.0	03.05

(Contd.)

AM species	Frequency of Occurrence (%)	Relative Abundance (%)
Glomus macrocarpum	62.5	06.33
Glomus maculosum	37.5	08.61
Glomus microaggregatum	37.5	00.62
Glomus sp.	25.0	26.56
Gigaspora margarita	62.5	01.38
Acaulospora elegans	25.0	01.71
Acaulospora foveata	25.0	04.14
Acaulospora nicolsoni	12.5	00.81
Acaulospora scrobiculata	37.5	02.28
Acaulospora spinosa	50.0	07.71
Acaulospora sp.	12.5	00.05

At Miramar site, 14 AM fungal species were identified. The identified species include *Scutellospora coralloidea* Walker and Sanders, *Scutellospora pellucida* Walker and Sanders, *Scutellospora verrucosa* Walker and Sanders, *Scutellospora weresubiae* Koske and Walker, *Sclerocystis coremioides* Berk. and Broome, *Sclerocystis sinusa* Gerdemann and Bakshi, *Glomus heterosporum* Smith and Schenck, *Glomus deserticola* Trappe, Bloss and Menge, *Glomus macrocarpum* Tul. and Tul., *Glomus microaggregatum* Koske, Gemma and Olexia, *Gigaspora margarita* Becker and Hall, *Acaulospora delicata* Walker, Pfeiffer and Bloss, *Acaulospora foveata* Trappe and Janos and *Acaulospora spinosa* Walker and Trappe. Maximum number of AM fungal species were recorded in *Lantana camara* (9) followed by *Acacia auriculiformis* and *Cocos nucifera* with 8 AM fungal species each, while minimum number of AM fungal species were recorded in *Panicum sp.* (2) **(Table-11)**. *Acaulospora spinosa* was the most frequently occurring AM fungal species (80%) followed by *Glomus macrocarpum* (70%), *Scutellospora coralloidea* (70%) *Scutellospora pellucida* (60%) and *Glomus deserticola* (60%). Frequency of occurrence was observed to be above 25% in 10 of the identified AM fungal species **(Table-13)**.

At Varca site, among the identified species *Glomus fasciculatum* (25.13%) was the most abundant followed by *Glomus maculosum* (8.61%), *Acaulospora spinosa* (7.71%), *Glomus macrocarpum* (6.33%) and *Scutellospora calospora* (5.14%) **(Table-12)**.

Table-13: Frequency of Occurrence and Relative Abundance of AM fungi in severely disturbed site (Miramar).

AM species	Frequency of Occurrence (%)	Relative Abundance (%)
Scutellospora coralloidea	70.0	03.26
Scutellospora pellucida	60.0	13.40
Scutellospora sp	10.0	00.19
Scutellospora verrucosa	40.0	02.42
Scutellospora weresubiae	10.0	00.09
Sclerocystis coremioides	30.0	00.47
Sclerocystis sinuosa	30.0	00.65
Glomus heterosporum	40.0	03.72
Glomus deserticola	60.0	19.63
Glomus macrocarpum	70.0	23.16
Glomus microaggregatum	20.0.	00.19

(Contd.)

AM species	Frequency of Occurrence (%)	Relative Abundance (%)
Glomus sp.	30.0	01.40
Gigaspora margarita	30.0	02.60
Acaulospora delicata	10.0	00.28
Acaulospora foveata	10.0	00.19
Acaulospora sp.	30.0	06.42
Acaulospora spinosa	80.0	21.95

At Miramar site, among the identified species *Glomus macrocarpum* (23.16%) was the most abundant followed by *Acaulospora spinosa* (21.95%), *Glomus deserticola* (19.63%), *Scutellospora pellucida* (13.4%) and *Glomus heterosporum* (3.72%) **(Table-13).**

The AM fungal species *viz., Scutellospora coralloidea, Scutellospora pellucida, Scutellospora verrucosa, Scutellospora weresubiae, Glomus heterosporum, Glomus macrocarpum, Glomus microaggregatum, Gigaspora margarita, Acaulospora foveata and Acaulospora spinosa* were common to both the sites but their abundance drastically varied at the two sites **(Table-12 and Table-13).**

The survey study revealed that the spore density showed a drastic reduction in severely disturbed site (424.4 spores + 5.6 sporocarps) compared to less disturbed site (1040.5 spores + 10 sporocarps /100g soil). **(Fig. 3).** Also, the species richness was lower in severely disturbed site (14 fungal species) and higher at less disturbed site (21 fungal species) **(Fig. 4).** The dominance of AM fungal species from undisturbed dunes were different than those from the disturbed dunes **(Fig. 5 & Fig. 6).**

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

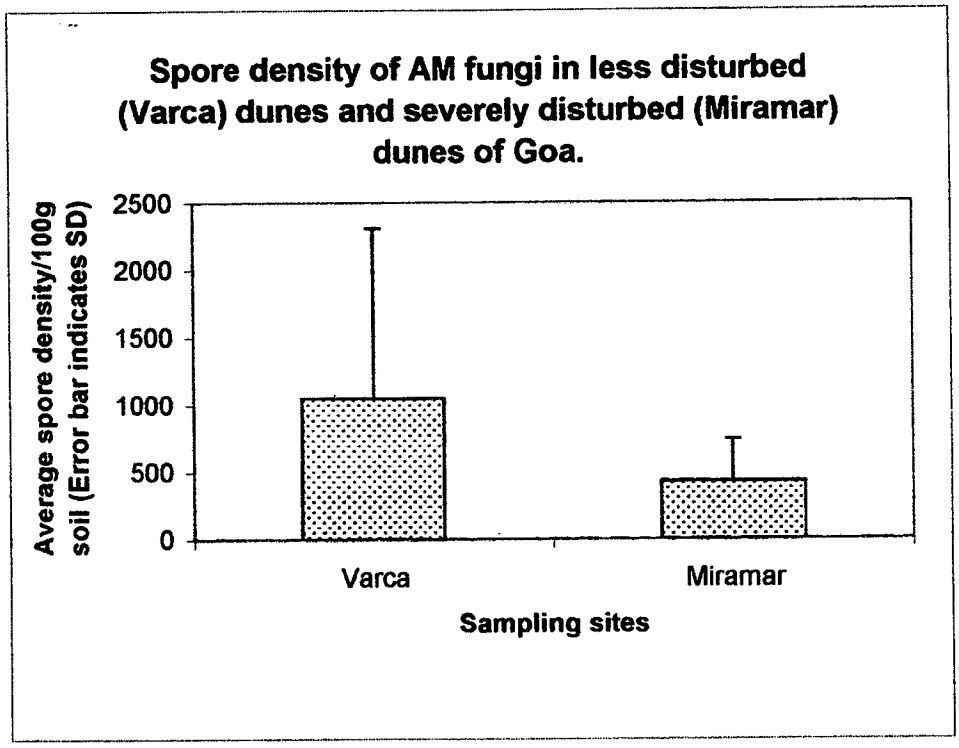

Spore density of AM fungi in less disturbed (Varca) dunes and severely disturbed (Miramar) dunes of Goa.
Average spore density/100g soil (Error bar indicates SD)
2500
2000
1500
1000
500
0
Varca
Miramar
Sampling sites

Fig.3

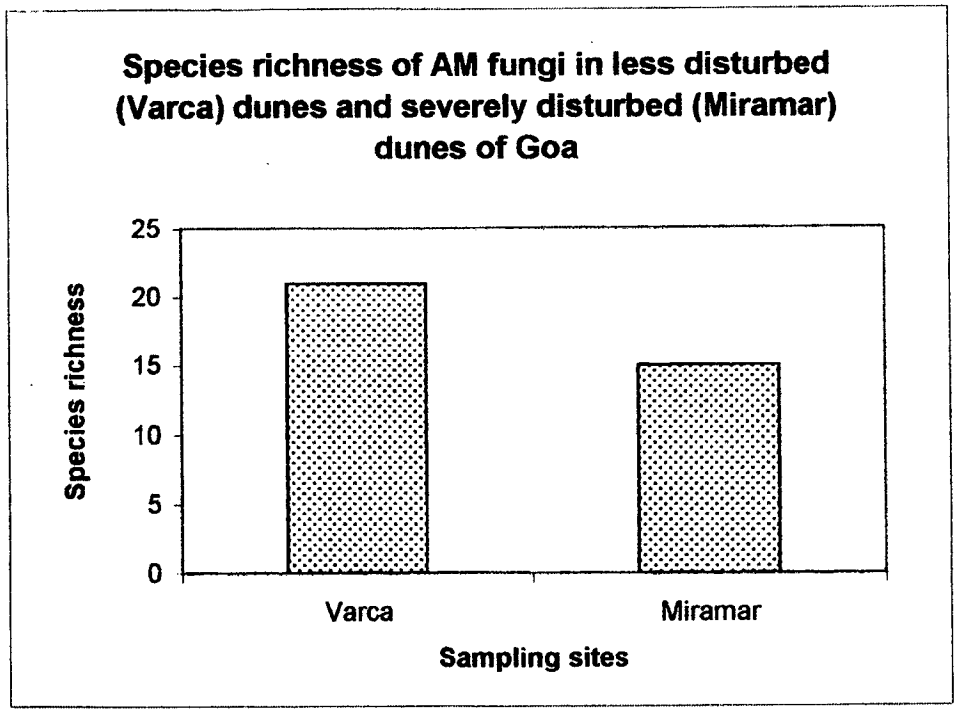

Species richness of AM fungi in less disturbed (Varca) dunes and severely disturbed (Miramar) dunes of Goa
Species richness
25
20
15
10
5
0
Varca
Miramar
Sampling sites

Fig.4

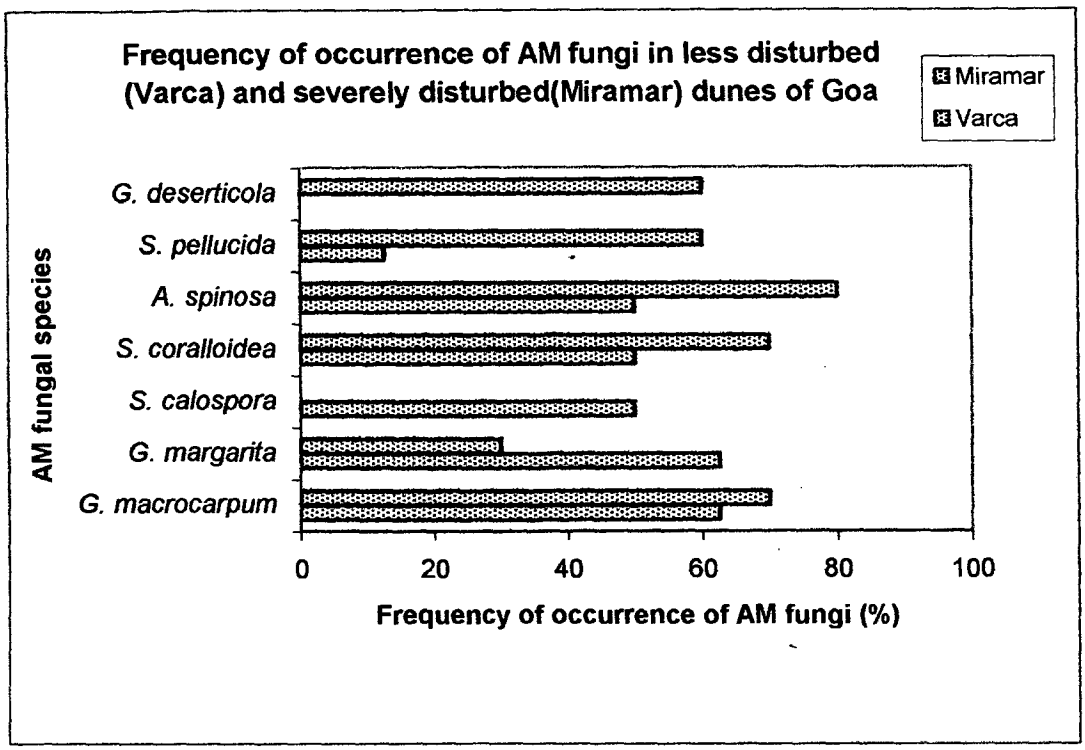

Frequency of occurrence of AM fungi in less disturbed (Varca) and severely disturbed(Miramar) dunes of Goa
Miramar
Varca
AM fungal species
G. deserticola
S. pellucida
A. spinosa
S. coralloidea
S. calospora
G. margarita
G. macrocarpum
0
20
40
60
80
100
Frequency of occurrence of AM fungi (%)

Fig.5

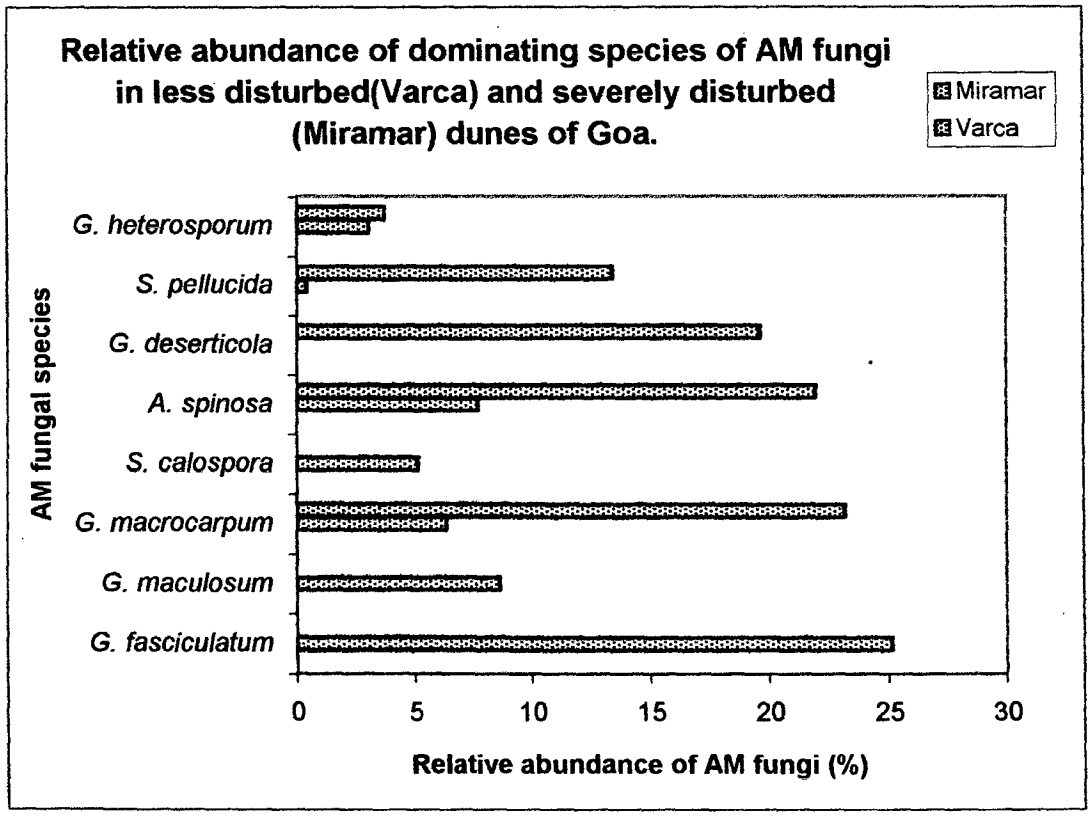

Relative abundance of dominating species of AM fungi in less disturbed(Varca) and severely disturbed (Miramar) dunes of Goa.
Miramar
Varca
AM fungal species
G. heterosporum
S. pellucida
G. deserticola
A. spinosa
S. calospora
G. macrocarpum
G. maculosum
G. fasciculatum
0
5
10
15
20
25
30
Relative abundance of AM fungi (%)

Fig.6

community between less disturbed and severely disturbed dunes reflects its response to disturbance.

In the present study our results suggest the theory of reduction of AM fungal propagation under severe disturbance which affects the natural plant community structure leading to the ecosystem instability.

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 his 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 mycorrhizal fungi. Mayr (1965) was of the opinion that disturbed habitats result in reduced number of mycorrhizal propagules because the hosts themselves are reduced.

It was also observed that available phosphorus was low in severely disturbed site. Among the edaphic features, available phosphorus is one of the major limiting factors in the severely disturbed dunes (Beena *et al.*,2000). Arbuscular mycorrhizal association increases the capacity of the plants to accumulate phosphates as it is released by other microorganism (Gupta and Rorison, 1975).

Nicolson (1967) suggested that mycorrhizal fungi may 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.

CHAPTER IV

DISTRIBUTION OF ARBUSCULAR MYCORRHIZAL (AM) FUNGI IN COASTAL SAND DUNE VEGETATION OF GOA.

INTRODUCTION

Sand dune systems are of great ecological significance. Stabilization of dunes by planting of vegetation has been recognized as a major means of slowing sand movement (Zak, 1965). The role of AM in dune ecology began to be perceived in the late 1950's (Nicolson, 1960). Nicolson (1967) regarded them as universal plant symbionts. The associations can affect nutrient uptake and ameliorate sand aggregation (Nicolson and Johnston, 1979) and further improve water relations (Hardie and Leyton, 1981). The available literature on the subject has been reviewed under the title " Review of Literature" at the beginning of the thesis. Investigations on AM fungi of coastal sand dunes of India are scarce (Mohankumar *et al.*, 1988; Kulkarni *et al.*, 1997). Coastal sand dune soils of Goa are unexamined for AM fungi. The present chapter deals with the qualitative and quantitative studies of AM spores in sand dune vegetation of Goa.

Six sites along the Goa coast were selected for the survey *viz.*, Mobor, Benaulim, Colva, Majorda (located in South Goa) and, Junes (Mandrem) and Bambolim (located in North Goa). Mobor was the least disturbed beach with profuse vegetation, while Benaulim, Colva, Majorda and Junes were moderately disturbed beach, with moderate to dense vegetation. Bambolim was severely disturbed beach with scanty vegetation. Climatic factors are almost uniform along the chosen coastal stretch. The climate is tropical, air temperature ranges between 19-34 ^{0}C. The annual rainfall is 368 cm, of which 74.6% occurs during the months of June and July. Relative humidity is high throughout the year especially

during monsoons (93-94%). The present study was conducted throughout the year.

The objectives of the present study were to determine the species richness and spore density of AM fungi in the rhizosphere soils of selected plants from all the six sites, and to determine the abundance of each species of AM fungi in the rhizosphere soils of selected plants from four sites *viz.*, Colva, Majorda, Junes (Mandrem) and Bambolim.

MATERIALS AND METHODS

In all, 29 plant species belonging to 20 different families were selected for the study. The selected plants included herbs, shrubs and trees. A maximum of 15 plant species were studied from the least disturbed site *i.e.* Mobor, while minimum of 5 plant species were studied from the most disturbed site *i.e.* Bambolim. The number of plant species studied from moderately disturbed sites ranged from 8-10, *viz.*, Junes (8), Benaulim (9), Colva (9) and Majorda (10) with number of plant species given in parenthesis. Collection of samples were done in different seasons, with samples from Colva and Bambolim sites in the month of March; from Majorda site in the month of July; from Benaulim and Mobor sites in the month of September and October respectively and from Junes (Mandrem) site in the month of December.

Rhizosphere soil samples were collected from the selected host plants at each site. For each plant species, rhizosphere soil samples were collected randomly from 3 individuals of the species. Later, a composite sample was made for each species and worked out. Rhizosphere soil samples were excavated from a depth of 0-25cm, placed in plastic bags, labeled and stored at 10^0 C until processed. While collecting soil samples, soil was dug at three places for each plant so as to cover the entire rhizosphere. Hundred grams of air dried composite sample was assayed for spore count using Wet Sieving and Decanting procedure (Gerdemann and Nicolson,1963). Spores were separated using sieves having mesh sizes ranging from 45μm to 300μm. Each composite sample was processed three times. Spores were observed under Leica Stereo-microscope. Estimation of Spore density was carried out as per the procedure given by Gaur and Adholeya (1994). Only healthy spores were enumerated, whereas, broken and parasitised spores were discarded. Spores were mounted in Polyvinyl Lacto Glycerol (PVGL), (Koske and Tessier, 1983), examined for their various characteristics using compound microscope (40x-1000x) and identified using the standard keys (Schenck and Perez, 1990; Morton and Benny, 1990; Wu, 1993). Spores in the rhizosphere soils were multiplied using *Eleusine coracana* (L.) Gaertn. as a host plant. The spores isolated from the pot cultures were later used for confirming the identification of the spores recovered from the dunes.

Voucher specimens are deposited in the Department of Botany, Goa University. Photographs of the identified spores were taken under Leica Compound Microscope WILD MP 32.

For soil analysis, rhizosphere soils of all the host plant species along with the sub samples were mixed to form a composite sample for each site and was analysed for pH, Total N, Total P and Total K.

Relative Abundance and Frequency of Occurrence of AM fungal species at the selected sites was calculated using the formulas given below:

$$\textbf{Relative Abundance (\%)} = \frac{\textbf{Number of AM fungal spores of particular species}}{\textbf{Total number of AM fungal spores of all species}} \times 100$$

$$\textbf{Frequency of Occurrence (\%)} = \frac{\textbf{Number of soil samples that possess spores of particular AM species}}{\textbf{Total number of soil samples screened}} \times 100$$

RESULTS AND DISCUSSION

The results of soil analysis indicated that the sandy soils were neutral to alkaline with pH of the soil ranging from 7.0-7.9. Sandy soils were poor in nutrients. Total Phosphate (P_2O_5) content varied from trace quantities to 0.130 %

while, Total Potassium (K_2O) content ranged from 0.008-0.060% and Total Nitrogen (N_2) content ranged from 0.040 to 0.080%. It was observed that the Electrical conductivity (E.C) ranged from 0.148 - 0.478 M mhos/cm **(Table-14)**.

All the soil samples analysed showed the presence of spores of arbuscular mycorrhizal fungi. Maximum average spore density was observed in Colva beach (725 spores + 15 sporocarps/100g soil), while minimum average spore density was observed in Mobor beach (233 spores + 18 sporocarps/100g soil) **(Fig. 7)**. At Mobor, maximum spore density was recorded in *Ixora coccinia* (848 spores /100g soil) followed by *Cocos nucifera* (628 spores /100g soil), while minimum spore density was recorded in *Spinifex litttoreus* (44 spores /100g soil). At Benaulim, maximum spore density was recorded in *Lantana camara* (700 spores /100g soil) while minimum spore density was recorded in *Ipomoea pes-caprae* (36 spores /100g soil). At Colva, maximum spore density was recorded in *Cocos nucifera* (1156 spores + 60 sporocarps /100g soil), while minimum spore density was recorded in *Lantana camara* (408 spores+ 16 sporocarps /100g soil). At Majorda, maximum spore count was recorded in *Cocos nucifera* (960 spores+38 sporocarps /100g soil) and minimum spore count was recorded in *Clerodendrum inerme* (20 spores + 2 sporocarps /100g soil). At Junes, maximum spore density was recorded in *Casuarina equisetifolia* (684 spores + 60 sporocarps /100g soil) followed by *Leucas aspera* (396 spores /100g soil) and *Cocos nucifera* (396 spores+ 4 sporocarps /100g soil), while minimum spore density was recorded in *Spinifex litttoreus* (32 spores /100g soil). At Bambolim, maximum spore density was recorded in *Vitex trifolia* (736

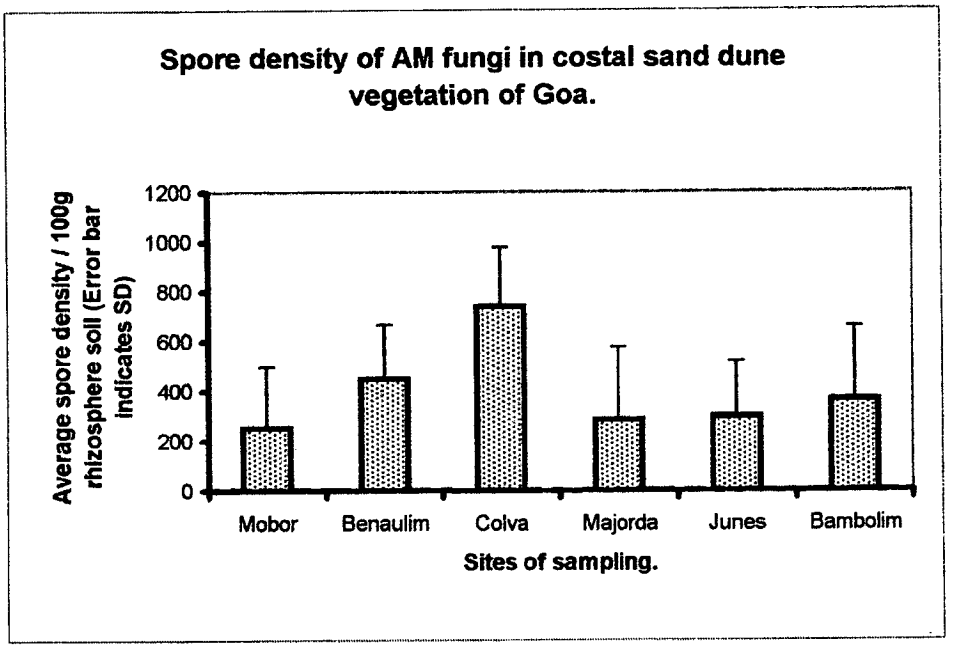

Spore density of AM fungi in costal sand dune vegetation of Goa.
Average spore density / 100g rhizosphere soil (Error bar indicates SD)
1200
1000
800
600
400
200
0
Mobor
Benaulim
Colva
Majorda
Junes
Bambolim
Sites of sampling.

Fig.7

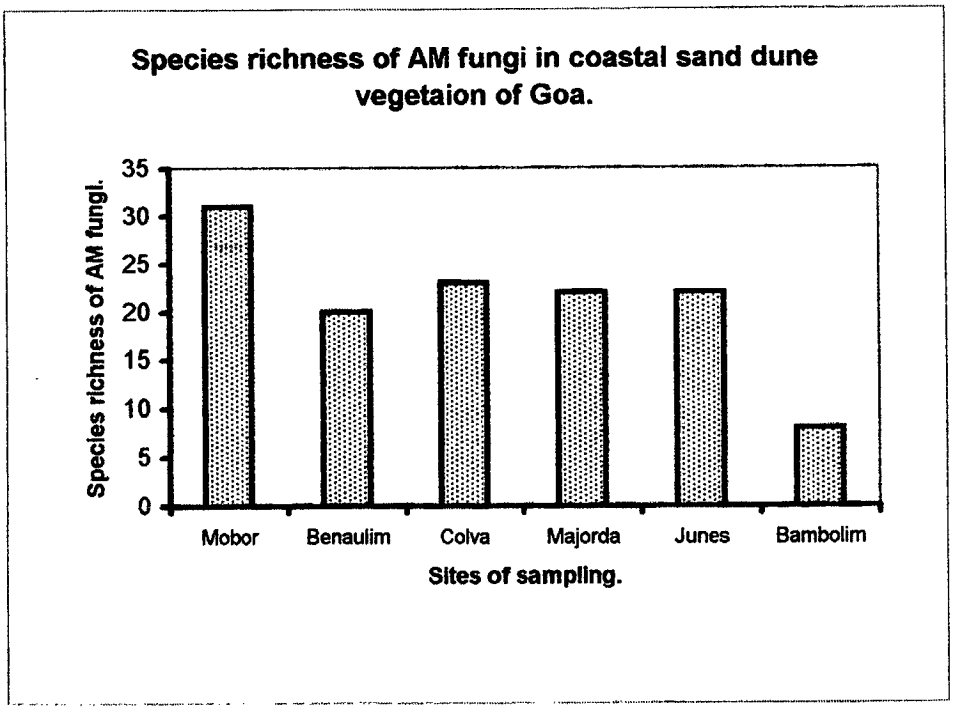

Species richness of AM fungi in coastal sand dune vegetaion of Goa.
Species richness of AM fungi.
35
30
25
20
15
10
5
0
Mobor
Benaulim
Colva
Majorda
Junes
Bambolim
Sites of sampling.

Fig.8

Table-14: Soil characteristics of the coastal sand dunes of Goa.

Site	pH*	E.C. * M mhos/cm	Total N_2* (%)	Total P_2O_5* (%)	Total K_2O* (%)
Mobor	7.0 (0.00)	0.148 (0.001)	0.079 (0.004)	0.061 (0.004)	0.012 (0.001)
Benaulim	7.7 (0.10)	0.165 (0.001)	0.080 (0.010)	0.123 (0.001)	0.008 (0.000)
Colva	7.6 (0.05)	0.478 (0.002)	0.040 (0.004)	Traces	0.040 (0.001)
Majorda	7.4 (0.10)	0.305 (0.001)	0.050 (0.005)	Traces	0.030 (0.000)
Junes	7.6 (0.05)	0.247 (0.002)	0.070 (0.001)	0.082 (0.002)	0.050 (0.001)
Bambolim	7.9 (0.00)	0.204 (0.001)	0.069 (0.001)	0.130 (0.002)	0.060 (0.003)

* Values are the mean of three readings.
Values in parenthesis indicates standard deviation from the mean value.

spores + 48 sporocarps /100g soil) and minimum spore density was recorded in *Crotolaria sp.* (48 spores /100g soil) **(Table-15).**

In the present study, it appears that the variation in spore population at different sites may be due to the influence of different environmental factors on AM sporulation and infection. Similar observation has been made by earlier workers (Mohankumar, 1985; Giovannetti and Nicolson,1983).

The study revealed that the samples collected from all the sites showed the presence of AM fungal spores which varied from 233 spores + 18 sporocarps/100g soil to 725 spores + 15 sporocarps/100g soil. However, Koske (1975) reported the presence of 0-110 AM spores/10g soil in Australian sand dunes. Kulkarni *et al.,* (1997) reported the presence of 0-1.6 AM spores/g soil in their survey of AM fungi in Mangalore coast of Karnataka, conducted during post-monsoon season. Koske (1981) ranked the factors affecting the occurrence and spore density of AM fungal species in the rhizosphere of dune plants in order of decreasing importance as follows: 1) Site physical characteristics; 2) Species of host plants, and 3) Other AM fungi present.

In all, 50 AM fungal species belonging to 5 genera *viz., Acaulospora, Gigaspora, Glomus, Sclerocystis* and *Scutellospora* were identified from the rhizosphere soil samples of all the six sites **(Table-16).** Identified AM fungi include *Scutellospora albarosea* Walker and Sanders, *Scutellospora calospora*

Table-15: Spore density of AM fungi in coastal sand dune vegetation of Goa.

Plant species	Spore density/100g soil*					
	Mobor	**Benaulim**	**Colva**	**Majorda**	**Junes**	**Bambolim**
VERBENACEAE						
Vitex trifolia Linn.	48 (13.87)	632 (150)+ 8(4) sporocarps	848 (188.33)	96 (14)+ 4 (4) sporocarps	216 (64)	736 (139.75)+ 48 (18.33) sporocarps
Lantana camara Linn.	64 (18.33)	704 (123.05)	408 (88.26)+ 16 (7.55) sporocarps	460 (102.59)	-	512(88)+ 24 (8.08) sporocarps
Premna integrifolia Linn.	140 (26.46)+ 128 (16) sporocarps	-	-	-	-	-
Clerodendrum inerme (Linn.) Gaertn.	-	-	-	20 (6.24)+ 2 sporocarps	-	-
POACEAE						
Spinifex litttoreus (Burm.f.) Merr.	44 (8)	400 (79.11)	-	-	32 (14.1)	-
Unidentified species	160 (27.84)	-	-	-	-	-
CONVOLVULACEAE						
Ipomoea pes-caprae (Linn.) Sweet	60 (15)	36 (16)	620 (130)+ 4 (4) sporocarps	408 (92)+ 8 (4) sporocarps	156 (34)	-

(Contd.)

Plant species	Spore density/100g soil*					
	Mobor	**Benaulim**	**Colva**	**Majorda**	**Junes**	**Bambolim**
ARECACEAE						
Cocos nucifera Linn.	628 (159.85)+ 8 (3) sporocarps	692 (188)	1156 (319.69)+ 60 (12) sporocarps	960 (240)+ 38 (13) sporocarps	396 (97.14)+ 4 (3) sporocarps	160 (60)+ 80 (24.44) sporocarps
CASUARINACEAE						
Casuarina equisetifolia Forst.	60 (17.56)+ 4 (2) sporocarps	-	864 (117.85)+ 4 (1) sporocarps	-	684 (165.91)+ 60 (16) sporocarps	-
ASCLEPIADACEAE						
Calotropis gigantea (Linn.) R.Br.	-	376 (47.29)	620 (141.07)+ 20 (12) sporocarps	236 (64)+ 4 (1) sporocarps	-	-
PANDANACEAE						
Pandanus tectorius Soland	-	-	-	304 9 (83.07)+ 4 (4) sporocarps	-	196 (31.8)+ 8 (4) sporocarps
ANACARDIACEAE						
Anacardium occidentale Linn.	256 (94)+ 4 (2) sporocarps	-	-	-	-	-

(Contd.)

Plant species	Spore density/100g soil*					
	Mobor	**Benaulim**	**Colva**	**Majorda**	**Junes**	**Bambolim**
RUBIACEAE						
Ixora coccinia Linn.	848 (242)	-	-	96 (25)	-	-
Neanotis sp.	284 (28.21)+ 44 (6) sporocarps	-	-	-	-	-
ASTERACEAE						
Vernonia sp.	92 (29.05)+ 40 (15) sporocarps	-	-	-	-	-
Acanthospermum sp.	48 (15)+ 20 (5) sporocarps	-	-	-	-	-
Launea fallax (Jaub. & Spach.) Kuntze	-	-	-	-	128 (45.15)	-
Chromolaena odorata (L.) King & Robinson	-	428(72)	-	-	276(75) + 16 (12)	-
CYPERACEAE						
Cyperus sp.	536 (97.14)+ 4 (1) sporocarps	-	-	-	-	-
TACCACEAE						
Tacca sp.	228 (48)+ 12 (10.82) sporocarps	-	-	-	-	-

(Contd.)

Plant species	Spore density/100g soil*					
	Mobor	**Benaulim**	**Colva**	**Majorda**	**Junes**	**Bambolim**
TILIACEAE						
Microcos paniculata Linn.	-	256 (64)	-	-	-	-
MALVACEAE						
Abutilon sp.	-	496 (144.04)+ 8 (2) sporocarps	-	-	-	-
LAMIACEAE						
Leucas aspera Spreng.	-	-	-	-	396 (104)	-
Hyptis suaveolens (Linn.)Poit.	-	-	852 (148)+ 12 (8) sporocarps	-	-	-
AMARANTHACEAE						
Achyranthes aspera Linn.	-	-	652 (148)+ 8 (1) sporocarps	-	-	-
EUPHORBIACEAE						
Fluggea sp.	-	-	508 (92)+ 12 (4) sporocarps	-	-	-

(Contd.)

Plant species	Spore density/100g soil*					
	Mobor	**Benaulim**	**Colva**	**Majorda**	**Junes**	**Bambolim**
SOLANACEAE						
Datura metel Linn.	-	-	-	104 (28.21)+ 8 (6) sporocarps	-	-
RHAMNACEAE						
Ziziphus rugosa Lamk.	-	-	-	80 (25)	-	-
FABACEAE						
Crotolaria sp.	-	-	-	-	-	48 (8.02)
Average spore density/100g	**233.07+ 17.6 sporocarps**	**446.67+ 1.78 sporocarps**	**725.33+ 15.11 sporocarps**	**276.4+ 6.8 sporocarps**	**285.5+ 10 sporocarps**	**330.4+ 32 sporocarps**

- = Plant species not worked out at the site.
* = Values are the mean of three readings.
Values in the parenthesis indicates Standard deviation.

Walker and Sanders, *Scutellospora coralloidea* Walker and Sanders, *Scutellospora gregaria* Walker and Sanders, *Scutellospora pellucida* Walker and Sanders, *Scutellospora persica* Walker and Sanders, *Scutellospora reticulata,* Walker and Sanders, *Scutellospora verrucosa* Walker and Sanders, *Scutellospora weresubiae* Koske and Walker, *Sclerocystis clavispora* Trappe, *Sclerocystis coremioides* Berk. and Broome, *Sclerocystis sinuosa* Gerdemann and Bakshi, *Sclerocystis taiwanensis* Wu and Chen, *Glomus aggregatum* Schenck and Smith emend. Koske, *Glomus constrictum* Trappe, *Glomus deserticola* Trappe, Bloss and Menge, *Glomus diaphanum* Morton and Walker, *Glomus fasciculatum* Gerdemann and Trappe emend. Walker and Koskep, *Glomus formosanum* Wu and Chen, *Glomus geosporum* Walker, *Glomus heterosporum* Smith and Schenck, *Glomus hoi* Berch and Trappe, *Glomus macrocarpum* Tul. and Tul., *Glomus maculosum* Miller and Walker, *Glomus microaggregatum* Koske, Gemma and Olexia, *Glomus multisubtensum* Mukerji, Bhattacharjee and Tewari , *Glomus occultum* Walker, *Glomus pubescens* Trappe and Gerdemann, *Glomus tenebrosum* Berch and Fortin, *Glomus tenerum* Tandy emend. McGee, *Gigaspora albida* Schenck and Smith, *Gigaspora candida* Bhattacharjee, Mukerji, Tewari and Skoropad., *Gigaspora decipiens* Hall and Abbott, *Gigaspora margarita* Becker and Hall, *Gigaspora ramisporophora* Spain, Sieverding and Schenck, *Gigaspora rosea* Nicolson and Schenck, *Acaulospora bireticulata* Rothwell and Trappe, *Acaulospora delicata* Walker, Pfeiffer and Bloss, *Acaulospora dilatata* Morton, *Acaulospora elegans* Trappe and Gerdemann, *Acaulospora foveata* Trappe and Janos, *Acaulospora laevis* Gerdemann and Trappe, *Acaulospora mellea* Spain and

Schenck, *Acaulospora morrowiae* Spain and Schenck, *Acaulospora myriocarpa* Spain Sieverding and Schenck *Acaulospora nicolsonii* Walker, Reed and Sanders, *Acaulospora rugosa* Morton, *Acaulospora scrobiculata* Trappe, *Acaulospora spinosa* Walker and Trappe and *Acaulospora undulata* Sieverding **(Table-16; Plates - 4 to 9).**

Acaulospora spinosa, Glomus microaggregatum and *Sclerocystis sinuosa* were the most dominating AM fungi being recorded from all the sites (100% frequency of occurrence). Other species with high frequency of recovery were *Gigaspora margarita, Scutellospora calospora, Glomus macrocarpum, Glomus geosporum, Acaulospora scrobiculata and Scutellospora weresubiae* (83.33% frequency of occurrence) **(Table-16).** Maximum species richness of AM fungi was observed at least disturbed site *i.e.* Mobor (31 AM fungal species). At moderately disturbed dunes, the species richness ranged from 20 to 23 AM fungal species, while at severely disturbed site *i.e.* Bambolim, the species richness was minimum with only 8 AM fungal species **(Fig. 8) (Table-17).**

At Mobor, maximum number of AM fungal species were recorded in unidentified grass sp. (8), while minimum number of AM fungal species were reported from *Vitex trifolia* (3), *Casuarina equisetifolia* and *Lantana camara* (2 in each case) and, *Spinifex litttoreus* (1) **(Table-18).** The most frequently occurring fungi at Mobor being *Gigaspora margarita* (53.33%) and *Acaulospora spinosa* (46.67%) **(Table-17).**

Table 16: Frequency of occurrence of AM fungal species in coastal sand dune vegetation of Goa.

AM fungal species	Mobor	Benaulim	Colva	Majorda	Junes	Bambolim	Frequency (%)
Scutellospora albarosea	+	-	-	-	-	-	16.67
Scutellospora calospora	+	+	+	+	+	-	83.33
Scutellospora coralloidea	+	-	+	+	+	-	66.67
Scutellospora gregaria	+	+	+	+	-	-	66.67
Scutellospora pellucida	+	-	-	+	+	-	50.00
Scutellospora persica	+	+	-	-	-	-	33.33
Scutellospora reticulata	-	-	-	+	-	-	16.67
Scutellospora verrucosa	-	+	+	+	-	+	66.66
Scutellospora weresubiae	-	+	+	+	+	+	83.33
Sclerocystis clavispora	+	-	+	+	-	+	50.00
Sclerocystis coremioides	+	-	+	+	+	-	50.00
Sclerocystis sinuosa	+	+	+	+	+	+	100.00
Sclerocystis taiwanensis	-	-	+	-	-	-	16.67

(Contd.)

AM fungal species	Mobor	Benaulim	Colva	Majorda	Junes	Bambolim	Frequency (%)
Glomus aggregatum	+	+	-	+	-	-	50.00
Glomus constrictum	+	-	+	-	-	-	33.33
Glomus deserticola	+	+	-	-	+	-	50.00
Glomus diaphanum	-	-	-	+	-	-	16.67
Glomus fasciculatum	-	+	+	-	-	-	33.33
Glomus formosanum	+	-	-	-	-	-	16.67
Glomus geosporum	+	+	+	-	+	+	83.33
Glomus heterosporum	+	+	+	+	-	-	66.67
Glomus hoi	-	-	+	-	-	-	16.67
Glomus macrocarpum	+	+	+	+	+	-	83.33
Glomus microaggregatum	+	+	+	+	+	+	100.00
Glomus maculosum	+	-	-	+	-	-	33.33
Glomus multisubtensum	-	-	-	+	-	-	16.67
Glomus occultum	+	-	-	-	-	-	16.67
Glomus pubescens	+	-	-	-	-	-	16.67
Glomus tenebrosum	-	-	+	-	+	-	33.33
Glomus tenerum	-	-	+	-	-	-	16.67

(Contd.)

AM fungal species	Mobor	Benaulim	Colva	Majorda	Junes	Bambolim	Frequency (%)
Gigaspora albida	-	-	-	+	-	-	16.67
Gigaspora candida	+	-	-	-	-	-	16.67
Gigaspora decipiens	-	-	-	-	+	-	16.67
Gigaspora margarita	+	+	+	+	+	-	83.33
Gigaspora ramisporophora	-	-	-	-	+	-	16.67
Gigaspora rosea	+	+	-	-	+	-	50.00
Acaulospora bireticulata	-	-	+	-	-	-	16.67
Acaulospora delicata	-	-	-	-	+	-	16.67
Acaulospora dilatata	-	-	-	+	+	-	33.33
Acaulospora elegans	+	+	+	-	+	-	66.67
Acaulospora foveata	+	-	-	-	-	-	16.67
Acaulospora laevis	+	-	-	-	-	-	16.67
Acaulospora mellea	+	-	-	-	-	-	16.67
Acaulospora morrowiae	-	-	-	-	+	-	16.67
Acaulospora myriocarpa	-	+	-	-	+	-	33.33
Acaulospora nicolsonii	+	+	-	-	-	-	33.33

(Contd.)

AM fungal species	Mobor	Benaulim	Colva	Majorda	Junes	Bambolim	Frequency (%)
Acaulospora rugosa	+	-	-	-	-	-	16.67
Acaulospora scrobiculata	-	+	+	+	+	+	83.33
Acaulospora spinosa	+	+	+	+	+	+	100.00
Acaulospora undulata	+	-	-	-	-	-	16.67

\+ = AM species recorded.
\- = AM species not recorded.

At Benaulim, maximum AM fungal species richness was observed in *Cocos nucifera* (10) and minimum was recorded from *Ipomoea pes-caprae, Microcos paniculata* and *Spinifex litttoreus,* (2 in each case) **(Table-18)**. The most frequently occurring fungi were *Acaulospora spinosa* (77.78%), *Gigaspora margarita* (55.56%) and *Glomus heterosporum* (44.44%) **(Table-17)**.

At Colva, maximum number of AM fungal species were recorded in *Hyptis suaveolens* (14), followed by *Fluggea sp.* (11), while minimum number of AM fungal species (4) were recorded in *Casuarina equisetifolia* **(Table-18)**. The most frequently occurring fungi were *Sclerocystis sinuosa* and *Acaulospora spinosa* (88.89% in each case), followed by *Scutellospora coralloidea* and *Glomus macrocarpum* (77.78%) **(Table-17)**.

At Majorda, maximum number of AM fungal species were recorded from *Pandanus tectorius* (10), followed by *Vitex trifolia* (8), while minimum number of AM fungal species (1) was recorded in *Clerodendrum inerme* **(Table-18)**. The most frequently occurring fungi were *Acaulospora spinosa* (70%) and *Gigaspora margarita* (60%), followed by *Scutellospora coralloidea, Scutellospora gregaria* and *Acaulospora scrobiculata,* (50%) in each case **(Table-17)**.

Table 17: Frequency of occurrence of AM fungal species at each site.

AM fungal species	Frequency (%)					
	Mobor	**Benaulim**	**Colva**	**Majorda**	**Junes**	**Bambolim**
Scutellospora albarosea	6.67	-	-	-	-	-
Scutellospora calospora	26.67	33.33	22.22	20	12.5	-
Scutellospora coralloidea	6.67		77.78	50	37.5	-
Scutellospora gregaria	20.00	33.33	22.22	50		-
Scutellospora pellucida	6.67		-	10	25.0	-
Scutellospora persica	6.67	11.11	-	-	-	-
Scutellospora reticulata	-	-	-	10	-	-
Scutellospora verrucosa	-	11.11	55.56	10	-	40
Scutellospora weresubiae	-	11.11	22.22	10	50.0	100
Sclerocystis clavispora	6.67	-	-	10	-	60
Sclerocystis coremioides	33.33	-	11.11	10	25.0	-
Sclerocystis sinuosa	20.00	33.33	88.89	40	12.5	40
Sclerocystis taiwanensis	-	-	11.11	-	-	-

(Contd.)

AM fungal species	Frequency (%)					
	Mobor	Benaulim	Colva	Majorda	Junes	Bambolim
Glomus aggregatum	6.67	11.11		10	-	-
Glomus constrictum	6.67	-	22.22	-	-	-
Glomus deserticola	6.67	11.11	11.11	-	25.0	-
Glomus diaphanum	-	-	-	10	-	-
Glomus fasciculatum	-	11.11	55.56	-	-	-
Glomus formosanum	13.33	-	-	-	-	-
Glomus geosporum	13.33	11.11	33.33	-	12.5	20
Glomus heterosporum	6.67	44.44	66.67	40	-	-
Glomus hoi	-	-	11.11	-	-	-
Glomus macrocarpum	26.67	33.33	77.78	30	50.0	
Glomus maculosum	13.33	-	-	10	-	-
Glomus microaggregatum	20.00	22.22	11.11	30	25.0	20
Glomus multisubtensum	-	-	-	10	-	-
Glomus occultum	6.67	-	-	-	-	-

(Contd.)

AM fungal species	Frequency (%)					
	Mobor	Benaulim	Colva	Majorda	Junes	Bambolim
Glomus pubescens	6.67	-	-	-	-	-
Glomus tenebrosum	-	-	22.22	-	-	-
Glomus tenerum	-	-	11.11	-	12.5	-
Gigaspora albida	-	-	-	30	-	-
Gigaspora candida	6.67	-	-	-	-	-
Gigaspora decipiens	-	-	-	-	12.5	-
Gigaspora margarita	53.33	55.56	44.44	60	25.0	-
Gigaspora ramisporophora	-	-	-	-	12.5	-
Gigaspora rosea	13.33	11.11	-	-	12.5	-
Acaulospora bireticulata	-	-	33.33	-	-	-
Acaulospora delicata	-	-	-	-	12.5	-
Acaulospora dilatata	-	-	-	10	12.5	-
Acaulospora elegans	20.00	11.11	44.44	-	25.0	-
Acaulospora foveata	13.33	-	-	-	-	-

(Contd.)

AM fungal species	Frequency (%)					
	Mobor	**Benaulim**	**Colva**	**Majorda**	**Junes**	**Bambolim**
Acaulospora laevis	13.33	-	-	-	-	-
Acaulospora mellea	26.67	-	-	-	-	-
Acaulospora morrowiae	-	-	-	-	12.5	-
Acaulospora myriocarpa	-	11.11	-	-	12.5	-
Acaulospora nicolsonii	6.67	11.11	-	-	-	-
Acaulospora rugosa	20.00	-	-	-	-	-
Acaulospora scrobiculata	-	33.33	44.44	50	12.5	60
Acaulospora spinosa	46.67	77.78	88.89	70	75.0	20
Acaulospora undulata	26.67	-	-	-	-	-
Total No. of AM fungal sp. recovered	31	20	23	22	22	8

(- = AM species not recorded)

At Junes, maximum AM fungal species (10) were recorded in *Casuarina equisetifolia* and minimum in *Ipomoea pes-caprae* (1) **(Table-18)**. The most frequently occurring fungi were *Acaulospora spinosa* (75%), followed by *Scutellospora weresubiae* and *Glomus macrocarpum,* (50%) in each case **(Table-17)**.

At Bambolim, maximum AM fungal species (6 each) were recorded in *Vitex trifolia* and *Lantana camara,* while minimum was recorded in *Crotolaria sp.* (1) **(Table-18)**. The most frequently occurring fungus was *Scutellospora weresubiae* (100%) followed by *Sclerocystis clavispora* and *Acaulospora scrobiculata* (60%) **(Table-17)**.

The study of the relative abundance at the four selected sites revealed that species of *Acaulospora* and *Glomus* dominated the rhizosphere soils **(Table-19).** *Acaulospora spinosa* was most abundant species at three sites *viz.*, Colva (25.03%), Majorda(15.54%), and Junes (28.26%), while *Acaulospora scrobiculata* (26.05%) was the most abundant at Bambolim site. At Colva site the abundance of *Acaulospora spinosa* was followed by *Glomus fasciculatum* (20.83%) and, *Glomus macrocarpum* (12.24%). At Majorda site, the abundance of *Acaulospora spinosa* was followed by *Glomus heterosporum* (12.43%), and *Acaulospora scrobiculata*(10.17%). At Junes site, the abundance of *Acaulospora spinosa* was followed by *Gigaspora margarita* (5.92%) and *Glomus macrocarpum* (5.41%).

Plant species	Number of AM fungal species/plant species					
	Mobor	**Benaulim**	**Colva**	**Majorda**	**Junes**	**Bambolim**
ARECACEAE						
Cocos nucifera Linn.	-	10	9	6	4	3
RUBIACEAE						
Ixora coccinia Linn.	7	-	-	4	-	-
Neanotis sp.	7	-	-	-	-	-
CASUARINACEAE						
Casuarina equisetifolia Forst.	2	-	4	-	10	-
ASTERACEAE						
Vernonia sp.	6	-	-	-	-	-
Chromolaena odorata (L.) King & Robinson	-	5	-	-	6	-
Acanthospermum sp.	4	-	-	-	-	-
Launea fallax (Jaub. & Spach.) Kuntze	-	-	-	-	2	-

(Contd.)

Plant species	Number of AM fungal species/plant species					
	Mobor	**Benaulim**	**Colva**	**Majorda**	**Junes**	**Bambolim**
AMARANTHACEAE						
Achyranthes aspera Linn.	-	-	9	-	-	-
EUPHORBIACEAE						
Fluggea sp.	-	-	11	-	-	-
SOLANACEAE						
Datura metel Linn.	-	-	-	7	-	-
RHAMNACEAE						
Ziziphus rugosa Lamk.	-	-	-	4	-	-
PANDANACEAE						
Pandanus tectorius Soland	-	-	-	10	-	3
FABACEAE						
Crotolaria sp.	-	-	-	-	-	1

- = Plant species not worked out at the site.

Table-19: Composition of AM fungal floras by Genus in coastal sand dune vegetation of Goa.

AM fungi	Relative abundance (%)			
	Colva	**Majorda**	**Junes**	**Bambolim**
Scutellospora	3.36	22.44	4.9	10.15
Sclerocystis	1.98	2.11	3.05	8.62
Glomus	49.27	31.77	25.72	8.38
Gigaspora	0.84	8.75	11.33	-
Acaulospora	44.29	34.89	55.01	72.85

- = Not recorded

At Bambolim site, the abundance of *Acaulospora scrobiculata* was followed by *Scutellospora weresubiae* (9.71%) and *Acaulospora spinosa* (6.18%) **(Table-20)**.

Thus, *Acaulospora spinosa, Glomus macrocarpum, Acaulospora scrobiculata, Gigaspora margarita and Scutellospora weresubiae* are dominating species in terms of frequency as well as abundance in coastal sand dune vegetation of Goa **(Fig. 9 & 10)**. The occurrence of AM spores as the regular components of the soil microflora indicates that there is no host specificity. Koske and Tews (1987) recovered *Glomus geosporum* and *Glomus macrocarpum* as commonly occurring AM fungi in Wisconsin sandy soils of North America. Rose (1988) recovered *Glomus fasciculatum, Scutellospora coralloidea* and *Scutellospora calospora* from sand dunes of Northern California. He found that distribution of spores and species paralleled plant succession and vascular plant diversity. 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. According to him there was no evidence that spores were limited to association with a particular host, dune, stage or pH level in sand. 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 *Gigaspora ramisporophora, Scutellospora gregaria, Glomus fasciculatum* from Mangalore coast of Karnataka. They recovered 16 species of AM fungi with *Gigaspora ramisporophora, Glomus*

Table-20: Relative abundance of AM fungal spores in coastal sand dune vegetation of Goa.

AM fungal species	Relative abundance (%)			
	Colva	Majorda	Junes	Bambolim
Scutellospora calospora	0.6	3.81	0.51	-
Scutellospora coralloidea	0.84	8.19	0.51	-
Scutellospora gregaria	0.24	9.04	-	-
Scutellospora pellucida	-	0.42	2.19	-
Scutellospora reticulata	-	0.14	-	-
Scutellospora verrucosa	1.32	0.42		0.44
Scutellospora weresubiae	0.3	0.14	1.69	9.71
Scutellospora sp.	0.06	0.28	-	-
Sclerocystis clavispora	-	0.14	-	2.43
Sclerocystis coremioides	0.06	0.07	2.37	-
Sclerocystis sinuosa	1.68	0.35	0.68	4.64
Sclerocystis taiwanensis	0.06	-	-	-
Sclerocystis sp.	0.18	1.55	-	-
Glomus aggregatum	-	1.41	-	-
Glomus constrictum	0.54	-	-	-
Glomus deserticola	1.08	-	2.03	-
Glomus diaphanum	-	0.14	-	-
Glomus fasciculatum	20.83	-	-	-
Glomus geosporum	1.56	-	0.51	2.87
Glomus heterosporum	5.22	12.43	-	-

(Contd.)

AM fungal species	Relative abundance (%)			
	Colva	Majorda	Junes	Bambolim
Glomus hoi	0.72	-	-	-
Glomus macrocarpum	12.24	7.63	5.41	-
Glomus maculosum	-	2.4	-	-
Glomus microaggregatum	0.06	0.28	0.34	0.22
Glomus multisubtensum	-	0.42	-	-
Glomus tenebrosum	2.1	-	0.17	-
Glomus tenerum	0.42	-	-	-
Glomus sp.	4.5	7.06	17.26	5.29
Gigaspora albida	-	6.92	-	-
Gigaspora decipiens	-	-	0.34	-
Gigaspora margarita	0.72	1.55	5.92	-
Gigaspora ramisporophora	-	-	0.17	-
Gigaspora rosea	-	-	2.71	-
Gigaspora sp.	0.12	0.28	2.19	-
Acaulospora bireticulata	0.9	-	-	-
Acaulospora delicata	-	-	1.02	-
Acaulospora dilatata	-	2.4	0.34	-
Acaulospora elegans	6.78	-	0.51	-
Acaulospora foveata	-	-	-	-
Acaulospora laevis	-	-	-	-
Acaulospora morrowiae	-	-	0.51	-

(Contd.)

AM fungal species	Relative abundance (%)			
	Colva	Majorda	Junes	Bambolim
Acaulospora myriocarpa	-	-	0.34	-
Acaulospora scrobiculata	7.44	10.17	0.34	26.05
Acaulospora spinosa	25.03	15.54	28.26	6.18
Acaulospora sp.	4.14	6.78	23.69	40.62

- = AM species not recorded.

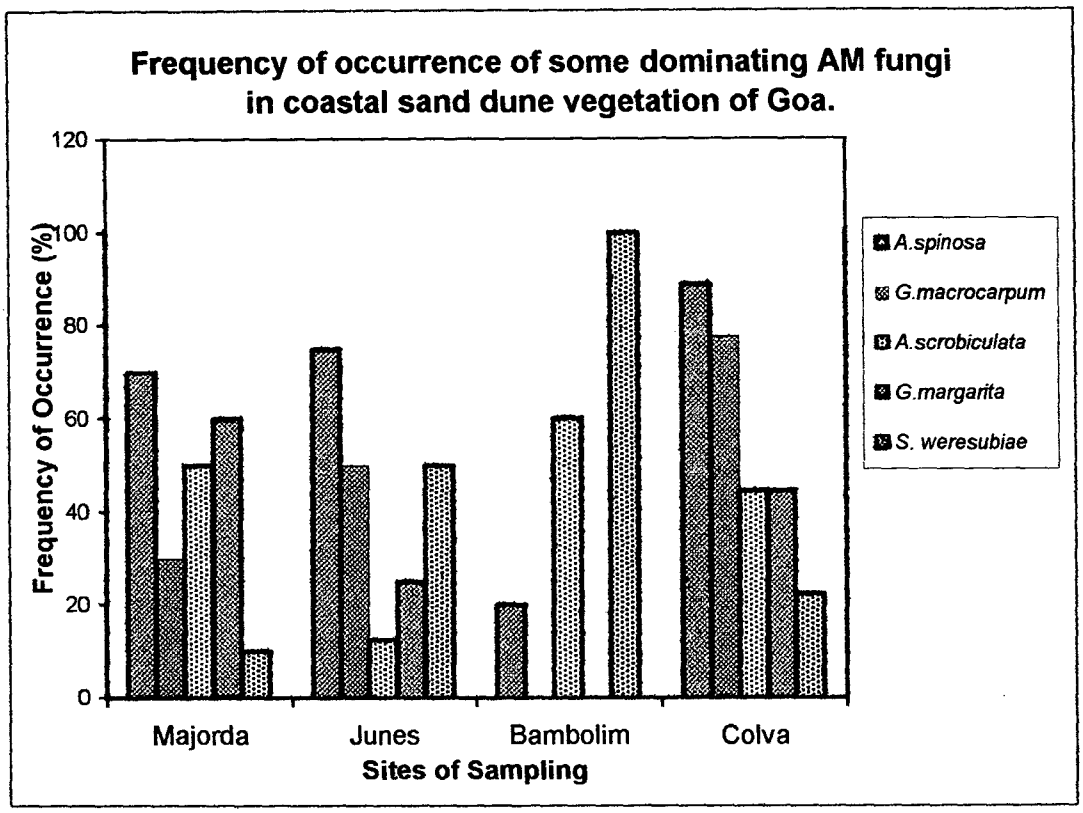

Frequency of occurrence of some dominating AM fungi in coastal sand dune vegetation of Goa.
Frequency of Occurrence (%)
120
100
80
60
40
20
0
Majorda
Junes
Bambolim
Colva
Sites of Sampling
A.spinosa
G.macrocarpum
A.scrobiculata
G.margarita
S. weresubiae

Fig.9

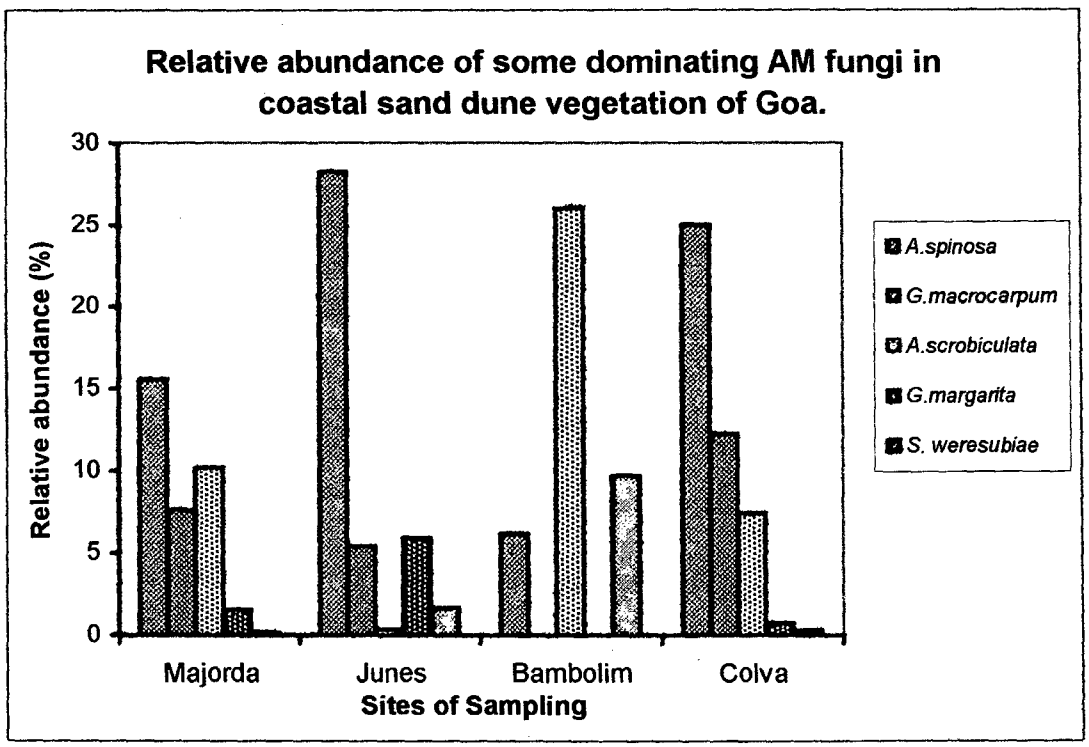

Relative abundance of some dominating AM fungi in coastal sand dune vegetation of Goa.
Relative abundance (%)
30
25
20
15
10
5
0
Majorda
Junes
Bambolim
Colva
Sites of Sampling
A.spinosa
G.macrocarpum
A.scrobiculata
G.margarita
S. weresubiae

Fig.10

clarum, Glomus albidum and Scutellospora gregaria as dominant species. *Glomus macrocarpum, Acaulospora scrobiculata* and *Scutellospora weresubiae* which were dominating species on the Goa coast are not reported from West Coast of India. Host species in concert with different (pH) may account for the differences in species distribution among the different sand dune communities.

The presence of AM fungal species with in spores of other AM fungi was noticed in the sand dune soils. The incidence of spore occupation is reported in many earlier studies from sand dunes (Koske, 1975; 1984). Koske (1984) reported the occurrence of 1-5 different species with in a single dead spore. Our observations are in accordance with him **(Plate-10)**. The dead spores of *Gigaspora sp.* and *Scutellospora sp.* were frequently occupied by *Glomus microaggregatum* which is in agreement with Koske *et al.*, (1986). Dead AM fungal spores may provide favourable microhabitat for spore formation (Koske, 1984).

The survey of dune soils of coastal vegetation in Goa confirms the universal occurrence of AM spores. The spores of *Acaulospora spinosa, Glomus macrocarpum, Acaulospora scrobiculata, Gigaspora margarita* and *Scutellospora weresubiae* were numerous and widely distributed in coastal sand dune vegetation of Goa. An increase in efficiency of phosphorus uptake (Daft and Nicolson, 1966) and drought resistance (Safir *et al.*, 1972) are characteristics of mycorrhizal plants. Sandy soils with a deficiency in phosphorus and subjected to high

temperature and water deficit conditions will favour plants that are mycotrophic. Such plants are of considerable significance in reclamation of disturbed sand dunes. Arbuscular mycorrhizal association may be developed not only to control soil erosion but to evolve a green cover on the coastal sand dunes.

CHAPTER V

SEASONAL DYNAMICS OF ARBUSCULAR MYCORRHIZAL FUNGI IN COASTAL SAND DUNE VEGETATION.

INTRODUCTION

Coastal sand dunes are natural defense structures against shore erosion, protecting the coast by absorbing energy from wind tide and wave action (McHarg, 1972). A few perennial grasses and other plants occurring in the dunes play a major role in stabilizing the coastal sand dunes.

Arbuscular Mycorrhizal (AM) fungi appear to play a vital role in the establishment and survival of dune colonizing plants. The benefits to the host plants in association with arbuscular mycorrhizal fungi, especially in low nutrient environments are well established (Mosse *et al.*,1981). In a nutrient poor environment such as sand dunes, arbuscular mycorrhizal fungi contribute not only to plant nutrition but also to the process of dune stabilization by binding sand grains into wind resistant aggregates (Koske *et al.*,1975; Sutton and Sheppard, 1976; Forster and Nicolson,1981).

There has been constant pressure on coastal sand dune vegetation due to tourism and hotel industries. Dune colonizing plants are routinely employed in the revegetation of eroding dune systems. Because of exposed nature of the habitat and instability of the substrate, young transplants are often subjected to environmental extremes. Wind, salt spray, excessive sand erosion or accretion and extremes in temperature, moisture and nutrient levels affect transplant survival and growth (Wagner, 1964; Woodhouse, 1982). To lessen environmental stresses and obtain rapid growth, the pioneer dune plants may depend on beneficial rhizosphere

microorganisms such as arbuscular mycorrhizal fungi (Koske and Polson, 1984). Thus, arbuscular mycorrhizal fungi contributes to the establishment of these plants in the dunes.

A more ecologically sound approach to dune restoration and management may be encouraged by a better understanding of the seasonal dynamics of arbuscular mycorrhizal fungi in sand dunes. Studies on seasonal changes of arbuscular mycorrhizal fungal spore populations in agricultural soils have reported maximum spore abundance at the end of the growing season (Mason, 1964; Hayman, 1970; Sutton and Barron, 1972 and Saif and Khan, 1975). These studies involved annual host plants, while the species of of arbuscular mycorrhizal fungi associated with perennials in sand dunes may sporulate at different times throughout the year (Sylvia, 1986). Ecological studies on AM fungi from the dune soils are scarce in Tropical and subtropical regions, with the exception of studies in Australia (Koske, 1975; Logan *et. al.*, 1989), Hawaii (Koske, 1988; Koske and Gemma 1990); Brazil (Sturmer and Bellei, 1994) and India (Bhaskran and Selvaraj, 1997).

The present chapter deals with the studies on seasonal variation of AM fungi with respect to Root Colonization, Total Spore density and Species diversity in selected plant species in dune soils. At one of the study sites, Seasonal changes of each of the AM fungal species (with reference to spore abundance) in association with selected plant species was also examined.

MATERIALS AND METHODS

Study site

Sand dunes at two sites *viz.*, Colva and Majorda located in South Goa were taken up for the study.

At Colva site, *Cocos nucifera* L. (Arecaceae), *Casuarina equisetifolia* Forst. (Casuarinaceae), and *Ipomoea pes-caprae* (L.) Sweet, (Convolvulaceae) formed the dominant vegetation. Other plant species present include *Calotropis gigantea* (L.) R.Br. (Asclepiadaceae), *Lantana camara* L. (Verbenaceae), *Vitex trifolia* L. (Verbenaceae), *Fluggea sp.* (Euphorbiaceae), *Hyptis suaveolens* (L.) Poit. (Lamiaceae) and *Achyranthes aspera* L, (Amaranthaceae).

At Majorda site, the vegetation was dominated by *Cocos nucifera* L. (Arecaceae), *Pandanus tectorius* Soland. (Pandanaceae), *Ipomoea pes-caprae* (L.) Sweet, (Convolvulaceae) and *Vitex trifolia* L. (Verbenaceae). Other plant species present include *Lantana camara* L. (Verbenaceae), *Ziziphus rugosa* Lam. (Rhamnaceae), *Bougainvillea spectabilis* Willd. (Nyctaginaceae), *Chromolaena odorata* (L.) King & Robinson (Asteraceae), *Spinifex littoreus* (Burm. f.) Merr. (Poaceae), *Clerodendrum inerme* (L.) Gaertn. (Verbenaceae), *Calotropis gigantea* (L.) R.Br. (Asclepiadaceae) and *Ixora coccinea* L. (Rubiaceae).

Sampling Method

Six plant species were selected for the study from each site. Plants selected from Colva site included *Cocos nucifera, Vitex trifolia, Ipomoea pes-caprae, Lantana camara, Casuarina equisetifolia* and *Calotropis gigantea,* while from Majorda site, *Ipomoea pes-caprae, Cocos nucifera, Lantana camara, Vitex trifolia Clerodendrum inerme* and *Pandanus tectorius* were selected.

Roots and rhizosphere soil samples were collected at the end of every four months (November, March and July). For each plant species, rhizosphere soil samples were collected randomly from 3 individuals of the species. Later, a composite sample was made for each species and worked out. Rhizosphere soil samples were excavated from a depth of 0-25cm, placed in plastic bags, labeled and stored at 10^0 C until processed. While collecting soil samples, soil was dug at three places for each plant so as to cover the entire rhizosphere. While collecting root samples, herbs were dug out entirely. For shrubs and trees the roots were dug and traced back to the plant, which ensured that the roots belonged to the intended species.

Soil analysis

For soil analysis, rhizosphere soils of all the host plant species along with the sub samples were mixed to form a composite sample for each site and was analysed for pH, Moisture content Total N, Total P and Total K.

Estimation of root colonization

Root samples were washed thoroughly under tap water. Feeder roots were cut into 1cm fragments. The composite sample of each species was cleared in 10% KOH, stained with 0.05% Trypan blue (Phillips and Hayman, 1970). Stained roots were mounted in glycerol and observed under Leica Compound Microscope. Percent root colonization was estimated by using Slide technique (Giovanetti and Mosse, 1980). For a sample to be regarded as possessing AM association, it had to be seen to have vesicles, arbuscules or both.

Recovery of AM fungal spores and their identification

Hundred grams of air dried composite sample was assayed for spore count using Wet Sieving and Decanting procedure (Gerdemann and Nicolson,1963). Spores were separated using sieves having mesh sizes ranging from 45μm to 300μm. Each composite sample was processed three times. Spores were observed under Leica Stereomicroscope . Estimation of Spore density was carried out as per the procedure given by Gaur and Adholeya (1994). Only healthy spores were enumerated; broken and parasitised spores were discarded. Spores were mounted in Polyvinyl Lacto Glycerol (PVGL), (Koske and Tessier, 1983), examined for their various characteristics and identified using the standard keys (Schenck and Perez, 1990; Morton and Benny, 1990; Wu, 1993). Spores in the rhizosphere soils were multiplied using *Eleusine coracana* L. as a host plant. The spores

isolated from the pot cultures were later used for confirming the identification of the spores recovered from the dunes.

For Colva site, the Relative abundance of spores for each of the AM fungal species was calculated, while the Frequency of occurrence of AM fungal species was determined for both the sites. Relative abundance and Frequency of occurrence was calculated using the formulas given below:

$$\text{Relative Abundance (\%)} = \frac{\text{Number of AM fungal spores of particular species}}{\text{Total number of AM fungal spores of all species}} \times 100$$

$$\text{Frequency of Occurrence (\%)} = \frac{\text{Number of soil samples that possess spores of particular AM species}}{\text{Total number of soil samples screened}} \times 100$$

RESULTS AND DISCUSSION

The results of soil analysis indicated that the sandy soils of both the sites were alkaline with pH of the soil ranging from 7.1-7.9. Sandy soils of both the sites were poor in nutrients. Total Phosphate (P_2O_5) content obtained was in trace quantities. Total Potassium (K_2O) content ranged from 0.03-0.040%, while Total Nitrogen (N_2) content ranged from 0.040 - 0.096%. It was observed that the Electrical conductivity ranged from 0.168 - 0.478 M mhos/cm. Moisture content in

the soil was maximum in July (5.15-5.95%) and minimum in March (0.41-0.60%) **(Table-21)**.

In both the sites, the maximum average spore density was recorded in early monsoon collections (July), *i.e.* 1154.67 spores + 8.67 sporocarps /100g of soil for Colva site and 374.67 + 9.33 sporocarps/100g of soil for Majorda site, followed by early summer collections (March), *i.e.* 752.67 spores + 17.8 sporocarps/100g of soil for Colva site and 209.33 spores + 2 sporocarps/100g of soil for Majorda site, while minimum average spore density was observed in post monsoon collections (November) *i.e.* 488 spores + 8 sporocarps/100g of soil for Colva site and 146.67 spores + 4.33 sporocarps/100g of soil for Majorda site **(Table-22; Fig. 11 & 12)**.

Root colonization was observed throughout the year. Average percentage root colonization was maximum in post monsoon collections *viz.,* 95.79 % for Colva site and 94.92 % for Majorda site. This was followed by early monsoon collections *viz.,* 77.16 % for Colva site and 86.83 % for Majorda site, while minimum percentage root colonization was recorded in early summer *viz.,* 54.02 % for Colva site and 60.61 % for Majorda site. **(Table-22; Fig. 11 & 12)**. In the sand dunes, variation in spore density of AM fungi and AM infection are attributed to seasonal changes (Koske, 1981; Mohan Kumar *et al.*, 1988). Seasonal variations can have remarkable influence on the occurrence of AM spores (Nicolson and Johnston, 1979).

Table-21: Chemical characteristics of sand dune soils in different seasons.

Site	Month	pH	Moisture content (%)	E. C. M mhos/cm	Total N_2 (%)	Total P_2O_5 (%)	Total K_2O (%)
Colva	July	7.2 (0.05)	5.95 (0.04)	0.168 (0.001)	0.096 (0.002)	Traces	0.030 (0.001)
	November	7.1 (0.01)	0.72 (0.02)	0.202 (0.002)	0.080 (0.005)	Traces	0.035 (0.002)
	March	7.6 (0.05)	0.60 (0.0)	0.478 (0.002)	0.040 (0.005)	Traces	0.040 (0.004)
Majorda	July	7.4 (0.1)	5.15 (0.08)	0.305 (0.001)	0.050 (0.005)	Traces	0.030 (0.0)
	November	7.7 (0.1)	0.56 (0.05)	0.168 (0.001)	0.068 (0.003)	Traces	0.030 (0.0)
	March	7.9 (0.05)	0.41 (0.01)	0.333 (0.002)	0.058 (0.004)	Traces	0.030 (0.0)

Values are the mean of 3 readings.
Values in parenthesis indicate Standard deviation.

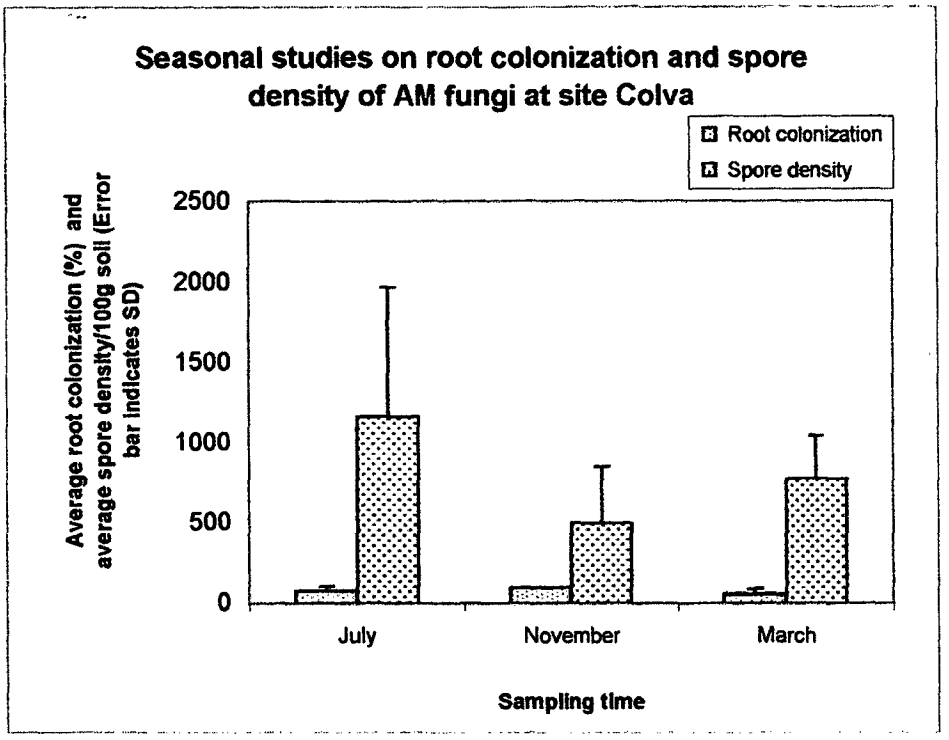

Seasonal studies on root colonization and spore density of AM fungi at site Colva
Root colonization
Spore density
Average root colonization (%) and average spore density/100g soil (Error bar indicates SD)
2500
2000
1500
1000
500
0
July
November
March
Sampling time

Fig.11

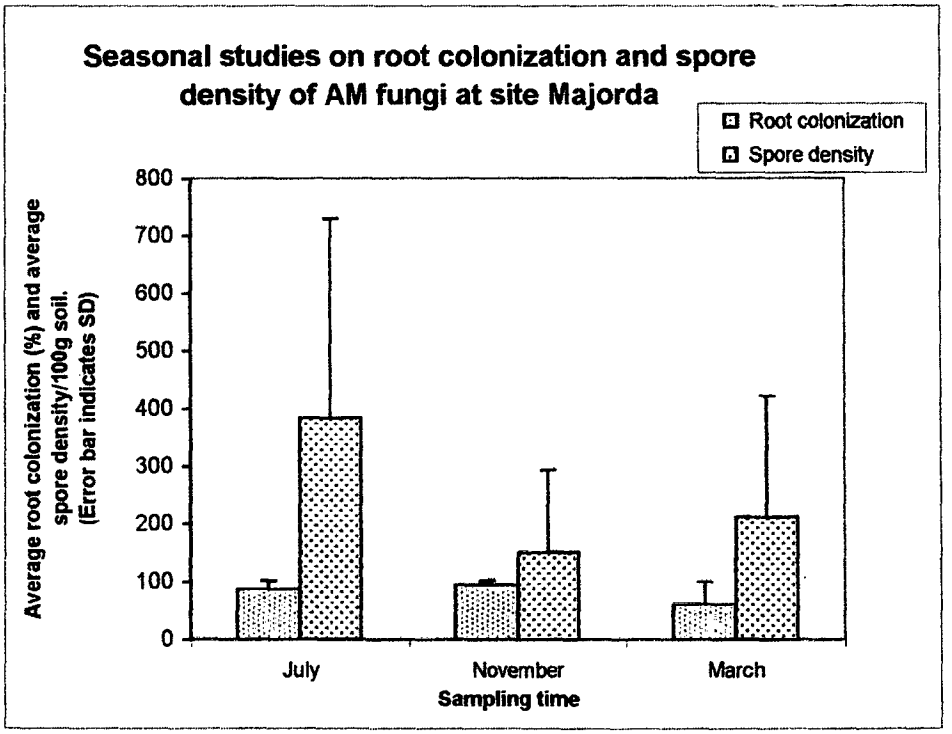

Seasonal studies on root colonization and spore density of AM fungi at site Majorda
Root colonization
Spore density
Average root colonization (%) and average spore density/100g soil. (Error bar indicates SD)
800
700
600
500
400
300
200
100
0
July
November
March
Sampling time

Fig.12

In the present study spore population increased from March to July; Thereafter, it declined and was minimum in November. This is in accordance to Mohankumar and Mahadevan (1988), who reported similar observation in seasonal studies in Tropical forest soils. Bhaskaran and Selvaraj (1997) also reported minimum number of spores in November and thereafter increase from January onwards in saline soils. Percent root colonization was minimum in March, thereafter it increased in July to November. Earlier studies have shown that rainfall or soil wetting could stimulate AM fungal spore germination (Gemma and Koske, 1988) which may result in an inverse relation as found in the present study. Newman *et al.* (1986) found a reduction in percentage of infection intensity in dry season. Redhead (1975) found that AM infection tended to decrease under low rainfall.

In *Casuarina equisetifolia* maximum spore density (864 spores + 4 sporocarps/ 100g soil) was recorded in summer and minimum was recorded in early rains (28 spores), while in *Calotropis gigantea* and *Clerodendrum inerme* there was not much fluctuation in spore population and root infection with change in season **(Table-22)**. Differences in host plants and soil fertility stimulates differential sporulation by AM fungal species in the field (Schenck and Kinlock, 1980). The spore population is found to have seasonal variation and also varies according to different hosts and also at different sites (Sparling and Tinker, 1975).

Table-22a: Seasonal studies on root colonization and spore density of AM fungi in dune vegetation of site Colva.

Month=>	July				November				March			
Host plant	**I%**	**V**	**A**	**Spore Density/ 100g soil**	**I%**	**V**	**A**	**Spore Density/ 100g soil**	**I%**	**V**	**A**	**Spore Density/ 100g soil**
Cocos nucifera	32.76 (1.08)	+	-	2144 (435.67) spores +28 (4) sporocarps	93.00 (7)	+	+	372 (49.03) spores +16 (8) sporocarps	83.33 (9.51)	+	-	1156 (319.69) spores +60 (12) sporocarps
Vitex trifolia	67.20 (6.9)	+	+	1860 (255.34) spores +4(4) sporocarps	93.33 (15.49)	+	-	156 (22.61) spores	20.59 (0.52)	+	-	848 (188.13) spores
Ipomoea pes-caprae	100.00 (0)	+	+	940 (60) spores +8 (4) sporocarps	97.51 (3.27)	+	+	984 (72.04) spores +20 (8) sporocarps	36.36 (3.47)	+	-	620 (130) spores +4 (4) sporocarps
Lantana camara	68.00 (5)	+	-	1396 (155) spores +8 (12.22) sporocarps	100.00 (0)	+	+	720 (160.93) spores +4 (1) sporocarps	87.09 (2.87)	+	+	408 (88.26) spores +16 (7.55) sporocarps

(Contd.)

Month=>	July				November				March			
Host plant	**I%**	**V**	**A**	**Spore Density/ 100g soil**	**I%**	**V**	**A**	**Spore Density/ 100g soil**	**I%**	**V**	**A**	**Spore Density/ 100g soil**
Casuarina equisetifolia	95.00 (2)	+	-	28 (6) spores	90.91 (11.4)	+	-	76 (17.78) spores	10.71 (1.12)	+	-	864 (117.85) spores +4 (4) sporocarps
Calotropis gigantea	100.00 (0)	+	+	560 (87.18) spores +4 (1) sporocarps	100.00 (0)	+	-	620 (135) spores +8 (2) sporocarps	86.04 (10.06)	+	-	620 (141.07) spores +20 (12) sporocarps
Average	**77.16**			**1154.67 spores +8.67 sporocarps**	**95.79**			**488 spores + 8 sporocarps**	**54.02**			**752.67 spores +17.8 sporocarps**

I = AM fungal Infection; V = Vesicular Infection; A = Arbuscular Infection.
Values are the mean of 3 readings.
Values in the parenthesis indicate Standard deviation.

Table-22b: Seasonal studies on root colonization and spore density of AM fungi in dune vegetation of site Majorda.

Month=>	July				November				March			
Host plant	**I%**	**V**	**A**	**Spore Density/ 100g soil**	**I%**	**V**	**A**	**Spore Density/ 100g soil**	**I%**	**V**	**A**	**Spore Density/ 100g soil**
Ipomoea pes-caprae	87.00 (3)	+	+	408 (92) spores +8 (4) sporocarps	100.0 0 (0)	+	+	90 (10) spores +2 (2) sporocarps	46.66 (4.05)	+	+	284 (46) spores
Cocos nucifera	64.00 (8)	+	-	960 (240) spores +38 (13) sporocarps	92.73 (8.08)	+	+	396 (91.15) spores +6 (3) sporocarps	27.27 (1.21)	+	-	528 (89.35) spores +6 (3) sporocarps
Lantana camara	95.00 (4)	+	-	460 (102.59) spores	97.78 (6)	+	+	144 (16) spores +4 (1) sporocarps	92.72 (4.96)	+	-	336 (64) spores +6 (3) sporocarps
Vitex trifolia	75.00 (7)	+	+	96 (14) spores +4 (4) sporocarps	82.00 (6)	+	+	12 (4) spores +2 (2) sporocarps	7.00 (1)	+	-	12 (1) spores

(Contd.)

ıth=>	July				November				March			
lost lant	**I%**	**V**	**A**	**Spore Density/ 100g soil**	**I%**	**V**	**A**	**Spore Density/ 100g soil**	**I%**	**V**	**A**	**Spore Density/ 100g soil**
odend n me	100.00 (0)	+	+	20 (6.24) spores +2 (2) sporocarps	100.00 (0)	+	+	28 (9.16) spores +2 (1) sporocarps	100.00 (0)	+	+	44 (10.58) spores
danus rius	100.00 (0)	-	+	304 (83.07) spores +4 (4) sporocarps	97.00 (3)	+	+	210 (70) spores +10 (4) sporocarps	90.00 (7.63)	+	+	52 (18.03) spores
erage	**86.83**			**374.67 spores +9.33 sporocarps**	**94.92**			**146.67 spores +4.33 sporocarps**	**60.61**			**209.33 spores +2 sporocarps**

I = AM fungal Infection; V = Vesicular Infection; A = Arbuscular Infection
Values are the mean of 3 readings.
Values in the parenthesis indicate Standard deviation.

In all, a total of 27 species of AM fungi belonging to 5 genera *viz.*, *Acaulospora, Gigaspora, Glomus, Sclerocystis* and *Scutellospora* were recorded from the rhizosphere soil samples of both the sites. Identified AM fungi include *Scutellospora calospora* Walker and Sanders, *Scutellospora coralloidea* Walker and Sanders, *Scutellospora gregaria* Walker and Sanders, *Scutellospora pellucida* Walker and Sanders, *Scutellospora verrucosa* Walker and Sanders, *Scutellospora weresubiae* Koske and Walker, *Sclerocystis clavispora* Trappe, *Sclerocystis coremioides* Berk. and Broome, *Sclerocystis sinuosa* Gerdemann and Bakshi , *Sclerocystis taiwanensis* Wu and Chen, *Glomus constrictum* Trappe, *Glomus fasciculatum* Gerdemann and Trappe emend. Walker and Koske, *Glomus geosporum* Walker, *Glomus heterosporum* Smith and Schenck, *Glomus hoi* Berch and Trappe, *Glomus macrocarpum* Tul and Tul, *Glomus maculosum* Miller and Walker, *Glomus microaggregatum* Koske, Gemma and Olexia, *Glomus multisubtensum* Mukerji, Bhattacharjee and Tewari, *Glomus tenebrosum* Berch and Fortin, *Gigaspora albida Schenck and Smith, Gigaspora margarita* Becker and Hall, *Acaulospora bireticulata* Rothwell and Trappe, *Acaulospora dilatata* Morton, *Acaulospora elegans* Trappe and Gerdemann, *Acaulospora scrobiculata* Trappe, *Acaulospora spinosa* Walker and Trappe (**Table-23 and 24**). At both the sites, *Scutellospora coralloidea* was the most frequently recovered fungus in all the seasons.

Table-23: Seasonal study on frequency distribution of AM fungal spores at site Colva.

AM Fungi	Months																		Frequency %		
	July						November						March								
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	July	Nov.	Mar.
Scutellospora coralloidea	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	83.33	100	83.33
S.calospora	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	33.33	-	-
S. gregaria	-	-	-	-	-	+	-	-	-	-	+	-	-	-	+	-	-	-	16.67	16.67	16.67
S.verrucosa	-	-	+	-	-	+	-	-	-	+	-	+	-	-	+	+	-	+	33.33	33.33	50
S. weresubiae	-	-	-	-	-	-	+	+	+	+	-	-	-	+	-	-	-	-	-	66.67	16.67
Scutellospora sp.	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	16.67
Sclerocystis sinuosa	-	-	+	+	-	+	-	-	+	+	-	-	+	-	+	+	+	+	50	33.33	83.33
S .taiwanensis	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	16.67
Sclerocystis sp.	+	+	-	-	-	-	+	-	-	-	-	+	-	-	+	-	-	-	33.33	33.33	16.67
Glomus fasciculatum	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	+	+	-	16.67	-	50

(Contd.)

AM fungi	Month																		Frequency %		
	July						November						March								
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	July	Nov.	Mar.
G. geosporum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	16.67
G. heterosporum	+	+	+	+	-	+	-	-	-	+	+	-	+	+	-	+	-	+	83.33	33.33	66.67
G. hoi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	16.67
G. macrocarpum	+	-	+	+	-	+	-	-	-	+	+	+	+	-	+	+	-	+	66.67	50	16.67
G. maculosum	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	33.33	-	-
G. tenebrosum	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	33.33
Glomus sp.	+	+	+	+	-	+	+	+	+	-	-	+	+	+	+	-	-	+	83.33	66.67	66.67
Gigaspora margarita	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	+	16.67	16.67	66.67
Gigaspora sp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	16.67
Acaulospora bireticulata	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	33.33
A. elegans	-	-	+	-	-	-	+	-	+	+	-	+	-	+	+	-	-	-	16.67	66.67	33.33

(Contd.)

AM fungi	Month																		Frequency %		
	July						November						March								
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	July	Nov.	Mar.
A. Scrobiculata	-	+	-	-	-	-	-	-	+	-	-	-	+	+	+	-	-	+	16.67	16.67	66.67
A. spinosa	-	-	+	-	-	+	+	+	-	-	+	+	+	+	+	+	-	+	33.33	66.67	83.33
Acaulospora sp.	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	+	-	+	16.67	33.33	33.33

Legend:

1. = *Lantana camara* 2. = *Vitex trifolia* 3. = *Calotropis gigantea*
4. = *Cocos nucifera* 5. = *Casuarina equisetifolia* 6. = *Ipomoea pes-caprae*

+ = Present; - = Absent

At Colva site, *Scutellospora coralloidea* was most frequent in all the seasons with frequency of occurrence being 83.33% in July and March and !00% in November respectively. Among the other AM species, the presence of *Sclerocystis sinuosa, Scutellospora gregaria, Scutellospora verrucosa, Glomus macrocarpum, Glomus heterosporum, Gigaspora margarita, Acaulospora elegans, Acaulospora scrobiculata* and *Acaulospora spinosa* were observed in all the seasons. *Acaulospora spinosa ,Acaulospora elegans* and *Scutellospora weresubiae* were more frequently occurring fungi in post monsoon collections, each with frequency of 66.67%. *Acaulospora spinosa* and *Sclerocystis sinuosa* were more frequently occurring fungi in early summer collections, each with frequency of 83.33%. *Glomus heterosporum* and *Glomus macrocarpum* were more frequently occurring fungi in early monsoon with frequencies of 83.33% and 66.67% respectively (**Table- 23; Fig. 13**).

At Majorda site, the frequency of occurrence of *Scutellospora coralloidea* was 50% in post monsoon collections. In early monsoon and early summer collections its frequency was 66.67% and 83.33% respectively. Among the other species, *Scutellospora gregaria, Sclerocystis clavispora, Glomus macrocarpum* and *Acaulospora spinosa* were present in all the seasons. In early monsoon samples, *Scutellospora gregaria* and *Acaulospora scrobiculata* were more frequent each with frequency of 66.67%. In post monsoon samples *Scutellospora gregaria* was more frequent with frequency of 66.67% **(Table-24)**.

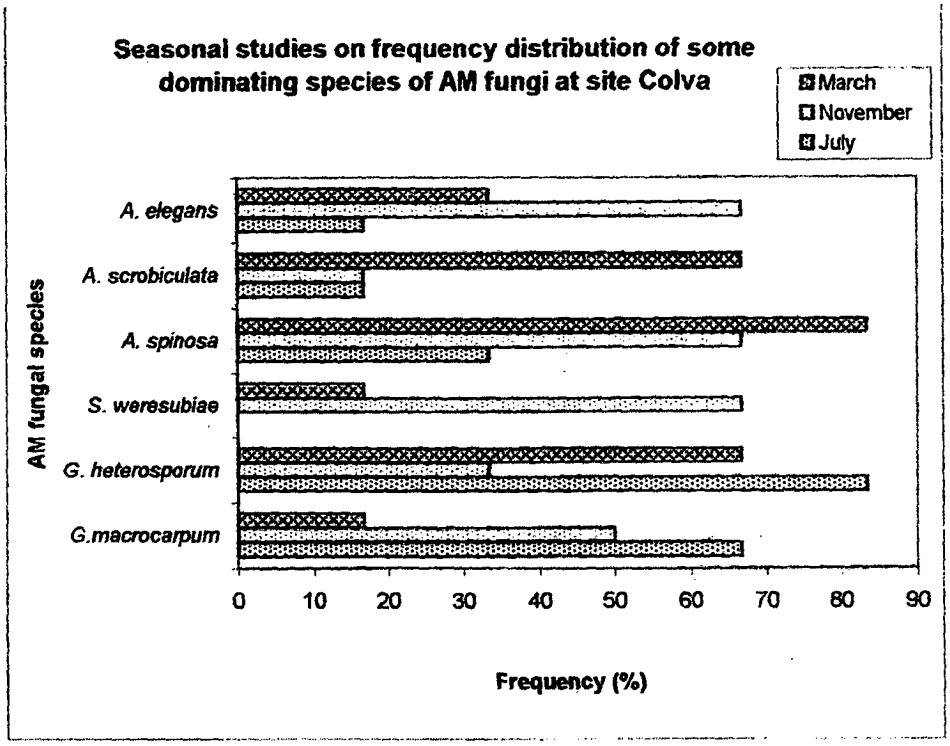

Seasonal studies on frequency distribution of some dominating species of AM fungi at site Colva
March
November
July
A. elegans
A. scrobiculata
A. spinosa
S. weresubiae
G. heterosporum
G.macrocarpum
AM fungal species
0
10
20
30
40
50
60
70
80
90
Frequency (%)

Fig.13

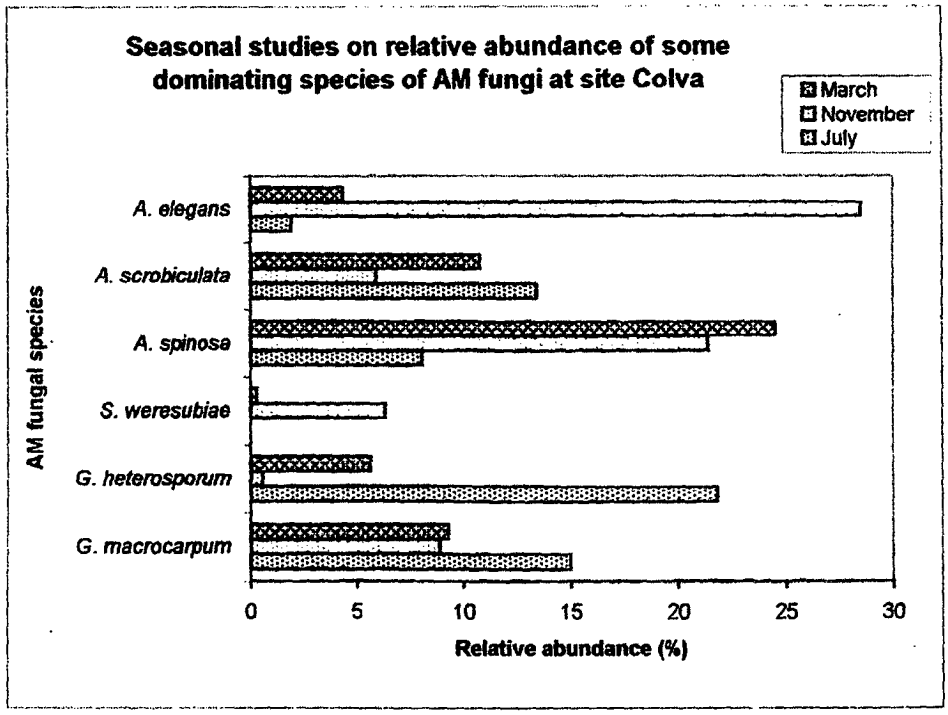

Seasonal studies on relative abundance of some dominating species of AM fungi at site Colva
March
November
July
A. elegans
A. scrobiculata
A. spinosa
S. weresubiae
G. heterosporum
G. macrocarpum
AM fungal species
0
5
10
15
20
25
30
Relative abundance (%)

Fig.14

Table-24: Seasonal study on frequency distribution of AM fungal spores at site Majorda.

AM Fungi	Months																		Frequency %		
	July						November						March								
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	July	Nov.	Mar.
Scutellospora coralloidea	+	+	-	-	+	+	-	+	+	-	-	+	+	+	+	+	-	+	66.67	50	83.33
S. calospora	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	16.67	-	-
S. gregaria	+	+	+	-	-	+	+	-	+	+	+	-	-	-	-	-	+	-	66.67	66.67	16.67
S. pellucida	-	-	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	33.33	-	16.67
S. verrucosa	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	16.67	-	16.67
S. weresubiae	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	33.33	-
Scutellospora sp.	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	16.67	16.67	16.67
Sclerocystis clavispora	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	16.67	33.33	16.67
S.coremioides	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	16.67	-	-

(Contd.)

AM Fungi	Month																		Frequency %		
	July						November						March								
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	July	Nov.	Mar.
S. sinuosa	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	33.33	16.67	-
Sclerocystis sp.	-	-	-	+	+	-	+	+	+	+	-	+	-	-	+	-	-	-	33.33	83.33	16.67
Glomus constrictum	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	16.67	-
G. fasciculatum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	16.67
G. heterosporum	-	+	+	-	+	-	-	-	-	-	-	-	-	+	+	-	+	+	50	-	50
G. macrocarpum	+	-	+	-	-	-	+	-	+	-	+	-	-	+	-	-	-	-	33.33	50	16.67
G. maculosum	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	16.67	-	-
G. micro-aggregatum	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	33.33	-	-
G. multisubtensum	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16.67	-	-

(Contd.)

AM Fungi	Month																		Frequency %		
	July						November						March								
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	July	Nov.	Mar.
Glomus sp.	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	+	66.67	100	50
Gigaspora albida	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	33.33	-	-
G. margarita	+	-	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	50	16.67	-
Gigaspora sp	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	33.33	-	-
Acaulospora dilatata	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	16.67	-	-
A. elegans	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-	-	33.33	16.67
A. scrobiculata	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	66.67	16.67	-
A. spinosa	+	-	-	-	+	+	-	-	+	-	+	-	-	-	+	-	+	-	50	33.33	33.33
Acaulospora sp.	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	33.33	-	16.67

Legend:

1. = *Lantana camara* 2. = *Vitex trifolia* 3. = *Calotropis gigantea*
4. = *Cocos nucifera* 5. = *Casuarina equisetifolia* 6. = *Ipomoea pes-caprae*

+ = Present; - = Absent

For Colva site, quantitative analysis of different spore types obtained revealed that the spores belonging to genera *Glomus* and *Acaulospora* were most abundant in all the seasons. *Glomus* species was most abundant in early monsoon collections (70.37%), followed by early summmer collections (53.68%) and minimum in post monsoon collections (26.21%). *Acaulospora* species were abundant in November and March collections with relative abundance 60.48% and 42.42% respectively and minimum in July (25.96%). *Scutellospora* species was most abundant in November (11.42%). *Sclerocystis* species was most abundant in March (2.25%). The relative abundance of *Gigaspora species* remained low throughout the season (0.26-0.29%), **(Table-25)**.

At the species level *Acaulospora spinosa* was most abundant in March (24.50%), *Acaulospora elegans* was most abundant in November (28.5%) and *Acaulospora scrobiculata* was most abundant in July (13.41%), **(Table-26; Fig. 14)**. Our result shows that high spore abundance of a species is associated with low abundance of other species. This is in accordance with Gemma *et al.*,1989. Interspecific competition appeared to be a major factor determining the abundance of spores of the AM species. Sylvia (1986) reported that while the overall populations of AM fungi increased as plants matured, sporulation by individual species was not synchronous. This phenomenon was more prominent in host plants *Vitex trifolia, Calotropis gigantea* and *Cocos nucifera* **(Table-26)**. The different sporulation patterns present among the species appear either to minimize the extent of simultaneous competition for substrate from the host during sporulation

Table-25: Seasonal studies on relative abundance of AM fungal spores of different genera at site Colva.

AM species	Total Spore density / Season			Relative Abundance		
	July	November	March	July	November	March
Acaulospora	1812	1800	1960	25.96	60.48	42.42
Gigaspora	20	8	12	0.29	0.27	0.26
Glomus	4912	780	2480	70.37	26.21	53.68
Sclerocystis	52	48	104	0.74	1.61	2.25
Scutellospora	184	340	64	2.64	11.42	1.39

Table-26: Seasonal study on quantitative analysis of AM fungal spores and there relative abundance at site Colva

Month=>	Spore density per 100g soil sample																		Relative Abundance (%)		
	July						November						March								
Fungi`	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	July	Nov.	Mar.
tellospora ılloidea	4	8	28	8	16	-	16	4	68	4	12	12	8	8	4	4	4	0	0.92	3.89	0.61
ılospora	-	-	-	-	12	64	-	-	-	-	-	-	-	-	-	-	-	-	1.09	-	-
regaria	-	-	-	-	-	8	-	-	-	-	8	-	-	-	4	-	-	-	0.11	0.27	0.09
errucosa	-	-	20	-	-	16	-	-	-	8	-	20	-	-	8	4	-	4	0.52	0.94	0.35
veresubiae	-	-	-	-	-	-	80	32	68	8	-	-	-	12	-	-	-	-	-	6.32	0.26
tellospora	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	0.09
erocystis ıosa	-	-	4	28	-	8	-	-	8	16	-	-	12	-	8	60	4	4	0.57	0.81	1.90
ıiwanensis	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	0.09
erocystis sp.	8	4	-	-	-	-	4	-	-	-	-	20	-	-	12	-	-	-	0.17	0.81	0.26

(Contd.)

ıth=>	Spore density per 100g soil sample																		Relative Abundance (%)		
	July						November						March								
Fungi	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	July	Nov.	Mar.
mus iculatum	-	-	-	280	-	-	-	-	-	-	-	-	104	-	-	344	840	-	4.01	-	27.88
eosporum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	-	-	-	0.35
rosporum	464	300	84	532	-	140	-	-	-	12	4	-	104	76	-	64	-	16	21.78	0.54	5.63
ioi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	48	-	-	-	-	1.04
enebrosum	-	-	-	-	-	-	-	-	-	-	-	-	-	96	-	-	-	44	-	-	3.03
crocarpum	464	-	84	356	-	140	-	-	-	32	8	224	96	-	40	180	-	112	14.96	8.87	9.26
naculosum	-	300	-	432	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10.48	-	-
mus sp.	464	296	80	356	-	140	60	12	132	-	-	296	20	112	136	-	-	32	19.14	16.80	6.49
aspora rgarita	-	20	-	-	-	-	8	-	-	-	-	-	-	-	-	4	-	4	0.29	0.27	0.17
aspora sp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	0.09

(Contd.)

nth=>	Spore density per 100g soil sample																		Relative Abundance (%)		
	July						November						March								
Fungi	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	July	Nov.	Mar.
reticulata	-	-	-	-	-	-	-	-	-	-	-	-	-	8	20	-	-	-	-	-	0.61
legans	-	-	132	-	-	-	268	-	176	188	-	216	-	20	180	-	-	-	1.89	28.5	4.33
ulospora biculata	-	936	-	-	-	-	-	-	176	-	-	-	40	336	20	-	-	100	13.41	5.91	10.74
spinosa	-	-	132	-	-	432	268	108	-	-	44	216	32	180	208	464	-	248	8.08	21.37	24.50
ulospora sp.	-	-	-	180	-	-	20	-	-	120	-	-	-	-	-	44	-	60	2.58	4.7	2.25

Legends:

1. = *Lantana camara* 2. = *Vitex trifolia* 3. = *Calotropis gigantea*
4. = *Cocos nucifera* 5. = *Casuarina litorea* 6. = *Ipomoea pes-caprae*

- = Absent

or to result from individual species being able to outcompete other species at certain times for host photosynthate or cortical cells.

Some associations between a specific fungus and a host obviously favoured spore production by certain fungi. Spore production by *Scutellospora coralloidea* was greatly stimulated in presence of *Calotropis gigantea* over that with other plants. Spore production of *Acaulospora spinosa, Acaulospora elegans* and *Acaulospora scrobiculata* was greatest with *Ipomoea pes-caprae*, *Calotropis gigantea* and *Vitex trifolia,* respectively **(Table-26).**

Quantitative assessment of fungal diversity currently relies solely on enumeration of the spores of the organisms forming mycorrhizae in plant communities (Koske, 1987). Seasonal changes and phenological state of the host influence mycorrhizal colonization as well as sporulation (Giovannetti, 1985), although vegetative growth of the fungi and their capacity to sporulate are not necessarily corelated (Abbott and Robson, 1991). Thus, the absence of sporulation does not indicate the absence of fungal organism. The presence of spores does indicate, however, that mycorrhizal biomass of detected organism has reached critical mass of sporulation and quantity of spores may provide some measure of fungal fitness.

CHAPTER VI

TAXONOMY OF ARBUSCULAR MYCORRHIZAL (AM) FUNGI OCCURRING IN COASTAL SAND DUNE VEGETATION OF GOA.

INTRODUCTION

Until recently, the order Endogonales (Zygomycotina) has consisted of only one family, the Endogonaceae (Benjamin, 1979; Morton, 1988) **(Table 27)**. Six genera *(Acaulospora* Gerdemann and Trappe emend. Berch, *Entrophospora* Ames and Schneider, *Gigaspora* Gerdemann and Trappe, *Glomus* Tulasne and Tulasne, *Sclerocystis* Berkeyley and Broome and *Scutellospora* Walker and Sanders were included whose members formed an arbuscular mutualistic symbiosis with many terrestrial plant families (Trappe, 1987) and one Genus (Endogone Link:Fr.) whose members were saprobic (Gerdemann and Trappe, 1974) or formed putative ectomycorrhizal associations (Chu-Chou and Grace, 1979; Fassi *et al.*, 1969). Pirozynski and Dalpe (1989) described a new family Glomaceae Pirozynski and Dalpe containing two Genera, *Glomus* and *Sclerocystis.* Morton and Benny (1990) emended the family Glomaceae and erected a new order Glomales and two new families Acaulosporaceae and Gigasporaceae **(Table 27)**.

CLASSIFICATION OF ARBUSCULAR MYCORRHIZAL (AM) FUNGI (ZYGOMYCETES) (MORTON AND BENNY, 1990).

Order: Glomales

Fungi, forming arbuscular endomycorrhizae in mutualistic symbiosis with living plants. Sexual reproduction rare. Asexual reproduction by spores resembling

chlamydospores and azygospores. Spores formed singly, but also in aggregates or in sporocarps.

Table-27: Classification of AM fungi.

Old Classification (Gerdemann and Trappe,1974) (Benjamin, 1979, Warcup,1990)	**Present Classification (Morton and Benny, 1990)**
Order: Endogonales	**1. Order: Endogonales**
Family: Endogonaceae	Family: Endogonaceae
Genera: *Endogone*	Genera: *Endogone*
Sclerogone	*Sclerogone*
Glomus	
Sclerocystis	**2. Order: Glomales**
Acaulospora	Suborder: Glomineae
Entrophospora	i)Family: Glomaceae
Gigaspora	Genera: *Glomus*
Scutellospora	*Sclerocystis*
	ii)Family: Acaulosporaceae
	Genera: *Acaulospora*
	Entrophospora
	Suborder: Gigasporineae
	Family: Gigasporaceae
	Genera: *Gigaspora*
	Scutellospora

I) Suborder: Gigasporineae

Only arbuscules formed in mycorrhizal roots; Azygospores produced on the apex of a sporogenous cell of a fertile hypha; auxiliary cells formed.

I A) Family: Gigasporaceae

Germ tubes produced directly through the wall or from a germination shield.

I Aa) Genus: *Gigaspora*

Germ tubes produced directly through spore wall; inner flexible wall groups absent; auxiliary cells finely papillate or echinulate.

Ab) Genus: *Scutellospora*

Germ tubes from germination shield; inner flexible wall group always present; auxiliary cells knobby, broadly papillate or smooth.

II) Suborder: Glomineae

Arbuscules and vesicles formed in mycorrhizal roots. Spores produced terminally or laterally on or within fertile hyphae.

II A) Family: Glomaceae

Chlamydospores formed apically from fertile hyphae.

II Aa) Genus: *Sclerocystis*

Fruiting body of a sporocarp composed of spores with lateral walls adherent to one another; connecting hyphae embedded in a central hyphal plexus; chlamydospores in a single layer except at the base; base composed of a sterile hyphae.

II Ab) Genus: *Glomus*

Fruiting structure of a sporocarp not formed as mentioned above; spores also produced singly or in loose to tight aggregates in soil less commonly in roots.

II B) Family: Acaulosporaceae

Azygospores formed or within the neck of a sporiferous saccule.

II Ba) Genus: *Acaulospora*

Spores arise laterally from the neck of a sporiferous saccule.

II Bb) Genus: *Entrophospora*

Spores formed in the neck of the sporiferous saccule.

The previous chapters deal with the survey studies of AM fungi in Coastal sand dune vegetation of eight different sites in Goa *viz.*, Varca, Mobor, Benaulim, Colva and Majorda (in the South) and Bambolim, Miramar and Junes (Mandrem) in the North. A total of 51 AM fungal species were recovered and identified during the study. The objective of the present chapter is to describe the morphological features of the AM fungal spores recovered and identified from the dunes. Spelling of scientific names of the identified AM fungi are in accordance with (Walker and Trappe, 1993)

DISTINCT MORPHOLOGICAL FEATURES OF THE IDENTIFIED AM FUNGAL SPECIES.

***Acaulospora bireticulata* Rothwell and Trappe**

Spores formed singly in soil, subhyaline-light brown, globose, 150-155 μm in diam. Spore surface ornamented with a polygonal reticulum, the ridges 2 x 1.5-2 μm with sinuous, dark greyish – green sides and a paler, depressed central stratum; ridges occasionally branched towards the center of polygons or forming irregular, isolated projections at polygon centers. Polygon 6 -18 μm long, the enclosed spore surface beset with round – tipped, 4- to 6- sided processes 1 x 1 μm to give the appearance of an inverted reticulum. Spore walls of three layers, each 1 μm thick, the outer layer dark greyish green to greyish brown, the inner layers hyaline.

***Acaulospora delicata* Walker, Pfeiffer & Bloss. (Plate 4, Fig. a)**

Spores formed singly in soil, hyaline to pale yellowish - cream, globose to subglobose, 80-125(-150) x 80-110(-140) μm. Spore wall structure of 4 walls in two groups (A & B). Wall group A consisting of a thin, hyaline, outer evanescent wall (wall 1) 1μm thick, closely attached to wall 2 which is relatively thick (2.5-3.5 μm) laminated wall with up to 6 sub equal laminations. Wall group B with 2 thin, hyaline membranous walls (wall 3 & 4) 0.5 μm and 0.75-1 μm thick respectively. Wall 3 covered by minute granular excrescences.

PLATE-4

SPORES OF ARBUSCULAR MYCORRHIZAL FUNGI

a) Spore of *Acaulospora delicata* (x 400).

b) Spore surface ornamentation of *Acaulospora foveata* (x 400).

c) Spore of *Acaulospora rugosa* (x 400)

d) Spore surface ornamentation of *Acaulospora scrobiculata* (x 1000).

e) Spore of *Acaulospora spinosa* (x 400).

f) Spore of *Acaulospora undulata* (x 400).

a
b
c
d
e
f

***Acaulospora dilatata* Morton.**

Spores formed singly in soil, deep yellow, globose to subglobose, (78-) 106 (130) μm in diam. Spore wall structure consisting of 5 walls in three groups. (A, B & C). Wall group A an outer yellow laminated wall, 2.6-4.5 μm thick, outer spore surface roughened with minute pits. Wall group B of two adherent rigid hyaline walls (walls 2 & 3) each 0.5-1 μm thick. Wall group C with 2 hyaline walls (wall 4 & 5); wall 4 membranous 0.5-1 μm thick; wall 5 amorphous and elastic.

***Acaulospora elegans* Trappe & Gerdemann**

Spores formed singly in soil, dull, dark brown, globose to subglobose, 140-156 (-330) μm in diam. Spore surface ornamented with crowed light brown spines 2 x 0.5 μm, soon developing an alveolate reticulum of hyaline ridges, 5-6 x 1μm super imposed on the spines, alveoli 4-8 μm long. Spore wall with an outer layer up to 12 μm thick (including spines and ridges), enclosing 3 hyaline walls which total up to 15 μm thick.

***Acaulospora foveata* Trappe & Janos (Plate 4, Fig. b)**

Spores formed singly in soil, yellowish brown to reddish brown, globose to subglobose, 185-195(-410) x 215-240(-480) μm. Spore surface uniformly pitted with round to oblong or occasionally irregular depressions 4-8(-12) x 4-16 μm, with rounded bottoms, separated by ridges 1-12 μm broad. Outer spore wall

yellowish or reddish brown, 11-15 μm thick, with an adherent but separable, hyaline inner layer 3 μm thick. Spore contents of small hyaline guttules.

***Acaulospora laevis* Gerdemann & Trappe**

Spores formed singly in soil, sessile, dull yellow to yellow-brown to red-brown in colour, smooth, globose to subglobose, 119- 125(-300) x 119-130(-520) μm in diam. Spore wall consisting of 3 layers; A rigid, yellow-brown to red-brown outer wall 2-4 μm thick, and two hyaline inner membranes. Spore contents globose to somewhat polygonal.

***Acaulospora mellea* Spain & Schenck**

Spores formed singly in soil, honey-coloured to yellow-brown, subglobose, 96-130 x 78-92 μm. Spore wall 4-8(-11) μm thick, consisting of 3 separable walls; the outermost wall, wall 1, yellow-brown to dark brown, 2-6 μm thick, laminate, inseparable from wall 2, 0.5 μm thick; wall 3 hyaline to light yellow, membranous, 0.5-1 μm thick: wall 4 and 5 membranous.

***Acaulospora morrowiae* Spain & Schenck**

Spores formed singly in soil, light yellow, globose or subglobose, (63-)79-92(-120) μm in diam. Spore wall 2-4(-6) μm thick consisting of several wall layers; outer wall 0.5-1 μm thick; wall 2 yellow, 1.5-3 μm thick; wall 3 brittle, hyaline, 0.5 μm thick; wall 4 and wall 5 membranous, each 0.5 μm thick .

***Acaulospora myriocarpa* Spain, Sieverding & Schenck**

Spores found singly in soil, hyaline to yellow, subglobose, (23-)104-114 x (21-)80-96 μm. Spore wall structure of 3 walls (1-3) in one group; Wall1 rigid, 0.75-2 μm; wall 2 rigid, 0.3-1.5 μm; wall 3 membranous (< 0.3 μm thick) closely appressed to wall 2.

***Acaulospora nicolsonii* Walker, Reed & Sanders**

Spores formed singly in soil, hyaline to pale yellow brown, globose to subglobose, 99- 108 x 109-120 (-218) μm. Spore wall an outer, brittle, wall group (Group A) (walls 1-3) enclosing an inner, membranous wall (Group B) (wall 4). Wall group A with an outer thin, hyaline evanescent wall (wall 1), 0.5-1μm thick, tightly adherent to a thick, brittle, hyaline to pale yellow-brown laminated wall (wall 2) 3-10 μm thick, with up to 13 subequal laminae, enclosing a loosely adherent, pale yellow, brittle, unit wall, 0.5-1.5 μm thick (wall 3); wall 1 smooth or roughened as it breaks up and sloughs, leaving granular fragments attached to wall 2 which may crack in an irregular network. Inner wall (Group B, wall 4) very thin, hyaline, membranous (<0.5 μm), Spore contents vacuolate or reticulate.

***Acaulospora rugosa* Morton (Plate 4, Fig. c)**

Spores formed singly in soil, sub-hyaline to straw-colored, mostly globose to sub-globose (49-)102-112(-118) μm in diam. Spore wall structure consisting of 5 walls in 3 groups (A, B & C). Wall group A with two walls (1 & 2); hyaline

outer wall 1-1.5 μm thick in lactophenol based- mountants, often forming folds 2(-10) μm deep surrounding intact spores, separating readily from wall 2 in crushed spores; in water, walls 1 & 2 adherent, appearing wrinkled or rugose; wall 2 pale yellow, laminated, 1.2-3 μm thick. Wall group B a semirigid hyaline wall (wall 3), 1-1.3μm thick. Wall group C two hyaline walls (4 & 5); wall 4 membranous; wall 5 in water rigid, 1-2.5 μm thick, in PVGL of indeterminate thickness, exhibiting some elasticity.

***Acaulospora scrobiculata* Trappe (Plate 4, Fig. d)**

Spores formed singly in soil, hyaline to light brown, globose to broadly ellipsoid, 100-149(-240) x 100-154(-220) μm. Spore surface evenly pitted with depressions 1-1.5 x 1-3 μm, separated by ridges 2-4 μm thick, the mouths of the depressions circular to elliptical or occasionally linear to Y - shaped. Spore wall 4-8.5 μm thick, consisting of four layers; a rigid, pitted, outer layer 3-6 μm thick and 3 inner smooth layers (<1μm). Spore contents of small, relatively uniform guttules.

***Acaulospora spinosa* Walker & Trappe (Plate 4, Fig. e)**

Spores formed singly in soil, dull yellowish brown to dark reddish brown, globose to subglobose, 100-240(-298) x 100-225(-335) μm. Spore surface ornamented with crowded blunt spines 1-4 μm high, 1 μm in diam. at the polygonal base, tapering to 0.5 μm at the tip, separated by ± 0.2 μm, some times adhering in lines to form an irregular, partial reticulum on parts of the spore surface. Spore wall 3

layered; outer layer yellowish brown to reddish brown, 4-10 μm thick including spines, enclosing 2 membranous hyaline walls, each 0.2- 1 μm thick.

***Acaulospora undulata* Sieverding (Plate 4, Fig. f)**

Spores formed singly in soil, hyaline to subhyaline, globose-subglobose, 55-85 μm in diam. Spore wall structure of 3 walls in two groups (A & B). Wall group A of walls 1 & 2; wall 1 is hyaline, evanescent, 0.5 μm thick, appressed to wall 2; wall 2 hyaline or white in water, 1-1.5 μm thick; wall 2 undulated by regular depressions, 1-2.5 μm deep; each depression circular, ovulate or slightly irregular, 4-9 x 4-9 μm wide at the periphery of spores; ridges between depressions about 1 μm wide. Wall group B of wall 3, a hyaline unit wall, 0.5-1 μm thick.

***Gigaspora albida* Schenck & Smith**

Spores formed singly in the soil, dull white, mostly spherical, (143-)162-222(-350) μm. Spore wall 4-12 μm thick, with 1-6 walls; outer wall thin, smooth, 1-2 μm thick, readily cracking under light pressure; usually 2 or 3 but occasionally 4-5 inner walls of varying thickness. Sporogenous cell (24-) 38-51 μm broad borne on a septate subtending hypha with fine hyphal branches.

PLATE-5

SPORES OF ARBUSCULAR MYCORRHIZAL FUNGI

a) Spore of *Gigaspora margarita* (x100).

b) Spore wall of *Gigaspora margarita* (x 400).

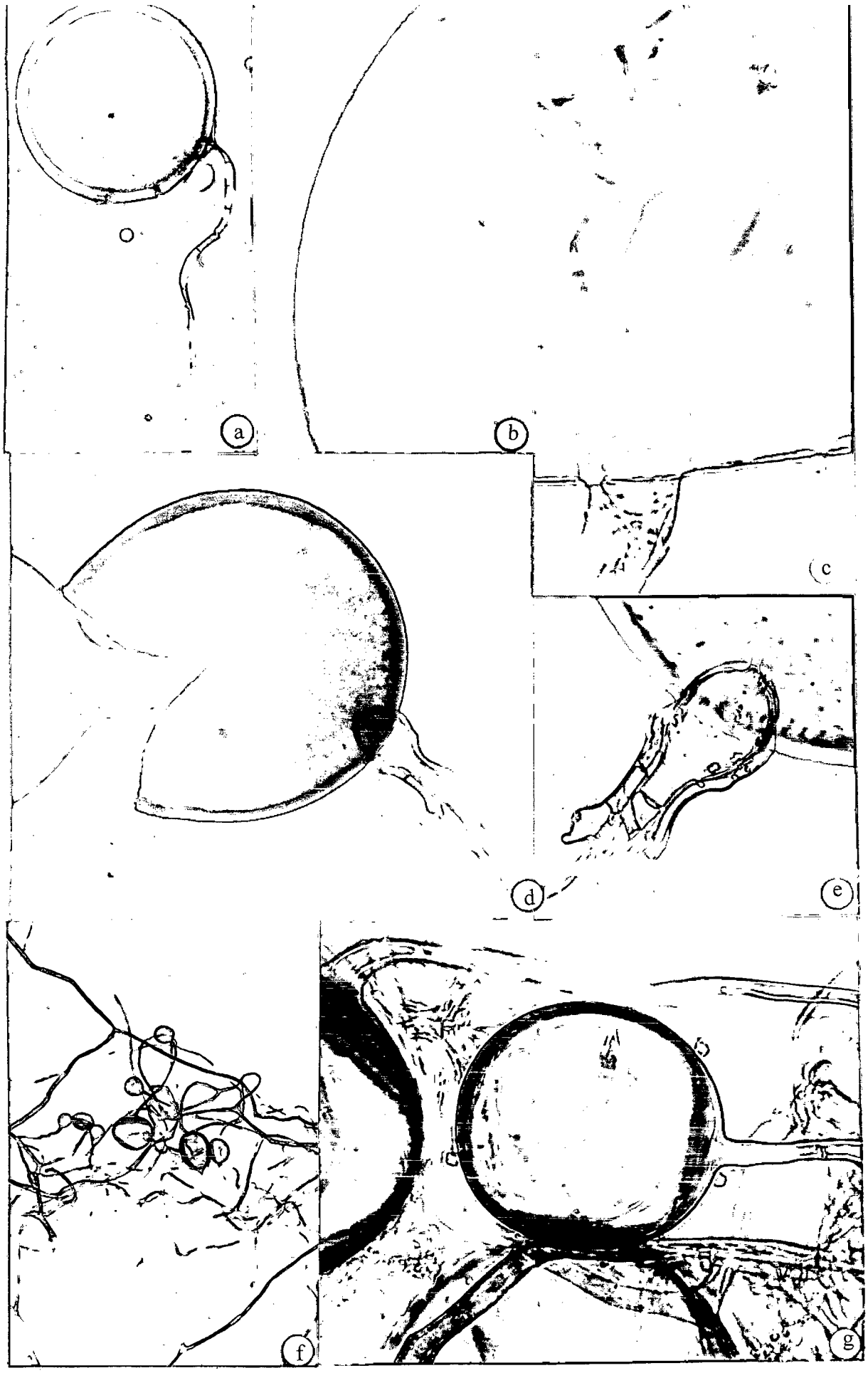
c) Sporogenous cell of *Gigaspora margarita* (x 400).

d) Spore of *Gigaspora ramisporophora* (x 100).

e) Sporogenous cell of *Gigaspora ramisporophora* (x 200).

f) Spores of *Glomus deserticola* (x 40).

g) Spores of *Glomus deserticola* (x 400).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***Gigaspora candida* Bhattacharjee, Mukerji, Tewari & Skoropad**

Spores found singly in soil, white, globose, 200-240(-300) µm. Spore wall smooth, 2 layered, outer layer 1 µm thick, inner layer up to 6 µm thick. Sporogenous cell, 30-50 µm diam.

***Gigaspora decipiens* Hall & Abbott**

Spores found singly in soil, globose to subglobose, 320-490 µm in diam. Spore wall 34-47 µm thick with 11-15, subequal laminations. Sporogenous cell 65 µm wide.

***Gigaspora margarita* Becker & Hall (Plate 5, Fig. a - c)**

Spores formed singly in soil, globose to subglobose, 260-384(-480) x 270-396 (-480) µm. Spore wall smooth, composed of 4-8, rarely 10, fused laminations; spore wall 5-24 µm thick, each lamination 1.5-4 µm thick. Contents of the spores white, composed of many small oil droplets. Sporogenous cell 27-51(-58) µm broad borne on a subtending hypha; subtending hypha generally septate below the suspensor like cell.

***Gigaspora ramisporophora* Spain, Sieverding & Schenck (Plate 5, Fig. d & e)**

Spores smooth, golden yellow, globose, (96-)200-273(-567) µm in diam. Spore wall structure of 3 walls in a single group with total wall thickness 9-12(-31) µm; wall 1, unit wall, hyaline, brittle, 1.4-4(-5.7) µm thick, continuous with outer wall

of sporogenous cell and adherent to wall 2; wall 2 laminate, yellow to yellowish brown, 4-10(-28) µm thick, adherent to wall 3 yellow, 1 µm thick. Sporogenous cell 60-83 µm broad; sporogenous cell develop a branch (sporogenous cell with connecting hypha) which gives rise to another spore; sporogenous cell has thick-walled, 3-4 µm hyphal protrusion, 8-10 µm in diam. Sporophores simple or branched, formed of specialized septate hypha, 9.3-13.9 µm in diam., with 1-3 sporogenous cells.

***Gigaspora rosea* Nicolson & Schenck**

Spores produced singly in soil, predominantly globose, 230-294(-305) µm diam., white to cream colour with a rose-pink tint on the spore wall near the hyphal attachment encompassing up to half the spore. Pink colour variable from distinctly rose pink to barely detectable. Spore wall thickness 2.4-7.5 µm, with 2-5 inseparable layers 1-2µm thick; outer wall layer smooth. Sporogenous cell 28-40 µm diam. borne on a subtending hypha; subtending hypha 7-14 µm wide, hyphal walls 1-2 µm thick, septate.

***Glomus aggregatum* Schenck & Smith emend. Koske**

Spores found in loose aggregation, pale yellow, globose to subglobose, (20-) 40-120 µm in diam. Spore wall of 1 or 2 separable, colored laminated walls, each 1-3(-5) µm thick, thicker near point of attachment; if 2 walls present, the outer is thicker. Subtending hypha straight, curved, swollen or irregular, up to 12µm wide

at the spore base; walls 1-3.5 μm thick. Pore open or closed by a curved septum in the subtending hypha by an inner wall near the spore base.

***Glomus constrictum* Trappe**

Spores formed singly in soil, dark brown to black, shiny–smooth, subglobose to globose, 150- 213(-330) μm in diam. Spore walls (7-) 12-15 μm thick, dark brown, one layered; base straight or occasionally with a short funnel-shaped projection; attachment occluded by wall thickenings; contents of oil globule of widely varying sizes. Attached hypha straight to recurved with the following features appearing in sequence away from the spore; point of attachment with dark brown walls 3-6 μm thick; just beyond the point of attachment the hypha constricted to 10-17(-22) μm diam.; just beyond the constriction the hypha inflated to 15-30 μm with yellow to yellow-brown walls 2-3 μm thick, from which often grow several hyaline to yellow, fragile, thin - walled hyphae, 5-7 μm in diam; just beyond the inflated segment often with a thick walled septum; and beyond the inflated segment the hypha dichotomously forked.

***Glomus convolutum* Gerdemann & Trappe**

Chlamydospores yellow to brown, globose to obovoid, 81-193 x 67-193 μm. Each spore tightly enclosed in a mantle of intertwined thin walled hyphae 1.5-5 μm broad. Spore wall 8-15 μm thick, laminate. Spores filled with deep yellow oil globules. Hyphal attachment 6-13 μm in diam.

***Glomus deserticola* Trappe, Bloss & Menge (Plate 5, Fig. f & g)**

Spores borne singly or in loose fascicles in soil, reddish brown, smooth, globose to subglobose, (47-)81-108 x (38-)75-96 µm, with a single some times laminated wall (1.5-)2-2.5(-4) µm thick. Attached hypha 6-9(-12) µm in diam., cylindric to occasionally some what funnel shaped, the walls thickened and reddish brown, especially thick adjacent to the spore but not occluding the hypha. Interior of the spore wall at the hyphal attachment thickened at maturity to form an inner mounded collar, which appears to be closed by membranous septum.

***Glomus diaphanum* Morton et Walker**

Spore formed singly, or in loose clusters, hyaline, gobose to subglobose, (39-)100-121 µm in diam. Spore wall structure consists of two wall (wall 1 and 2) in a single group (A). Wall 1 (2)-4.4(6.5) µm thick, laminate. Wall 2 membranous, (0.2-) 0.8(-1.3) µm, extends 5-12 µm in the subtending hypha and forms a septum enclosing the spore contents. Spore contents are hyaline and contain one to many oil globules. Subtending hypha 5.4-11 µm in diam. at the spore base; hyphal wall (1.4-) 3 (-3.7) µm thick.

***Glomus dimorphicum* Boyetchko and Tewari.**

Single spores yellow to reddish brown, globose to subglobose, 90-300 µm. Spore wall made up of 3 walls organized in 2 wall groups; wall 1 hyaline, laminate, 2-8 µm thick, ephemeral; wall 2 light yellow to reddish brown, laminate 2-8 µm thick; wall 3 light yellow to reddish brown membranous, 1 µm thick. Subtending hypha

PLATE-6

SPORES/SPOROCARPS OF ARBUSCULAR MYCORRHIZAL FUNGI

a) Spore of *Glomus fasciculatum* (x 200).

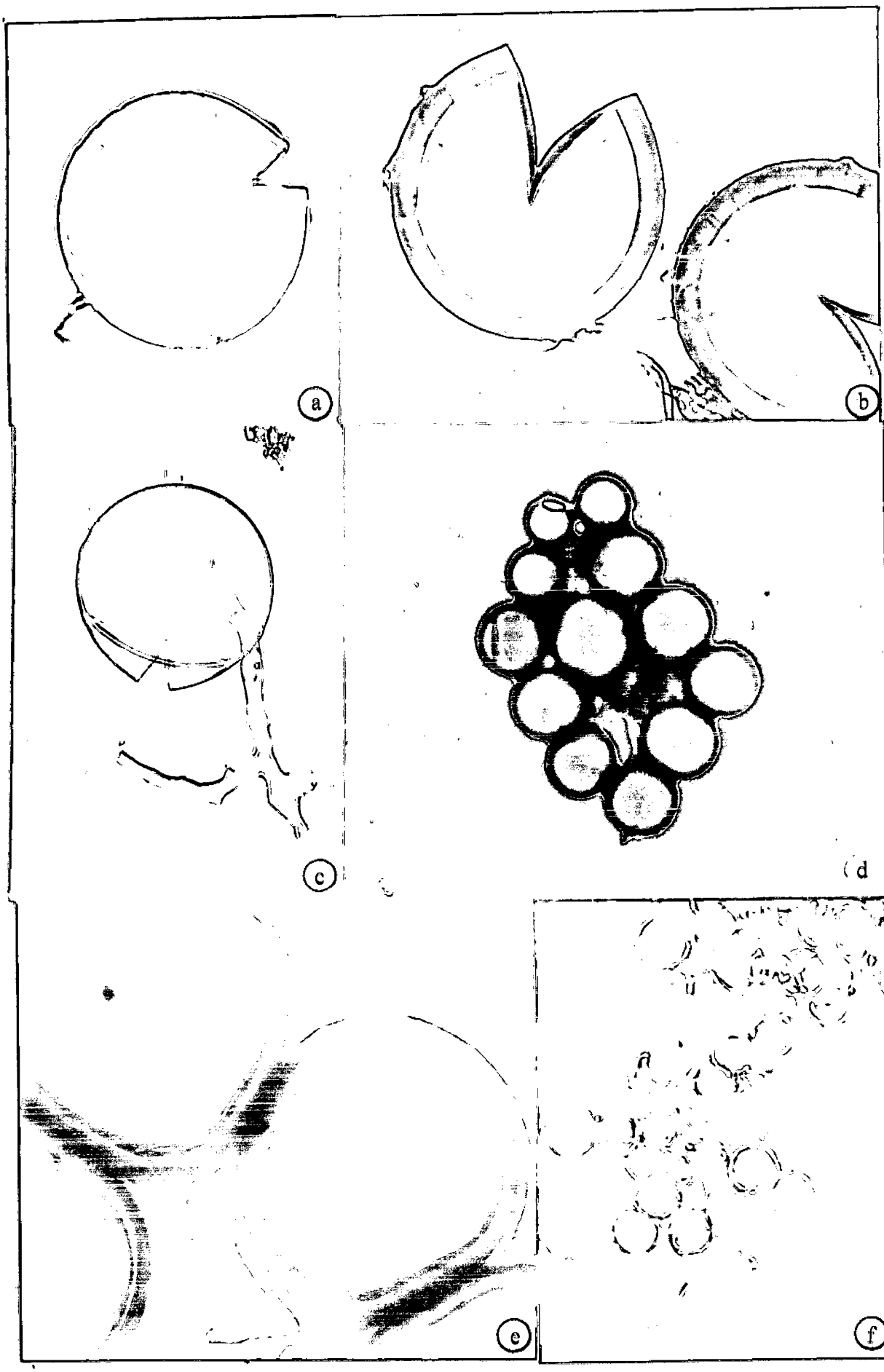
b) Spores of *Glomus heterosporum* (x 200).

c) Spore of *Glomus hoi* (x 200).

d) Sporocarp fragment of *Glomus macrocarpum* (x 100).

e) Spores of *Glomus macrocarpum* (x 400).

f) Spores of *Glomus microaggregatum* (x 400).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straight to slightly curved, light yellow to light brown; wall 1.5-9 μm thick and consisting of wall 1-3. Point of attachment occasionally possessing a septum, often occluded, flared or cylindric, 10-34 μm thick.

***Glomus fasciculatum* (Thaxter) Gerdemann & Trappe emend. Walker & Koske (Plate 6, Fig. a)**

Spores formed singly or in loose aggregation in soil, pale yellow to pale yellow-brown, globose to subglobose. Spore wall structure of 3 walls in one group. Wall 1 a smooth hyaline unit wall, 0.2-1(-1.8) μm thick. Wall 2 pale yellow to pale yellow-brown, laminated (2-)5-10(-14.3) μm thick. Wall 3, a hyaline membranous wall, 0.1-0.9 μm thick. Subtending hypha flared, straight or slightly constricted proximally, (3.5-)9-15(-19) μm broad at the spore base. Pore open or closed by thickening of wall 2.

***Glomus formosanum* Wu & Chen**

Spores usually formed in aggregates without peridium in soil, yellow-brown to reddish brown, smooth, globose to subglobose, 82-125 x 95-120(-135) μm. Spore wall single, yellow - brown or reddish brown, 5.5-12.5 μm thick but thickest at attachment (<20μm).

Spores with 1-4, sometimes branched attached hypha. Frequently two nearby attached hypha fused together or closely separated at attachment. Attached

hyphae 7-17.5μm diam, with opening at attachment nearly occluded by spore wall thickness, with thick wall, 5-6 μm at attachment.

Glomus geosporum (Nicolson and Gerdemann) Walker

Sporocarps unknown. Spores formed singly in soil, light yellow-brown to dark yellow-brown, globose to subglobose, 110-225(-290) μm. Spore walls 4-6(-18) μm in thickness, 3 - layered with a thin hyaline, tightly adherent outer wall (<1μm), a yellow- brown to red-brown laminated middle wall and a yellow to yellow-brown inner wall (<1μm) that forms a septum. Spore contents granular in appearance, cut off by a thick septum that protrudes slightly into the subtending hypha. Spores with one straight to recurved, simple to slightly funnel shaped subtending hypha up to 200 μm long ,10-18(-24) μm diam., with yellow to dark yellow-brown wall thickening that extends 30-100 μm along the hypha from the spore base.

***Glomus heterosporum* Smith & Schenck (Plate 6, Fig. c)**

Spores produced in sporocarps, light to dark brown, obovoid to ellipsoid, occasionally globose, (99-) 114 – 206 x (61-) 111-201 μm. Spores with two distinct walls. Inner wall laminate brown 3-10 μm thick. Outer wall smooth, evanescent, hyaline, 2-7 μm thick. Spore contents hyaline, non-globular and separated from hyphal attachment by a septum.

Hypha at the point of attachment is 5-15(-31) µm wide. Spores frequently with multiple hyphal attachments.

***Glomus hoi* Berch & Trappe (Plate 6, Fig.c)**

Spores borne singly in soil, globose to subglobose, (50-)80-120(-140) µm, light brown. Wall of spores composed of two distinct, separable layers; outer layer orange- yellow, 2-6(-8) µm thick, with an outer surface that fractures and sloughs; inner layer hyaline – light yellow, membranous 1µm thick. Subtending hypha (5-)8-12(-13) µm wide, with a single wall 2.5-5 µm thick. Pore in the subtending hypha occluded by a fine curved septum at or somewhat below its point of attachment to the spore.

***Glomus macrocarpum* Tul. & Tul. (Plate 6, Fig. d & e)**

Sporocarps are fragmentary. Spores yellow to brown, usually longer than wide, subglobose or globose, (90-)120(-140) x (70-)110(-130) µm. Spore wall is composed of two distinct layers; outer layer is thin, 1-2 µm and hyaline; inner wall layer is yellow, 6-12µm thick, with laminations. Spore taper to the point of attachment of the single persistent hypha. Hyphal width at this point varies from 12-18µm. The inner wall at maturity thickens to occlude the pore of the attached hypha, and the wall thickening continues into the subtending hypha for up to 90 µm from the spore. Infrequently, the pore seems to be closed by a septum that is thinner than the normal occluding wall thickening. Spores characteristically bear a

straight, long subtending hypha which may extend up to 100 μm before branching or breaking.

***Glomus maculosum* Miller & Walker**

Sporocarps unknown. Spores formed singly in the soil, hyaline to pale straw-colored, globose- subglobose, 95-220 μm in diam. Spore wall (4.6-) 6-15(-19.6) μm thick, of three walls in two wall groups (A & B). Wall group A of an outer, thin, hyaline unit wall (wall 1), 0.3-1.0 μm thick, tightly adherent to wall 2, a brittle pale straw-coloured laminated wall, 4-13 μm thick, with 4-16 laminae. Inner wall group (group B, Wall 3), membranous (<1μm). Wall 3 may bear domed, scalloped ingrowths, 6-15 μm diameter and up to 12 μm deep. Spore contents of crowded oil droplets. Spores formed on one to three subtending hypha; subtending hypha concolorous with spore wall 2, straight to sharply recurved, parallel sided, sometimes constricted at the spore base, 5-13 (-25) μm long and 5-13 μm wide proximally, and 5-7 μm wide at the point of connection to a thin walled hyaline parent hypha. Walls of the subtending hypha 1.5-3 μm thick proximally, tapering distally to 1μm.

***Glomus microaggregatum* Koske, Gemma & Olexia (Plate 6, Fig. f)**

Spores formed in clusters inside dead spores, hyaline, globose or subglobose to irregular, 15-30(-50) μm in diam. Spore wall structure of 1-2 walls, in one group. Wall 1, a smooth, brittle unit wall, hyaline, 0.5-1.2(-2) μm thick. Wall 2 if

present, a membranous unit wall, < 0.5-1.2(-2) µm thick. Attachment hypha concolorous with wall 1, straight or infundibuliform, 1.8-3 (-4.5) µm wide at spore base, wall up to 1.5(-2) µm thick. Pore open, or closed by septum formed by wall 2 distending into the attachment hypha.

***Glomus multisubtensum* Mukerji, Bhattacharjee & Tewari**

Spores found singly in soil, light brown, globose, 100-150 µm in diam. Spore wall 10-15 µm thick with two inseparable layers; outer layer 10-12 µm thick, brown; inner layer 1-4 µm thick, pale yellow brown. Subtending hypha 2-4 in number, attached at one end of the spore, hyaline – pale yellow, thin walled, 10-15 µm wide at the point of attachment.

***Glomus occultum* Walker**

Spores borne singly in soil, hyaline-white, ovoid to obovoid, 15-100 x 20-120 µm. Spore wall 1-2 layered, rough. Outer wall when present less than 1 µm thick. Inner wall (1-) 1.5-2.5(-5) µm thick. Subtending hypha funnel shaped to simple, 3-10 µm wide at spore base.

***Glomus pubescens* (Sacc. & Ellis) Trappe & Gerdemann**

Spores found singly in soil, hyaline, smooth, subglobose to ellipsoid, 20-48 x 18-45 µm, Spore walls 3-6 µm thick, hyaline to light yellow, the opening may be occluded by wall thickening. Spores filled with oil globules of variable size.

Hyphal attachments ± 2μm in diam, the attached hypha hyaline, thin walled and inconspicuous.

***Glomus tenebrosum* (Thaxter) Berch. & Fortin.**

Spore found singly in soil, yellow to dark brown, globose, 200- 230 (-270). Spore wall single, thick, (13-)15(-26) μm; the thick wall may appear laminated near the subtending hypha. The hypha is 16(-32) μm at the point of attachment to the spore and fades from dark brown or yellow near the spore to yellow or hyaline within approximately 100 μm of the attachment. The pore in subtending hypha open.

***Glomus tenerum* Tandy emend. McGee**

Chlamydospores not aggregated, yellow, pale orange or brown, globose, 44-130 μm in diam. Spore wall in two thin layers, a rough, hyaline, thin (1μm) exospore and a smooth hyaline endospore thickened up to 7 μm. There is no septum at the base of the spore. Spore with very dense granular contents, sometimes with a few oil globules. Subtending hyphae 8-12 μm wide.

***Sclerocystis clavispora* Trappe**

= *Sclerocystis microcarpus* Iqbal & Bushra

Sporocarps brownish black to black, globose to subglobose, 320-750 x 340-780 μm, minutely verrucose from exposed tips of spores formed radially, side by side

in a single, tightly packed layer around a central plexus of tightly woven hyphae. Peridium lacking. Chlamydospores brown to dark brown, clavate to subcylindric (80-)120-160(-185) x (20-)25-40(-50) µm. Spore wall laminate, 1.5-5 µm thick on the side walls, thickened to 9.5-27 µm at the apex and to 6-10 µm at the base with a small pore open or occluded.

According to Almeida and Schenck (1990), *Sclerocystis clavispora* is variable in spore and sporocarp size. Thus, *Sclerocystis microcarpus* can be considered synonymous with *Sclerocystis clavispora*. The only distinction between the two is size of the spores and sporocarps. Wu (1993b) was of similar opinion. According to him, *Sclerocystis clavispora* produces two distinctly different sized spores, one large and other small. The sporocarps producing smaller spores were formerly identified as *Sclerocystis microcarpus*. Both type of spores could be found in the same sporocarps or in different sporocarps isolated from the same rhizosphere.

***Sclerocystis coremioides* Berk. & Broome (Plate 7, Fig. a & b)**

= *Sclerocystis coccogena* (Pat.) von Hohn

= *Sclerocystis dussii* (Pat.) von Hohn

Sporocarps dull brown, subglobose, 340-600 µm broad. Peridium of thickwalled interwoven hyphae. Chlamydospores tightly grouped in a hemisphere around a central plexus of hyphae. Chlamydospores obovoid to ellipsoid, (50-)75-102 x 35-63(-82) µm, septa may or may not be present at the spore base. Spore wall 1.5-2.5 µm and frequently thickened at base up to 6 µm.

PLATE-7

SPORES/SPOROCARPS OF ARBUSCULAR MYCORRHIZAL FUNGI

a) Sporocarp of *Sclerocystis coremioides* (x 200).

b) Sporocarp of *Sclerocystis coremioides* (x 200).

c) Sporocarp of *Sclerocystis sinuosa* (x 200).

d-g) Variations in spores of *Sclerocystis sinuosa.*

d) (x400),
e) (x 200),
f) (x 400),
g) (x100).

a
b
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d
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Sclerocystis coccogena, Sclerocystis dussii and *Sclerocystis coremoides* sould be treated as one species, as the sporocarps of these species are unique, resembling a coremium with a short stalk and a head with spores produced side by side, around a plexus of hyphae, but never being formed at the base of the sporocarp (Almeida & Schenck, 1990).

***Sclerocystis sinuosa* Gerdemann & Bakshi (Plate 7, Fig. c - g)**

= *Sclerocystis pakistanica* Iqbal & Bushra

Sporocarps reddish-brown to dull brown, globose to subglobose, 220-650 μm in diam. Peridium tightly enclosing a sporocarp, composed of thick walled interwoven hyphae. Peridial hyphae sinuous. Sometimes sinuous feature may be indistinct. Chlamydospores globose, subglobose, obovoid, clavate or irregular, 45-150 x 30-83 μm, walls single with walls evenly thickened or unevenly thickened, usually thickened at the apex or lateral side, (1.5-) 5-22.5(-30) μm, with 1-2 attached hyphae.

The spores and sporocarps of *Sclerocystis sinuosa* exhibit wide range of morphological variations. The heterogenous spores with uneven wall thickness and indistinct sinuous features have been illustrated by Wu & Chen (1993). Almeida and Schenck (1990) noted a great variation in the sporocarp size and peridial nature in *Sclerocystis sinuosa*. They also considered *Sclerocystis pakistanica* synonymous to *Sclerocystis sinuosa* as the descriptions of the two

PLATE-8

SPORES/SPOROCARPS OF ARBUSCULAR MYCORRHIZAL FUNGI

a) Sporocarp of *Sclerocystis taiwanensis* (x 400).

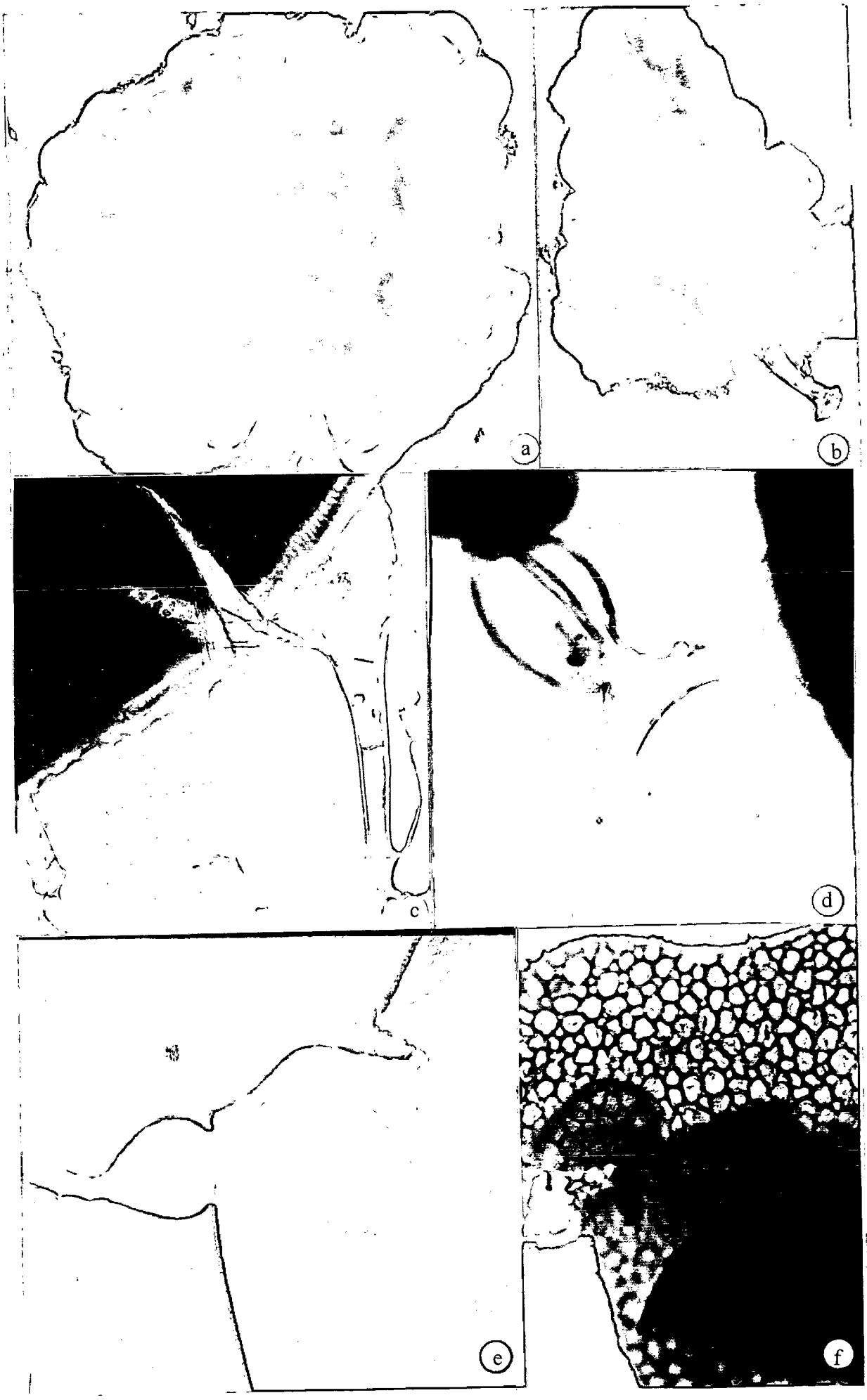
b) Sporocarp of *Sclerocystis taiwanensis* (x 400).

c) Spore of *Scutellospora coralloidea* (x 200).

d) Branched hypha of *Scutellospora coralloidea* (x 400).

e) Spore of *Scutellospora gregaria* (x 400).

f) Spore of *Scutellospora reticulata* (x 200).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species were almost identical except for the sporocarp size and nature. Observations in the present study are in accordance with the above reports.

***Sclerocystis taiwanensis* Wu & Chen (Plate 8, Fig. a & b)**

Sporocarps reddish brown, brown or dark brown, globose to subglobose, 200-300 x 180- 280 μm, with chlamydospores formed radially in a single, tightly packed layer around a central plexus of hyphae. Peridium lacking. Chlamydospores clavate, cylindro-clavate, 40-57(-105) x 22-28(-55) μm, with or without a septum at the spore base. Chlamydospore wall laminate or single, with a hyaline separable outer layer (1μm thick), yellow -brown inner layer (4-) 12-15 (25) μm thick at the apex, 1.5-3(-5) μm thick at sides, generally thickest at the apex.

***Scutellospora albarosea.* (Ferr. & Herr.) Walker & Sanders**

Spores formed free in soil, hyaline-pink or pinkish–brown, globose to subglobose, 204-287 μm diam. Wall composed of an exospore of two layers and a membranous endospore. The external layer of the exospore is thick, pinkish, 4-11 μm thick; internal layer yellow, 1.5-5.5 μm thick. Endospore 0.7-2.1μm thick enclosing reticulate contents. Sporogenous cell 21-25(-50)μm broad.

***Scutellospora calospora* (Nicolson & Gerdemann) Walker & Sanders**

Spores found singly in the soil, terminally on a bulbous sporogenous cell. Spores hyaline to pale yellow; globose to subglobose, (114-)189-360(-511) x (110-)189-396(-511) μm diam. Spore wall structure of four walls (1-4) in two groups (A & B). Group A of a thin hyaline unit wall (Wall-1) (1-2) μm thick tightly appressed to laminated wall, hyaline to pale yellow, (Wall-2) (3-5) μm thick. Group B of two hyaline membranous walls (wall 3 & 4) each 0.5-1 μm thick. Sporogenous cell (33-48) μm broad, borne terminally on a septate subtending hyphae. Germination shield oval (35-)70 X (35-)90 μm.

***Scutellospora coralloidea* (Trappe, Gerd. & Ho) Walker & Sanders (Plate 8, Fig. c & d)**

Spores formed singly in soil, terminally on a bulbous suspensor like cell. Spores globose to subglobose, 308 – 393 x 294-420(-454) μm, dark brown. Spore wall structure of 3 walls (1-3) in two groups (A & B). Group A with an outer ornamented unit wall (wall-1) tightly adherent to an inner laminated wall (wall-2); wall 1 brittle, dark brown, 3-5 μm thick excluding widely spaced patch like warts; warts hyaline to pale yellow, 1-2 μm high, 2-12 μm across, with subangular margins; wall 2 brittle, yellow brown to orange brown 4.5-8 μm thick. Group B consisting of a hyaline, membranous wall (wall-3) 0.5 μm thick, enclosing spore contents. Sporogenous cell pale brown, 55-64 μm wide, with a peg-like, narrow,

yellow-pale brown hypha up to 20 x 5 µm. Sporogenous cell borne terminally on a septate or aseptate subtending hypha.

***Scutellospora gregaria* (Schenck & Nicolson) Walker & Sanders (Plate 8, Fig. e)**

Spores found singly in the soil, terminally to some what eccentrically on a bulbous sporogenous cell. Spores red brown to dark brown, globose to subglobose, 250-334(-448) x 250-334(-480) µm. Spore wall structure of four walls (1-4) in two groups (A & B). Wall group A composed of three closely appressed walls, an outer unit wall (wall-1) and two laminated walls (wall-2 & wall-3); wall-1 brittle, brown, (1-3) µm thick excluding the closely packed warts situated on its outer surface, warts pale brown, 1- 2(-5) µm high with rounded tips, 2-7(-10) µm diam at the base, crowded together in groups; wall-2 brittle, yellow (3-5) µm thick; wall-3, brittle, pale yellow to nearly colourlesss, (5-13) µm. thick. Group B of a single membranous hyaline wall (wall-4) 1-2 µm thick. Sporogenous cell pale brown, 39-45(-80) µm wide, with 1 or 2 thick or thin walled hyaline projections, borne terminally. Germination shield globose - subglobose 135 x 144 µm diam.

***Scutellospora pellucida* (Nicol. & Schenck) Walker & Sanders (Plate 9, Fig. a)**

Spores found singly in the soil, terminally on a bulbous sporogenous cell. Spores hyaline to pale grey, glistening with oil droplets, globose ellipsoid, or irregular,

PLATE-9

SPORES OF ARBUSCULAR MYCORRHIZAL FUNGI

a) Spore of *Scutellospora pellucida* (x 200).

b) Spore of *Scutellospora verrucosa* (x 100).

c) Spore of *Scutellospora verrucosa* (x 400).

d) Spore of *Scutellospora weresubiae* (x 100).

a
b
c
d

(58-)180-220(-410) µm diam. Spore wall structure of six walls (1-6) in three groups (A, B & C). Group A of a brittle, hyaline unit wall (wall-1) (1-2) µm thick, closely appressed to an inner brittle, hyaline, laminated wall (wall-2) (2-) 5(-18) µm thick. Group B of a hyaline membranous (wall-3) less than 1 µm thick, closely adherent to two hyaline unit walls (wall 4 and 5) measuring 1-2 µm each. Group C consists of a single amorphous wall (wall-6) (2-5) µm thick. Sporogenous cell (10-)18 –51 µm broad, borne terminally on a septate subtending hyphae. Germination shield oval to spherical, (60-) 60(-110) x (110-) 110(-130) µm, margins with invaginations.

***Scutellospora persica* (Koske & Walker) Walker & Sanders**

Spores found singly in the soil, terminally on a bulbous sporogenous cell. Spores brownish orange, globose to subglobose to ellipsoid, (270-)291-354 x (281-)312-288 (-384) µm diam. Spore wall structure of three walls (1-3) in two groups (A & B). Group A has an outer ornamented hyaline wall (wall-1) (0.5-0.8) µm thick, with rounded warts 0.25- 0.5 µm high and 0.5 µm diam. at the base, tightly adherent to (wall-2)(2-10) µm thick, laminated, brittle, pinkish-orange to brown . Group B of hyaline membranous wall (wall 3) 0.5- 1 µm thick. Sporogenous cell 45(-125) µm wide borne terminally on a septate subtending hyphae. Germination shield subcircular,150-156(-180) x (60-) 80-120(150) µm diam.

PLATE-10

SPORES OF ARBUSCULAR MYCORRHIZAL FUNGI

a) Spore in spore syndrome (x 100)

b) Spore of *Acaulospora spinosa* in spore of *Scutellospora gregaria* (x 400).


a


b

Scutellospora reticulata **(Koske, Miller & Walker) Walker & Sanders (Plate 8, Fig. f)**

Spores found singly in the soil, laterally on a bulbous sporogenous cell. Spores dark red- brown, globose to subglobose (208-470) x (188-340) µm diam. Spore wall structure complex, consisting of two separate groups of wall layers overlain by an alveolate reticulum. Outer wall group three layered. Outer layer (0.5-1) µm thick, orange brown to red-brown, supporting raised, straight to sinuous interconnecting ridges that form a reticulum 0.5-1 µm high with 4-8 sided meshes 2-24 x 2-30 µm across. Spore surface between ridges covered with polyhedral, conical or subcylindrical spines, or narrow straight, curved, or angular ridges 0.5-1 µm high and 0.25-0.5(<1µm) apart; middle layer hyaline to pale yellow, 5-11 µm thick; inner layer hyaline 0.3-0.7µm thick. Reticulate ridges on outer wall supporting a detachable alveolate reticulum 0.5-2 µm wide and 2-6 µm high. Inner wall group 3 layered, totaling to 3 µm thickness. Suspensor like cell 45 (-87) x 84(-140) µm.

Scutellospora verrucosa **(Koske & Walker) Walker & Sanders (Plate 9, Fig. b & c)**

Spores found singly in the soil, terminally or somewhat laterally on a bulbous sporogenous cell. Spores pale straw to yellow, globose to subglobose, (220-)280-

320(-476) µm. Spore wall structure of three walls (1-3) in two groups (A & B). Group A with 2 walls (wall 1 and 2); wall 1, hyaline to pale yellow brittle, ornamented unit wall (2-3.5) µm thick including crowded, low, rounded warts mostly 0.5-1.5 x 0.5-1.5 µm at the base, 0.5-1.5µm high, spaced 0.5-2(-4) µm apart on the surface, tightly adherent to an inner pale yellow to orange-brown laminated wall (Wall-2) (3-12.5) µm thick. Group B of a single membranous hyaline wall (Wall-3), 0.5-1 µm thick. Sporogenous cell, yellow-brown, often darker than the spore, (55-)39-45(-100) x (35-)39-54(-70) µm diam with 1 or 2 thick walled peg like hyphal projections (22-)23(-50) x (8-)5(10) µm. Sporogenous cell borne terminally on a coenocytic to sparsely septate subtending hyphae. Germination shield dark brown, often darker than the spore, oval (80-) 69-90 x (150-) 130-156(-210) µm, from which germ tube emerges.

***Scutellospora weresubiae* Koske & Walker (Plate 9, Fig. d)**

Spores found singly in the soil, terminally on a bulbous sporogenous cell. Spores translucent, glistening, pale pink, globose to subglobose, (125-)156-265 x 135-294(-414) µm diam. Spore wall structure of six walls (1-6) in three groups (A,B & C). Group A often with an outer brittle, hyaline, unit wall (wall-1) up to 0.5 µm thick, tightly adherent to an inner brittle, pink, laminated wall (wall-2) (3-)12(-15) µm thick. Group B of two membranous walls (3 & 4), each 1µm thick. Group C formed of a thin hyaline coriaceous wall (wall-5) (2-8 µm thick), surrounding a

hyaline membranous innermost wall (wall-6) 0.5 μm thick. Sporogenous cell, hyaline to pale brownish-yellow, (32-50) μm wide, with 1 or 2 hyphal pegs 27 μm long and 3-8 μm wide, projecting towards the spore base. Sporogenous cell, borne terminally on a sparsely septate or aseptate subtending hyphae.

CHAPTER VII

PREPARATION OF PURE ARBUSCULAR MYCORRHIZAL INOCULUM.

INTRODUCTION

Arbuscular Mycorrhizal fungi are obligate biotrophs and play an important role in growth and survival of dune plants. A scarcity of prime inoculum has limited the broad use of AM fungi. Various soil free methods such as soil-less growth media (Jarstfer and Sylvia, 1992), hydroponics (Elmes and Mosse, 1984; Mosse and Thomson, 1984), aeroponic techniques (Hung and Sylvia, 1988; Sylvia and Jartsfer, 1992) and axenic cultures of AM fungi with transformed or non transformed living roots of various hosts (Chabot *et al.*, 1992; Diop *et al.*, 1994), have recently been used successfully to produce AM fungal colonized root inocula. These methods look promising but many basic questions relating to infectivity and effectiveness of the species maintained for long periods *in vitro*, the persistence of these fungi in field situations and competition with others soil microorganisms including indigenous AM fungal populations remains largely unanswered. Thus, one is forced to collect the spores from the soils and multiply them in the green house conditions on living host plants. Clearly, the production and collection of propagules from soil remains an important time consuming and fundamental part of AM research for inoculum preparation.

Propagules of AM fungi can consist of chlamydospores or azygospores, soil borne vesicles and mycelium or infected roots pieces. Used together, as they occur in soil, these propagules may be termed mixed inculum as compared with spores which have been separated from soil and represent a 'purer' inoculum.

In a pure inoculum, with extracted spores one can ensure that only the desired species is present, asses spore viability and reduce the possibility that a plant pathogen or a AM hyperparasite is carried in the inoculum.

The survey of the distribution of AM fungal spores in coastal sand dune vegetation of Goa revealed that *Acaulospora spinosa*, *Glomus macrocarpum*, *Acaulospora scrobiculata* , *Gigaspora magarita*, *Glomus fasciculatum* and

Scutellospora coralloidea are the dominating species on the moderately disturbed dunes. Isolation and multiplication of these dominating AM fungi is important for mass inoculum production and for tailoring roots of tree seedlings suitable for rehabilitation and reclamation of the disturbed sites.

The objective of the present study was to prepare pure inoculum of the dominating AM species recovered from the coastal sand dune vegetation of Goa.

MATERIALS AND METHODS

The seeds of *Eleusine coracana* (L) Gaertner were sterilized in 0.1% $HgCl_2$ for 5 minutes. Turgid and unbroken spores were considered viable and were sterilized in chloramine T for 15 minutes. Sand was sterilized in an autoclave at 121° C for 2 hrs. daily for a period of 3 days. Surface sterilized pots were filled with sterilized sand. Sterilized spores of each AM fungal species were pipetted seperately on a small piece of filter paper and placed in the pot. A layer of 2 cm of soil was spread gently over it. Now the sterilized seeds were placed on and around the spore placement. A thin layer of soil was spread on the top. The pots received sunlight and were watered daily. After 20 days the pots were fertililzed with Hoagland solution which was lacking in phosphorus. After 60 days the roots were checked for infection by methods described by Phillips and Hayman (1970). At the end of 100 days the plants were stopped watering and dried shoot portions in each pot were harvested and soil based inoculum was collected in polyethylene bags and stored in deep freezer at -10° C for further use.

RESULTS AND DISCUSSION

Limited space, contamination by pathogens, interference of insect, fungal and bacterial pests, and rodents were common problems encountered during the inoculum preparation. However, pure inoculum of *Glomus macrocarpum* and *Glomus fasciculatum* were successfully obtained. Spores of *Scutellospora coralloidea* could also be successfully multiplied in the roots of *Eleusine coracana*

but the inoculum obtained was a inoculum containing only spores of *Scutellospora coralloidea* and *Glomus aggregatum*. This may be due to inoculation of some *Scutellospora coralloidea* spores occupied with spores of *Glomus aggregatum*. The dynamics of inoculum production, the nature of host specificity, the activity of hyperparasites are crucial factors affecting inoculum production. Attempts to obtain pure cultures of other AM fungal species dominating the coastal dune vegetation of Goa is a promising area in future research.

CHAPTER VIII

GROWTH RESPONSE OF *ANACARDIUM OCCIDENTALE* (L.) TO ARBUSCULAR MYCORRHIZATION IN SAND DUNE SOILS.

INTRODUCTION

Coastal sand dunes are immensely valuable for sea defense. Stabilization of large, mobile dune fields by planting of vegetation has long been recognized as an effective means of slowing the inland movement of sand (Jagschitz and Bell, 1966; Hewett, 1970; Ranwell, 1972; Woodhouse, 1982). Arbuscular mycorrhizal fungi are of vital importance to the establishment, growth and survival of the dominant plant species that colonizes dunes (Koske and Polson, 1984; Puppi and Reiss, 1987). Arbuscular mycorrhizal fungi appear to benefit by improving the uptake of phosphate and other nutrients from the soil, conferring increased drought and diseases tolerance to the host plant and contributing to the soil structure and stability (Koske *et al.*, 1975; Sutton and Sheppard, 1976; Harley and Smith, 1983; Nelson, 1987; Newsham *et al.*, 1995). The phosphate solubilizing microorganisms (PSM) interacts well with AM fungi in phosphorus deficient soils. The PSM can release some phosphate ions from otherwise sparingly soluble phosphate sources and it was postulated that AM fungal hyphae can trap these ions and translocate them to the plant (Krishna et al., 1982). Because species of plant differ in their reponse to AM fungi in the soil the presence or absence of AM fungi has been linked to the composition of the plant communities that developed in dune sites (Read, 1989; Koske and Gemma, 1990,1992; Francis and Read, 1994,1995).

While AM fungi have been found to be present in almost all natural sites (Harely and Smith, 1983), their propagules are rare or lacking from unvegetated dune sites (Sylvia and Will, 1988). The fungi are obligate symbionts, able to grow

and reproduce only when attached to living roots (Harely and Smith, 1983). The large spores of AM fungi are found under ground and appeared not to be easily dispersed to new sites and move below the soil surface in barren sites. Further, if the vegetation of an area is destroyed, the population of viable propagules of AM fungi declines steadily (Miller, 1979; Reeves *et al.*, 1979) until it is too low to contribute to the successful establishment of plant species that can benefit from the symbiosis.

As soil erosion tends to decrease the number of mycorrhizal propagules it could be critical to re-introduce such propagules to improve the recovery rate of ecosystem. However, it is not feasible to inoculate large tracts of lands with AM fungi, as there are currently too many problems associated with the mass production of these fungi. Transplantation of AM inoculated nursery seedlings may prove advantageous on reclamation sites, as limited nutrients in the soil would be more efficiently extracted (Jasper *et. al,* 1989, Sylvia, 1990).

Anacardium occidentale is a hardy and drought resistant plant that thrives best in sandy soil and is the dominating plant in the backshores of Goa coast. Planting trees with deep rooting species like *Anacardium occidentale* could be effective means of stabilizing disturbed coastal sand dunes of Goa. A wide variation among and within different species of AM fungi in the ability for stimulating plant growth has been observed (Abbott and Robson, 1978; Govinda Rao *et al.*, 1983)

In the present study, two AM fungi *viz.*, *Glomus macrocarpum* Tul. and Tul. and *Glomus intraradices* Schenck and Smith, were screened for symbiotic response with *Anacardium occidentale*. *Glomus macrocarpum* was a local isolate that dominated the vegetation on coastal sand dunes of Goa, while *Glomus intraradices* is a species tolerant to high temperature and salinity and is reported from sand dune of West Coast of India (Beena *et al.*, 2000).

MATERIALS AND METHODS

Spores of *Glomus macrocarpum* were isolated and multiplied on the roots of *Eleusine coracana* (L.) Gaertn. *Glomus intraradices* inoculum was procured from Tata Energy Research Institute (TERI), New Delhi and maintained as pot cultures, using *Eleusine coracana* as the host **(Plate-11; Fig. a)**.

Sand was sterilized in an autoclave at 121^{0}C for 2 hrs. daily, for a period of two days, to eliminate naturally occurring endophytes and other contaminants. Seeds of *Anacardium occidentale* were surface sterilized for 20 min. in 70% ethanol, soaked overnight in sterile water, then placed in moist sterile sand at room temperature until germinated.

The experiment comprised following 3 treatments. 1) Unsterilized sand (Control); 2) Unsterilized sand +*Glomus macrocarpum* inoculum 3) Unsterilized sand + *Glomus intraradices* inoculum.

For each treatment polyethylene bags were filled with 4.5 Kg of unsterile sand dune soil and 1 germinated seed per bag was sown. For treatments 2 and 3, 20g of the inoculum containing 200-215 AM fungal spores were applied separately as a thin layer 5cm below the soil surface. There were 6 replicates for each treatment.

All the polyethylene bags containing plants were labeled and kept separately. Plants received sunlight, were watered to field capacity as required and was fertilized with Hoagland solution lacking in phosphorus biweekly. The plants were harvested at the end of 180 days after transplanting.

Measurement of shoot and root length, number of leaves, length and breadth of third leaf and fresh weights of shoot and root portions were recorded. The shoot and root portions of plants were dried separately at 70°C for 72 hrs and dry weights were recorded. Data were analysed by Analysis of Variance. Significant differences were determined at $\alpha = 0.05$. Mycorrhizal inoculation effect (MIE) was calculated by using the formula (Bagyaraj, 1995).

$$MIE\ (\%) = \frac{\text{Dry wt. of inoculated pt.} - \text{Dry wt. of uninoculated}}{\text{Dry wt .of inoculated plant}} \times 100$$

RESULTS AND DISCUSSION

A significant positive response to AM inoculation was observed in the growth of *Anacardium occidentale* seedlings. However, the magnitude of the response varied with the two AM fungal species **(Table –28)**. Root and shoot fresh weights significantly varied between the three treatments with C.D, 0.55 and 1.91 respectively at $P< 0.05$; so also the root and shoot dry weights with C.D, 0.27 and 0.77 respectively at $P< 0.05$. Root length also significantly varied between the treatments (CD = 5.62; $P< 0.05$).

The significant increase in dry matter through mycorrhizal inoculation could be attributed to formation of a better root system leading to tapping of a larger volume of soil for water and nutrients. Shoot length did not vary significantly between the three treatments. However, there was significant increase in stem girth through mycorrhizal inoculation, thus making the plant strong enough to face wind breaks.

There was a significant increase in number of leaves through mycorrhizal inoculation (C.D = 3.23; $P< 0.05$) indicating greater photosynthetic activity. The leaf length did not significantly vary between the three treatments. However,

Table-28: Influence of *Glomus macrocarpum* and *Glomus intraradices* on growth of *Anacardium occidentale.*

Treatment	Root Dry wt. (g)	Shoot Dry wt. (g)	Root Fresh wt. (g)	Shoot Fresh wt. (g)	Root Length (cm)	Shoot Length (cm)	No. of Leaves	Leaf Length (cm)	Leaf Breadth (cm)	Stem Girth (cm)
Control	0.17	0.94	0.41	3.29	19.33	15.42	6.00	10.00	3.00	2.50
	(0.03)	(0.17)	(0.07)	(0.75)	(3.94)	(2.65)	(2.9)	(1.87)	(0.54)	(0.18)
Glomus	1.05	2.69	2.37	7.35	34.27	21.5	5.67	12.73	3.98	2.92
macrocarpum	(0.45)	(1.32)	(1.01)	(2.91)	(7.29)	(7.78)	(2.58)	(1.73)	(0.51)	(0.20)
Glomus	0.81	2.43	1.84	6.99	32.98	22.27	5.33	12.83	3.75	2.63
intraradices	(0.20)	(0.56)	(0.52)	(1.53)	(4.54)	(3.22)	(3.61)	(2.71)	(0.67)	(0.23)
C.D at 0.05%	**0.27**	**0.77**	**0.55**	**1.91**	**5.62**	**N. S**	**3.23**	**N. S**	**0.60**	**0.21**

Values are the mean of 6 replicates.
Values in parenthesis indicates standard deviation.
N.S = Not significant.

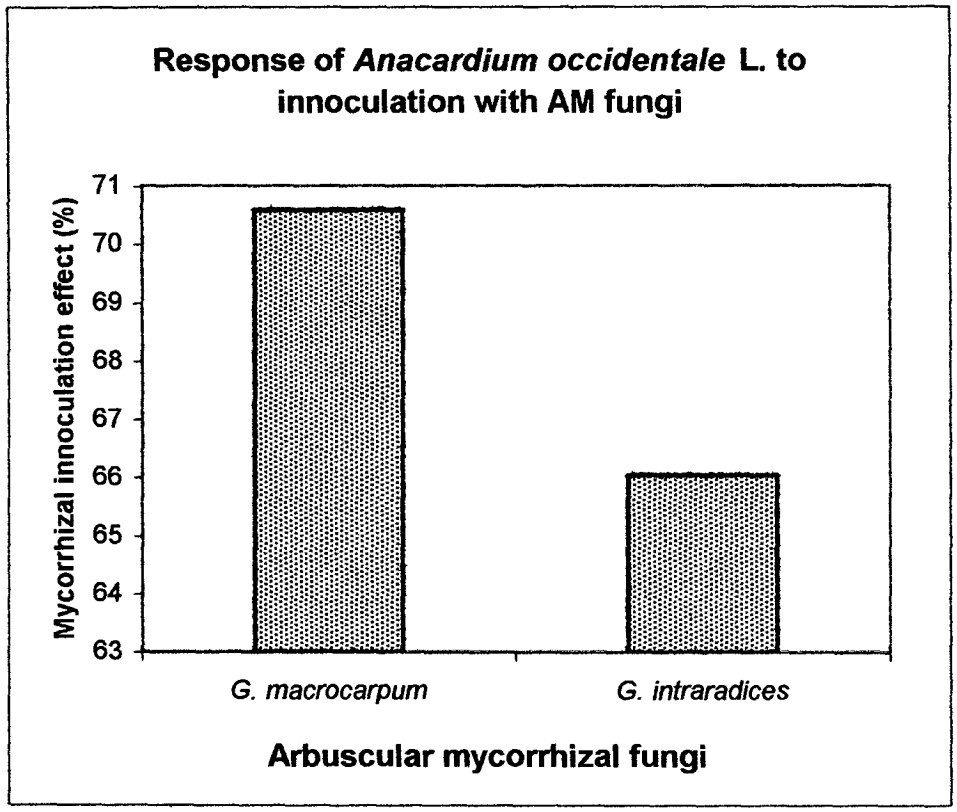

Response of *Anacardium occidentale* L. to innoculation with AM fungi
Mycorrhizal innoculation effect (%)
71
70
69
68
67
66
65
64
63
G. macrocarpum
G. intraradices
Arbuscular mycorrhizal fungi

Fig.15

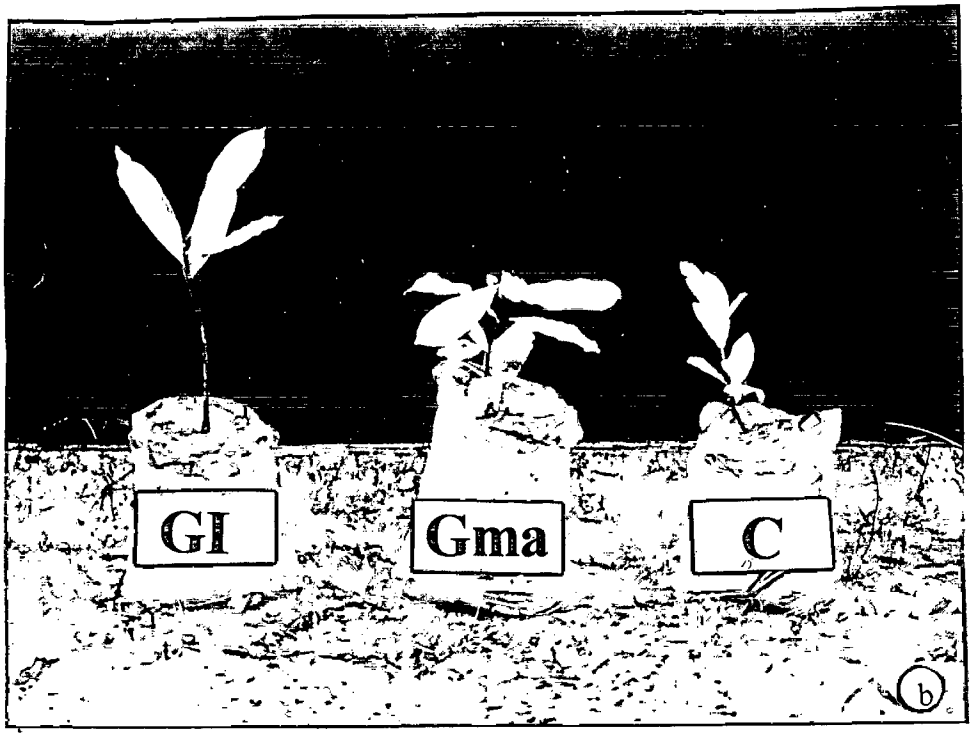
PLATE-11

MULTIPLICATION OF ARBUSCULAR MYCORRHIZAL (AM) FUNGI AND RESPONSE OF AM FUNGI TO PLANT SPECIES.

a) Single spore cultures of some AM fungi using *Eleusine coracana* (L.) Gaertn. and *Sesbania bispinosa* (Jacq.) Steud. as host plants.

b) Response of *Ancardium occidentale* L. to arbuscular mycorrhization.


a


GI
Gma
C
b

there was significant increase in leaf breadth through mycorrhization (C.D = 0.60; P< 0.05) indicating increase in photosynthetic area.

Hayman *et al.*, (1981) pointed the need for selection of VA endophytes suitable for a particular host/soil/climate combination. In the present study AM inoculation with both the AM inoculants was efficient in stimulating the growth of *Anacardium occidentale.* However, both the AM inoculants showed variation in their response. The local isolate, *Glomus macrocarpum* gave a better response over *Glomus intraradices* **(Plate-11; Fig. b)**. Mycorrhizal inoculation effect was observed to be 70.59% with *Glomus macrocarpum* and 66.05% with *Glomus intraradices* **(Fig. 15)**. This shows that the extent to which *Glomus intraradices* could compete with native endophyte to bring about plant growth response was lower to that of *Glomus macrocarpum.* Although there was no host specificity, a host preference for *Glomus macrocarpum* was observed. This study is in agreement with earlier study (Bagyaraj *et al.*,1989). Bagyaraj *et al.*, (1989) screened seven AM fungi for symbiotic response with *Leucaena leucocephala,* of which a local isolate *Glomus mosseae* was found to be the best mycorrhizal fungus for inoculation of *Leucaena leucocephala.*

The present study thus suggests the importance of isolation of local mycorrhizal fungi and including them during screening for efficiency, in order to select an efficient strain of mycorrhizal fungus best suited for a particular host plant.

SUMMARY

Coastal sand dunes are of great ecological significance. Dune vegetation stabilizes existing sand surfaces and speeds further accretion by reducing wind speed over the surface of sand. In the sand dunes, sand movement, scarcity of nutrients and organic matter, low water retention, high evaporation rates and other factors contribute to make the dunes a harsh and unfavorable medium for plant growth.

Arbuscular mycorrhizae improves nutrient uptake by plants, helps to stabilize the sandy substratum by binding sand grains into wind resistant aggregates and are thus important for the survival of plants and in sustaining their growth in the physiologically demanding dune environment.

The coast of Goa which extends approximately 120 Km in length has beautiful stretches of sandy shores and beaches. The dimension of hotel industries and tourism development on the beaches is surely affecting the dune system. Any perturbation to an existing ecosystem that includes physical removal of plants and changes in soils physical and chemical characteristics has a major impact on symbiotic association.

An understanding of mycorrhizal associations in sand dunes and their distribution in soil is essential in any attempt to use them in environmental conservation. The work carried out from the present investigation can be highlighted as follows:

1. In the present study, the status of arbuscular mycorrhizal fungi in Coastal sand dune vegetation of Goa is reported for the first time.

2. Depending upon the magnitude of disturbance, the various sites surveyed under the present study are classifed into three categories viz., Least disturbed, moderately disturbed and Severely disturbed.

3. The least disturbed sites include sand dunes from Varca and Mobor; The moderately disturbed sites include sand dunes from Colva, Benaulim, Majorda and Junes (Mandrem), while the severely disturbed sites include sand dunes from Miramar and Bambolim.

4. The survey of the Coastal sand dune vegetation revealed presence of 55 plant species belonging to 33 families.

5. A total of five plants species *viz.*, *Ipomoea pes-caprae, Spinifex littoreus, Vitex trifolia, Cocos nucifera* and *Casuarina equisetifolia* were the most dominant and common at all the eight study sites.

6. A survey of extent of root colonization and AM fungal spore density in the Coastal sand dune vegetation of Goa carried out from the three sites *viz.*, Mobor, Colva and Majorda indicated that all the plants selected for the study

were mycorrhizal. Hoever, the extent of colonization in these plant species varied. Arbuscular mycorrhizal colonization in plants ranged from 52-100%. Arbuscular mycorrhizal spore number in rhizosphere soil exhibited great variation. It ranged from 12 spores + 2 sporocarps/ 100g soil to 984 spores + 20 sporocarps/ 100g soil.

7. Study on the occurrence and distribution of AM fungi in less disturbed (Varca) and severely disturbed (Miramar) sand dune ecosystem indicated that the average spore density was higher in less disturbed dunes (1040.5 spores + 10 sporocarps/ 100g soil) than severely disturbed dunes (424.4 spores + 5.6 sporocarps/ 100g soil). The AM fungal species richness was higher in Varca (21 sp.) than Miramar (14 sp.). The dominance of AM fungal species varied at both the sites.

8. The qualitative and quantitative studies of AM fungal spores in the sand dune vegetation of Goa carried out from six sites *viz.*, Mobor, Benaulim, Colva, Majorda, Bambolim and Junes (Mandrem) confirms the universal occurrence of AM fungal spores. Average spore density varied from a minimum of 233 spores + 18 sporocarps/ 100g soil per site to a maximum of 725 spores + 15 sporocarps/ 100g per site. In all, 50 AM fungal species belonging to five genera *viz.*, *Acaulospora, Gigaspora, Glomus, Sclerocystis* and *Scutellospora* were identified. The species richness varied from a minimum of 8 AM fungal species per site to a maximum of 31 AM fungal species per site. *Acaulospora*

spinosa, Glomus macrocarpum, Acaulospora scrobiculata, Gigaspora margarita and *Scutellospora weresubiae* were dominating species in terms of frequency of occurrence as well as abundance in coastal sand dune vegetation of Goa.

9. The studies on seasonal variation of AM fungi with respect to root colonization, Total spore density and Species diversity taken up at two sites *viz.* Colva and Majorda revealed that the seasonal variations have a remarkable influence on the occurrence of spores. Average spore density recorded was maximum in early monsoon (July) followed by early summer (March) and minimum in post monsoon (November). Average percentage root colonization was maximum in November followed by July and minimum in March. At both the sites, *Scutellospora coralloidea* was the most frequently recovered fungus in all the seasons. The frequency of occurrence and relative abundance of AM fungal species varied with the seasons.

10. The various types of studies on AM fungi in coastal dune vegetation of Goa revealed a rich diversity of AM fungal species. A total of 51 AM fungal species were recorded from the dunes. The most frequently occurring AM fungi from the less disturbed to moderately disturbed dunes include *Acaulospora spinosa, A. scrobiculata, A. elegans, Gigaspora margarita, Glomus macrocarpum, G. heterosporum , Sclerocystis sinuosa, S. clavispora, Scutellospora coralloidea, S. calospora, S. gregaria* and *S. weresubiae;* while

the most abundant AM fungi include *Acaulospora spinosa, A. scrobiculata, A. elegans, Gigaspora margarita, Glomus macrocarpum, G. fasciculatum, G. heterosporum, G. maculosum* and *Scutellospora calospora.*

11. Six of the dominating species of AM fungi on Coastal sand dunes of Goa were multiplied on the roots of *Eleusine coracana* (L.) Gaertn. Three of the AM fungal species could be successfully multiplied.

12. Study of growth responses of *Anacardium occidentale* L., a dominating plant species in back shores of Goa coast, to inoculation with *Glomus macrocarpum*, a local isolate and *Glomus intraradices*, an introduced species tolerant to high temperature and salinity revealed that the plant showed better growth response with the local isolate (MIE=70.59%) as compared to introduced species (MIE=70.59%).

13. Transplantation of AM inoculated nursery seedlings may prove advantageous on reclamation sites, as limited nutrients in the soils would be more efficiently extracted. It is essential to isolate local AM fungi and include them during screening for efficiency, in order to select an efficient strain of AM fungi best suited for a particular host plant.

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* Originals not seen.

SYNOPSIS

Introduction

Coastal dune systems are dynamic natural features of vital economic and ecological significance. They function as flexible barriers to storm, tides and waves (Woodhouse, 1982). Dune vegetation plays an important role in the formation and stabilization of coastal sand dunes. It keeps the coastal land free from erosion and also prevents internal desertification.

The common constraints associated with sandy soils are normally related to nutrient deficiencies as well as low precipitation and high evapo-transpiration regime. The fungus – plant mutualistic symbiosis termed as "Mycorrhiza" play an important role in nutrient deficient sites. The growth advantages attributed to AM fungi are believed to be associated with an increase in nutritional status of the plant brought about by increased phosphorus uptake (Daft and Nicolson, 1969) and water transport (Safir *et al.*, 1972).

A mycorrhiza is a type of endophytic, biotrophic, mutualistic symbiosis prevalent in many cultivated and natural ecosytems. Arbuscular mycorrhiza is formed by non-septate zygomyceteous fungi. These endophytes can be identified by vesicles and arbuscules formation in roots (Abbot and Robson, 1979), dimorphic branching of extramatrical hyphae and production of large number of chlamydospores and azygospores in the soil. They show a little host specificity (Baylis, 1975).

The AM fungi invade host roots and proliferate inter- and intra-cellularly in the root cortex where they form vesicles and arbuscules. The main effect of mycorrhizal infection on plant growth is stimulation of phosphorus uptake due to exploration by the external hyphae of the soil beyond the root hair and phosphorus depletion zone. Absorbed phosphorus is converted into polyphosphate granules and made available to the plant (Callow *et. al.*, 1978). Enhanced uptake of other immobile elements can occur as well. Infection can influence plant- water relation by reducing plant resistance to water transport. Infection causes carbohydrate drain on the host, but in low phosphorus environment, the exchange of carbohydrate for inorganic minerals can be highly favourable to the host.

The coast of Goa has beautiful stretches of sandy shores and beaches. The coast line of Goa extends to 120 Km in length. The coastal sand dunes are susceptible for natural as well as human disturbances which affect the dune plant community, structure and stability. The re-establishment of functional ecosystems presumes a knowledge of both the important macro- and micro- elements of the system *i.e.*, both the above and below ground elements constituting the system. Arbuscular mycorrhizae are among the ubiquitous components in below ground ecosystems (Wilheim, 1966; Gerdemann, 1968,1971,1975; Smith, 1974; Read, Kaucheki and Hodgson, 1976); the fungal symbionts appear to be essential in most ecosystems (Hacskaylo, 1972; Mosse, 1973; Gerdemann, 1975). These fungi, therefore must be studied to understand the ecosystem changes. No effort seem to be have been directed to isolate and identify the native AM fungi in the sand dune

soils of Goa. The present study is taken up to determine the status of AM fungi in the sand dune vegetation of Goa.

Aims and Objectives

1. To study the diversity of the AM species in natural and disturbed sites of sand dunes.
2. To find AM species diversity in rhizosphere of each host plant species.
3. Determination of colonization of AM in infected roots and spore density in rhizosphere of dune plants.
4. To determine the seasonal fluctuations in AM fungal colonization, spore density and diversity.
5. Preparation of pure inoculum of AM fungi in association with dune plants.

Methodology

A survey of the beaches in Goa having sand dunes was carried out and the disturbed and undisturbed sites were identified. The dune vegetation was surveyed to find out the dominant plant species. Rhizosphere soil samples and root samples were collected. The roots were examined for percent infection using Phillips and Hayman method, (1970). Arbuscular mycorrhizal fungi were isolated from the rhizosphere soil samples by Wet sieving and decanting method (Gerdemann and Nicolson, 1963). Spores and sporocarps of AM species in rhizosphere soil samples were quantified (Gaur and Adholeya, 1994). Spores and sporocarps isolated were identified using Manual for identification of AM fungi by Schenck and Perez, (1990). Pure cultures of isolated AM fungi were prepared.

Observations

The first chapter deals with the survey studies of coastal sand dune vegetation in Goa. The objective of the work was to study the diversity of vegetation and to find out the dominant plant species existing on the dunes. It was observed that among all the beaches selected for the study, the beaches in South Goa comprised well developed sand dunes with rich sand dune vegetation whereas, the beaches in North Goa possessed highly disturbed dunes with scanty vegetation. The dunes were found to be covered with different plant species belonging to thirty three families. It was observed that a total of five plant species *viz., Ipomoea pes-caprae* (L.) Sweet, *Spinifex littoreus* (Burm.f.) Merr., *Vitex trifolia* L., *Cocos nucifera* L. and *Casuarina equisetifolia* Forst. were the most dominant and common at all the study sites.

The second chapter deals with the preliminary survey of mycorrhizal association in sand dune vegetation. The survey revealed that all the plants were mycorrhizal but, the extent of colonization varied. The mycorrhizal infection consisted of hypha, vesicles and arbuscules. Mycorrhizal infection in plants ranged from 52 to 100%.

The third chapter deals with the occurrence and distribution of mycorrhiza in natural and disturbed sand dunes of the Goa coast. The objective of the study was to compare the spore density and diversity of AM fungi in an undisturbed verses disturbed sand dune vegetation. The study reveals that the spore density and

species richness were high in undisturbed site. Th AM fungi *viz., Scutellospora weresubiae* Koske et Walker, *Scutellospora pellucida* Walker and Sanders, *Scutellospora verrucosa* Walker and Sanders, *Scutellospora coralloidea* Trappe, Gerdemann and Ho. *Gigaspora margarita* Becker and Hall, *Glomus macrocarpum* Tul. and Tul., *Glomus microaggregatum* Koske, Gemma and Olexia, *Glomus heterosporum* Smith and Schenck, *Acaulospora spinosa* Walker and Trappe and *Acaulospora foveata* Trappe and Janos, were common at both undisturbed and disturbed sites. *Glomus macrocarpum* and *Gigaspora margarita* were the most frequent in undisturbed site whereas *Acaulospora spinosa* was the most frequent in disturbed site followed by *Scutellospora coralloidea* and *Glomus macrocarpum.*

The fourth chapter deals with the study of diversity of AM fungi in coastal sand dune vegetation of Goa. The study reports a rich diversity of AM fungi associated with the dune plants. The AM fungi recorded, belonged to five genera *viz., Scutellospora, Sclerocystis, Glomus, Gigaspora* and *Acaulospora. Scutellospora weresubiae* Koske et Walker, *Scutellospora calospora* Walker and Sanders, *Sclerocystis sinuosa* Gerdemann and Bakshi, *Glomus microaggregatum* Koske, Gemma and Olexia, *Glomus macrocarpum* Tul. and Tul., *Gigaspora margarita* Becker and Hall, *Acaulospora spinosa* Walker and Trappe and *Acaulospora scrobiculata* Trappe, were the most frequently occurring AM fungi in sandy soil of Goa coast.

The fifth chapter deals with the seasonal dynamics of AM fungi in coastal sand dune vegetation. The aim of the study was to understand the pattern of colonization, spore density and species richness in relation to seasonal changes. Maximum average spore density per 100g soils was recorded in early monsoon (July), followed by early summer (March), where as the minimum average spore density per 100g soils was observed in post monsoon collections. Root colonization was observed throughout the year. Percent root colonization was maximum in post monsoon followed by early monsoon. Minimum percent colonization was recorded in summer season. *Scutellospora coralloidea* was the most frequently occurring AM fungi in all the seasons.

Conclusions

Our study of the native vegetation of coastal sand dunes indicates that AM fungi is a well developed feature of sand dunes. Most plant species on the dunes are mycorrhizal. There is a rich diversity of AM fungi in the sandy soil. Disturbance due to human interference on the dunes has deleterious effects on AM fungal spore density in the soil. Arbuscular mycorrhizal fungi in the dunes vary in their spore density and intensity of colonization with the changes in the seasons.

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APPENDIX

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Occurrence and distribution of arbuscular mycorrhizal fungi in coastal sand dune vegetation of Goa

Coastal sand dunes are of great ecological significance. They act as barriers against the action of waves and tides[1]. Dune vegetation helps in the formation and stabilization of sand dunes. The beaches of Goa are famous for scenic beauty. The dimensions of tourism development on the beaches are surely affecting the dune system and any further damage to the fragile dune system will cause disaster by erosion or accretion of the coastal belt. Arbuscular mycorrhizal (AM) fungi play a very important role in dune stabilization, uptake of water and nutrient by plants and binding sand grains[2]. Mycorrhizal plants are effective colonizers of disturbed habitats and the lack of mycorrhizal fungi exerts profound influence on species composition[3]. Through close mutual interaction between plants and soil organisms these ecosystems create conditions that allow the systems to persist. Severing the close links between plants and soil has contributed to degradation of many ecosystems and restoring these links is an important step towards rehabilitation[4]. Since native AM fungal flora is well adapted to the conditions of its natural occurrence due to its long process of evolution, it can be of immense use for various plants compared to exotic AM species. The understanding of mycorrhizal association with dune plants and their distribution in the soil is necessary for wise management of fragile habitats.

The present study represents an attempt to establish the qualitative and quantitative distribution of AM fungal spores in sand dune vegetation. The study was taken up in one of the smallest states of India, Goa, situated along the west coast of India lying between latitude 15°48′00″ and 14°43′54″N and longitude 74°20′13″ and 73°40′33″E (ref. 1). The coast is approximately 120 km in length. Colva beach, a famous tourist site located in south Goa, was selected for the survey. Rhizosphere soil samples were collected from nine host plants, viz. *Achyranthus aspera* L., *Calotropis gigantea* R. Br., *Casuarina equisetifolia* Forst., *Cocos nucifera* L., *Fluggea* sp., *Hyptis suaveolens* Poit., *Ipomoea biloba* Forsk., *Lantana camara* L. and *Vitex trifolia* L. selected for the study. Samples were collected in March 1999.

Routinely three root zones of each plant were sampled at a depth of 0–25 cm. Soil samples collected from individuals of a species were mixed to form a composite sample. These composite soil samples were used for the enumeration of spores. Hundred grams of composite sample was assayed for spore count using wet sieving and decanting procedure[5]. Each composite sample was processed three times. Estimation of spore density was carried out according to the procedure given by Gaur and Adholeya[6]. Spores were mounted in polyvinyl alcohol-lacto-glycerol (PVLG), examined for their various characteristics and identified using the standard keys[7]. For soil analysis, rhizosphere soils of all the host plant species along with the sub samples were mixed to form a composite sample and this was analysed for pH, total N, total P and total K.

Physio-chemical properties of soil samples indicated that the soil was slightly alkaline (pH 7.6) with 0.04% total N, 0.016% total P, and 0.045% total K.

Average mean spore count was 725 spores + 15 sporocarps/100 g rhizosphere soil. Maximum mean spore density recorded was in *C. nucifera* (1156 spores + 60 sporocarps/100 g soil), followed by *C. equisetifolia* (864 spores + 4 sporocarps/100 g soil). Minimum mean spore density was observed in *L. camara* (408 spores + 16 sporocarps/100 g soil).

The AM fungal spores obtained belonged to the five genera, viz. *Acaulospora*, *Gigaspora*, *Glomus*, *Sclerocystis* and *Scutellospora*. The identified species of AM fungi include *Acaulospora bireticulata* Rothwell and Trappe, *A. scrobiculata* Trappe, *A. spinosa* Walker and Trappe, *Gigaspora coralloidea* Trappe, Gerdemann and Ho, *G. gregaria* Schenck and Nicol., *G. margarita* Becker & Hall, *Sclerocystis sinuosa* Gerdemann and Bakshi and *Scutellospora verrucosa* Walker and Sanders. *G. coralloidea* was found to be the most frequently occurring fungus (89%), followed by *S. verrucosa* (67%),

Table 1. Frequency distribution of AM fungi in selected host plants

M fungi	*Vitex trifolia*	*Cocos nucifera*	*Ipomoea biloba*	*Fluggea* sp.	*Lantana camara*	*Achyranthes aspera*	*Casuarina equisetifolia*	*Hyptis suaveolens*	*Calotropis gigantea*	Frequency (%)
aulospora birelculata	+	–	–	+	–	–	–	+	+	44
aulospora scrobiculata	+	–	–	–	+	–	–	–	+	33
aulospora spinosa	+	+	+	+	+	+	–	+	+	89
gaspora coralloidea	+	+	–	+	+	+	+	+	+	89
gaspora gregaria	–	–	–	+	–	–	–	–	+	22
gaspora nargarita	–	+	+	+	–	–	–	+	–	44
'erocystis inuosa	–	–	–	–	–	+	+	+	+	44
itellospora errucosa	–	+	+	+	–	+	–	+	+	67

= Present; – = absent.

margarita and *S. sinuosa* (44%), ible 1). Maximum number of AM igal species was observed in root zone l of *C. gigantea* (6 spp.) and least nber of species was found in *C. equi-ifolia* (2 spp.); (Table 1). Spores of *aulospora* sp. dominated the root zone ls of six selected host plants with per-tage spore density/100 g soil ranging n 33 to 51%.

The coast of Goa has a beautiful tch of sandy shores and beaches ch attract a large number of tourists n home and abroad. A well-planned elopment of beaches is essential for ecological importance of sand dune etation.

is now widely accepted that AM gi contribute significantly to plant wth and survival[8]. The prevalence of fungal propagules in temperate mari-sand dunes has also shown to con-ute to the effectiveness of mycorrhizal ts as pioneer dune colonizers, thereby ributing to the stabilization of the dunes[9]. It is apparent that the orrhizal status of early successional ts is governed by AM fungal species lability, composition and inoculum res, external mycelia and mycorrhizal i) potential.

Stabilization of disturbed ecosystems like coastal dunes is dependent upon successful establishment of the most effective plant community. As mycorrhizal plants serve this purpose, there is great potential for land reclamation programme in manipulating symbiotic association to accelerate the success of more desirable plants[10]. Our study shows the presence of rich diversity of AM fungi in the rhizosphere soils of coastal dune vegetation. *A. spinosa* and *G. coralloidea* are the most frequently occurring species. Successful revegetation would depend on selecting and maintaining these essential mycorrhizal fungi and developing methods to re-inoculate these fungi in disturbed beaches in south Goa.

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ARBUSCULAR MYCORRHIZAL (AM) FUNGI FROM COASTAL SAND DUNE VEGETATION OF GOA

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Abstract

Arbuscular mycorrhizae (AM) may be used for rebuilding the vegetation of coastal region under threat. Prior to exploiting the reclaiming potential of these organisms, it is necessary to examine their occurrence and distribution in sand dunes. The occurrence and the number of AM propagules in the rhizospheric soils of six selected saline host plant species was worked out. Arbuscular mycorrhizal propagules were observed in rhizospheric soils of all the selected host plant species. Average spore count was 327.5 spores/100 g rhizospheric soil. Fifteen species of AM fungi were identified from the soil samples. Species of *Glomus* dominated the rhizospheric soils of the sand dune ecosystem.

Introduction

Sand dunes throughout the world have been recognized for their ecological significance. The dune vegetation helps in keeping the coastal land free from erosion and also prevents internal desertification. There has been a continuous pressure on sand dunes. The dimension of hotel industries and tourism development is surely affecting the dune system. The presence of fungus plant mutualistic symbiosis termed 'Mycorrhizae' is critical for the regeneration of the coastal ecosystem. Arbuscular mycorrhizae that participate in the uptake of phosphorus are involved in pioneer colonization of nutrient deficient sites. There is a need to identify the AM species from existing vegetation so that the same may be used for rebuilding the vegetation of coastal area under threat. No efforts seem to have been directed to isolate and identify the native AM fungi in saline soils in the state.

Materials and Methods

The coast of Goa which is approximately 120 km in length has beautiful stretches of sandy shores and beaches. Two adjacent coastal beaches were selected for the survey of the distribution of AM spores, viz., Bogmalo beach (0.70 km) and Hansa beach (0.60 km). Soil samples were collected from the two locations and were analysed for pH, P and K content. Rhizospheric soil samples were collected from six selected host plants from 0-25 cm depth. The collection was done in between the months of July to October 96. Soil samples (100 g) were assayed for spore count using the Wet Sieving and Decanting procedure (Gerdemann & Nicolson, 1963). The spore counting was done for the estimation of spore density (Gaur & Adholeya, 1994). The spores and the sporocarps were examined for their various characteristics for identification (Schenck & Perez, 1988). Single spore pot cultures of the isolated spores were prepared using *Eleusine coracana* (Linn.) Gaertn. and *Sesbania bispinosa* (Jacq.) Steud. as host plants. Later, the roots were examined for infection (Phillips & Hayman, 1970).

Results and Discussion

Physio-chemical properties of soil samples from the two locations of coastal areas taken for the present study indicated that the soils were alkaline. The pH ranged from 8.3 to 8.6. The sandy soil had low available phosphorus (6.73 kg/ha - 17.95 kg/ha).

Potassium content was also low (22.4 kg/ha - 44.9 kg/ha). The rhizosphere of all the six plant species examined exhibited AM spores. Average

spore count was 327.5 spores/100 g of rhizospheric soil. The soil characteristics and the spore count in rhizosphere of each plant species is presented in Table-1. Number of AM spores was found to be less where soil phosphorus content was higher. The maximum spore count was 427/100 g at P content 6.73 kg/ha and minimum spore count was 311/100 g and 170/100 g at P content 17.95 kg/ha. This shows that the higher concentration of P limits AM fungal spore population. Earlier studies of AM fungi isolated in soil samples collected from different parts of the country have indicated that the P content was found to be negatively correlated with the number of spores in the soil (Mukerji & Rekha Rani, 1989). The spore count did not show any definite correlation with the K content.

Table -1 : Soil characteristics and spore density in the rhizospheric soil of some plant species (Mean of three samples)

Family & Plant species (source of sampling)	Depth of sampling (cm)	No. of spores/ 100 g rhizo-spheric soil	Soil pH	Characteristics P kg/ha	K kg/ha
Cyperaceae					
Cyperus sp.	0-25	427	8.6	6.73	33.7
Liliaceae					
Urgenia indica	0-25	370	8.5	9.00	22.4
Asclepiadaceae					
Calotropis gigantea	0-25	355	8.5	11.2	44.9
Poaceae					
Spinifex squarrosa	0-25	332	8.5	11.2	44.9
Arecaceae					
Cocos nucifera	0-25	311 (+11 sporocarps)	8.3	18.0	22.4
Verbenaceae					
Lantana camara	0-25	170 (+4 sporocarps)	8.3	18.0	22.4

Fifteen species of AM fungi were recorded from the rhizospheric soils of the six selected plant species. Most of the AM fungi were isolated as chlamydospores and in few cases sporocarps were recorded. The isolated sporocarps were identified as *Sclerocystis sinuosa* Gerd. & Bakshi. Species of *Glomus* dominated the soils. Very few spores of *Gigaspora* and *Acaulospora* species were recorded. It has been reported that *Gigaspora* and *Acaulospora* species are more tolerant to acidity and *Glomus* species favour neutral and alkaline soils (Mosse, 1973). Present study showed absence of *Entrophospora* and *Scutellospora* species in the rhizosphere of the plant species taken up for the study. Different AM apecies isolated from the rhizosphere of each of the host plant species are listed in Table-2.

The most frequent endophyte recorded was *Glomus fasciculatum* which was present in three of the hosts, viz., *Calotropis gigantea, Cocos nucifera* and *Lantana camara. Glomus citricolum* and *Sclerocystis sinuosa* occupied the next position being present in two hosts, viz., *Urginea indica* and *Spinifex squarrosa,* and *Cocos nucifera* and *Lantana camara* respectively. *Cyperus* spp.

Table -2 : Arbuscular mycorrhizal fungi identified from the rhizospheric soils of some plant species

Family & Plant species	VAM fungi species
Cyperaceae *Cyperus* sp.	*Glomus occultum.*
Liliaceae *Urgenia indica*	*Glomus citricolum* & *Acaulospora bireticulata.*
Asclepiadaceae *Calotropis gigantea*	*Glomus deserticola,* *G. fasciculatum* & *G. intraradices.*
Poaceae *Spinifex squarrosa*	*Glomus citricolum,* *Gigaspora margarita,* *Acaulospora spinosa* & *A. scrobiculata.*
Arecaceae *Cocos nucifera*	*Sclerocystis sinuosa,* *Glomus etunicatus,* *G. fasciculatum,* *G. reticulatum,* *G. versiforme,* *G. hoi* & *Gigaspora coralloidea.*
Verbenaceae *Lantana camara*	*Sclerocystis sinuosa* & *Glomus fasciculatum.*

was exclusively associated with *Glomus occultum.* All the AM spores associated with *Calotropis gigantea* belonged to various species of *Glomus,* viz., *G. deserticola, G. fasciculatum* and *G. intraradices.* In remaining hosts different combinations of AM species were observed (Table-2). *Cocos nucifera* and *Spinifex squarrosa* showed maximum species diversity of AM fungi. *Cocos nucifera* was associated with *Sclerocystis sinuosa, Gigaspora coralloidea* and five species of *Glomus,* viz., *G. etunicatum, G. fasciculatum, G. reticulatum, G. versiforme* and *G. hoi. Spinifex squarrosa* showed presence of *Glomus citricolum, Gigaspora margarita* and two species of *Acaulospora,* viz., *A. spinosa* and *A. scrobiculata.* The difference in the species may be attributed to the edaphic factors, host plant interactions at a particular site and host species compatibility with AM fungi.

The roots analysed from single spore pot cultures showed the presence of vesicles and arbuscules confirming that these spores belonged to AM fungi and were potentially viable to bring about infection.

Our study confirms the contention that most plants grown under natural conditions possess AM and phosphorus content in the soil may be the major edaphic factor which determines the abundance of AM fungal spores. AM may be of considerable significance to the success of any coastal environment stabilization programme. Isolation and multiplication of efficient strains of AM fungi is important for mass inoculum production and for adaptation of tree seedlings suitable for rehabilitation and reclamation of these saline and nutrient deficient soils.

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