STUDIES ON ARBUSCULAR MYCORRHIZAL (AM) FUNGI IN MANGROVES OF GOA

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1.1: Introduction

Mangrove forests are open 'interface' ecosystems connecting upland terrestrial and coastal estuarine ecosystems (Lugo and Snedaker 1974). The mangrove plant species are facultative halophytes, characterized by regular tidal inundation and fluctuating salinity (Gopal and Chauhan 2006). They are highly adapted to the coastal environment, thriving in intertidal zones of tropical and sub-tropical regions (Ball 1996; Naidoo *et al.* 2002) and exhibit a number of unique morphological and eco-physiological adaptations to the coastal environment (Kathiresan and Bingham 2001).

The diversity of mangroves has recently become increasingly important as a result of the Convention on Biological Diversity (http://www.cbd.int/convention/text), and because mangrove ecosystems are among the most threatened by global climate changes, particularly the rise in sea levels (Macintosh and Ashton 2002). Their floral diversity which is comprised of only 65 to 69 species is relatively well known. Mangroves are taxonomically diverse. True mangroves include about 54 species in 20 genera belonging to 16 families (Hogarth 1999). Global patterns of mangrove biodiversity present an interesting picture where the latitudinal pattern of mangrove flora is normal *i.e*. highest species richness of plants occurs around the Equator and declines at higher latitudes both north and south (Duke *et al.* 1998; Ellison *et al.* 1999), the longitudinal distribution is 'anomalous' with high concentrations in the Eastern hemisphere between 90º E and 135º E (Ellison and Farnsworth 2001). Interestingly, the mangrove-inhabiting molluscs follow a similar pattern (Ellison *et al.* 1999). Mangrove habitats have relatively low levels of species richness compared with other high biomass tropical habitats such as rain forest and coral reef (Ricklefs and Latham 1993). Despite the relatively low biodiversity, plants in these forests have a broad range of structural and functional attributes which promote their survival and

propagation in the hostile conditions of the intertidal zone. The richest mangrove communities occur in tropical and sub-tropical regions where water temperature is greater than 24^0C and annual rainfall exceeds 1250 mm.

Mangrove distribution is governed by topography, tidal height, substratum and salinity. Extreme variation is displayed in plant composition, forest structure and growth rate. Mangrove forests can vary from a narrow fringe along the banks of an estuary to dense stands covering many square kilometres **(Plate I & II)**. The total global area of mangroves is estimated at only 18.1 million ha (Spalding *et al.* 1997), as against over 570 million ha of freshwater wetlands (Spiers 1999). In India total mangrove area is 6740 km^2, 80% of which is found along the east coast and 20% on the west coast. Deltaic environments on India`s east coast support extensive mangrove forest formations due to intertidal slope and heavy impact of siltation. The western coastline has narrow intertidal belts which support fringe mangroves, a restricted cover due to the peculiar coastal structure and the nature of estuaries formed by relatively small and non-perennial rivers except Narmada and Tapi. All the estuaries in Goa are classified as microtidal estuary as tidal level is below two meters (Ahmad 1972). Although mangroves have been exploited for many centuries, our understanding of these wetland forests remained poor until the 1970s (Lugo and Snedaker 1974; Chapman 1976).

1.2: Morphology and Anatomy

Mangroves are highly adapted to the coastal environment, with exposed breathing roots, extensive support roots, buttress roots, salt-excreting leaves and viviparous water-dispersed propagules. These adaptations vary among taxa and with the physico-chemical nature of the habitat (Duke 1992). One of the most remarkable of

these adaptations are the stilt *i.e.* support roots of *Rhizophora*. In *Avicennia, Sonneratia* and *Lumnitzera*, pneumatophores from lateral roots in mud and often project 20-30cm above soil transmitting oxygen to reach submerged roots. The density, size and number of pneumatophores vary depending on plant species. The specialized roots are important sites of gas exchange for mangroves living in anaerobic substrata. The exposed surfaces of roots may have numerous lenticels (loose, air-breathing aggregations of cells), (Tomlinson 1986). *Sonneratia, Avicennia, Xylocarpus, Bruguiera* and *Ceriops* exhibit a system of air-filled cable roots. These are horizontal roots, lying 20-50 cm below soil surface. These cable roots give off anchoring roots downwards for nutrients absorption and negatively geotrophic aerial roots upwards that may be human-knee shaped. Besides, *Avicennia* possesses lenticels equipped pneumatophores (upward directed roots) through which oxygen passively diffuse.

1.2.1: Leaf anatomy - Mangroves have xerophyte adaptations to live physiologically in dry and saline environments. Water storage tissue present in the leaves help in filtering the solar radiations. The leaves are a moderate size and are arranged in a modified decussate pattern with each pair at an angle less than 180^0C to the preceding pair. This arrangement reduces self-shading and produces branch systems that fill space in the most photosynthetically efficient way. Mangrove leaves are leathery; the cuticle is thick and smooth with small hairs, giving the plant a glossy appearance. In general, sunken stomata are present in mangrove leaves. Some genera such as *Aegiceras, Avicennia* and *Acanthus* possess salt glands structure in their leaves enhancing tolerance to salinity (Tomlinson 1986).

1.3: Physiology

Salt regulation- Mangroves are physiologically tolerant of high salt levels (Ball 1996). They require salt in their tissue to maintain gradient of water flow from soil, through roots to shoots. Water tends to flow from a region of lower salt concentration to a region of higher salt concentration. Because of this, the concentration of salt in the plant tissue has to be higher than that around the roots, for water to flow from soil to roots. The mangroves regulate salt concentration in plant tissue through a combination of salt exclusion, salt excretion and salt accumulation. For example, *Rhizophora, Bruguiera* and *Ceriops* may possess ultra filters in their root system. The ultra filters exclude excessive salts while extracting water from the soil (Kathiresan and Bingham 2001).

1.3.1: Accumulation of compatible solutes and osmolytes - One of the important biochemical mechanisms by which mangroves counter the high osmolarity of salt is accumulation of compatible solutes (Takemura *et al.* 2000; Parida *et al.* 2004). In mangroves the vacuole is the main site for salt accumulation. This high concentration of salt in the vacuole must be balanced with chemical concentration in the cytoplasm. If there is no balance the water will flow from cytoplasm to vacuole and damage the cell. To accommodate ionic balance in the vacuoles, cytoplasm accumulates low-molecular-mass compounds termed compatible solutes that do not interfere with normal biochemical reactions; rather they replace water in biochemical reaction (Hasegawa *et al.* 2000; Ashihara *et al.* 2003). Maintaining an osmotic balance between the two components is a remarkable adaptation of mangroves to saline environment thus regulating the loss of water vapour from the leaf or transpiration stream. In the wet season, the fine root biomass increases in response to decreased

salinity of the surface waters, directly enhancing the uptake of low-salinity water (Lin and Sternberg 1994). Water use becomes increasingly conservative with increasing salinity in the environment and with increasing salt tolerance of mangrove species. The restriction of transpiration of water vapour through stomatal leaf openings may, however reduce entry of CO_2, photosynthesis and growth.

1.3.2: Photosynthesis - Mangroves show characteristic of C_3 photosynthesis. Chloroplasts of mangroves are in fact sensitive to high salts. Hence, excessive salts are excluded from the chloroplasts. Photosynthetic rates of some species are strongly affected by environmental conditions and show saturation at relatively low light levels even though they are inhibited in high sunlight tropical environments. To prevent damage to the photosystems, mangroves dissipate excess light energy *via* the xanthophyll cycle (Gilmore and Bjorkman 1994) and through the conversion of O_2 to phenolics and peroxidases (Cheeseman *et al.* 1997). Mangroves show maximum growth when there is a plentiful supply of fresh water into their saline environment. Growth differs with the fluctuating soil salinities leading to significantly lower intercellular CO_2 concentration and reduced photosynthesis (Lin and Sternberg 1992). The stunted mangroves in these habitats have much lower canopies and smaller leaves than mangroves in fringe forests that experience less salinity variability.

1.4: Reproduction, Dispersal and Establishment

Mangroves reproduce sexually by means of flowers generally of a small size. There are four methods of mangrove reproduction; vivipary, crypto-vivipary, normal germination in soil, and vegetative propagation. Vivipary, the continuous growth of offspring while still attached to the mother plant, is a unique adaptation to shallow

marine habitats. True viviparous species remain attached to the mother plant for a full year, while crypto viviparous offspring's are only attached for 1-2 months (Bhosale and Mulik 1991). Viviparous reproduction allows seedlings to develop some salinity tolerance and a store of nutrients before being released from the parent tree (Smith and Snedaker 1995). It also helps to develop buoyancy for the aquatic distribution of seedlings and structural stability to protect a seedling from damage. In non-viviparous mangroves, seeds or fruits are larger which assist flotation. The timing of mangrove reproduction is dependent on local environmental conditions and may differ greatly over a range of species.

Dispersal of propagules depends on buoyancy, longevity and the activity of tides and currents. Mangroves are also vulnerable during establishment and early growth. The mortality can be attributed to the failure to establish before seed viability is lost, predation, or desiccation (Farnsworth and Ellison 1991). After establishment, survival and growth are strongly influenced by encountered physiochemical stresses. Experimental work with *Rhizophora* species demonstrates that propagule length, planting depth, soil type, salinity, pH and light intensity are important determinants of growth (Kathiresan and Thangam 1989; Kathiresan and Ramesh 1991; Kathiresan and Moorthy 1993; Kathiresan *et al.* 1995, Kathiresan 1999).

1.5: Biochemistry

Mangroves are biochemically unique, producing a wide array of novel natural products. E*xcoecaria agallocha*, for example, exudes acrid latex that is injurious to the human eye, hence its common name the blinding tree, and are rich in polyphenols and tannins (Kathiresan and Ravi 1990). The levels of these substances may vary seasonally. Phenols and flavonoids in mangrove leaves serve as UV-screen

compounds enhancing tolerance to UV radiation propagating an UV-free, under-canopy environment (Moorthy 1995). Several recent studies have examined issues concerning the ecology, management and conservation of mangroves (Ricklefs and Latham 1993; Ellison *et al.* 1999; Kathiresan and Bingham 2001; Ellison 2002).

Mangroves have become the center of many conservation and environmental issues because of loss of beneficial effects on the coastal environment. Anthropogenic pressure is constantly increasing and hence immediate protection and conservation of the ecosystem is necessary. Mangrove forests are important for biomass production because of their contribution to the geo-aquatic food chain. Reforestation of mangrove is a promising solution to restoration.

Mycorrhiza literally means “fungus root” and refers to the relationship that most plant species have with fungi. Arbuscular mycorrhizae (AM) are obligate fungal symbionts of estimated 80–90% vascular plants and some nonvascular plants, such as mosses (Smith and Read 1997). The fungus receives carbon from the plant while facilitating plant uptake of phosphorus (P) and other nutrients of low mobility in soil (Rhodes and Gerdemann 1978). Within the diversity of mycorrhizal associations, this symbiosis is the most prevalent and is a type of endomycorrhiza in which the fungus penetrates cortical cell walls. They are characterized by specialized intercellular hyphae and unique branching hyphal arbuscles and coils which form inside the cells. The fungi responsible are classified in the phylum Glomeromycota, order Glomales. Arbuscular mycorrhizae used to be classified as Vesicular Arbuscular Mycorrhizae (VAM) but research uncovered that a major sub-order did not form thin-walled, lipid-filled vesicles, so they are referred to as AM associations today. There is no evidence for specificity between plants and fungi in AM. Fungal colonization starts from source inocula that include spores, colonized root fragments and hyphae. The fungal

spores tend to be thick walled and contain several thousand nuclei. Both hyphae and spores can survive harsh conditions including plant dormancy, plant death and seasonally severe environmental circumstances. Carbon is used by the fungus to produce vegetative and reproductive structures and respiration for the energy needed in nutrient uptake. At this stage, the plant has increased capabilities for absorbing elements like P, N, Zn, C, Ni and is more efficient at gathering nutrients than when roots are not colonized (Smith and Read 1997). The efficiency of P and other crucial nutrients absorption is dependent on many ecological variables, including the ability of extra-radical hyphae to explore greater soil volume, smaller diameter hyphae having greater surface area than plant roots, carbon availability, bacterial community and various fungal chemicals needed for the process (Büching and Shachar-Hill 2005).

Arbuscular mycorrhizal fungi occur in natural plant communities, help to increase plant tolerance to adverse soil conditions, influencing response to severe climatic conditions and increasing plant productivity. These ecological functions are a feature of natural ecosystems (Brundrett and Kendrick 1996). Availability of nutrients is a primary factor affecting abundance and composition of plant species communities (Klironomos 2003). Such close linkages are necessary in tropical habitats, as available nutrient pools of the major nutrients, such as phosphorus (P) and nitrogen (N) are deficient in mangrove ecosystems (Carr and Chambers 1998) and likely to limit the growth of mangrove plant species. Microorganisms such as phosphate solubilizing, N fixing microorganisms and AM fungi are known to interact in the rhizosphere soils and can solubilise the bound P into available form. Arbuscular mycorrhizae fungal hyphae aid in transport of nutrients by extending beyond the depletion zone (Cui and Cladwell 1996). Besides, AM fungi play significant role in physiological processes

such as water use efficiency (Ruiz-Lozano *et al.* 1996), modify the structure and function of plant communities and are useful indicators of ecosystem change (Miller and Bever 1999). It is now recognized that the high rates of photosynthesis and primary productivity of many mangrove forests depend on not only unique and highly evolved physiological mechanisms (Ball 1988) but also on highly evolved and energetically efficient interrelationships among soil nutrient pools, microbes, and trees (Alongi 2005).

1.6: AM and salt stress amelioration

Soil salinity significantly reduces the absorption of mineral nutrients, especially phosphorus (P) as, phosphate ions precipitate with calcium (Ca_2), Magnesium (Mg) and Zinc (Zn) ions in the salt stressed conditions. AM fungi have been shown to have a positive influence on the composition of mineral nutrients of plants grown in salt-stress conditions (especially poor mobility nutrients such as P) by enhancing selective uptake of nutrients (Al-Karaki and Clark 1998). This is primarily regulated by the supply of nutrients to the root system (Giri and Mukerji 2004) and increased transport (absorption and/or translocation) by AM fungi (Sharifi *et al*. 2007). Zuccarini and Okurowska (2008) investigating the role of AM fungi and salt stress have demonstrated several mechanisms by which AM symbiosis alleviates salt stress in host plants. Under salt stress, non-mycorrhizal plant growth and biomass suffered a setback. The reasons may be the non-availability of nutrients and the expenditure of energy to counteract the toxic effects of NaCl. Mycorrhization was found to increase the fitness of the host plant by enhancing its growth through accumulation of an osmoregulator with increase in photosynthetic rate and water-use efficiency, suggesting that salt-stress alleviation by AM fungi results from a combination of

nutritional, biochemical and physiological effects. Enhanced growth of AM plants has been partly attributed to mycorrhizically mediated enhanced nutrient acquisition, especially better P nutrition (Sharifi *et al.* 2007).

1.7: AM in Phytoremediation and Land Reclamation

Bioremediation is the biochemical technology that uses organisms to alter polluted environments back to their healthy conditions. Phytoremediation is a form of bioremediation where plants are used to detoxify soil and AM has great promise in this area (Khade and Adholeya 2007). Because of the nutrient absorption capability of AM and resistance to heavy metals in certain fungal species, interest in using AM to detoxify polluted soils is growing. Gaur and Adholeya (2004) conclude that AM can play important roles in plant survival on metal contaminated soils by serving as filtration barriers to the transfer of heavy metals to shoots. They suggested that it will be important to use only indigenous heavy metal tolerant fungi with appropriate plant species in order to get the best results which require a basic understanding of the ecology.

Mangrove ecosystem is a natural unit where the intricate relationships between a community of organisms and their physical environment occur. Their responses in ecosystems are extremely difficult to measure because the complexities require a consideration of many variables, including climate, soil type, seasonal plant requirements, stage of plant growth and other community members. However, based on the current data, AM fungi are the most important microbial symbioses under conditions of P-limitation because they influence the development of plant communities, increase nutrient uptake and above ground productivity, improve water relations, and act as bio-protectants (Jeffries *et al.* 2003).

Arbuscular mycorrhizal fungi play important role in competition and redistribution of nutrients in the ecosystem. There is also evidence that AM provides seedlings with nutrients from hyphal network connected to established plants. These relationships may minimize competition with other plants, allow seedlings to experience considerable energy savings, and give them a greater chance of establishment (Smith and Read 1997).

Arbuscular mycorrhizal fungi relationships with other community members are continuously being revealed. Animals can act as vectors of AM inocula which affects plant succession. Allen and Allen (1988) report that animals transported AM propagules over long distance, facilitating succession. There is interesting evidence that AM can work synergistically with organisms such as P solubilizing bacteria and fungi to further increase available P in the rhizosphere (Smith and Read 1997).

Previous studies on mangroves have shown that these fungi are either absent (Mohankumar and Mahadevan 1986), rare (Kothamasi *et al.* 2006) or ubiquitous (Sengupta and Chaudhuri 2002; Kumar and Ghose 2008) in mangrove ecosystems. Although most of the studies on mangroves and AM fungi have mostly been carried out along the east coast where mangroves grow in deltaic habitats, the studies on the west coast of India that mostly supports fringing and riverine habitats with fewer number of mangrove species are scarce. In any attempts for afforestation, established AM fungal plant species may serve as important sources of inocula for initially non-mycorrhizal conspecifics. This may affect regeneration and contribute patchy distribution of species within the community (Koide *et al.* 2000). Hence, it is necessary to study the diversity of native AM fungal species and assess the potential of both AM fungal species and host plant species for afforestation of mangrove habitats. The aim of the present study was to determine the AM fungal diversity in

selected mangroves sites from Goa and to identify the dominant AM fungal species found therein.

Aims and Objectives

- ✓ To study AM fungal root colonization in mangrove plant species of Goa.
- ✓ To isolate and identify spores of AM fungi from the rhizosphere soils of mangrove of Goa.
- ✓ To assess the AM fungal spore density in the rhizosphere soils of mangrove of Goa.
- ✓ To study the mycorrhizal status of selected plant species as influenced by its phenology.
- ✓ To produce monospecific cultures of dominant AM fungal species and their mass multiplication.
- ✓ To evaluate the effect of dominant AM fungal species on growth of selected mangrove plant species.

2.1: History of Arbuscular Mycorrhizal (AM) Fungi

The term mycorrhiza (*mykes* = fungus, *rhiza* = root) was first coined by Frank (1885) to describe the symbiosis between a soil fungus and plant roots. Based on the type of fungus involved and the resulting structures produced by the root fungus combination, various mycorrhizal associations *viz.,* ectomycorrhiza, ectendomycorrhiza, ericoid, arbutoid, orchid, monotropoid and Arbuscular Mycorrhizal (AM) fungi have been identified (Smith and Read 1997). The AM fungi are the most common mycorrhiza and it has been estimated that they colonize about 80% of plant families from all terrestrial plants (Schüßler *et al*. 2001). The AM fungi have undergone changes to their name in recent years, from endomycorrhiza to vesicular-arbuscular mycorrhiza (VAM) to AM. Recently VAM was replaced by AM because VAM do not resemble other types of endomycorrhiza that penetrate the root cells, such as *Rhizoctonia* (mycorrhizal with orchids) and ascomycetes (ectendomycorrhiza). The name also changed from VAM to AM because not all VAM form vesicles, *e.g.* members of the Gigasporaceae (Morton and Benny 1990). Hence the term AMF is preferred because of the formation of highly branched intracellular fungal structures or 'arbuscules' by almost all members. The success of mycorrhizal evolution has been attributed to the role that mycorrhizal fungi play in the capture of nutrients from the soil of all ecosystems (Bonfante and Perotto 2000). The symbiosis is characterised by the exchange of nutrients where carbon in the form of hexose sugars flows to the fungus and inorganic nutrients are passed to the plant, thereby providing a linkage between the plant root and the soil (Sylvia *et al*. 1998). Mycorrhizal fungi provide inorganic nutrients mainly phosphorus (P) and other complex compounds to the plant through the extensive network of their hyphae that forage for soil nutrients more effectively than plant roots (Van der Heijden *et al*. 1998). For this association to occur there must

be a host plant (the phytobiont), an ecological habitat (the soil) and a suitable fungus (the mycobiont). Mycorrhizal fungi differ from other plant–fungus associations because of their ability to create an interface for nutrient exchange which occurs within living cells of the plant (Brundrett 2002, 2004).

2.2: Classification of AM Fungi

In earlier classifications, the AM fungi were placed in the order Glomales within the division Zygomycota. They have non-septate hyphae, a similar characteristic to that found in hyphae of most Zygomycota. However, AM fungi are distinguished from the Zygomycotan lineages due to some specific characteristics, *e.g.* mutualistic symbiotic nutritional habit and lack of formation of characteristic zygospores. The rDNA analysis exposed a clear separation of AM fungi from other fungal groups and they are now placed in a separate phylum, Glomeromycota (Schüßler *et al.* 2001). To date, more than 214 AM fungal species have been described. They are classified in four orders, 10 families and 14 genera **(Table 1)** with *Glomus* being the largest genus containing 54.8% of all described species. In phylogenetic tree based on rDNA, Glomeromycota are sister group to Asco- and Basidio-mycota. Traditionally, glomeromycotan taxonomy is mainly based on the morphology of spores. The way the spore is formed on the hypha (mode of spore formation) has been important to circumscribe genera, families, and spore wall structure to distinguish species (Walker 1983; Morton 1988). Distinguishing mycorrhizal spore characteristics used in classification include wall morphologies, size, shape, colour, hyphal attachment and reaction to staining compounds (Wright *et al.* 2006).

Table 1: Classification of AM fungi.

GLOMEROMYCOTA C. Walker & Schuβler

Glomeromycetes Cavalier-Smith, emend. Oehl, G.A. Silva, B.T. Goto & Sieverd.

Diversisporales C. Walker & Schuβler, emend. Oehl, G.A. Silva & Sieverd.

Diversisporaceae C. Walker & A. Schüβler, emend. Oehl, G.A. Silva & Sieverd.

Tricispora Oehl, Sieverd., G.A. Silva & Palenz.

Otospora Oehl, J. Palenzuela & N. Ferrol

Diversispora C. Walker & A. Schüβler, emend. G.A. Silva, Oehl & Sieverd.

Redeckera C. Walker & A. Schüβler, emend. Oehl, G.A. Silva & Sieverd.

Acaulosporaceae J.B. Morton & Benny

Kuklospora Oehl & Sieverd

Acaulospora Gerd. & Trappe emend. S.M. Berch

Sacculosporaceae Oehl, Sieverd., G.A. Silva, B.T. Goto, I.C. Sánchez & Palenz.

Sacculospora Oehl, Sieverd., G.A. Silva, B.T. Goto, I.C. Sánchez & Palenz.

Pacisporaceae C. Walker, Blaszk., Schuβler & Schwarzott

Pacispora Oehl & Sieverd.

Gigasporales Sieverd., G.A. Silva, B.T. Goto & Oehl

Scutellosporaceae Sieverd., F.A. Souza & Oehl

Orbispora Oehl, D.K.A. Silva, Maia, Sousa, Vieira & G.A. Silva

Scutellospora C. Walker & F.E. Sanders. emend. Oehl, F.A. Souza & Sieverd.

Gigasporaceae J.B. Morton & Benny emend. Sieverd., F.A. Souza & Oehl

Gigaspora Gerd. & Trappe emend. C. Walker & F.E. Sanders

Dentiscutataceae F.A. Souza, Oehl & Sieverd.

Dentiscutata Sieverd., F.A. Souza & Oehl

Quatunica F.A. Souza, Sieverd. & Oehl

Fuscutata Oehl, F.A. Souza & Sieverd.

Racocetraceae Oehl, Sieverd. & F.A. Souza

Cetraspora Oehl, F.A. Souza & Sieverd.

Racocetra Oehl, F.A. Souza & Sieverd.

Glomerales J.B. Morton & Benny, emend. Oehl, Palenz., G.A. Silva & Sieverd.

Claroideoglomeraceae C. Walker & A. Schüβler, emend. Oehl, G.A. Silva & Sieverd.

Viscospora Sieverd., Oehl & G.A. Silva

Claroideoglomus C. Walker & A. Schüβler, emend. Oehl, Sieverd., B.T. Goto & G.A. Silva

Entrophosphora R.N. Ames & R.W. Schneid., emend. Oehl, Sieverd., Palenz. & G.A. Silva

Albahypha Oehl, G.A. Silva, B.T. Goto & Sieverd.

Glomeraceae Piroz. & Dalpé emend. Oehl, G.A. Silva & Sieverd.

Simiglomus Sieverd., G.A. Silva & Oehl

Funneliformis C. Walker & A. Schüβler, emend. Oehl, G.A. Silva & Sieverd.

Rhizophagus P.A. Dang.

Septoglomus Sieverd., G.A. Silva & Oehl

Glomus Tul. & C. Tul. emend. Oehl, G.A. Silva & Sieverd.

Archaeosporomycetes Sieverd., G.A. Silva, B.T. Goto & Oehl

Archaeosporales C. Walker & Schuβler, emend. Sieverd., G.A. Silva, B.T. Goto & Oehl

Ambisporaceae C. Walker, Vestberg & Schuβler

Ambispora (= *Appendicispora*) Spain, Oehl & Sieverd.

Geosiphonaceae Engler. & E. Gilg emend. Schuβler

Geosiphon (Kütz.) F. Wettst.

Archaeosporaceae J.B. Morton & D. Redecker emend. Oehl & Sieverd.

Intraspora Oehl & Sieverd.

Archaeospora J.B. Morton & D. Redecker

Paraglomeromycetes Oehl, G.A. Silva, B.T. Goto & Sieverd.

Paraglomerales C. Walker & Schuβler

Paraglomeraceae J.B. Morton & D. Redecker

Paraglomus J.B. Morton & D. Redecker

Classical spore morphology and more recently PCR-based molecular approaches are generally used for identification of AM fungal communities, but there are problems with both these approaches. In the case of spore morphology, it is not always easy to identify all spores when sieved directly from field soil. There are variations in spore development and sometimes AM fungi colonising the plant roots are not found as spores (Clapp *et al.* 1995, 2002). The main problem with molecular approaches is that most are based on rDNA sequences, whereas AM fungal species have polymorphic rDNA sequences (Sanders 2002; Redecker *et al.* 2002). Thus, it is normal to recover multiple sequences by PCR amplification from a single spore because a single spore can contain a thousand or more nuclei (Antoniolli *et al.* 2000;

Pawlowska and Taylor 2004). Hence, in order too is in conflict with previous morphology-based analyses that placed *Glomus* and Acaulosporaceae together (Morton and Benny 1990). The fungi of the Glomeromycota have coenocytic to sparsely septate mycelia. They reproduce asexually through blastic development of the hyphal tips and form symbiotic relationships with photoautotrophs.

2.3: Phylogenetic Relationships

The Glomeromycota are very old group with an estimated origin of at least 600 to 620 million years ago. The ancient phylogenetic origin of Glomales is confirmed by fossil findings, with symbiotic structures within fossil roots from Devonian (Remy *et al.* 1994; Taylor *et al.* 1995) and fossilized glomalean spores from the Ordovician, about 460 million years ago (Redecker *et al.* 2000). Since molecular phylogenetic methods have been used to elucidate the phylogenetic relationships among these fungi, their classification has been in a rapid transition.

In the recent classification, the phylogeny erected is based entirely on analyses of the small subunit RNA gene. rDNA phylogenies have shown that the genus *Glomus* is several times polyphyletic (Redecker *et al.* 2000; Schwarzott *et al.* 2001). Species forming *Glomus*-like spores can be found in six different lineages within the Glomeromycota. *Paraglomus* appears to be the earliest-diverging glomeromycotan lineage in rDNA phylogenies, although sometimes receiving relatively weak bootstrap support. The separation of *Pacispora* and the *Diversispora* clade from other "*Glomus*" lineages is well supported by rDNA data. *Glomus* groups A and B are exemplified by the well-known species of *Glomus mosseae* and *G. claroideum* respectively. The two groups are genetically relatively distant but still form a monophyletic group in rDNA phylogenetic trees (Schwarzott *et al.* 2001).

The formation of a "sporiferous saccule" was once thought to be characteristic of Acaulosporaceae (*Acaulospora* and *Entrophospora*), but now it is known to occur in at least one additional lineage, namely *Archaeospora*. The Gigasporaceae (*Scutellospora* and *Gigaspora)* are distinguished by the formation of their spores on a "bulbous suspensor" and are well supported by molecular data. Gigasporaceae and Acaulosporaceae form a clade in most rDNA phylogenies, which assess the total community present at a specific site, and hence the use of both methods is recommended because they complement each other (van der Heijden and Scheublin 2007).

Unusual polymorphism of ribosomal RNA in individual spores has led to the concept of inter-nuclear variation in single spores, defining AM fungi as heterokaryotic organisms (Trouvelot *et al*. 1999; Kuhn *et al*. 2001). Heterokaryosis has been assumed to be of importance to ecology and application of AM fungi. This concept however has recently been challenged by experiments suggesting that single spores contain uniform population of nuclei characterized by intra-nuclear polymorphism (Pawlowska and Taylor 2004).

2.4: Taxonomy

Peyronel (1923) discovered that the regular occurrence of associations of spores and sporocarps of the Endogonaceae with AM fungi of plants and suggested fungi to be the originators of the mycorrhizae. Valuable data on the biology of fungi of the family Endogonaceae has been obtained from studies using pot cultures. The mode of germination of spores of these fungi, their life cycles, sub-cellular spore structures and the manner of colonization of roots has been recognized (Mosse 1959, 1970). Mosse

and Bowen (1968) prepared the first key for the recognition of the types of isolated endogonaceous spores.

Prior to 1974, most AM fungi were in the genus *Endogone*. Gerdemann and Trappe (1974) revised the family Endogonaceae in the order Mucorales, where 44 species belonging to seven genera were characterized. Among them, many taxa were redefined, and two genera (*Acaulospora*, *Gigaspora*) and 12 species were described as new. The genus *Endogone* contained 11 species with zygospores arranged in sporocarps. Phylogenetic analysis of the nuclear small subunit ribosomal RNA strongly suggest that *Endogone* (Endogonales) and the Glomeromycota do not form a clade (Gehrig *et al*. 1996).

Tulasne and Tulasne (1845) erected the genus *Glomus* with 19 species with two varieties of *Gl. macrocarpus* Tul. & Tul., i.e. *Gl. macrocarpus* var. *macrocarpus* and *Gl. macrocarpus* var. *geosporus*, and also the genus *Sclerocystis* with four taxa containing species forming chlamydospores blastically at hyphal tips. In contrast to sporocarpic *Sclerocystis* species, the chlamydospores of members of the genus *Glomus* have been considered to occur mainly in loose aggregates or singly in the soil, although the genus also included species forming compact sporocarps with or without a peridium. The distinctive property of genus *Sclerocystis* was the production of chlamydospores arranged in a single layer around a central plexus. However, molecular phylogenetic analysis has shown that the species which form complex sporocarps formerly placed in the genus *Sclerocystis* are actually phylogenetically nested within well characterized *Glomus* species with simple spores (Redecker *et al*. 2000).

Ames and Schneider (1979) erected a new genus in Endogonaceae, *Entrophospora* with *E. infrequens*, a species earlier existing in genus *Glomus*, as *Gl.*

infrequens. Spores of *E. infrequens* were formed inside the neck of a sporiferous saccule.

Walker and Sanders (1986) separated the genus *Gigaspora*, containing species with spores lacking an inner wall having no physical contact with their main structural wall, from genus *Scutellospora* with fungi forming spores having at least one inner wall.

Morton and Benny (1990) located soil-borne fungi forming arbuscules in roots of terrestrial plants in new order, Glomales consisting of two suborders, Glomineae and Gigasporineae. The former suborder consisted of the type family Glomaceae with genera *Glomus* and *Sclerocystis* and Acaulosporaceae comprising of the genera *Acaulospora* and *Entrophospora*. The latter suborder was proposed to include the Gigasporaceae with the genera *Gigaspora* and *Scutellospora*. These families were characterized by the mode of spore formation and were initially supported by molecular data (Simon *et al*. 1993).

Almeida and Schenck (1990) concluded that except for *Sclerocystis coremioides* a continuum of morphological properties exists between sporocarpic *Glomus* species and the other members of the genus *Sclerocystis*. As a result, the five-species genus *Sclerocystis* was reduced to single-species.

The genera *Acaulospora* and *Gigaspora* have been defined by Gerdemann and Trappe (1974) as forming azygospores singly in the soil, although no parthenogenetical process of spore development was observed. Species of *Acaulospora* produced spores laterally on the neck of a sporiferous saccule and species of the genus *Gigaspora* formed spores terminally at the tip of a bulbous sporogenous cell. Spores of *Glomus* and *Acaulospora* types were reported to be produced by several distinct, deeply divergent lineages (Redecker *et al*. 2000)

subsequently, described as two new genera *Archaeospora* and *Paraglomus* (Morton and Redecker 2001) and placed in separate families. Since some species in *Archaeospora* were dimorphic, members of this genus were classified originally in separate families (Morton *et al*. 1997).

The genus *Pacispora* comprising of some former *Glomus* species was erected by Oehl and Sieverding (2004). The spores of *Pacispora* have characteristics intermediate between *Glomus* and Gigasporaceae. Another emerging genus split off from *Glomus* is *Diversispora* (Morton and Benny 1990). Only one *Glomus* species has been renamed so far mainly based on ribosomal small subunit signatures (Walker and Schuβler 2004). The new classification includes the Geosiphonaceae; order Archaeosporales, which presently contain one fungal species that forms endosymbiotic association with the cyanobacterium *Nostoc punctiforme* and produce spores typical to AM fungi (Schuβler 2002).

Redecker *et al*. (2000) utilizing both morphological and molecular data transferred *S. coremioides* to genus *Glomus* and thereby eliminated the genus *Sclerocystis* from Kingdom Fungi. Morton and Redecker (2001) erected two new families in the order Glomales, *i.e.* Archaeosporaceae and Paraglomaceae based on data from molecular, morphological and biochemical investigations. Each of these families were phylogenetically distant from other glomalean families, despite similarities in mycorrhizal morphology. The family Archaeosporaceae contained one genus, *Archaeospora* with three species forming typical *Acaulospora*-like spores from the neck of a sporiferous saccule. Two of these species, *Ar. gerdemannii* and *Ar. leptoticha* were considered to dimorphic, forming *Glomus*-like spores. The genus *Paraglomus* in the family Paraglomaceae consisted of two species producing spores indistinguishable from those of *Glomus* species.

The fungi of the order Archaeosporales form endocytosymbioses with photoautotrophic prokaryotes *Geosiphon pyriformis* (Kütz.) Wettstein emend. Schüßler produce mycorrhizae with arbuscules with or without vesicles. Their spores are colourless and do not react in Melzer's reagent. Glomoid spores (identical to those of fungi of the genus *Glomus*) form singly or in clusters on or under the soil surface. Acaulosporoid spores (similar to those of members of the genus *Acaulospora*) develop singly in the soil. They differ from other AM fungi by the possession of the rRNA SSU gene signature YCT**AT**CYKYCTGGTGAKRCG, corresponding to homologous position 691 of *Saccharomyces cerevisiae* SSU rRNA sequence J01353, with the nucleotides being specific for the taxon. The order Archaeosporales contains two families, Archaeosporaceae with the genera *Appendicispora*, *Archaeospora* and *Intraspora* and Geosiphonaceae with the genus *Geosiphon*.

Members of the order Diversisporales form mycorrhizae with arbuscules, frequently lacking vesicles, with or without auxiliary cells. Spores develop either inside (entrophosporioid spores of the genera *Entrophospora* and *Kuklospora*) or laterally on the neck of a sporiferous saccule (acaulosporioid spores of the genus *Acaulospora*), from a bulbous base on the sporiferous hypha (gigasporioid spores of the genera *Gigaspora* and *Scutellospora*), or blastically at the tip of a sporogenous hypha (glomoid spores of the genera *Diversispora* and *Pacispora*). They differ from other AM fungi by the possession of the rRNA SSU gene sequence signature YV**R**RYW/1-5/NGY**Y**YGB, corresponding to homologous position 658 of *S. cerevisiae* SSU rRNA sequence J01353 SSU rRNA, GTYARDYH**M**HYY/2-4/GRADRKKYGWCRAC, corresponding to homologous position of *S. cerevisiae* SSU rRNA sequence position 1346 of *S. cerevisiae* SSU rRNA sequence J01353, TTATCGGTTR**A**ATC, corresponding to homologous position 650 of *S. cerevisiae*

rRNA SSU sequence J01353, and A**CT**GAGTTMATYT, corresponding to homologous position 1481 of *S. cerevisiae* rRNA SSU sequence J01353 with the nucleotides being specific for the taxon. The order Diversisporales is represented by five families, Diversisporaceae with the genus *Diversispora*, Acaulosporaceae with the genera *Acaulospora* and *Kuklospora***,** Entrophosporaceae with the genus *Entrophospora*, Gigasporaceae with the genera *Gigaspora* and *Scutellospora* and Pacisporaceae with the genus *Pacispora*.

Fungi of the order Glomerales usually are hypogeous, rarely epigeous. They produce mycorrhizae with arbuscules, vesicles and spores. Spores form either blastically at the tip of a sporogenous hypha or intercalary inside them. Spores occur singly, in clusters or sporocarps having a peridium. They differ from other AM fungi by the possession of the rRNA SSU gene sequence signature **YTRRY**/2-5/R**YYA**RGTYGNCARCTTCTTAGAGGGACTATCGG**T**GTYTA**A**CCGRTGGcorresponding to homologous position 1353 of *S. cerevisiae* SSU rRNA sequence J J01353, with the nucleotides being specific for the taxon. The order Glomerales includes one genus, *Glomus*.

Species of the order Paraglomerales form arbuscular mycorrhizae, rarely with vesicles. Spores are glomoid and colourless. The fungi differ from other AM fungi by the possession of rRNA SSU gene sequence signature G**C**GAA**G**CGTCATG**G**CCTTAAC**C**GG**C**CGT, corresponding to homologous position 703 of *S. cerevisiae* SSU rRNA sequence J01353, with the nucleotides being specific for the taxon. The order Paraglomerales is represented by one family Paraglomeraceae containing one genus, *Paraglomus*.

2.4.1: Genera of Arbuscular Mycorrhizal fungi - Arbuscular mycorrhizal fungi are placed in four orders, *viz.,* Archaeosporales, Diversisporales, Glomerales and Paraglomerales belonging to the class Glomeromycetes of the phylum Glomeromycota (Schußler *et al*. 2001) under 14 genera *viz., Acaulospora, Archaeospora, Ambispora, Diversispora, Entrophospora, Gigaspora, Glomus, Intraspora, Kuklospora, Pacispora, Paraglomus, Scutellospora, Otospora* and *Geosiphon*. The different genera of AM fungi are described below:

***Acaulospora* Gerdemann & Trappe emend. Berch.** Berch SM.1985. *Mycotaxon* 23: 409-418.

Etyomolgy: Greek, *a*-(without), *caulos* (stem), and *spora* (spore) - referring to the sessile spores.

Spores of fungi of the genus *Acaulospora* develop laterally from the neck of a sporiferous saccule (Morton and Benny, 1990; Morton 2000). The spores are sessile, *i.e.* no pedicel (a short branch of the sporiferous saccule neck) is formed. The wall of the most juvenile spores consists of only one layer continuous with the wall of a sporiferous saccule hypha. Spores produced singly in soil, generally globose to sub-globose with oily contents. Spore composed of two distinct, separable wall groups; outer wall is continuous laminated; variously ornamented, inner wall composed of one or more walls that are membranous, hyaline, laminated and ornamented. Spore walls are continuous except for a small-occluded pore. Spores of the genus *Acaulospora* germinate by germ tubes emerging from a plate-like germination orb formed by centrifugally rolled hyphae (Blaszkowski 1994). The germ tubes penetrate through the spore wall. The mycorrhizae of *Acaulospora* species consist of (1) arbuscules with cylindrical or slightly flared trunks (2) irregular and knobby vesicles, and (3) straight and coiled intra-radical hyphae with coils mostly concentrated at entry points (Morton 2000).

***Gigaspora* Gerdemann & Trappe emend. Walker & Sanders**. Walker C, Sanders FE. 1986. *Mycotaxon*. 27: 169-182.

Etymology: Greek, *giga* (giant) and *spora* (spore). Referring to the exceptionally large spores typically produced by the members of the genus.

Azgospores produced singly in soil, generally globose to sub-globose, with oily contents, usually with a narrow hypha extending from the suspensor cell to the pore.

Spores of *Gigaspora* develop blastically from a bulbous sporogenous cell formed at the end of a fertile hypha connected with mycorrhizal roots (Bentivenga and Morton 1995; Walker and Sanders 1986). The wall of the most juvenile, expanding spores consists of two layers of equal thickness. The inner layer thickens due to the synthesis of new sub layers (laminae). At the end of ontogeny, a warty or knobby one-layered germination wall is formed, from which germ tubes arise. This wall tightly adheres to the inner surface of the laminate spore wall layer. The outermost spore wall layer of all the *Gigaspora* species is smooth. Apart from spores, *Gigaspora* species also form clusters of auxiliary cells. They are echinulate with spines. The mycorrhizae of *Gigaspora* species consist of only arbuscules and hyphae staining darkly in trypan blue; no vesicles are produced (Bentivenga and Morton 1995). Arbuscules generally form fine branches directly from a swollen basal hypha. Intra-radical hyphae are straight to coiled and vary in diameter because of the presence knob-like projections and inflated areas.

***Scutellospora* Walkers & Sanders.** Walker C, Sanders FE. 1986. *Mycotaxon*. 27: 169-182.

Etymology: Latin *scutellum*- small shield and *spora,* spore referring to the production of germination shield in spores of members of the genus.

Spores are produced singly in soil and are variable in shape, usually globose or sub-globose often ovoid, obovoid, pyriform or irregular borne on a bulbous sporogenous cell formed at the end of a fertile hypha connected with mycorrhizal roots (Walker and Sanders 1986) usually with a narrow hypha extending from one or more peg-like projections towards the spore. Spore wall structure consists of two wall groups of equal thickness. The inner layer thickens due to addition of new sub layers (laminae).

The formation of spore wall ends the differentiation of a third thin, flexible layer, which is tightly adherent to the laminate layer. Germination by means of one or more germ tubes produced from the spore base through the germination shield formed upon or within a flexible inner wall. The mycorrhizae of *Scutellospora* species consists of only arbuscules and hyphae staining darkly in trypan blue and no vesicles are produced (Morton 2000). Arbuscules develop from swollen basal hyphae. Intra-radical hyphae are straight or coiled and vary in diameter because of the presence of knob-like projections and inflated areas. Thin-walled, knobby or broadly papillate auxiliary cells borne in soil on straight or coiled hyphae, formed singly or in clusters.

Glomus **Tulasne & Tulasne.** Gerdemann JW, Trappe JM. 1974. *Mycologia Memoir* No.5: 76.

Etymology: Latin, glomus (a ball of yarn), possibly in reference to sometimes rounded and cottony appearance of the species.

Spores of *Glomus* species develop blastically at the end of sporogenous hyphae, although intercalary spore formation has also been reported (Declerck *et al*. 2000). In most species, the sporogenous hyphae develop from extra-radical hyphae of mycorrhizal roots. The surface of spores of *Glomus* species may be smooth (in most species) or ornamented. Some species produce spores enveloped in a hyphal mantle 'Gleba' consisting of interwoven 'Peridium'. The wall layers of a subtending hypha are continuous with spore wall layers. At the end of spore development, the lumen of the subtending hypha usually becomes closed by either (1) a curved septum continuous with the innermost lamina of the laminate spore wall layer (2) an invaginated flexible innermost layer (3) an amorphous plug and (4) thickening subtending hyphal wall. Spores of the genus *Glomus* germinate by emergence of the

germ tube through the lumen of the subtending hypha (most species) or the spore wall. Most species of the genus *Glomus* produce spores singly in the soil. Other taxa form more or less compact spore aggregates consisting of spores and a peridium. The mycorrhizae of *Glomus* species consist of arbuscules, vesicles (not always formed), and intra- and extra-radical hyphae. Arbuscules have cylindrical or slightly flared trunks with branches progressively tapering in width toward tips. Vesicles are usually thin-walled and ellipsoid. Intra-radical hyphae usually spread along roots and frequently form Y-shaped branches, H-shaped connections and coils mainly occur at entry points.

***Intraspora* Oehl & Sieverd.** Sieverding E, Oehl F. 2006. *Journal of Applied Botany and Food Quality*. 80: 69-81.

Spores occur singly in the soil or in roots. The spores develop inside the neck of a sporiferous saccule at some distance from the saccule. The sporiferous saccule originates terminally or intercalary in extra- and intra-radical hyphae. The spores are globose to sub-globose and frequently pyriform. Their sub cellular structure consists of two walls, a spore wall and an inner germination wall. The spore wall is composed of two layers, of which the outer layer sloughs with age and is continuous with the wall of the neck of the sporiferous saccule. The inner layer of this wall is persistent, semi flexible and closes two opposite pores of spores. The inner germination wall is semi-flexible and laminate. The mycorrhizae comprise of arbuscules, vesicles as well as intra- and extra-radical hyphae. Vesicles form rarely and all the mycorrhizal structures stain faintly in trypan blue.

***Kuklospora* Oehl & Sieverd**. Sieverding E, Oehl F. 2006. *Journal of Applied Botany and Food Quality*. 80: 69-81.

Spores develop inside the neck of a sporiferous saccule at some distance from this saccule and originate from the neck and saccule contents. The sporiferous saccule originate terminally or intercalary inside mycorrhizal extra-radical hyphae by their swelling and are globose to sub-globose. The sub cellular structure consists of a 3-layered, coloured spore wall and two inner colourless germination walls. The outermost spore wall layer is colourless, and is continuous with the wall of the sporiferous saccule neck. The second structural layer of this wall consists of coloured, tightly adherent, thin sub layers (laminae). This layer occasionally develops towards the saccule, forming a stalk supporting the wall of the sporiferous saccule neck. The first inner germination wall consists of two adherent flexible to semi-flexible layers. The second germination wall is composed of three layers, of which the outermost one is ornamented with small granules. The spores forms typical mycorrhizae intensively stained in trypan blue.

***Pacispora* Oehl & Sieverd.** Sieverding E, Oehl F. 2006. *Journal of Applied Botany and Food Quality*. 80: 69-81.

Spores of fungi of the genus *Pacispora* develop blastically at the end of cylindrical sporogenous hyphae (subtending hyphae) continuous with extra-radical hyphae of AM fungi. The spores of members of this genus consist of three wall layers. The ontogenetic development of spores of *Pacispora* species is by the formation of a uniform, plate-like germination shield on the surface layer of the inner germination wall. A germ tube grows from this shield and penetrates through the spore wall. The mycorrhizae consist of arbuscules, vesicles, intra- and extra-radical hyphae, as well as of auxiliary cells. The arbuscules, vesicles and hyphae morphologically resembled

those of *Glomus* species and stained intensively in trypan blue. The auxiliary cells occur both outside and inside roots and are knobby.

***Paraglomus* Morton & Redecker.** Morton JB, Redecker D. 2001 *Mycologia* 93: 181-195.

Etymology: Resembling "*Glomus*" with identical spore morphotypes.

Spores of species of the genus *Paraglomus* develop blastically at the tip of extra-radical hyphae. The spores of the known species of this genus occur singly in the soil. They are globose to irregular and colourless to pale in colour. The sub cellular structure of spores of *Paraglomus* consists of a spore wall comprising two to three layers continuous with those of their subtending hyphae. Spores of *Paraglomus* species germinate by germ tubes emerging from both the lumen of the subtending hypha and the spore wall (Morton and Redecker 2001). Arbuscules of *Paraglomus* species are cylindrical or slightly flared trunks with branches progressively tapering in width towards the tips (Morton 2002; Morton and Redecker 2001). The mycorrhizae of *Paraglomus* species do not contain vesicles and their intra-radical hyphae are frequently coiled within and between cortical cells. The main visible evidence of mycorrhizae of *Paraglomus* species is their light staining or the lack of any staining reaction in trypan blue or other stains.

Archaeospora Morton & Redecker. Morton JB, Redecker D. 2001 *Mycologia*. 282-285.

Etyomology: Greek, "archaios" = ancient, referring to the ancient position of this genus in Glomales.

Archaeospora is dimorphic, forming both acaulosporioid and glomoid spores (Morton and Redecker 2001; Sieverding and Oehl 2006; Spain *et al*. 2006). Acaulosporioid spores develop laterally, directly on the neck of a sporiferous saccule and are sessile. Two-layered glomoid spores origin blastically at the tip of or intercalary in fertile hyphae, as spores of *Glomus* species. Germination of *Archaeospora* spores is by a germ tube emerging from an irregular germination structure (Spain 2003). Mycorrhizae of *Archaeospora* (1) do not contain intra-radical vesicles or they form rarely, (2) have intra-radical hyphae with many coils located within and between cortical cells, (3) stain lightly or not at all in trypan blue and other stains, and (4) are patchily distributed along roots (Morton 2002).

***Diversispora* Walker & Schüßler.** Walker C, Schußler A. 2004. *Mycological Research*. 108: 979-982.

Spores of *Diversispora* develop blastically at the tip of cylindrical to slightly flared sporogenous hyphae continuous with extra-radical hyphae of AM fungi. The mycorrhizae of most *Glomus* species consist of arbuscules, vesicles and hyphae staining intensively in trypan blue whereas those of *D. spurca* lack vesicles and stain variably, from almost no staining to intensive staining (Morton 2002).

***Entrophospora* Ames & Schneid. emend. Oehl & Sieverd**. Ames RN, Schneider RW. 1979. *Mycotaxon* 8: 347-352.

Spores occur singly in the soil or inside roots (Blaszkowski *et al*. 1998; Sieverding and Oehl 2006). The spores develop inside the neck of a sporiferous saccule directly at or at a short distance from the saccule originating from the neck. The sporiferous saccule originate terminally or intercalary inside extra- and intra-radical hyphae by

their swelling. The spores are globose to sub-globose and coloured; their sub cellular structure consists of a multilayered, coloured spore wall and one inner 3-layered, colourless germination wall. In spores lacking the sporiferous saccules, two opposite cicatrices resembling small rings with a slightly raised border are visible. The cicatrices are frequently accompanied by stalks developed from the permanent spore wall layers. The mycorrhizae of *Entrophospora* showed intense staining in trypan blue (Sieverding and Oehl 2006).

***Ambispora* Walker Vestberg & Schüβler.** Walker C, Vestberg M, Schuβler A. *Mycological Research*. 111: 253-255.

Species of the genus *Ambispora* are dimorphic producing both acaulosporoid and glomoid spores i.e. spores originating similarly to those of *Acaulospora* and *Glomus* species (Morton and Redecker 2001; Spain *et al*. 2006). The acaulosporoid spores occur singly in the soil and the glomoid ones are formed singly or in loose clusters in the soil and develop terminally from the thin walled hyphae grown from either the wall of a pedicel or branched germ tubes (Spain *et al*. 2006). In contrast to the sessile acaulosporoid spores of the genus *Acaulospora* and *Archaeospora,* those of *Ambispora* species develop blastically at the tip of a short branch formed at the distal end of the neck of a sporiferous saccule. This branch is called appendix or pedicel. The sporiferous saccule of *Ambispora* species orginate terminally from mycorrhizal extra-radical hyphae by their swelling. The spores of the known species of *Ambispora* are globose to sub-globose and coloured. The sub cellular structure consists of three layered, coloured spore wall and two inner colourless germination walls. The outer spore wall completes development subsequent to the formation of the outer layer of the first inner germination wall. The spore wall and the outer layer of the first inner

germination of the spores of *Ambispora* species are continuous with the pedicel wall layer. The mycorrhiza of the species *Ambispora* consists of arbuscules, vesicles as well as intra- and extra-radical hyphae. All these structures stain faintly in trypan blue (Spain *et al*. 2006).

***Archaeospora* Morton & Redecker.** Morton JB, Redecker D. 2001 *Mycologia*. 93: 181-195.

Archaeospora trappei the only member of the genus *Archaeospora* is a dimorphic fungus, producing both acaulosporoid and glomoid spores (Morton and Redecker 2001; Sieverding and Oehl 2006; Spain 2003; Spain *et al.* 2006). Acaulosporoid spores develop laterally on the neck of a sporiferous saccule, are sessile similarly as most spores of the genus *Acaulospora.* Two layered glomoid spores originate blastically at the tip of intercalary in fertile hyphae as spores of *Glomus* species. The sub cellular structure of acaulosporoid spores *Archaeospora trappei* comprises of a spore wall and one inner germination wall, each consisting of two to three layers. Germination of *Archaeospora trappei* spores is by germ tube emerging from an irregular germination structure (Spain 2003).

Mycorrhizae of *Archaeospora trappei* 1) do not contain intra-radical vesicles or they form rarely, 2) have intra-radical hyphae with many coils located within and between cortical cells 3) Stain lightly or not at all in trypan blue and other stains and 4) are patchily distributed along roots (Morton 2002).

2.5: Life Cycle of AM Fungi

The life cycle of AM fungi generally starts from the spores present in soil or from adjacent mycorrhizal plant roots. The germinating hyphae from spores or mycorrhizal

roots grow towards the plant root. At the root surface, the tip of the hyphen swells and forms a specific structure called the appressorium (Mandelbaum and Piche 2000). From these appressoria, infective pegs penetrate the adjacent epidermal root cell walls. The particular point at which hyphae from any propagule first enter the root is called the entry point. The number of primary entry points formed on a root surface by a fungus is equivalent to its inoculum potential (Garrett 1963). Inside the root, hyphae grow inter-cellularly to the inner cortical layers and in the inner cortex region hyphae start to grow inside the cells. After that the host cell membrane invaginates and envelopes the fungus and forms a new compartment called the apoplastic space. This space allows the efficient transfer of nutrients between the two symbionts but prevents direct contact between plant and fungal cytoplasm (Sylvia *et al.* 2001). The hyphae form different structures such as hyphal coils, arbuscules and vesicles inside the cortical cells but outside the cytoplasm. Arbuscules are highly dichotomously branched intracellular structures and could be the site of exchange of P, carbon, water and other nutrients (Smith and Read 1997). Vesicles are lipid-filled and thought to be carbon storage structures but they can also serve as reproductive propagules (Sylvia *et al.* 2001). Not all the AM fungi form vesicles *e.g.* Gigasporaceae (Morton and Benny 1990). Formation of the vesicles depends on the fungal symbiont as well as on the environmental conditions (Smith and Read 1997). The intra-radical hyphae colonize the root in different patterns. Based on its structure, the mycorrhiza is separated into *Arum-*, *Paris-* and *Intermediate*-type (Gallaud 1905). In the *Arum*-type, intercellular hyphae grow in a longitudinal manner along the root and penetrate the cortical cells to form arbuscules. Arbuscules arise from these inter-cellular hyphae on short side branches, typically at right angles to the main root axis (Smith and Smith 1997). The *Arum-* type morphology is abundant in crop plants (Smith and Smith 1997; Ahulu *et*

al. 2005). In the *Paris*-type, the hyphae are entirely intra-cellular and irregularly coiled, some of them forming arbuscules that are not terminal but are localised in definite layers. The arbuscules are formed as intercalary structures and called arbusculate coils (Gallaud 1905; Yawney and Schultz 1990; Cavagnaro *et al*. 2001). The *Paris*- type morphology is more often seen in plants in natural ecosystems (Brundrett and Kendrick 1988; Ahulu *et al.* 2005).

Sometimes, both types of structures are formed in the same root system and this has been termed the *Intermediate* type (Smith and Smith 1997). The extra-radical mycelium associated with the root radiates out into the soil. Hyphae are two distinct types, runner and absorbing (Friese and Allen 1991). The runner hyphae are thicker and grow in the soil to find host roots. The hyphae that penetrate the roots are initiated from the runner hyphae. The absorbing hyphae develop from the running hyphae and form a network of thinner hyphae extending into the soil and absorb the nutrients to transport to the host. In certain AM fungi, *e.g. Gigaspora* and *Scutellospora* species, typical clustered swellings are formed on extra-radical hyphae called 'auxiliary cells' and the function of these structures is yet to be identified.

Finally reproductive structures, spores can be formed as hyphal swellings either in the roots or, more commonly, in the soil. Spores may be formed singly or in clusters. Spores function as storage structures, resting stage and propagules. Generally spores are formed when nutrients are remobilized from roots where the AM associations are senescing (Brundrett *et al*. 1996). Several factors such as host dependence, age of host, sporulation ability of AM fungal species, presence of other AM fungal species or composition of indigenous soil micro-flora, spore dormancy and the distribution patterns of AM fungal spores in soils, seasonal influence and

other biotic factors can affect AM fungal sporulation in different plant rhizospheres (Walker *et al*. 1982; Koske 1987).

2.5.1: Structures - Arbuscular mycorrhizae consist of intra- and extra-radical structures. The intra-radical structures are arbuscules, vesicles and intra-radical hyphae. The extra-radical structures are extra-radical hyphae, spores and auxiliary cells. The latter are formed only by members of the genera *Gigaspora*, *Pacispora* and *Scutellospora*.

2.5.1.1: Inter- and intra-cellular hyphae - in roots contain storage materials and take part in transportation of the substances absorbed by extra-radical hyphae from the soil to arbuscules or directly to root cells of the host plant (Bieleski 1973). Intra-radical hyphae may be straight or with H- or Y-shaped branches. They may also form coils, whose frequency of occurrence depends on their location in a root and the generic affiliation of the arbuscular fungal species (Morton 2000). Generally, coils more abundantly occur at entry points. Intra-radical hyphae of *Glomus* species are infrequently coiled in the other regions of a mycorrhizal root. In contrast, coils produced by species of the other genera of AM fungi usually are abundant and evenly distributed along mycorrhizal roots.

2.5.1.2: Extra-radical hyphae - significantly increase the absorptive area of roots (Bieleski 1973), form hyphal bridges transferring nutrients between co-occurring plants (Newman 1988), and bind sand grains into aggregates (Koske and Polson 1984). They are also important fungal propagules colonizing plant roots (Jasper *et al.* 1989, 1991).

2.5.1.3: Arbuscules - Haustorium-like arbuscules are the main sites of nutrient exchange between a plant host and a fungus (Gianinazzi *et al.* 1979). They are formed within the cells of the inner root cortex (Mosse 1973) and are indicators of active mycorrhizae. Arbuscules differ in morphology, depending on the generic affiliation of the arbuscular fungal species (Morton 2000). Fungi of the genera *Acaulospora*, *Archaeospora*, *Ambispora*, *Diversispora*, *Entrophospora*, *Glomus*, *Intraspora*, *Kuklospora*, *Pacispora* and *Paraglomus* produce arbuscules with cylindrical or slightly flared, narrow trunks, whose branches progressively taper in width towards tips. Arbuscules of members of the genera *Gigaspora* and *Scutellospora* generally have swollen trunks with branches tapering abruptly at tips. The characters of mycorrhizae of *Otospora bareai*, the only member of the genus *Otospora*, have not been recognized to date (Palenzuela *et al.* 2008).

2.5.1.4: Vesicles - Globose or ovoid, thin-walled vesicles are storage organs filled with lipids and glycolipids (Mosse 1981). They originate by an intercalary or terminal swelling of a mycorrhizal intra-radical hypha of an arbuscular fungus. Vesicles may be inter- or intra-cellular and may be found in both the inner and the outer layers of the cortical parenchyma. In *Glomus* species, vesicles generally are ellipsoid, whereas those of *Acaulospora*, *Entrophospora* and *Kuklospora* highly vary in shape and frequently have knobs and concavities on their surface (Morton 2000). Not all *Glomus* species form vesicles (Morton and Redecker 2001). They are never produced by members of the genera *Gigaspora* and *Scutellospora*.

2.5.1.5: Auxiliary cells - are swollen structures produced by extra-radical hyphae of only *Gigasporaceae* species, *i.e.* species belonging to the genera *Gigaspora* and

Scutellospora. The cells are spiny in *Gigaspora* species and those of the genus *Scutellospora* are smooth or knobby (Blaszkowski 2003; Morton 2002).

Arbuscular mycorrhizae also differ in the degree of evenness of distribution along roots and the intensity of staining. The distribution of mycorrhizal structures of members of the genera *Ambispora*, *Archaeospora*, *Acaulospora*, *Diversispora*, *Entrophospora*, *Intraspora*, *Kuklospora* and *Paraglomus* is patchy, whereas that of mycorrhizae of the genera *Gigaspora*, *Glomus*, *Pacispora* and *Scutellospora* is usually continuous. The intensity of staining of mycorrhizae of fungi of the genera *Ambispora, Archaeospora*, *Diversispora*, *Intraspora*, and *Paraglomus* is very faint, those of *Acaulospora*, *Entrophospora* and *Kuklospora* faint to moderate, those of *Glomus* dark, and those of *Gigaspora*, *Pacispora* and *Scutellospora* are very dark (Morton and Redecker 2001; Sieverding and Oehl 2006).

2.5.1.6: Spores - Spores are multinucleate single cells mainly produced blastically at the tip of extra-radical hyphae. Sometimes spores also occur inside roots (Koske 1985), on the soil surface (Berch and Fortin 1983), and on plants or their decaying fragments (Blaszkowski *et al.* 1998). Arbuscular mycorrhizal fungi form spores ranging from 22 to 1050μm in diameter (Schenck and Perez 1990). The number of spores produced depends on the fungal species (Blaszkowski 1993), the plant species and its variety (Blaszkowski 1993; Hetrick and Bloom 1986), soil fertility and fertilizer application (Hayman 1970), host phenology (Giovannetti 1985), light intensity (Daft and El Giahmi 1978) and competitive abilities of co-occurring AM fungal species (Gemma *et al.* 1989). The reproduction of AM fungi is stated to be clonal (Morton 2000) and the role of spores is to sequester the genetic information of a given fungal species, disperse the information to new habitats, and initiate new

individuals spatially separated from the parent organisms (Morton 1993). Since many components of the sub-cellular structure of spores are stable in different environmental conditions, they are the most important structures considered in classification of AM fungi.

2.6: Distribution of AM Fungi

Although AM fungi are widespread and are distributed in different parts of the world especially in the tropics, little functional information revealed about them, until the mid 1950s (Smith and Read 1997). They are reported to be found in diverse land areas such as calcareous grasslands, arid/semi arid grasslands, several temperate forests, tropical rain forests and shrub lands in diverse parts of the world (Renker *et al*. 2005; Oehl *et al*. 2003; Muthukumar and Udaiyan 2002). Recently, AM fungi have received more attention especially in African countries such as Namibia, Cameroon, Kenya, Morocco, Nigeria, Senegal, Zambia and South Africa. These studies have concentrated on AM fungal diversity in various regions and soil types or the mycorrhizal status of indigenous crop and plant species (Bouamri *et al*. 2006; Hawley and Dames 2004; B*â* *et al*. 2000; Dalpé *et al*. 2000; Stutz *et al*. 2000; Diop *et al*. 1994). Results from these studies reveal that different species of AM fungi are obtained depending on plant species and geographic location. Amongst AM fungal species, *Glomus* species were consistently isolated while others species belonging to the genera *Acaulospora*, *Gigaspora* and *Scutellospora* were either absent or found in few numbers (Bouamri *et al*. 2006; Uhlmann *et al*. 2004; Stutz *et al*. 2000; Dames 1991).

2.6.1: Spore dispersal - Arbuscular mycorrhizal fungi depend on passive means of spore dispersal. Wind and animal are good vectors of spore dispersal (Warner *et al.* 1987; MacMohan and Warner 1984). In soil, spores of AM fungi are protected by rhizomes, leaves and scales which carry spore with them to new sites. Evidence clearly indicates that germinating spores and active AM mycelial webs colonize plant roots within the immediate mycorrhizospere and that each web overlaps and interacts with all others in the vicinity, spatially becoming a 'global' network. A strategy for dissemination of viable AM fungal propagules over distance is less clear. There are reports of spores being vectored by insects and small mammals, and possibly by water and wind over long distances.

2.7: AM Fungal Interaction With Plants

About 90% of plant families from all phyla of land plants are estimated to be hosts of AM fungi and AM fungi usually colonise the host roots by forming inter-cellular and intra-cellular hyphae and intra-cellular arbuscules. The remaining plant species are either non-mycorrhizal or non hosts of AM fungi. Plant species belonging to the Brassicaceae and Chenopodiaceae are not known to form AM fungal symbiosis (Smith and Read 1997). Giovannetti and Sbrana (1998) suggested that this is due to the lack of any recognition leading to the establishment of a functional symbiosis. Glenn *et al.* (1985) reported *Brassica* roots to be colonised by *Glomus mosseae* but only when root cells were dead, *i.e.* when no plasma membrane was present. The AM-crucifer association appears mostly non-functional with regard to nutrient exchange between plant and fungus (Ocampo *et al*. 1980).

Association with AM fungi has generally been assumed to have no, or at least very low, host specificity because many species have been shown to colonise a wide

range of hosts and the same plant root can be colonised by a mixture of AM fungi species (Helgason *et al*. 1999; Klironomos 2000). However, Van Heijden *et al*. (1998) indicated that plants might select the AM fungus and Vandenkoornhuyse *et al*. (2002) demonstrated that distinct AM fungal communities are associated with different host plants. The degree of host specificity could be under the genetic control of the host, the AM fungus, or more likely a complex interaction of both symbionts with the soil environment (Chanway *et al.* 1991; Sylvia *et al*. 2003).

2.8: Abiotic Factors Affecting AM Fungi

2.8.1: Light - In nature, the exposure of soil borne AM fungi to light is an extremely unlikely event as underground roots are colonized. Light treatments affect the growth pattern of axenically growing hyphae. Light induced hyphal branching was observed in developing germ tube of *Gigaspora rosea* and *Glomus intraradices* (Nagashi *et al.* 2000). Light availability is positively correlated with AM fungal formation (Tester *et al.* 1986), P uptake (Smith and Gianinazzi-Pearson 1990) root soluble carbohydrates levels and plant growth response (Graham *et al.* 1982). As AM fungi are significant carbon sink in mycorrhizal plant system (Haris *et al.* 1985) the influence of light environment on mycorrhizal colonization is indirectly mediated by the carbon status of host plant. Several studies have documented that lower levels of soluble carbohydrates affect mycorrhizal colonization (Graham *et al.* 1982; Hayman 1974).

2.8.1.2: pH - The efficiency is influenced by the properties of the soil. The AM fungi have been found in the soils from pH 2.7- 9.2 but different fungal isolates have varied pH tolerances (Sequeira *et al.* 1982). Optimal pH for spore germination seems to be linked to pH of soil, where the AM fungi are isolated (Giovannetti 2000).

2.8.1.3: Carbon dioxide (CO_2) - Although released by roots, CO_2 cannot be regarded as plant specific for AM fungi as CO_2 level in soil can also be increased from sources such as respiration of soil organisms. When initiating AM fungal monoxenic culture and enriched CO_2 atmosphere developed in Petri plates due to root organism probably activates spore germination and asymbiotic hyphal growth (Vierheilig and Bago 2005).

2.9: Disturbance - Physical disturbance of the top 20-30 cm of soil drastically affects inoculum potential in the short term (Jasper *et al*. 1989) as the AM fungal mycelial web is fragmented. With the possible exception of fragile, low nutrient-available systems such as sand dunes and arid regions, the recovery period appears to be rapid. Hyphal repair, inoculum potential of root fragments, and re-connection of viable hyphal fragments *via* anastomosis may accelerate recovery. Arbuscular mycorrhizal species diversity recovery response to restoration after fire was rapid where mycotrophic understorey herbaceous plants were re-planted (Korb *et al*. 2003). Winter freezing had little impact on the inoculum potential of AM fungal hyphae (Addy *et al*. 1997). Diversity of AM fungi also recovers rapidly where herbaceous plants re-established on newly created islands after flooding in a European alpine river (Harner *et al*. 2011). Recovery of diversity in agricultural soils in flood plains and deltas is also rapid, possibly due to the relatively large diversity of spores at 50-75 cm in agricultural soils (Oehl *et al*. 2005). Alluvial deposits are also a possible source of viable inoculum replenishment. Miller and Bever (1999), in their study of AM fungal species variation in a wetland grass species along a dry-to-wet gradient, found certain species in the drier regions only. There were no species found only in the wet regions. There are flooding effects reported that reduce AM colonization and spore

numbers in paddy fields but sufficient inoculum survives to colonize (19-33%) subsequent non-flooded crops (Wangiyana *et al*. 2006).

In agricultural practices of continuous tillage disturbance and in increasing land use intensity, AM fungal spore numbers and morphologically assessed species diversity were reported to be consistently reduced (Douds and Millner 1999; Oehl *et al*. 2003). Continuous monoculture of maize and to a much lesser extent crop rotation also showed reduction in diversity. Soils left fallow or under sustainable or organic agricultural systems showed significantly greater diversity (Oehl *et al*. 2003, 2004). The greatest diversity was found in semi-natural grasslands, where disturbance is severe such as removal and stockpiling of topsoil, AM fungal propagule viability is considerably reduced after just three to four years of storage (Gould and Liberta 1981).

2.9.1: Agrochemicals - Applications of agricultural chemical fertilizers, fungicides and pesticides has shown to have both negative and positive effects upon AM fungal population characteristics. Increase in levels of available P by fertilizer application almost always promotes a negative feedback, reducing diversity and abundance in AM fungal community. Residual levels of P were found to inhibit AM fungal root colonization even after conversion to organic systems (Hijri *et al*. 2004). An interesting exception is described by Johnson (1984) where *G. intraradices* colonization of *Citrus aurantium* was unaffected after 26 weeks following weekly application of P, and more root cortex sporulation at the highest of concentrations.

2.9.2: Fungicides - Captan and benomyl were observed to decrease metabolic activity in AM fungal tissue three days after treatment (Kough *et al*. 1987). Schreiner and

Bethlenfalvay (1996) investigated the effects of captan, benomyl and PCNB on *G. etunicatum*, *G. mosseae* and *Gi. rosea* in pea plants. All three depressed percentage root colonization. They found *Gi. rosea* spore abundance significantly reduced in captan treated soils, *G. etunicatum* spore abundance increased in all three fungicide treated soils, and *G. mosseae* spore abundance increased only in captan treated soils.

2.9.1.3: Pesticides - Show variable effects on AM fungi, generally decreasing colonization, sometimes significantly. Abd-Alla *et al*. (2000) found significant inhibition of AM fungal root colonization and spore production in an investigation of the effect of five different pesticides applied to three legume crops. Sreenivasa and Bagyaraj (1989) in their assessment of the effects of five insecticides on *G. fasciculatum* in pot culture found all were deleterious at recommended dosage but two applied at half that rate significantly increased root colonization and spore density.

2.10: Symbiotic Benefits of AM Fungi

The major benefits of AM fungi to symbionts includes enhanced nutrient uptake, increased tolerance to root pathogens, drought resistance, tolerance to toxic heavy metals and improved soil aggregation and structure.

2.10.1: Nutrient uptake - Macro- and micro-nutrients are required for plant growth in varying amounts. Micro-nutrients are required in moderate quantities and could result in toxicity disorders when present in high levels or deficiencies when present in very low levels (Ashman and Puri 2002). Various levels of micro-nutrients have been reported to affect the yield of crops (Johnson *et al*. 2005). These fungi are known to enhance the uptake of P from the soil, which is then translocated to the host plant

through hyphal network in the soil. Also, their ability to uptake other micro-nutrients such as Zn, Cu, Fe and Mn has been demonstrated with different host plants and soil types (Pawlowska and Charvart 2004; Gonzalez-Chavez *et al*. 2002; Caris and Hordt 1998; Weissenhom *et al*. 1995).

Arbuscular mycorrhizal fungi also have the ability to sequester these nutrients and minimise transfer to the plant roots when present in high concentrations. However, the mechanism of this ability has not been proved (Turnau *et al*. 1993). Phosphorus is the essential nutrient required for plant growth and is found in many soils in organic and complex inorganic forms. Due to its low solubility and mobility, plants cannot readily utilise P in an organic or complex inorganic form (Schachtman *et al*. 1998). Inorganic P present in soluble forms in the soil can be utilised by plants but usually in limited amounts. Thus, AM fungi intervene to enhance nutrient uptake through the spread of extra-radical hyphae into the surrounding soil and hydrolysing any unavailable sources of P with the aid of secreted enzymes such as phosphatase (Carlile *et al*. 2001; Koide and Kabir 2000; Amaranthus 1999; Schachtman *et al*. 1998). The enzyme phosphatase, produced by the extra-radical hyphae hydrolyses and releases P from organic P complexes and facilitates the absorption of P and other nutrients thereby creating a depletion zone around the roots (Li *et al*. 1991). These depletion zones limits the rate of P uptake by non-mycorrhizal plants but gives mycorrhizal plants a greater advantage because of the ability of extra-radical hyphae to extend past this nutrient depletion zone (Sylvia *et al*. 2001; Liu *et al*. 2000). Sylvia *et al*. (2001) reported that under nutrient deficient conditions the effectiveness of AM fungi is exercised by the ability of the extra-radical hyphae to bridge the nutrient depletion zones of host plant roots. However, when the nutrients are available to the

plant, root length growth is increased and the mycorrhizal dependency of the plant to take up nutrient is reduced.

Although P is the main nutrient transported by AM fungi to plants, N is of great importance for plant growth and should not be over-looked (Onguene and Habte 1995). Nitrogen is obtained by the extra-radical hyphae of AM fungi in different forms ranging from amino acids, peptides, ions ($NO3^-$ or $NH4^+$) to recalcitrant organic nitrogen forms (Hawkins *et al*. 2000; Lipson *et al*. 1999; Tobar *et al*. 1994; Ames *et al*. 1983). It has been recorded that the extra-radical hyphae of different *Glomus* species can assimilate and metabolise both organic and inorganic sources of nitrogen perhaps by glutamate synthetase activity (Hawkins *et al*. 2000; Ames *et al*. 1983). It can be stated therefore that the concentration of P and N in the soil can determine the rate of other micro- (Fe, Cu, Mn, Zn) and macro-nutrient (K, Ca) uptake by mycorrhizal plants (Azcón *et al*. 2003). Liu *et al*. (2000) confirmed this in their study which determined the role of AM fungi in the uptake of Cu, Zn, Mn and Fe in maize which showed that the uptake of these nutrients was significantly influenced by soil P nutrition. Due to the potential of AM fungi to enhance nutrient uptake, this benefit has however brought about the suggested use of AM inoculum instead of chemical fertilisers for plant productivity, growth and restoration of polluted soils or in revegetation (Cardoso and Kuyper 2006; Khan 2006; Quilambo 2003).

2.10.2: Drought tolerance - Along with accessing soil nutrients, the hyphae of AM fungi allows greater access to water through mechanisms such as stomatal regulations, increased root hydraulic conductivity, osmotic adjustments, maintenance of cellular water pressure and cell wall elasticity changes (Augé, 2000; Davies *et al.* 1993).

Recent studies observed that the mycorrhizal colonization of maize with *Gl. mosseae* and *Gl. intraradices* helped the plant to maintain higher leaf water potential compared to non-mycorrhizal plants (Amerian and Stewart 2001). The ability of AM fungi to effectively alleviate drought stress has been studied in terms of P uptake, photosynthesis and cytokinins (Goicoechea *et al*. 1997; Tobar *et al*. 1994). Allen *et al*. (1981) observed that colonization of *Bouteloua gracilis* by *G. fasciculatum* enhances water translocation, nutrient uptake and rate of photosynthesis.

2.10.3: Plant pathogens - Colonization of plant roots by AM fungi is known to increase plants tolerance to pathogens thereby acting as a bio-control agent (Azcón-Aguilar and Barea 1996; Chhabra *et al.* 1992). Several mechanisms or combination of mechanisms could account for the observed bio-protection of plants by AM fungi. Primarily, the ability of AM fungi to enhance plant vigour due to increased nutrient uptake enables it to resist pathogen infection (Smith 1988). Chhabra *et al*. (1992) reported that increased nutritional status of plants by AM fungi increase tolerance to root pathogens. Besides, AM fungi were found to increase *Zea mays* tolerance to leaf rust with control plants having 80% leaf rust as compared to AM inoculated plants, which had less than 5% leaf rust (Dames 1991). Arbuscular Mycorrhizal fungi have direct access to plant photosynthetic products while pathogens, which are not obligate biotrophs, can only obtain C from decomposing organic sources. This automatically gives AM fungi a growth advantage over pathogens like *Fusarium* that must access organic sources for carbon on their own. In addition, microbial changes in the mycorrhizosphere and anatomical changes in the root induced by AM formation causes stimulation of specific functional groups in the microbiota that are antagonistic towards pathogens (Azcón-Aguilar *et al*. 2002; Sylvia *et al.* 1998; Azcón

Aguilar and Barea 1996; Linderman 1994). However, these mechanisms are said not to be effective for all pathogens and are influenced by soil and environmental conditions (Azcón-Aguilar and Barea 1996). A study on the biocontrol potential of AM fungi on *Fusarium* using different cultivars of maize proved to increase the plant's tolerance to the pathogen when used as an inoculant (Mukasa-Mugerwa 2005). The actual mechanism by which AM fungi confers protection against pathogens to plants remains unidentified (Dumas-Gaudot *et al*. 2000). Lignification caused by AM fungal colonization makes penetration of pathogenic hyphae difficult (Cordier *et al*. 1996) and hence pathogens that target plants are unable penetrate and infect the plant root due to anatomical changes in root structure (Dumas-Gaudot *et al*. 2000). Similarly, Zhu and Yao (2004) suggested that release of phenolic compounds in response to AM fungal colonization inhibit growth of pathogens (*Ralstonia solanacearum*).

2.10.4: Soil aggregation - Soil structure is improved by AM fungi through the secretion of a glue-like, proteinaceous, water-soluble and heat stable substance from their hyphae called glomalin (Steinberg and Rillig 2003). This compound aids in soil aggregation by binding soil particles, thereby influencing soil porosity, which promotes aeration and water movement, essential for good root growth, root development and microbial activity (Amaranthus 1999). Soil micro-aggregates are bonded more tightly than macro-aggregates (>250 μm) (Smith and Read, 2008), perhaps suggesting greater glomalin deposition from the larger surface areas of small diameter hyphae. The colonization of soil by microbiota and subsequent incorporation of detritus organic materials develops and maintains a structurally water-stable living soil. Glomalin C fraction can range from 9-22%, residence time in soil from 6-42

years, and may represent >5% of total soil C (Rillig *et al*. 2001). Glomalin, a recalcitrant, iron-containing glycoprotein is indeed responsive to ecosystem fluctuations such as elevated atmospheric CO_2 concentrations, global warming and agricultural practices. Due to the positive correlation observed between glomalin, land-use and soil carbon-nitrogen ratio, this glycoprotein can be used to assess changes in soil C in various land-use types (Rillig *et al*. 2003). Hence, glomalin can be regarded as an indicator for soil aggregation and stability. Glomalin is easily assayed and cannot be produced from uncolonized plant roots as it is AM fungal specific. Therefore, it can be used to determine AM hyphal growth and activity in the soil (Lovelock *et al*. 2004; Rillig *et al*. 2001; Wright *et al*. 1998). AM fungi thus have immense influence upon soil C cycles. Rillig (2004) conducted long-term research with single AM fungal species isolates. They reported differences in aggregation, and attributed it to the influences of different associative microbiotic communities.

2.10.5: AM symbiosis and osmotic stress alleviation - Arbuscular mycorrhizal symbiosis occurs in all the natural habitats, and aids in ecosystem restoration (Barea and Jeffries 1995; Khade and Adholeya 2007; Miransari 2010). It is a specialized system for nutrient uptake, and is more efficient than roots (Smith and Read 1997; Azcón-Aguilar *et al.* 1999). These beneficial organisms can alleviate the unfavourable effects of stresses on plant growth due to the formation of extensive hyphal networks (Garg and Chandel 2010). This unique symbiosis reduces oxidative damage and enhances tolerance to salinity (Azcon *et al.* 2009), drought (Ruiz-Lozano *et al*. 1995) and soil-borne pathogens (Azcon-Aguilar and Barea 1996). Salt stress can pose several problems for plant growth and development as well as inhibit leaf photosynthesis (Shafi *et al.* 2010). One important physiologic and basic strategy

employed by higher plants to resist salt stresses is the adjustment of osmosis through production and subsequent transportation of organic osmoprotectants in plant cells (Xu *et al*. 2010). Several eco-physiological studies investigating the role of AM symbiosis in protection against osmotic stresses have demonstrated that the symbiosis often results in altered rates of water movement of the host plants (Auge 2001). Safir *et al*. (1972) reported that the AM symbiosis probably affected the water relations of plants indirectly through improved P nutrition. Moreover, it is likely that the contribution of the AM symbiosis to plant drought tolerance results from a combination of physical, nutritional, physiological and cellular effects. Studies carried out so far have suggested several mechanisms by which AM symbiosis can alleviate drought stress in host plants. As soil dries out and soil water potential becomes more negative, plants must decrease their water potential to maintain a favourable gradient for water flow from soil into roots (Ruiz-Lozano 2003). The most important mechanism which achieves such an effect, known as osmotic adjustment or osmoregulation, is a decrease in the plant osmotic potential by active accumulation of organic ions or solutes (Hoekstra *et al.* 2001). Osmotic adjustment is common to all cellular organisms (Csonka and Hanson 1991). It allows cells to maintain turgor and the processes that depend on it, such as cellular expansion and growth, stomatal opening and photosynthesis, as well as keeping a gradient of water potential favourable to water entrance into the plant. The solutes which participate in osmotic adjustment are inorganic ions or uncharged organic compounds like proline or glycine betaine, as well as carbohydrates like sucrose, pinitol or mannitol.

Azcon *et al.* (1996) reported an increase in proline accumulation in mycorrhizal plants subjected to drought. It has also been shown that mycorrhizal colonization and drought interact in modifying free amino acid and sugar pools in

roots (Auge *et al*. 1992). Kubikova *et al*. (2001) reported higher osmotic adjustment in leaves of mycorrhizal basil plants than in non-mycorrhizal ones during drought stress. A number of studies have demonstrated that, during soil drying, mycorrhizal plants often maintain higher gas exchange rates and nutrient status compared to non-mycorhhizal plants (Bethlenfalvay *et al.* 1987; Goicoechea *et al.* 1997). Plants colonized by AM fungi maintained higher stomatal conductance, transpiration rate and shoot water than non-mycorrhizal plants (Duan *et al*. 1996). The mechanism by which the AM symbiosis achieves such an effect is not clear, however it is suggested that AM fungi probably increase the ability of the root system to scavenge water in dried soil, resulting in less strain on foliage, and hence higher stomatal conductance and shoot water content at a particular soil water potential (Duan *et al*. 1996). The pioneering studies of Allen (1983) and Hardie (1985) indicated a possible role of AM fungal hyphae in water uptake and transfer to the host plant. Hyphae with a diameter of 2 -5mm can penetrate soil pores inaccessible to root hairs (10-20mm diameter) and so absorb water that is not available to non-mycorrhizal plants. Allen (1991) estimated that the rate of water transport by extra-radical hyphae to the root was sufficient to modify plant water relations.

2.10.6: Ecological restoration - The use of AM fungi in ecological restoration projects has been shown to enable their host plant establishment on degraded soil and improve soil quality and health (Jeffries *et al.* 2003). A relatively new approach to restore land and protect against desertification is to inoculate the soil with AM fungi with the reintroduction of vegetation. A long term study done by Jeffries *et al.* (2003) demonstrated a significantly greater long term improvement in soil quality parameter was attained when the soil was inoculated with a mixture of indigenous AM fungi

species compared to the non inoculated soil and soil inoculated with a single exotic species of AM fungi. The benefits observed were an increased plant growth, higher soil organic matter content and soil aggregation. Inoculation with native AM fungi increased plant uptake of phosphorus improving plant growth. The results support the use of AM fungi as a biological tool in the restoration of self-sustaining ecosystems.

Literature data indicate that AM fungi increase the root absorptive area and hence the plant nutrition (Bieleski 1973), influence succession of plant communities (Janos 1980), their competitiveness (Allen and Allen 1984) and phenology (Allen and Allen 1986), equalize the level of nutrition of co-existing plants by formation of hyphal bridges transferring nutrients between them (Newman 1988), and improve soil structure through binding sand grains into aggregates by extra-radical hyphae (Sutton and Sheppard 1976). The requirement of AM fungi for up to 20% of host photosynthate for establishment and maintenance is well accepted (Graham 2000; Jakobsen and Rosedhal 1990).

3.0: Introduction

Mangroves are facultative halophytes, characterized by regular tidal inundation and fluctuating salinity (Gopal and Chauhan 2006). Mangrove plant species are highly adapted to coastal environments, both morphologically and physiologically, and thrive in intertidal zones of tropical and sub-tropical regions (Ball 1996; Naidoo *et al.* 2002). They exhibit exposed breathing roots, extensive support roots and buttresses, salt-excreting leaves and viviparous water dispersed propagules. These adaptations vary among taxa and with physico-chemical variations of habitat (Duke 1990). Distribution is governed by topography, tidal height, substratum and salinity. Mangroves display extreme variations in species composition, forest structure and growth rate. Mangrove forests can vary from a narrow fringe along the banks of an estuary to dense stands covering many square kilometers. Total mangrove area in India is 6740 km^2, of which 80% is found along the east coast and 20% on the west coast. Deltaic environments on Indian east coast support extensive mangrove forest due to intertidal slope and the heavy impact of siltation (MOEF 1994). The western coastline has narrow intertidal belts which support only fringe mangroves. All estuaries in Goa are classified as microtidal because the tidal level is below two meters (Ahmad 1972). Mangroves have become the center of many conservation and environmental issues because of loss of effects on the coastal environment. Anthropogenic pressure is constantly increasing and immediate protection and conservation of the ecosystem is necessary. Reforestation of mangrove is a promising solution to restoration (Badola and Hussain 2005; Danielsen *et al*. 2005).

Ecological functions attributable to AM fungi include helping to increase plant tolerance of adverse soil conditions, influencing response to severe climatic conditions and increasing plant productivity in natural plant communities (Brundrett

and Kendrick 1996). Arbuscular mycorrhizal fungi enhanced availability of nutrients is described as a primary factor affecting abundance and composition of plant species communities (Klironomos 2003). The major nutrients phosphorus (P) and nitrogen (N) are deficient in mangrove ecosystems (Carr and Chambers, 1998) and likely to limit the growth of mangrove plant species.

Arbuscular mycorrhizal fungi play significant role in physiological processes such as water use efficiency (Ruiz-Lozano *et al.* 1996) modifying the structure and function of plant communities, and are useful indicators of ecosystem change (Miller and Bever 1999). Burke *et al.* (2003) demonstrated that inoculation with AM fungi improves growth of plants under salinity stress. Previous studies have shown that these fungi are either absent (Mohankumar and Mahadevan 1986), rare (Kothamasi *et al.* 2006) or ubiquitous (Sengupta and Chaudhuri 2002; Kumar and Ghose 2008) in mangrove ecosystems. In India, most studies on mangroves and AM fungi were carried out along the east coast while studies on the west coast are scarce. In addition to their use in afforestation, established AM fungal plant species might serve as important sources of inocula for initially non-mycorrhizal conspecifics, thereby affecting their regeneration and contributing to overcome the patchy distribution of species within communities (Koide *et al.* 2000). Hence, it is important to study the diversity of AM fungal species and identify their potential for use in afforestation using native species of mangrove habitats. The aim of the present study was to determine the AM fungal diversity in selected mangrove sites from Goa and to identify the dominant AM fungal species found therein.

3.1: Materials and Methods

3.1.1: Study sites and sample collection

Goa is located on the west coast of India. The state has 12,000 ha of estuaries, 2000 ha of which are occupied by mangrove forest. Seven sites covering all the major estuaries of Goa **(Fig. 1; Table 2)** were selected for the study. Tropical weather at the sites is warm and humid, and soils are marshy. The mean temperature range is 22-35°C and average annual rainfall is 2500mm. In total, 17 mangrove species of 8 families *viz.,* Acanthaceae, Rhizophoraceae, Euphorbiaceae, Myrsinaceae, Salvadoraceae, Sonneratiaceae, Ceratopteridaceae and Fabaceae were investigated **(Table 3).** Of these, 14 were true mangroves (TM) species and 3 were mangrove associates (MA), identified following Rao (1985). Root and rhizosphere soil samples were randomly collected from June 2007 to September 2009 from all the study sites. During collection, care was taken to ensure that the collected roots belonged to the same plant. Fine roots of mature trees were traced by digging, and removed with adhering soil. The samples were collected in polyethylene bags and brought to the laboratory. The roots were separated from adhering soil, washed gently under tap water and fixed in FAA (formalin-acetic acid-alcohol) for estimation of AM colonization. Rhizosphere soil of individual plants was air dried at room temperature, sieved (mesh size 720μ) and divided into two parts, one for isolation, enumeration and identification of AM spores, the other as inoculum for trap culture.

3.1.2: Soil analyses

Three soil samples from each of the study site were separately collected in polyethylene bags from a depth of 0-25 cm, air-dried in the laboratory before passing through a 2-mm sieve, and mixed thoroughly to obtain a composite sample. Soil pH

was measured in soil-water (1:2) suspension using pH meter (LI 120 Elico, India). Electrical Conductivity (EC) was measured at room temperature in 1:5 soil suspension, using a Conductivity meter (CM-180 Elico, India). Standard soil analysis techniques *viz.*, rapid titration method (Walkley and Black 1934) and Bray and Kurtz method (1945) were employed for determination of organic carbon and available P, respectively. Available potassium was estimated by ammonium acetate method (Hanway and Heidel 1952) using a flame photometer (Systronic 3292). Available zinc (Zn), copper (Cu), manganese (Mn) and iron (Fe) were quantified by DTPA-$CaCl_2$-TEA method (Lindsay and Norvell 1978) using Atomic Absorption Spectrophotometer (AAS 4139).

3.1.3: Estimation of AM fungal root colonization

Fixed roots were placed in 2% KOH, heated at 90° C, acidified with 1% HCl and stained with trypan blue (Koske and Gemma 1989). The stained roots were examined on a compound microscope (100X-1000X) for AM fungal structures and percent root colonization was estimated using the slide method (Giovannetti and Mosse 1980). A segment was considered mycorrhizal when it showed the presence of hypha and arbuscule or vesicle.

3.1.4: Trap culture, isolation and taxonomic identification of AM fungal spores

For identification of AM species, trap cultures were prepared in pots using field soil and sterile sand (1:1). *Solenostemon scutellarioides* (L.) Codd was used as the catch plant and the pots were maintained in a polyhouse at 27^oC. All cultures were provided a 14h day/10h night photoperiod for six months. The pots were watered as and when required and Hoagland's solution minus P was added fortnightly. Intact and non-

parasitized spores used for identification, obtained from both rhizosphere soil samples and trap cultures, were isolated using wet sieving and decanting technique (Gerdemann and Nicolson 1963). Intact and crushed spores mounted in Poly vinyl-lacto Glycerol (PVLG) (Koske and Tessier 1983) were examined under an Olympus BX 41 compound microscope. Identification was based on spore morphology and sub-cellular characteristics (Schenck and Perez 1990; Rodrigues and Muthukumar 2009; http: //invam.caf.wvu.edu.).

3.1.5: Diversity studies

Diversity studies were conducted for each site separately by calculating Simpson's Index of Diversity $1\text{-}D$ (Simpson 1949), $D=1-\Sigma\ (Pi)^2$ where $Pi=ni/N$ (ni, the relative abundance (RA) of the species calculated as the proportion of individuals of a given species to the total number of individuals in a community, N). Shannon diversity index (H') is commonly used to characterize species diversity in a community, accounting for both abundance and evenness of the species present, $H= -\Sigma\ (Pi \ln (Pi)$ (Shannon and Weaver 1949). Species richness (SR) is number of species present. Species evenness (E), which indicates the distribution of individuals within species was calculated by using the formula: $\Sigma\ (H') = H'/H'_{max}$ where $H'_{max} = ln\ (SR)$. Isolation Frequency (%) of each species was calculated as (si/S) 100, where si is the number of soil samples containing spores of the ith species and S is the total number of soil samples examined.

3.1.6: Statistical analysis

Pearson's correlation coefficient was calculated to assess the relationships between root colonization and spore density, isolation frequency and relative abundance and spore density and species richness at each site, using WASP software (Web Based Agricultural package) 2.0 ($P \leq 0.05$). By using WASP software, relative abundance of AM fungal species common to all sites was correlated with soil pH, P and EC ($P \leq 0.05$).

3.2: Results

3.2.1: Soil analysis - Results of the soil physico-chemical analyses are shown in **Table 2.** Soils were acidic (pH range of 5.5-6.7). Electrical Conductivity (EC) ranged from 2.19 to 8.49 dSm^{-1}. Maximum organic carbon content was recorded in Terekhol site (5.21%) and minimum was recorded in Chapora site (0.92%). Soils at all study sites were deficient in available P. Levels of micronutrients such as Cu, Zn, Mn and Fe varied at all study sites.

3.2.2: AM colonization - Arbuscular mycorrhizal colonization was recorded in 16 out of 17 mangrove plant species selected for the study **(Table 3)**. With the exception of *Salvadora persica,* all investigated plant species were mycorrhizal. Colonization was characterized by presence of hyphae, arbuscules and vesicles. Hyphal coils **(Plate III - a, b & c)**, arbuscular colonization **(Plate III - d, e & f)** and vesicular colonization **(Plate IV - a, b, c, d & e)** were observed. Auxiliary cells were observed in rhizosphere of *A. ilicifolius* **(Plate IV - f)**. Arbuscular mycorrhizal colonization varied by species and the situation of their occurrence. Percent colonization was maximal for *E. agallocha* (77.29%) and minimal in *A. marina* (6.21%) **(Table 3)**.

Both *Arum*- and *Paris*-type morphologies were observed, the latter type was dominant, observed in 74% of the plant species. 18% plants recorded both *Arum*- and *Paris*-type of morphologies while 6% plants exhibited only *Arum*- type of morphology. *Acanthus ilicifolius* was common to all study sites and showed variation in AM colonization. Site wise average root colonization was maximum in Galgibagh and minimum in Chapora site **(Fig. 2)**.

3.2.3: Spore density and species richness - Spore density varied in the rhizosphere soils of selected plant species, with maximum spore density for *A. ilicifolius* (324 spores100g^{-1}) and minimum for *D. heterophylla* (8 spores100g^{-1}) **(Table 4).** Maximum spore density was recorded at the Sal site (184 spores100g^{-1}) and minimum spore density was recorded at Talpona (54 spores100g^{-1}) **(Fig. 3)**. *Acanthus ilicifolius* supported maximum species richness with seven AM fungal species of four genera. Zuari site showed highest species richness (16) with lowest richness (5) at Talpona **(Fig. 4)**.

3.2.4: Diversity and distribution of AM fungi - Twenty eight AM fungal species of five genera, *viz., Glomus*, *Acaulospora*, *Scutellospora*, *Gigaspora* and *Entrophospora* were recovered from rhizosphere soils of all study sites. *Glomus* (16 species) was the most dominant genus followed by *Acaulospora* (6), *Scutellospora* (4), *Gigaspora* (1) and *Entrophospora* (1) with species number given in parenthesis.

RA and IF were greatest for *Glomus* and least for *Entrophospora.* Within AM species, highest IF was recorded for *G. intraradices* followed by *G. geosporum, A. scrobiculata, A. laevis* and *A. bireticulata*, and lowest in *G. clarum* followed by *G. nanolumen, G. rubiforme*, *A. foveata, S. weresubiae* and *E. infrequens*. Maximum RA

was recorded for *G. intraradices* followed by *A. scrobiculata* and *A. laevis* while minimum RA was recorded for *Gi. albida* followed by *S. calospora* and *A. delicata*. *Glomus mosseae* (25.4 % IF, 2.02% RA) and *A. scrobiculata* (58.3% IF, 2.98% RA) at Mandovi and *G. aggregatum* (28.5% IF, 3.09% RA) at Sal had low relative abundance but were widely distributed (high IF). In contrast, *G. fasciculatum* (8.3% IF, 17.1% RA) at Mandovi and *G. clarum* (7.1% IF, 25.9 % RA) at Zuari showed lower IF but were dominant in sporulation in comparison to other species. *Glomus intraradices* and *A. laevis* were recovered from all seven sites **(Fig. 5)**. However, RA of both species showed no significant correlation with soil pH, P or EC ($p \leq 0.05$) **(Table 5)**.

Species evenness was highest at Talpona and lowest at Zuari. Shannon-Wiener diversity (*H'*) was highest at Talpona and lowest at Zuari. Simpson`s dominance index (*D*) ranged from 0.97 to 0.99 **(Table 6)**. Spore density was significantly correlated with species richness at six sites while at Chapora (r=0.720; $p \leq 0.05$) there no significant correlation. Six of the seven sites, showed significant positive correlation between RA and IF, while Talpona (r=0.834; $p \leq 0.05$) showed no significant correlation. There was no significant correlation between spore density and root colonization at any site **(Table 7)**.

3.3: Discussion

Recorded fluctuation in pH and EC levels of mangrove soils might be attributable to the constant flushing of water that leads to deposition of salts (Rodrigues and Anuradha 2009). Padma and Kandaswamy (1990) reported that nearly 80%−85% of P is made unavailable to plants due to fixation and immobilization.

The root hair of the studied mangrove species was small and poorly developed. This feature is known to create potential plant mycotrophy, enhancing nutrient acquisition in stressed environments (Baylis 1975). This study revealed that 94% of mangrove species were mycorrhizal. Similar observations were recorded by Sengupta and Chaudhuri (2002); Kumar and Ghose (2008) and Wang *et al*. (2010). But this study contradicts the earlier findings of Mohankumar and Mahadevan (1986) who reported absence of AM fungi in mangrove vegetation of the Pitchavaram forest at Tamil Nadu in India.

Paris-type morphology was present in 74% of the plant species, which is in agreement with the finding of Kubota *et al.* (2005) who reported dominance of *Paris*-type morphology in natural ecosystems. Brundrett and Kendrick (1990) suggested that slow growth coupled with long root life span and gradual AM fungal colonization exhibited in *Paris*-type morphology may be the best growth strategy under low nutrient and high stress conditions that often prevail in mangrove ecosystems. Bedini *et al.* (2000) suggested that the genotypic physiology of the host may also contribute to the type of colonization. In *A. ilicifolius*, AM fungal colonization showed insignificant variation by site.

Newsham *et al.* (1995) suggested overall functionality in AM communities remains fairly constant regardless of species variation. Variation in AM fungal root and spore density was observed. However, no significant correlation was established which is in agreement with Miller (2000). Arbuscular mycorrhizal fungal colonization is known to depend on soil moisture and P availability (Ruotsalainen *et al*. 2002; Wang *et al.* 2010), physiology, growth rate and turnover of plant roots (Lugo *et al.* 2003). Smith and Read (1997) reported that the extent to which typical AM fungi colonize root system varies with plant species and is known to be influenced by age of

plant and the viability of AM propagules in the soil. Similarly, variation in spore density at the seven study sites might be due to environmental fluctuations playing a key role in influencing AM symbiosis. Zhao (1999) reported seasonality, edaphic factors, age of host plants and dormancy might be factors contributing to variation in spore density.

In present study, hyphae were the dominant AM fungal structures. Similar observations were reported by Sengupta and Chaudhuri (2002). It might possibly be explained by the high moisture levels in mangrove ecosystems. Miller and Bever (1999) identified the possible mechanisms by which AM fungal could survive in hypoxic conditions. Since AM fungi are aerobic micro-organisms, occurrence of AM fungal symbioses in mangrove ecosystems is related to the well developed aerenchyma and when flooded, AM fungi may survive by relying on the oxygen provided by the aerenchyma. The importance and functioning of AM fungi in mangroves is now being realized suggesting their potential to alter at least some aspects of plant morphology under stressful condition but further research is needed to understand their mechanisms in extreme environments like mangroves.

Based on RA and IF, *Glomus* and *Acaulospora* were the dominant genera and *G. intraradices* and *A. laevis* were the dominant species. Bever *et al.*, (1996) reported that *Glomus* and *Acaulospora* species usually produce more spores than *Gigaspora* and *Scutellospora* species within the same environment. Because of their smaller spore size, *Glomus* and *Acaulospora* species require less time to sporulate (Hepper 1984) and are therefore more adaptive in adjustment of sporulation pattern in varied environmental conditions (Stutz and Morton 1996).

Thus, it is important to consider the sporulation characteristics of AM fungi in determining dominance in mangrove communities. Stutz *et al*. (2000) reported that

Glomus species are known to be widely distributed and commonly found in different ecosystems and geographical regions. Since the studied mangrove soils were acidic, this might explain the dominance of *A. laevis*, as reported by Abbott and Robson (1991) for the occurrence of *Acaulospora* species in acidic soils. Climatic and edaphic factors, together with the host species and soil type (Muthukumar and Udaiyan 2002) and differential sporulation ability of AM fungal species (Barni and Siniscalco 2000), might influence AM fungal distribution. The species that produce more spores had wider distribution while species with a smaller geographical range produced fewer spores. Spore production of AM fungi is known to vary greatly by ecosystem type and is affected by many environmental and biological factors (Zhang *et al.* 2004).

Maximium AM species richness was recorded at Zuari (16) and minimum at Talpona (5) which may be correlated to plant species diversity occurring at the sites. More samples were examined at Zuari because it showed maximum mangrove diversity. Sturmer and Bellei (1994) reported that species richness is dependent on sample number. The more samples collected, the more species are likely to be recovered.

Correlation analysis between AM species richness and spore density showed a significant positive correlation at six sites while in Chapora no significant correlation was observed. Ferrol *et al.* (2004) and Radhika and Rodrigues (2010) reported highest species richness and spore density in terrestrial ecosystems in comparison to the wetland system studied here.

Six of the seven sites showed significant positive correlation between RA and IF. Talpona showed no significant correlation. Some AM species, *viz. G. fasciculatum* at Mandovi and *G. clarum* at Zuari, were not present at high IF but were dominant in sporulation compared to other species. In contrast, *G. mosseae* and *A. scrobiculata* at

Mandovi and *G. aggregatum* at Sal had low RA but were widely distributed (high IF). Clapp *et al.* (1995) suggested that wider distribution and lower RA signifies a strong mycelial network that spreads among host plants over a large area and produces fewer spores. Also, in the present study there was no significant correlation between spore density and root colonization at any site. Shannon Wiener diversity (*H'*) was highest at Talpona and lowest at Zuari. Distribution of AM species was more uniform at Talpona (E= 0.41) compared to other sites. Bever *et al.* (1996) reported that differences in sporulation ability of different AM fungal species can result in unevenness in spore density. Simpson's dominance *(D)* ranged from 0.97 to 0.99.

Absence of significant correlation between root colonization and spore density may be attributed to the adaptation of AM fungi to particular soil conditions (Dhar and Mridha 2003) and variations in timing of germination potential of AM fungi (Gemma *et al.* 1989). Miller *et al*. (1995) reported that spore numbers poorly reflects the colonization potential of soil and they are not always related to rate and extent of AM colonization (Abbott and Robson 1985). Similarly, He *et al.* (2002) observed increase in AM fungal colonization when soil conditions were favourable for spore germination and spore number decreased resulting in no correlation between AM colonization and spore density.

In this study 28 AM fungal species of five genera were recovered from the rhizosphere soils, more than the six species reported by Wang *et al.* (2010) for South China. Radhika and Rodrigues (2007) described only four AM fungal species of two genera of aquatic and marshy plant species at Goa, India. Dalpe and Aiken (1998) reported that the persistence of AM fungi in mangroves depends upon survival of propagules like spores, mycelia and colonized root systems and AM fungal mycelium seem to be morphologically and physiologically well adapted to extreme

environments (Klironomos *et al.* 2001), thus enabling long term survival and sporulation.

In general, the diversity of AM fungi observed in wetland ecosystem is lower than in terrestrial ecosystems (Radhika and Rodrigues 2010; Zhao and Zhao (2007). Spatial and temporal variations in mangrove ecosystems and the preference of different host plants may be reasons for lower diversity of AM fungi in wetlands (He *et al.* 2002).

Variation in functional diversity within one AM fungal species can be greater than between different AM fungal species or even genera (Munkvold *et al.* 2004). This may indicate host preference. Thus, ecological studies are needed to test differential mangrove plant performance in response to AM fungi. Considering their effect on mangrove species, AM fungi may be important drivers of plant community composition in mangrove ecosystems. The fungal species that occurred at all study sites showed different patterns of sporulation and distribution, suggesting differences in functional diversity. Further studies are needed to consider the combined effect of occurrence of AM fungi by season and phenological stage of mangroves.

4.0: Introduction

Mangroves are a type of coastal woody vegetation that fringes muddy saline shores and estuaries in tropical and subtropical regions (Kumar and Ghose 2008). They are characterized by high levels of productivity, and fulfill essential ecological functions, harbouring precious natural resources (Wang *et al*. 2010). Recent evidence suggests that growth of mangroves is limited primarily by phosphorus (P) availability as it is adsorbed and co-precipitated within carbonate-dominated environments (Lovelock *et al.* 2004). Phosphate solubilizers, N fixers and AM fungi are known to interact in the rhizosphere soils (Alongi 2002) where hyphae of AM fungi assist in accessing nutrients by extending beyond the root depletion zone (Cui and Caldwell 1996). These fungi also alleviate salt stress and aid physiological processes such as osmotic adjustment *via* accumulation of soluble sugars in root cells (Feng *et al.* 2002), and contribute to the nutritional status of plants (Zandavalli *et al.* 2004). They play a crucial role in determining plant diversity, production and species composition (van der Heijden *et al.* 1998). The seasons are a result of tilt of Earth's axis that causes variation in environmental conditions and spore density, and community composition of AM fungi are influenced by these changes. To understand the ecology and function of plant-fungus associations in natural ecosystems, it is necessary to clarify seasonal diversity of AM fungi, providing insight into the factors and processes regulating ecosystem development (Su *et al.* 2011). Studies on the occurrence and diversity of AM fungi from different mangrove plants have been documented (Wang *et al.* 2010; Kumar and Ghose 2008). However, no studies have been reported on the seasonal dynamics of AM fungi in mangroves. The objective of the present study is to determine patterns of AM colonization, spore density and species richness in relation to seasons.

4.1: Materials and Methods

4.1.1: Study sites and sample collection - Two study sites *viz.,* Terekhol (15° 72' 28'' N & 73° 72' 99''E) with a stretch of 28 Km and Zuari (15° 32' 56''N & 73° 89' 71'' E) having 67 Km were selected for the study. Three dominant plant species *viz.*, *Acanthus ilicifolius* L. (Acanthaceae), *Excoecaria agallocha* L. (Euphorbiaceae) and *Rhizophora mucronata* Poir. (Rhizophoraceae) common to the two sites, were undertaken for the study. Mangroves species were identified following Rao (1985). Rhizosphere soil samples were randomly collected in the pre-monsoon (March 2009 - May 2009), monsoon (July 2009 - September 2009) and post-monsoon (October 2009 - February 2010) seasons from the two sites. During collection, care was taken to ensure that the collected roots belonged to the designated plant and the traced fine roots removed with adhering soil. The samples were placed in polyethylene bags, transported to the laboratory and stored at 4^{0}C until processed. The roots were separated from adhering soil, washed gently under tap water and fixed in FAA (formalin-acetic acid-alcohol) for estimation of AM colonization. Rhizosphere soil of individual plants was air dried at room temperature, sieved (mesh size 720µ) and divided into two parts, one for isolation, enumeration and identification of AM spores, the other as inoculums for trap culture.

4.1.2: Soil analyses - Were carried out as described under **3.1.2**.

4.1.3: Estimation of AM fungal root colonization - Were carried out as described under **3.1.3**.

4.1.4: Trap culture, isolation and taxonomic identification of AM fungal spores - Were carried out as described under **3.1.4**.

4.1.5: Diversity studies - Simpson's Index of Diversity, Shannon diversity index, Species evenness and species richness were calculated as described under **3.1.5**.

4.1.6: Statistical analysis - Pearson's correlation coefficient was calculated to assess the relationships between spore density and species richness at each site, using WASP software (Web Based Agricultural package) 2.0 ($P \leq 0.05$). Relative abundance of AM fungal species common to all seasons was correlated with soil pH, P and EC ($P \leq 0.05$). Data on seasons and host co-affect the AM fungal spore density, species richness, and Shannon-Weiner diversity index was analyzed using multivariate analysis of variance (MANOVA). The statistically significant difference was determined at $P \leq 0.05$.

4.2: Results

4.2.1: Soils analyses - Results of the soil physico-chemical analyses are shown in **Table 8**. The study revealed acidic soils (pH range 5.5-6.8) at both sites. Electrical Conductivity (EC) ranged from 4.03 to 8.49 dSm^{-1}. Organic carbon content was higher in pre-monsoon season at both the study sites. Soils at both the sites were deficient in P. Levels of micronutrients such as Cu, Zn, Mn and Fe varied at the two study sites.

4.2.2: AM colonization - AM fungal colonization was recorded in all the plant species investigated and was characterized by the presence of hyphae, vesicles and

arbuscules. Site wise results of the seasonal variations in AM colonization in the three mangrove plant species undertaken for the study are depicted in **Table 9**. Average root colonization was higher in Terekhol than in Zuari site **(Fig. 6)**. Arbuscular mycorrhizal colonization varied between the seasons in each of the mangrove species.

Pre-monsoon season: At both the sites average AM colonization was significantly higher in the pre-monsoon season compared to monsoon and post-monsoon season **(Fig. 7)**. At Terekhol site, maximum root colonization (86%) was recorded in *E. agallocha* while minimum root colonization (26%) was recorded in *R. mucronata.* At Zuari site, maximum root colonization was recorded in *E. agallocha* (78%) while minimum root colonization (23%) was recorded in *R. mucronata* **(Table 9)**.

Monsoon season: *Excoecaria agallocha* recorded maximum root colonization at both the sites *i.e.* 69% in Terekhol and 58% in Zuari site. *Rhizophora mucronata* recorded minimum root colonization at both the sites *i.e.* 17% in Terekhol and 14 % in Zuari site **(Table 9)**.

Post-monsoon season: At Terekhol site, maximum root colonization (36%) was recorded in *A. ilicifolius* while minimum root colonization (14%) was recorded in *R. mucronata.* At Zuari site, maximum root colonization was recorded in *E. agallocha* (39%) while minimum root colonization (11%) was recorded in *A. marina* **(Table 9)**.

4.2.3: Spore density - Site wise results of the seasonal variations in spore density of AM fungi in the three mangrove plant species undertaken for the study are depicted in **Table 10**. Spore density in the rhizosphere soils of the selected plant species showed

variation ranging from 7-230 spores $100g^{-1}$. At Terekhol site, the mean spore density was 53 spores $100g^{-1}$ soil (range 7-230), while at Zuari site, mean spore density was 51 spores $100g^{-1}$ soil (range 12-186) **(Fig. 8)**. Spore density varied between the seasons in each of the mangrove species.

Pre-monsoon season: *Excoecaria agallocha* recorded maximum spore density at both sites *i.e.* 128 spores $100g^{-1}$ soil at Terekhol and 186 spores $100g^{-1}$ soil at Zuari site. At Zuari site minimum spore density was recorded in *A. marina* (32 spores $100g^{-1}$ soil) whereas at Terekhol site minimum spore density was recorded in *R. mucronata* (38 spores $100g^{-1}$ soil) **(Table 10)**.

Monsoon season: At both sites mean spore density was significantly higher in the monsoon season compared to pre-monsoon and post-monsoon seasons **(Fig. 9)**. At Terekhol site, maximum spore density was recorded in *A. ilicifolius* (230 spores $100g^{-1}$ soil) and minimum in *R. mucronata* (17 spores $100g^{-1}$ soil). At Zuari site, minimum spore density was recorded in *R. mucronata* (24 spores $100g^{-1}$ soil) and maximum in *A. ilicifolius* (149 spores $100g^{-1}$ soil) **(Table 10)**.

Post-monsoon season: At both sites mean spore density was significantly lower in the pre-monsoon season compared to monsoon and post-monsoon seasons **(Fig. 9)**. At Terekhol site, maximum spore density was recorded in *A. ilicifolius* (40 spores $100g^{-1}$ soil) and minimum in *R. mucronata* (7 spores $100g^{-1}$ soil). At Zuari site, minimum spore density was recorded in *S. alba* (12 spores $100g^{-1}$ soil) and maximum in *A. ilicifolius* (28 spores $100g^{-1}$ soil) **(Table 10)**.

4.2.4: Distribution and relative abundance (RA) of AM species - A total of 11 AM fungal species representing three genera were recorded. *Glomus* was the most dominant genus followed by *Acaulospora* and *Scutellospora.* In the present study two sporocarp species were recovered*, G. aggregatum* and *G. rubiforme*. Identification of AM fungal species was confirmed by trap culture method where no additional AM fungal species were recovered. Within AM species the highest RA was recorded in *G. intraradices* followed by *A. scrobiculata, A. laevis* and *A. bireticulata*, and lowest RA was recorded for *S. gregaria*. *Acaulospora laevis* was recovered in all three seasons, *G. intraradices* and *S. gregaria* recorded in two seasons from both the sites, while *G. nanolumen*, G. *fasciculatum* and *G. multicaule* were recorded in only one season from either site **(Table 11)**. In the present study RA of dominant AM fungal species showed no significant correlation with soil pH, P, or EC values ($p \geq 0.05$).

4.2.5: AM fungal species richness (SR) and species evenness (E) - Species richness in combined sites was maximum in monsoon (9 species) and minimum in the post-monsoon season (4 species) **(Fig. 10)**. Correlation analysis indicated that the spore density in pre-monsoon was significantly correlated with species richness in both the sites (Terekhol $r = 0.726$; Zuari $r = 0.645$; $P \geq 0.05$) while no significant correlation was observed in either post-monsoon or monsoon season in either site **(Table 12)**. Species evenness was maximum in post-monsoon season in both sites, and Shannon-Weiner (H) and Simpson`s indices showed variation between the different seasons **(Table 13)**.

Multivariate analysis revealed that seasons ($F_{2, 26} = 2.346$; $P < 0.001$) and host ($F_{2, 18} = 1.854$; $P < 0.001$) coaffected AM fungal spore density and species richness.

The seasons had a greater influence than host species as evidenced by higher F values; however the interaction was found to be non significant.

4.3: Discussion

Variation in pH and EC levels in the mangrove soils observed in the present study may be attributed to the constant flushing of tidal water, leading to deposition of salts (Rodrigues and Anuradha 2009). Soils at both the study sites were deficient in available P. It is reported that nearly 80-85% of P is made unavailable to plants due to fixation and immobilization (Padma and Kandaswamy 1990). Degradation of litter in mangrove ecosystem is active and continuous, resulting in the release of various acids during hydrolysis of tannins (Liao 1990) and the oxidation of iron sulfide (pyrite) that releases dissolved ferrous iron (Stumm and Morgan 1996), that are known to shift soils towards more acidic conditions (Liao 1990). The present study revealed a high organic carbon content in the mangrove sediments, its degradation resulting in low pH (Liao 1990). Concentration of Fe was higher at Zuari than at Terekhol. At the Zuari site, maximum concentration of Fe was recorded in the pre-monsoon season. Earlier study suggested that increased Fe content in mangrove soils could be attributed to the precipitation of the respective metal sulfide compounds in anaerobic sediments (Howarth 1979).

In present study, mangrove plant species exhibited higher root colonization levels in pre-monsoon and least in post- monsoon season. Similar seasonal patterns in AM colonization were observed in aquatic plants by (Khan 1974). These differences may be related either to the different behaviour of each AM fungal species and the influence of different environmental conditions (Klironomos *et al.* 1993). The evaluation of biotic and abiotic factors responsible for AM seasonal patterns has

shown that plant phenology is related to AM colonization. In this study, *E. agallocha,* and *A. ilicifolius* recorded maximum AM colonization in the pre-monsoon season and minimum in post-monsoon season at both sites. Flowering period in *E. agallocha* and *A. ilicifolius* was observed in pre-monsoon season, which explain the maximum AM colonization. Van Duin *et al.* (1989) suggested that the highest level of colonization corresponds to the maximum flowering period in wetlands plant species. The ability of specific AM fungi to colonize the root system is indicative of biological niche of each fungus in rhizosphere, where some fungi may allocate more carbon to colonizing the root surface; others allocate more to develop external hyphae in soil. Seasonal shift in AM colonization have been found which indicate that the benefits of mycorrhizal symbiosis for plant changes during the seasons (Fitter 1991). The results of seasonal dynamics in AM colonization in this study emphasize that host plant rather than environmental stress factors are responsible for AM fungal distribution. Like the plant species, AM fungi may have developed adaptive strategies to tolerate this stressful environment.The selected mangrove plant species showed variation in spore density. An earlier study reported that patterns observed in spore density may not reflect the activity of AM fungi in roots, but rather the tendency to sporulate along wide environmental conditions (Miller and Bever 1999). Spore density showed variation, maximum in monsoon and minimum in the post-monsoon season at both the sites. Similar observations have been reported in earlier studies (Dhillion and Anderson 1993; Gemma *et al.* 1989). Other studies suggested that P availability (Ruotsalainen *et al.* 2002) plant physiology, and turnover of plant roots (Lugo *et al.* 2003) are among the drivers of AM fungal seasonality. Furthermore, variation in spore density may be due to host efficiency in resource capture and utilization (Clark and Zeto 2000) and edapho-climate factors (Abbott and Robson 1991).

In the present study no significant correlation between relative abundance (RA) of dominant AM fungal species and soil pH, P or EC values suggests that AM fungi have a specific multi-dimensional niche determined by host plant species. This may effect variation between and within sites in AM fungal community composition (Ahulu *et al*. 2006). Some reports suggest that AM fungi are obligatly aerobic and flooding reduces sporulation (Aziz *et al*. 1995) and total spore density correlates negatively with soil moisture (Anderson *et al*. 1994). Others have found higher values of total spore density in wet soils than in dry soils (Rickerl *et al*. 1994) and have suggested that high sporulation is a stress response to adverse or extreme environmental conditions.

Glomus species are known to be widely distributed and are commonly found in different geographical regions (Stutz *et al.* 2000). Furthermore, *Glomus* species are more adaptable to adjustment of sporulation patterns in varied environmental conditions (Stutz and Morton 1996) resulting in dominance. In our study, *Acaulospora laevis* was recorded in all of the three seasons at both study sites. The acidic nature of mangrove soils explains the presence of *A. laevis* in all the seasons as *Acaulospora* species are known to occur in acidic soils (Abbott and Robson 1991). Based on RA, the dominant species *viz*., *A. laevis* and *G. intraradices* showed different patterns of sporulation and distribution. These differences in sporulation pattern may be attributed to plant phenological events including new root growth (Stenlund and Charvart 1994), flowering and fruiting (Van Duin *et al.* 1989). They may also suggest differences in AM functionality.

Multivariate analysis revealed that seasons and host co-affected AM fungal spore density and species richness, and the seasons have greater influence than host by analyses of F values. The seasons and host are important factors influencing AM

fungal spore density and species richness in natural ecosystems because the host plant can regulate carbon allocation to roots, produces secondary metabolites, and changes the soil environment during different seasons (Su *et al*. 2011).

Seasonal diversity observed in the present study is higher than that in an earlier mangrove study in South China, where only six AM fungal species were reported (Wang *et al.* 2010). Similarly, only four AM fungal species belonging to two genera in 16 aquatic and marshy plant species were reported from Goa, India (Radhika and Rodrigues 2007). In general the AM fungal diversity in wetland ecosystems is lower than terrestrial ecosystems (Radhika and Rodrigues 2010; Zhao 1999). Preference of different host plants and dormancy may be factors attributing to lower diversity of AM fungi in wetlands (He *et al.* 2002).

Mangrove plant communities interact with rhizosphere soil and can modify edaphic properties. Similarly edaphic factors interact with plant communities and modify their composition. Consequently in this study there was no clearly observed separation between the plant and soil factors influence on AM fungal sporulation. Our results suggest that the uneven spatial distribution of AM fungal spores and the complex structure of a mangrove ecosystem should also be considered as major factors affecting spore density of AM fungi. Seasonal studies of AM fungi help to predict the conditions crucial for development of AM fungi. Further targeted ecological studies are needed to consider the combined effect of occurrence of AM fungi in the different phenological stages of mangroves to provide an accurate picture of AM fungal development and function prevailing in the given ecosystem.

5.1: INTRODUCTION

Mangroves are climax formation of hydro halophytes inhabiting estuarine or marine salt marshes in the tropics and subtropics and are well adapted, both morphologically and physiologically, to survive under saline conditions (Naidoo *et al.* 2002). They are physiologically tolerant to high salt levels and have mechanisms to obtained fresh water despite the strong osmotic potential of the sediments (Ball 1996). Arbuscular mycorrhizal (AM) fungi are important partners in natural plant communities (Karagiannidis and Nikolaou 1999), which influences response to severe climatic conditions and increase plant productivity (Brundrett *et al*. 1996). Other benefits include increased tolerance to plant root diseases (Barea *et al.* 1996). They function as extension of the root system, increasing the absorptive area and enhancing inorganic P uptake through hyphal scavenging of soil volumes that are not accessed by roots (Joner *et al*. 2000). Occurrence of AM fungi under natural conditions is important to evaluate the inoculum potential. Despite their recognized importance, the factors controlling AM fungal community composition in mangroves are poorly understood. One such factor is phenology which may influence temporal pattern of nutrient demand (Rorison 1987).

Phenology is the study of the timing of vegetative, flowering and fruiting activities, and its relationship to environmental factors. Growing plant may experience different stages in mineral nutrition based on balance among internal and external nutrient supplies and plant demand for the nutrients. Plant requires adequate P from very early stages of growth. Although fruit and seed production is more expensive process than vegetative stage as far as minerals are concerned, root system development may be proportionally greater in relation to demand during early seedling growth stage (Grant *et al*. 2001). AM fungi benefit plants during the times of

P demand (Grant *et al*. 2005) and the need of P is not constant during the life cycle of most of plants (Fitter 1985). To understand the role of AM fungi in increasing the plant growth, nutrient uptake and other processes it is necessary to monitor AM symbiotic association during different phenological growth stages of the plant. Patterns and timing of AM colonization within the plant roots may vary and depending on edaphic factors and variation in nutrient levels (Sanders 1999). Hence, identifying the patterns of symbiotic effects between host plant communities and AM fungi in natural ecosystems is important as the AM fungi are known to influence plant diversity (Watkinson 1998). AM fungi are known to enhance the growth of mangrove plant species (Wang *et al*. 2010). However no previous studies have reported the variation in AM colonization and changes in AM fungal species in relation to phenology of mangrove plant species. The objectives of the present study were to determine the changes in abundance, composition and variation in colonization of AM fungi with respect to plant phenology in two selected mangrove habitats of Goa.

5. 2: Materials and methods

5.2.1: Study sites and sample collection - Two sites *viz.,* Chapora (15° 63’ 98’’N & 73° 73’ 61’’ E) and Mandovi (15° 48’ 64’’N 73° 86’ 52’’ E) were selected for the study. Seven mangrove species of 5 families *viz.,* Acanthaceae, Rhizophoraceae, Euphorbiaceae, Myrsinaceae and Salvadoraceae were investigated. Of these, 6 were true mangroves species (TM) and one mangrove associate (MA), identified following Rao (1985). Root and rhizosphere soil samples were randomly collected from November 2008 to December 2009 from both the study sites. For each investigated species, rhizosphere soil and root samples of three plant species in each of the three growing stages *viz*., vegetative, flowering and fruiting stage were collected from the

same locality (radius of 100-150m) **(Table 14)**. During collection, care was taken to ensure that the collected root samples belonged to the same plant, of similar age and uniform size. Fine roots of mature trees were traced by digging, and removed with adhering soil. The samples were collected in polyethylene bags and brought to the laboratory and kept in the refrigerator at 4^0C until processed. The roots were washed gently under tap water and fixed in FAA (formalin-acetic acid-alcohol) for estimation of AM colonization. One part of the rhizosphere soil collected from individual plant species was used for isolation, enumeration and identification of AM spores and the other as inoculum for trap cultures.

5.2.2: Soil analysis - Were carried out as described under **3.1.2**.

5.2.3: Estimation of AM fungal root colonization - Were carried out as described under **3.1.3**.

5.2.4: Trap culture, isolation and taxonomic identification of AM fungal spores - Were carried out as described under **3.1.4**.

5.2.5: Diversity studies - Simpson's Index of Diversity, Shannon diversity index, Species evenness and species richness were calculated as described under **3.1.5**. Isolation frequency and relative abundance of AM fungi was calculated using formula of (Beena *et al.* 2000).

Relative abundance of AM fungi (RA) = Spore number of species (genus)/Total number of identified spores x 100

Isolation frequency of AM fungi (IF) = Number of soil samples where species (genus) occurred/ Total number of soil samples x 100

5.2.6: Statistical analysis

Pearson correlation coefficient was calculated to assess the relationship between root colonization and spore density, colonization frequency and relative abundance at each site separately. Statistical analysis for correlation coefficient was carried out using WASP (Web Based Agricultural package: www.icar.goa.res.in/wasp/cor1.php) 1.0. For analysis difference were considered significant when $P \leq 0.05$.

5.3: Results

5.3.1: AM fungal colonization - AM fungal colonization was recorded in all the plant species investigated and was characterized by the presence of hyphae, vesicles and arbuscules. Average root colonization was higher in Chapora than in Mandovi site **(Fig. 11)**. Average root colonization was maximum in the flowering stage at both the sites **(Fig. 12)**. Three plant species common to the both the study sites showed variation in the number of hyphae, vesicles and arbuscules in different growth stages. Hyphal colonization was observed in all the three different stages, while arbuscular colonization was prominent in the flowering stage in all plant species at both the sites. Both *Arum*- and *Paris*-type morphologies were observed.

Vegetative stage: At both the sites, hyphal and vesicular colonization was observed in all the plants species while arbuscular colonization was recorded in only two plant species. *Exoecaria agallocha* recorded maximum root colonization at both the sites *i.e.* 68% in Chapora and 53% in Mandovi site. At Chapora site, minimum root

colonization was recorded in *A. marina* and *R. mucronata* (16%) while *S. persica* (6%) recorded minimum root colonization in Mandovi site **(Table 15 & 16)**.

Flowering stage: All the selected plants showed the presence of hyphal, vesicular and arbuscular colonization at both the sites. Average root colonization was significantly higher in the flowering stage compared to the vegetative and fruiting stage **(Fig. 12)**. In Chapora site, *E. agallocha* recorded maximum root colonization (90%) whereas in minimum root colonization (34%) was recorded in *A. marina* **(Table 15)**. In Mandovi site, *A. ilicifolius* recorded maximum root colonization (79%) while minimum root colonization (24%) was recorded in *S. persica* **(Table 16)**.

Fruiting stage: Average root colonization was significantly lower in the fruiting stage at both the sites **(Fig. 12)**. At Chapora (Agarwada) site, maximum root colonization (47%) was recorded in *A. ilicifolius* while minimum root colonization (12%) was recorded in *A. marina* **(Table 15)**. At Mandovi site, maximum root colonization was recorded in *Exoecaria agallocha* (32%) while minimum root colonization (13%) was recorded in *R. apiculata* **(Table 16)**. Except for *A. ilicifolius* in Mandovi site, all the mangrove plant species recorded arbuscular colonization at both the sites **(Table 15 & 16)**.

5.3.2: Spore density - Spore density in the rhizosphere soils of the selected plant species showed variation that ranged from 7-329 spores $100g^{-1}$. Variation in spore densities in various growth stages at both the sites was observed **(Table 15 & 16)**. At Chapora site, the mean spore density was 158 spores $100g^{-1}$ soil (range of 15 - 298), while at Mandovi site, mean spore density was 111 spores $100g^{-1}$ soil (range of 7-329)

(Fig. 13). At both the sites mean spore density was significantly higher in the fruiting stage compared to the other two stages *i.e.* vegetative and flowering stage **(Fig. 14)**.

Vegetative stage - At Mandovi site, maximum spore density was recorded in *A. ilicifolius* (53 spores $100g^{-1}$ soil) and minimum in *R. apiculata* (7 spores $100g^{-1}$ soil) **(Table 16)** while at Chapora site, maximum spore density was recorded in *E. agallocha* (84 spores $100g^{-1}$ soil) and minimum in *A. marina* (15 spores $100g^{-1}$ soil) **(Table 15)**.

Flowering stage - At Chapora site, maximum spore density was recorded in *A. ilicifolius* (43 spores $100g^{-1}$ soil) and minimum in *A. marina* (19 spores $100g^{-1}$ soil) **(Table 15)**. In Mandovi site, maximum spore density was recorded in *E. agallocha* (84 spores $100g^{-1}$ soil) and minimum in *S. persica* (14 spores $100g^{-1}$ soil) **(Table 16)**.

Fruiting stage - Maximum spore density was recorded in *E. agallocha* with 298 spores $100g^{-1}$ soil at Chapora site and 329 spores $100g^{-1}$ soil at Mandovi site **(Table 15 & 16)**. At Chapora site minimum spore density was recorded in *A. marina* (27 spores $100g^{-1}$ soil) **(Table 15)** whereas at Mandovi site, minimum spore density was recorded in *R. apiculata* (25 spores $100g^{-1}$ soil) **(Table 16)**.

5.3.3: Isolation frequency (IF) and Relative abundance (RA)

Vegetative stage - In all eight AM species belonging to 2 genera *viz., Glomus* and *Acaulospora* were recovered from both the sites. Six AM species were recovered from Chapora and four from Mandovi site. Two AM species *viz., A. scrobiculata and G. rubiforme* were common to both the sites. At Chapora site maximum RA was

recorded in *A. scrobiculata* and least in *G. rubiforme* whereas at Mandovi site maximum RA was recorded in *G. intraradices* and least in *G. rubiforme* **(Fig. 15)**. Isolation frequency (IF) showed similar trend at Chapora site. Maximum IF was recorded in *A. scrobiculata* and miminum in *G. rubiforme* whereas at Mandovi site, *G. intraradices* recorded highest IF and least in *G. rubiforme* **(Fig. 16)**. Based on RA and IF three AM fungal species *viz., G. intraradices*, *A. scrobiculata* and *A. laevis* were found dominant.

Flowering stage - At both sites, 8 AM species belonging to three genera *viz., Glomus*, *Acaulospora* and *Scutellospora* were recovered. Five AM species each were recorded at Chapora and Mandovi sites. Two AM species *viz., A. scrobiculata* and *G. taiwanense* were common to both the sites. In both the study sites RA was highest in *A. scrobiculata* (38.2% in Chapora, 35.5% in Mandovi) whereas at Chapora site lowest RA was recorded in *G. taiwanense* and at Mandovi site it was least in *S. calospora* **(Fig. 17)**. In both the study sites IF was highest in *A. scrobiculata* (72.4% in Chapora, 81.4% in Mandovi) whereas least IF at Chapora and Mandovi sites was recorded in *G. taiwanense* and *S. calospora* respectively **(Fig. 18)**. At both the sites, *Acaulospora* (4 species) was dominant genus followed by *Glomus* (2 species) and *Scutellospora* (2 species). Based on RA and IF three AM fungal species *viz., A. scrobiculata, A. spinosa* and *A. laevis* were found dominant.

Fruiting stage - At both the sites, 14 AM species belonging to four genera *viz., Glomus*, *Acaulospora*, *Scutellospora* and *Gigaspora* was recovered. Eight AM species in Chapora and 10 in Mandovi site were recorded. Four AM species *viz., A. scrobiculata, G. aggregetum, G. intraradices* and *G. taiwanense* were common to

both the sites. At Chapora site maximum RA was recorded in *G. intraradices* and least in *S. dipurpurscens,* whereas at Mandovi site maximum RA was recorded in *A. scrobiculata* and least in *S. calospora* **(Fig. 19)**. In both the study sites IF was maximum in *A. scrobiculata* (80.0% in Chapora, 93.3% in Mandovi) while minimum IF at Chapora and Mandovi sites was recorded in *G. taiwanense* and *S. calaspora* respectively **(Fig. 20)**. Based on RA and IF six AM fungal species *viz., G. intraradices*, *G. aggregatum, A. scrobiculata, A. mellea, A. spinosa* and *A. laevis* were found dominant. *Acualospora* (6 species) was dominant genus followed by *Glomus* (5 species), *Scutellospora* (2 species) and *Gigaspora* (1 species).

5.3.4: Diversity and distribution of AM fungi - Fourteen AM fungal species belonging to four genera *viz., Glomus*, *Acaulospora*, *Scutellospora*, and *Gigaspora* were recovered from the rhizosphere soils of both the study sites *Acaulospora* (6 species) and *Glomus* (5 species) were dominant genera followed by *Scutellospora* (2 species) and *Gigaspora* (1 species). In the present study three sporocarpic forms *i.e. G. rubiforme, G. taiwanense* and *G. aggregatum* were recovered. Seven AM species *viz., G. intraradices, G. aggregatum*, *G. taiwanense, A. scrobiculata, A. spinosa, A. laevis and A. spinosa* were recovered from both the sites **(Table 17)**.

Maximum species richness was recorded in the fruiting stage with 14 AM species, whereas in the vegetative and flowering stages 8 AM fungal species each were recorded **(Fig. 21)**. Distribution of AM fungal species was more uniform in flowering stage in both sites **(Fig. 22)**. Shannon Wiener diversity index (*H'*) was higher in flowering stage **(Fig. 23)**, while Simpson`s dominance index (*D*) was highest in fruiting stage **(Fig. 24)**. Correlation analysis showed a significant positive correlation between RA and IF, in all three growth stages. Except in fruiting stage, in

both sites there was no significant correlation between spore density and root colonization **(Table 18)**.

5.4: Discussion

Mangroves ecosystem is characterized as nutrient conservative system, where nutrients are rapidly synthesized between biotic parts and are not available (Khathiresan and Bingham 2001). Arbuscular Mycorrhizal fungi are critical in this type of ecosystem as AM fungal hyphae are more efficient in scavenging nutrients and channeling them to hosts plants. They also regulate composition and functioning of plant communities by regulating resource allocations and growth characteristics of interacting plants (Allen 1991).

In the present study, average root colonization in the three growth stages showed variation. Following the vegetative stage AM fungal colonization increased in flowering stage while in fruiting stage least colonization was observed. The variation in AM fungal colonization in different growth stages is attributed to dependence of AM fungi on plant growth rate and turnover (Lugo *et al*. 2003). Ruotsalainen *et al*. (2002) suggested that availability of P might be another factor affecting AM colonization. Lower levels of AM colonization in fruiting stage observed may be due to decrease rate of photosynthesis leading to lower photosynthate supply to the roots resulting in lower AM colonization (Kaschuk *et al*. 2009).

Type of AM colonization was influenced by the phenology of selected mangrove plant species. Three common mangrove species *viz*., *E. agallocha*, *A. ilicifolius* and *A. corniculatum* in both the sites recorded maximum arbuscular colonization in the flowering stage while vesicular colonization was more pronounced in the vegetative stage. Maximum arbuscular colonization in the flowering stage is

attributed to higher P demand in the flowering stage (Rorrison 1987; Khade and Rodrigues 2009). Harrison *et al*. (2002) reported that as arbuscules are nutrient transfer sites, higher arbuscular colonization in the flowering stage suggests facilitation of higher P uptake. Persistence of vesicles in the vegetative stage indicates the lower P demand compared to flowering stage and P requirements are met by diffusive transport (Dune and Fitter 1989).

The study indicates that the spore density is mainly influenced by growth stage and showed variation. Mean spore density decreased from vegetative to flowering stage and recorded increase in the fruiting stage. Variation in spore density may be due to inter-specific competition and differences in timing of spore production in associated host plants (Brundrett and Kendrick 1990) suggesting competition between AM fungi and their interaction with rhizosphere environment in natural communities. Zhao (1999) reported AM fungal dormancy and distribution patterns are other factors affecting sporulation in the rhizosphere.

At both the sites, *E. agallocha* recorded maximum spore density in the fruiting stage. The fruiting stage represents the end of optimum plant growth and slow root growth (Gaurdaramma and Sanchez 1999) which leads to resource remobilization in senescence root and often seen dependant on nutrient availability in natural communities (Auge 2001; Johnson *et al*. 2003) resulting in maximum AM fungal spore production. Except for fruiting stage in both the sites, there was no significant correlation between spore density and root colonization. This may be due to the germination potential of AM fungi that varies at different times of the year (Tommerup 1983; Gemma and Koske 1988). Miller *et al*. (1995), reported spore numbers poorly reflects the colonization potential of soil and they are not always related to rate and extent of AM colonization (Abbott and Robson 1982). Dhar and

Mridha (2003) reported adaptation of AM fungi to particular soil conditions may result in the absence of correlation between root colonization and spore density. Similarly, He *et al.* (2002) observed increase in AM fungal colonization when soil conditions were favourable for spore germination and spore number decreased resulting in no correlation between AM colonization and spore density.

Based on RA and IF, *Glomus* and *Acaulospora* were dominant genera in the present study. Bever *et al*. (1996) reported that *Glomus* and *Acaulospora* species usually produce more spores than *Gigaspora* and *Scutellospora* species within the same environment. Because of their smaller spore size, *Glomus* and *Acaulospora* species require less time to sporulate (Hepper 1984) and are therefore more adaptive in adjustment of sporulation pattern in varied environmental conditions (Stutz and Morton 1996). Composition and richness of AM fungal species showed variation in different phenological stages of mangrove plant species. In the vegetative stage, three *Acaulospora* and five *Glomus* species was recovered. In flowering stage, an additional A*caulospora* species (in addition to the three *Acaulospora* species recorded in the vegetative stage), and only two *Glomus* species *viz., G. flavisporum* and *G. taiwanese* besided two *Scutellospora* species were recovered. In the fruiting stage, there was an increase in AM fungal species in both genera *viz., Acaulospora* and *Glomus*. Six *Acaulospora* and five *Glomus* species were recovered. The reduction of *Glomus* species in the flowering stage and their subsequent increase in the fruiting stage revealed competition among AM fungi. Bever *et al*. (1996) suggested that inter-species fungal interactions and host preference may play a role in determining AM fungal composition and distribution of the AM fungal community during various growth stages.

Acaulospora scrobiculata was dominant species and recorded most frequently in all the growth stages. Other AM fungal species recorded include *G. intraradices, G. aggregatum* and *G. taiwanense* in the fruiting stage, *G. rubiforme* in the vegetative stage and *G. taiwanense* in the flowering stage. These species were common despite differences in the plant composition. The acidic nature of mangrove soils may explain the dominance of *A. scrobiculata* supporting an earlier study (Stutz *et al*. 2000).

In both the study sites, maximum Shannon Weiner index (H) was recorded in flowering stage and minimum in fruiting stage. Rosendahl and Stukenbrock (2004) suggested that the variation in the diversity index is due to the change in functional role of the symbiotic organism in the plant cycle. This emphasizes the importance of sampling in various developmental stages of mangrove plant species. Results in the present study indicated that mangrove plant communities harbour distinct AM fungal communities, but the composition of AM fungal communities depends on the interacting effects of different growth stages and host identity, indicating AM fungi play a role in the growth of mangrove plant species. Also, present study elucidates how complexity of plant community and phenology determine AM fungal community structure. Furthermore, the fungal species that occurred in all growth stages in both the study sites showed different patterns of sporulation and distribution suggesting differences in functional diversity. Diversity of AM fungi appears to be linked to specific molecules with soil and the host roots.

6.0: Introduction

The potential of AM fungi as biofertilizers and bio-protectors to enhance plant productivity has been widely recognized, but not fully exploited because of inadequate methods for large scale inoculum production. Some AM fungal species show spore dormancy, while newly formed spores showed a period of endogenous dormancy (Gemma and Koske 1988) which contribute to the survival of AM fungi in adverse environments.

The most widespread approach to measure and analyze species diversity of AM fungi is to recover, count and identify the spore from the field. However, spores collected directly from the field soil can be problematic as:

a. They appear healthy but are not viable, usually parasitized thereby hampering accurate species identification,
b. They change the appearance in their structural characteristics in response to root pigments, soil chemistry temperature and moisture and microbial activity,
c. They represent only those colonizing mycorrhizal activity, and
d. They are low in number and only those fungi sporulating in the rhizosphere of given plant at the time of sampling are recovered (Brundrett *et al*. 1999).

Trap culture provide non-molecular approach for baiting the cryptic species of AM fungi present in plant communities. Trapping is necessary to obtained many healthy spores of colonizing fungi required for identification and also establish mono-specific cultures. Trap culture using host plant grown in sterilized sand is most commonly used method to isolate AM fungi (Brundrett *et al*. 1999) and results in the isolation of greater number of spores than other methods (Watson and Milner 1996). It provides additional information on fungal diversity that complements spore

occurrence data obtained from same soil samples and may provide valuable new information about the biology of AM fungi (Brundrett *et al*. 1999).

Root-organ cultures were first developed by White *et al.* (1943) who used excised roots on synthetic mineral media supplemented with vitamins and a carbohydrate source. Pioneering work by Mosse and Hepper (1975) used root cultures and demonstrated for the first time that spores of an AM fungus could be successfully used to colonize excised roots growing on a mineral-based medium. Later, Strullu and Romand (1986) showed that it was also possible to re-establish mycorrhiza on excised roots using the intra-radical phase (*i.e*. entire mycorrhizal root segment) of several species of *Glomus* as inoculum. Transformed roots have a greater growth potential, which makes them more adaptable to different experimental conditions, and they can be generated from most dicotyledonous plants (Tepfer 1989).

Attempts to determine requirements for spore germination and germ tube growth on artificial media have met with variable results, probably due to variation in methodology, fungal species and the culture conditions employed. During this pre-symbiotic phase, AM spores germinate and develop a germ tube. Germ tube consists of a straight growing hypha (runner hyphae) exploring the media by successive branching into thinner-diameter filaments (Diop *et al*. 1994). Germ tube growth is dependent on the availability of spore reserves (Bécard and Fortin 1988; Sancholle *et al.* 2001). To achieve successful germination and hyphal elongation, protein content of the AM fungal spores needs to be sufficiently stable (Strullu *et al*. 1997). Besides, *in vitro* germination is affected by substrate (Maia and Yano-Melo 2001), and flavanols (Becard *et al*. 1992). However, the systematic information about effects of sucrose concentration in the substrate on AM fungal spore germination is limited.

The taxonomy of AM fungi is based almost entirely on spore morphology, with description, identification and classification to the species level being difficult. Isolation of AM fungi from pot cultures often produces spores lacking subtending hyphae and (or) with damaged spore wall layers (especially the outer evanescent layer). As a consequence, poor quality spore reference material has generated incomplete and sometimes unusable species descriptions. Moreover, the absence of living cultures of type specimens has dramatically reduced studies of spore ontogenesis. However, the root-organ culture system has renewed interest in AM fungal taxonomy. The contaminant-free cultures give constant access to clean fungal propagules, which can be observed and harvested at any stage during fungal development (Bécard and Piché 1992; Chabot *et al*. 1992; Strullu *et al.* 1997; Dalpé 2001). This material is much more appropriate for morphological, ultra-structural, physiological, biochemical and molecular studies than pot-cultured fungi. The *in vitro* grown AM fungi constitute a reliable material for species characterization and description (Declerck *et al.* 2001). Besides, *in vitro* culture systems provided useful information about AM fungal spore ontogeny (Pawlowska *et al*. 1999), and sporulation dynamics without disturbance of the symbionts (Declerck *et al*. 2001). These studies have greatly improved our understanding of AM fungal propagation and life cycles. Most of the AM fungal species successfully cultivated in monoxenic culture belong to genus *Glomus* and Gigasporaceae members. Besides *Glomus* and Gigasporaceae, *Acaulospora rehmii* (Dalpé and Declerck 2002) which is the first Acaulosporaceae representative to have been successfully cultivated *in vitro*.

In most cases, two types of fungal inoculum can be used to initiate monoxenic cultures either extra-radical spores or propagules from the intra-radical phase (*i.e.* mycorrhizal root fragments and isolated vesicles) of the fungus. Sporocarps of *G.*

mosseae have also been used in an attempt to establish *in vitro* cultures (Budi *et al.* 1999). Enzymatically extracted vesicles from roots have been used to establish pot cultures with *Glomus intraradices*, *G. versiforme*, and *G. macrocarpum* (Strullu and Romand 1986), but vesicles are rarely used for routine *in vitro* inoculation. Vesicles can be easily isolated by lacerating heavily colonized roots. Vesicles within roots may be less contaminated than the root surface and they aid in the completion of the AM fungal life cycle (Harley and Smith 1983), thus representing a potential source of inoculum.

In this chapter, the following aspects related to culturing of AM fungi have been studied:

1. Taxonomy of AM fungal spores isolated from the rhizosphere of mangrove plant species using trap cultures and production of mono-specific cultures of dominant AM fungal species and their mass multiplication by pot cultures.
2. Evaluation of *in vitro* germination and germ tube growth of *Glomus intraradices* in Modified MSR medium with and without sucrose.
3. Establishment of *in vitro* culture of *Glomus clarum* by using mature vesicles grown monoxenically with Ri T-DNA transformed *Cichorium intybus* L. roots.

6.1 Materials and Methods

6.1.1: Isolation of AM fungal spores, preparation of Trap- and mono-specific cultures and taxonomic identification - Trap culture were established using AM fungal species isolated from the rhizosphere soils of plant species collected by wet sieving and decanting technique (Gerdemann and Nicolson 1963) and identification of AM species was carried out as described under **3.1.4**.

Spores of AM fungal species recovered from trap cultures were further used for the preparation of mono-specific cultures. Pots (15cm diameter) were filled with sterilized sand (sterilized at 180^0C for 2 hours daily for 3 continuous days) in which surface sterilized spores (using 4% sodium chlorite and 30% ethanol for 5min) were placed 4 cm below the soil. Pots were planted with cuttings of *Solenostemon scutellarioides* (L.) Codd and maintained in the glass house at 27^0C (14h day light) and relative humidity (RH) 62% and were watered regularly with sterile distilled water **(Plate V a & b)**. . Hoagland`s solution (Hoagland and Arnon 1938) minus P was added every 15 days. After 90 days of growth watering was stopped and pots were harvested and analyzed for the recovery of the spores.

6.1.2: Inoculum preparation and sterilization process for *in vitro* germination - *Glomus intraradices* Schenck & Smith was propagated in the glasshouse using sand-based pot cultures and *Solenostemon scutellarioides* (L.) Codd as host. *Glomus intraradices* was originally obtained from the rhizosphere soils of *Rhizophora apiculata* Blume from a local estuary. Cultures were harvested after 8 weeks. Spores were extracted from soil by wet sieving and decanting method (Gerdemann and Nicolson 1963) and stored at 4°C before being used for sterilized. After surface sterilization, using a sterile micro-pipette, they were transferred to Petri plates containing a solution of 2% (w/v) streptomycin sulphate and stored overnight. MSR medium (Declerck *et al*. 1998) solidified with 5% Clarigel, with and without sucrose was used as substrate. An experimental design with 30 replicates was employed for the two treatments. A single spore was inoculated in each Petri plate and incubated in an inverted position in the dark at 26^oC. Hyphal growth was recorded after a time

interval of 12 hours. Statistical analysis was performed by using WASP (Web Based Agricultural package) 2.0 ($P \leq 0.05$).

6.1.3: Isolation, sterilization and inoculation of mature vesicles - Spores of *Glomus clarum* Nicolson & Smith were isolated from rhizosphere soil of *Acanthus ilicifolius* L. collected from Terekhol estuary in North Goa, and propagated in the greenhouse in pot culture with sand as substrate and *Solenostemon scutellarioides* (L.) Codd as host. Cultures were harvested after six weeks to obtain the mature vesicular stage **(Plate XI, a & b)**. Root fragments containing mature vesicles were extracted from soil by wet sieving and decanting method (Gerdemann and Nicolson 1963) and stored at 25°C before being used. Within 24 hours from extraction each root fragment was placed in a Petri plate containing sterile distilled water. This was followed by disinfection for 4 min in 2% chloramine-T, and then a 10 min bath in an antibiotic solution (Streptomycin sulfate 0.02% w/v and gentamycin sulfate 0.01% w/v) (Chabot *et al*. 1992). After the two disinfection steps the fragments were rinsed three times with sterile distilled water. Using fine non-magnetic Dumont tweezers (110mm length, tips 0.06 x 0.10mm) vesicles were separated under a stereo-microscope (Olympus SZ61), and selected vesicles were surface sterilized by using sodium hypochlorite (0.04% w/v) for 2 min. Vesicles found floating were discarded. This was followed by washing with sterile distilled water (5 times) with vigorous shaking of the Petri plate for five minutes each time. Using a micropipette, separated vesicles were transferred to a Petri plate containing a solution of 2% (w/v) ambistriyn-s and stored for 16 h at $4^{o}C$. Modified Strullu and Romand (MSR) medium solidified with 5% clarigel, adjusted to pH 5.5 was used as substrate **(Table 19)**. Excised Ri T-DNA transformed Chicory (*Cichorium intybus* L.) roots were used as host for *G. clarum*.

Following germination, vesicles were transferred to Petri plates containing actively growing 15 days old transformed chicory roots. Germinated vesicle was placed within 1mm of the roots with care taken to ensure that they touched the substrate. Petri plates were incubated in an inverted position in dark at 26°C. Observation of hyphal growth after transfer to the Petri plates containing actively growing Ri T-DNA roots was recorded every 14 hours.

Results

6.2.1: Isolation of AM fungal spores, preparation of Trap- and mono-specific cultures and taxonomic identification - In the present study 28 AM fungal species belonging to five genera *viz., Glomus*, *Acaulospora*, *Scutellospora*, *Gigaspora* and *Entrophospora* were recovered. *Glomus* (15 species) was the most dominant genus followed by *Acaulospora* (7 species), *Scutellospora* (4 species), *Gigaspora* (1 species) and *Entrophospora* (1 species). *Glomus intraradices* was most dominant species in present study. Four out of 28 species *viz., G. intraradices, G. clarum, A. scrobiculata* and *A. laevis* were successfully multiplied using pot culture method and *Solenostemon scutellarioides* as host plant. Extensive root colonization with hyphae, arbuscules and vesicles was observed in the host plant. Cultures of the selected AM species were multiplied and maintained in the poly house. Taxonomical descriptions of the identified AM fungal species are given below:

Acaulospora bireticulata Rothwell &Trappe. *Mycotaxon* **8**: 471-475, 1979 (**Plate VI b**).

Spores formed singly in the soil, sessile, borne laterally on a hyaline thin wall hypha near its terminus. Sporocarps unknown, spores sub-hyaline to light brown, globose to

sub-globose 150-156µm in diam. Spore surface ornamented with polygonal reticulum, polygons 6-18 µm long, enclosed spore surface give the appearance of inverted reticulum. Spore walls consisting of three layers, each one is 1 µm thick. Outer layer is dark green to grayish brown, inner layer is hyaline.

Acaulospora laevis Gerdemann & Trappe. *Mycologia Memoir*, **5**: 76, 1974 **(Plate VI f)**.

Spores 119-500µm in diam., dull yellow in colour. Spore wall with three layers, yellow brown outer wall, 2-4µm thick and inner two hyaline membranes. Spore contents dense, white in colour, globose to polygonal.

Acaulospora scrobiculata Trappe. *Mycotaxon* **6**:359-366, 1977 **(Plate VI a)**.

Spore hyaline to light brown, globose, 100-240µm in diam. Spore surface evenly pitted with depressions 1-1.5 x 1-3µm, separated by ridges 2-4µm thick at the mouth of depressions, circular to elliptical or occasionally linear to Y-shaped. Spore wall consisting of four layers: 1) Sub hyaline to light greenish yellow outer layer 3-6µm thick 2) An adhering smooth, hyaline layer, 0.2-0.5µm thick 3) Hyaline layer, 0.5-1.0µm thick and 4) Roughened, hyaline inner layer of 0.2-1.0µm thick.

Acaulospora delicata Walker, Pfeiffer & Bloss. *Mycotaxon* **25**: 621-628, 1986. **(Plate VI c)**.

Spores hyaline to pale yellow, globose, 80-150µm, with four wall layers. Outer thin hyaline wall 1µm thick, closely attached to wall 2 laminated with 6 sub equal laminations of 5-3.5µm thick. Wall 3 hyaline, 0.5µm thick, covered by minute granules. Wall 4 thin, hyaline, 0.75-1µm thick.

Acaulospora spinosa Walker & Trappe. *Mycotaxon* **12**: 515-521, 1981. **(Plate VI d)**.

Spores dull yellowish brown, 100-335µm diam., ellipsoid to reniform. Spore surface ornamented with crowded blunt spines 1-4µm high, 1µm diam. at the polygonal base, tapering to 0.5µm at the tip. Patches of hyaline to sub hyaline amorphous material upto 2µm thick, irregularly encrusting the spines and covering the whole spore surface. Spore wall continuous except for the occluded openings, three layered. Outer layer, light yellowish brown, 4-10µm thick including spines and encrustations enclosing two membranous hyaline walls of 0.2-1µm thick.

Acaulospora mellea Spain & Schenck. *Mycologia*, 76: 685–699, 1984. **(Plate VI e)**.

Spores formed singly in soil, honey-coloured to yellow-brown, sub-globose, 96–130 x 78–92 µm. Spore wall 4–8(–11) µm thick, consist 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.

Glomus constrictum Trappe. *Mycotaxon* **6**: 359-366, 1977.

Spores 150-330µm, dark brown to black in colour. Spore walls 7-15µm thick, straight with a short funnel shaped projection. Attached hyphae straight or recurved at the point of attachment with dark brown walls 3-5µm thick. Just beyond the point of attachment the hypha constricted to 10-22µm diam. Just beyond the constriction the hypha inflated to 15-30µm diam. with yellow-brown walls of 2-3µm thick.

Glomus etunicatum Becker & Gerdemann. *Mycotaxon* **6**: 29-32, 1977 **(Plate VII e)**.

Spores light brown in colour, globose 68-162µm in diam. Spore wall 4-13µm thick, composed of an outer wall 5µm thick, inner laminated wall 2-8µm thick, spore contents separated from attached hyphae by a thin curved septum.

Glomus fasciculatum (Thaxter) Walker & Koske. *Mycotaxon* **30**: 253-262, 1987 **(Plate VII a)**.

Spores light brown to reddish brown in colour, 75-149µm in diam. Spore walls highly variable in thickness 3-17µm diam., perforated with thickened inward projections. Wall 1 smooth hyaline unit 0.2-1.0(-1.8) µm thick. Wall 2 pale yellow to pale brown, laminated, 0.8-14.3µm thick. Wall 3 hyaline membranous wall, 0.1-0.9µm thick. Subtending hyphae often pale in colour than the spore, flattened, straight or slightly constricted proximally, tapering to 1.5-2.0µm, thick distally.

Glomus geosporum Walker. *Mycotaxon* **15**: 49-61, 1982 **(Plate VII b)**.

Spores 110-290µm in diam., ellipsoidal, light to dark brown in colour. Spore walls 4-8µm thick, 3 layered with thin tightly adherent outer wall (<1 µm), yellow brown to red brown laminated middle wall (3-16µm), yellow-brown inner wall (<1µm) that appears membranous and forms a septum separating the spore contents from the subtending hypha. Spores with straight to recurved funnel shaped subtending hyphae of 10-24µm in diam. Spore contents of uniform droplets, granular in appearance with age separated by a septum that protrudes slightly into subtending hypha.

Glomus intraradices Schenck & Smith. *Mycologia* **74**: 77-92, 1982 **(Plate VII e)**.

Spores 93-131µm diam., brown in colour. Spore walls 3-15µm thick, yellow to gray brown, with 4 laminated walls, inner walls 1-2µm thick, darker than outer walls. Spore contents globular, yellow to light brown. Wall of the spore extending into the hyphal attachment forming an apparent tubaeform flare at the juncture with the hyphal attachment. Hyphal attachment 9-33µm wide with wall thickness of 1.5-5.2µm at the base of the spore. Hyphal attachment constricted 2-3µm at the base of the spore.

Glomus clarum Nicolson & Schenck *Mycologia* **71**: 178-198, 1979.

Spores hyaline 68-290µm in diam., composed of two wall layers. Spore contents hyaline consisting of globules of variable size, outer wall consists of 5-20µm thick, inner wall of 2-9µm thick. Subtending hyphae 15-80µm wide, becoming thinner with increasing distance from the spore.

Glomus aggregatum Schenck & Smith emend. Koske. *Mycologia* **77**: 619-630, 1985 **(Plate VIII a)**.

Sporocarps 200-1400µm diam., lacking a peridium of loosely aggregate spores. Spores formed in sporocarps, 20-210µm diam., pale-yellow to yellow-brown. Spore wall laminated, 1-3(-5) µm thick. Subtending hyphae straight, constricted, swollen or irregular upto 12µm wide at the spore base.

Glomus hyderabadensis Rani, Kunwar, Prasad & Manoharachary. *Mycotaxon* **89**: 245-253, 2004. **(Plate VIII f)**.

Sporocarps unknown, spores formed singly, globose to sub-globose 97 - 136 µm in diam., honey coloured to orange brown. Spore wall as three wall in single group.

Composite spore wall smooth or roughened, dull yellow, 1.1-2.4 µm thick, perforated with aperture of 1.1-1.5 µm diam., middle wall single, non-layered, rigid, orange brown, 1.1-1.27 µm, inner wall layer rigid, dull yellow, 1.1- 1.21 µm. Subtending hypha single slightly flared toward the point of attachment, 15-32 µm, 136 - 223 µm long, rarely branched, pore in subtending hypha occluded by thick straight septum.

Glomus maculosum Miller & Walker. *Mycotaxon* **25**: 217-227. 1986.

Spores 95-220 µm, light brown to dark brown. Spore wall of three walls (1-3) in two groups. Group A, outer thin hyaline, unit wall (wall1), 0.3-1.0µm thick, tightly adherent to wall 2. Wall 2, pale straw-coloured, laminated, 4-13µm thick with 4-16 laminae. The innermost lamina appearing as separate unit wall often forming a septum at the spore base. Inner wall group (Group B, wall 3) thin (<0.3µm) and tightly adherent to wall 2. Wall 3 bearing dome shaped scalloped ingrowths, 6-15 µm diam., consisting of 2-8 concentric bulging discs increasing towards the inside of the spore. Subtending hyphae straight to sharply recurved parallel sided or funnel shaped constricted at the spore base, 5-25µm wide proximally 5-7µm.

Glomus formosanum Wu & Chen. *Taiwania* **31**: 65-88, 1986.

Spores 65-117.5µm in diam., 1-4 branched attached hyphae. Spore wall 5-6µm thick, single layer, reddish brown, 5.5-12.5µm thick. Frequently two nearby hyphae closely separated at the attachment. Hyphae 7-17.5µm diam., with an opening at the attachment.

Glomus multicaule Gerdemann & Bakshi. *Trans. Br. Mycol. Soc.* **66**: 340-343, 1976 **(Plate VII c & d)**.

Spores dark brown, 149-162µm, ellipsoidal, occasionally triangular with 1-4 hyphal attachments generally occurring at opposite end of spores. Spore wall 8.6-34µm, thickest at the point of attachment with rounded projections of 1.2-3.7µm long distributed all over the spore surface.

Glomus mosseae Gerdemann & Trappe. *Mycologia Memoir* No, **5**: 6, 1974.

Spores yellow to brown, globose to sub-globose, 105-305µm with one or two funnel shaped bases 20-50µm diam., divided from subtending hyphae by a curved septum, hyaline 2-7µm thick irregularly branched septate hyphae of 2-12µm wide.

Glomus nanolumen Koske & Gemma. *Mycologia* **81**: 935-938, 1989.

Sporocarps forms in soil, sub-globose to iregular 90-520 µm diam., composed of 5-10 loosely to tightly packed spores and sporogenous hyphae. Spores sub-globose, pyriform, ovoid to irregular 30-135µm translucent yellow to reddish-yellow or rose pink. Spore wall structure consisting of two walls (1 & 2) in one group. Outermost wall is golden yellow to brown, 0.5 µm thick, wall-2 laminated, pale yellow to nearly hyaline, 4-11 µm thick.

Glomus rubiforme Gerdemann and Trappe. *Mycologia Memoir*, **5**: 76, 1974 **(Plate VIII b)**.

Sporocarps dark brown, 180-675µm diam., surrounding a central plexus of hyphae. Peridium absent, individual spores partially enclosed in a thin network of tightly appressed hyphae. Spores dark brown, obovoid to ellipsoid or sub-globose, 37-125µm with a small pore opening in to thick walled subtending hypha. Spore wall laminate,

3-7.6µm thick up to 13.5µm thick at the spore base, perforated projections appears on the inner surface-walled subtending hyphae.

Glomus taiwanense (Wu & Chen) Almeida & Schenck. *Kew Bulletin*, **50**: 306. 1995. **(Plate VIII c & d)**.

Sporocarps reddish brown, brown or dark brown, globose to sub-globose, 200–300 x 180–280 µm, with chlamydospores formed radially in a single, tightly packed layer around a central plexus of hyphae. Peridium absent, 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 (1 µm 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.

Entrophospora infrequens (Hall) Ames & Schinder. *Mycotaxon* **8**: 347-352, 1979 **(Plate VIII e)**.

Sporocarps unknown, spores produced in soil by expansion within smooth and unbranched hyphae, which terminate in a sub-globose to ellipsoid spores. 156 - 227µm diam., dull orange to brown, surrounded by hyaline wall vesicular stalk 2.5 – 10 µm. Spore content is enclosed by separable membrane.

Gigaspora albida Schenck & Smith. *Mycologia* **74**: 77-92, 1982. **(Plate IX f)**.

Spores dull white to light greenish yellow, spherical 143-350µm diam. Spore wall continuous 4-12µm thick with 1 to 6 walls. Outer wall smooth, 1-2µm thick with 4 to 5 inner laminated walls. Germ tube produced directly through the spore wall near the

bulbous suspensor separating it from the spore contents. Hyaline to yellow, bulbous suspensor, 24-36µm diam., attached to septate hyphae with fine hyphal branches.

Scutellospora biornata Spain, Sieverding & Toro. *Mycotaxon* **35**: 219–227, 1989. **(Plate IX c)**.

Spores yellowish brown in colour, globose, 282 – 415 µm diam. Spore wall structure of six walls 8–15 µm thick, in two groups. Group A 0.5-12µm thick with three walls. Outer wall, 0.5 –1µm diam. at base fused to wall 2. Wall 2 hyaline laminated, 8–10 µm thick, adherent to wall 3. Wall 3 membranous, 0.5–1 µm thick ornamented on inside with blunt projections of < 0.5–1 µm thick. Germination sheild brown, 113-275µm thick. Greatest pigment concentration around the germ tube, initial aperture, Y & U onfiguration and other fissures. Germtube initials (6-17), 6–7µm diam. separated from each other by long fissure.

Scutellospora calospora (Nicolson & Gerdemann) Walker & Sanders. *Mycotaxon* **27**: 219-235, 1986. **(Plate IX b)**.

Spores formed terminally on a bulbous suspensor like cell, hyaline to pale greenish yellow, globose, 114-511µm diam. Wall structure of four walls (walls1-4) in two groups. Group A consisting of an inner brittle, hyaline to pale yellow, laminated wall (wall 2) 3-5µm thick, surrounded by thin, hyaline unit wall (wall1), 0.5-1µm thick. Group B of two hyaline walls (wall 3 and 4). Wall 3, 0.5-1µm diam., wall 4, 1-1.5µm thick. Germination shield oval, 35-90µm diam. with invaginations along the margin. Suspensor cell 33-48µm, borne terminally on a septate subtending hypha, broad, concolorous with the spore base.

Scutellospora dipurpurascens Morton & Koske. *Mycologia* **80**: 520-524, 1988.

Spores formed singly in the soil, borne terminally on a bulbous suspensor cell, usually shiny smooth, yellow to greenish-yellow globose to sub-globose, occasionally ellipsoid, 197 - 240µm in diam., spore wall composed of four walls (1-4) in two groups (A & B). Group A consist of smooth finely laminate yellow outer wall (wall-1) up to 3-5 µm thick, and an inner hyaline membranous wall (wall-2) <1 µm thick. Wall - 2 often adhering to wall-1, difficult to discern in young spores, separating more readily in older spores after germination shield is formed. Group B with two walls which separate only after pressure is applied. Wall-3 is semi-rigid hyaline unit wall 1 - 1.5 µm thick. Wall - 4 is amorphous, thickness varying from 2- 28 µm becoming semi-rigid unit wall, 1 - 1.5 µm thick. Suspensor cell 7 - 10µm thick, wall concolorous with wall -1, 2 - 4 µm thick near point of attachment to spore, thinning distally to 1-2 µm, sometime bearing single peg like projection 3 - 4 µm wide occluded by thickening of wall 1. Germination shield ovoid 60 - 85 x 90 -140µm in diam., forming on wall -2.

Scutellospora gregaria (Schenck & Nicolson) Walker & Sanders. *Mycologia* **77**: 702-720, 1979. **(Plate IX d & e)**.

Spores reddish brown in colour, 250-448µm in diam. Spore wall composed of four walls (1-4) in two groups. Group A composed of three closely appressed walls, an outer wall (1) and two laminated walls (2 and 3). Wall 1 brown, 1-5µm including the closely packed warts on its outer surface. Warts are brown 1-2µm high with rounded tips, 2-7(-10) µm. Wall 2, yellow, 3µm thick. Wall 3 pale yellow, 5-13µm thick. Group B, hyaline, membranous. Wall (4) 1-2µm thick, enclosing the contents. Suspensor cell 2-4µm thick, pale brown, borne terminally on a septate hypha, 39-80

μm wide. Thick or thin walled hyphal projections arise from the suspensor cell towards the spore. Germination shield ovoid 116μm in diam. with many convolutions appearing as warts at the margins.

Scutellospora weresubiae Koske & Walker. *Mycotaxon*, **27**: 219-235, 1986. **(Plate IX a)**.

Spores found singly in the soil, terminally on a bulbous sporogenous cell. Spores translucent, glistening, pale pink, globose to sub-globose, (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 1 μm 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.

6.2.2: Inoculum preparation and sterilization process for *in vitro* germination - Germinating spores produced germ tubes that grew through the subtending hyphae. It was observed that *in vitro* germination in MSR medium without sucrose commenced 38 hours after inoculation with a higher germination rate (90%) whereas in MSR medium with sucrose germination was observed after 60 hours with a relatively lower germination rate (75%) **(Fig. 25)**.

The study revealed differences in spore germination rate. *Glomus intraradices* in MSR medium without sucrose recorded highest germination rate which was significantly greater than that in MSR medium with sucrose ($r = 90$; $P \leq 0.05$). Germ tube length was significantly greater ($r = 658.4$; $P \leq 0.05$) in MSR medium without sucrose **(Fig. 26)** Average width of germ tube was higher in MSR medium without sucrose ($r = 10.0$; $P \leq 0.05$). The straight hyphae with fewer branches were longer in comparison to hyphae in MSR medium with sucrose **(Table 20) (Plate X a - d)**.

6.2.3: Isolation, sterilization and inoculation of mature vesicles - In the present study, vesicle germination was observed after 36 h of inoculation with a 90% germination rate. Germinating vesicles produced germ tubes that grew through the subtending hyphae. Formation of appressoria and characteristic right-angled branching pattern of hyphae was observed in the present study **(Plate XI c & Plate XII a)**. Contact between fungus and transformed roots occurred on the fourth day after transferring the germinating vesicles in the MSR medium. After initial root colonization, extensive development of extra-matrical hyphae was observed. The mycelial growth pattern of *G. clarum* consisted of long non-septate hyphae growing on the medium. Sporulation was observed after six weeks and continued until roots

were harvested at senescence (10 weeks). New spores were seen extending from the sporulating hyphae and appeared mostly in terminal or intercalary position **(Plate XII b)**, or more often in clusters containing 1-3 spores **(Plate XII c - e)**. The new spores thus obtained were globose, hyaline to creamish in their juvenile stage, becoming yellowish-brown at maturity with numerous lipid inclusions. The average size of the spores at maturity ranged from 100 to 140 µm.

Discussion

Pot cultures technique of AM fungi is the most widely adopted technique for AM fungal inoculum production because relatively low technical support is needed and consumables are cheap. Since AM fungi are obligate symbionts, most experiments have been done by using pot culture inocula derived from surface-disinfected spores of a single AM fungal species on a host plant grown in a sterilized medium. Single spore culture isolates of AM fungi can be a valuable resource for plant growth experiments, taxonomic and biochemical studies.

In the present study four out of 28 species of AM fungal species were multiplied using pot cultures. Bever *et al*. (1996) reported 23 AM fungal species from a 75m^2 region of a mown field with sorghum trap cultures. In an arid ecosystem, Stutz and Morton (1996) recovered 15 more AM fungal species than those detected in the field after three cycles of trap cultures.

In the present study, it was observed that a specific AM fungus is associated with different host plants. Moreira *et al*. (2007) reported that the effect of a single AM fungal species could differ in two different host plants, as each host plant would selectively produce a differentiated spore composition. However, depending on the growth conditions *i.e.* host plant and environmental conditions, there may be

qualitative and quantitative change in the spore composition, which may not reflect the original composition in the field (Carrrenho *et al*. 2002). Brundrett *et al*. (1999) reported that AM fungal species frequently forming spores in the field soil were not detected in traps as conditions in the pots are less favourable for their sporulation. The differences in spore number obtained from trap cultures may be due to the variations in host plant root type and morphology, carbon biomass, nutrient and endogenous hormonal levels. These factors may influence the richness of AM fungi isolated from soil in trap cultures (Brundrett *et al*. 1999; Cuenca and Meneses 1996; Stutz and Morton 1996). Host plant and soil factors can influence both diversity and overall levels of P in soil and plant are able to inhibit mycorrhiza formation (Douds and Schenck 1990) and influence the diversity of AM fungi in field soil (Cuenca and Meneses 1996).

In the present study MSR medium devoid of sucrose recorded faster germination. De Souza and Berbara (1999) reported germination after 7 days in *G. clarum*. Dalpe *et al*. (2005) reported germination rate as low as 10-12% in *Glomus intraradices* in MSR medium with sucrose. Sucrose is known to reduce *in vitro* germination (Carr *et al*. 1985) and prolong formation of appresorium (Becard and Fortin 1988) which leads to lower germination in MSR medium with sucrose. Furthermore, AM fungal germination is known to depend on availability of spore reserves (Sancholle *et al*. 2001) and not on the nutrients present in substrate suggesting that AM fungal spore germination is affected by exogenous sucrose, as the germination rate was lesser in medium with sucrose than in without sucrose. Lower germination rate in MSR with sucrose might be also attributed to nutrients toxicity in the substrate (Clark 1997). Germ tube length was significantly greater in MSR medium without sucrose. Also average width of germ tube was higher in MSR

medium without sucrose. Ramos *et al*. (2008) also reported similar observation where hyphal length was significantly lower in presence of sucrose in the medium. Although sucrose is one of the nutrients exchanged during symbiotic phase (Smith and Read 1997), higher concentration of sucrose is known to produce inhibitory effect on hyphal growth resulting in decreased hyphal length. This phenomenon can be correlated to the lowest H^+ effluxes found in hyphae grown on complete medium containing sucrose. Indeed, under these conditions, the lowest rate of hyphal branching and growth was observed. This was in agreement of the findings of Mosse (1959) where a negative effect of sucrose on germination and hyphal growth of *Glomus mosseae* and *Gigaspora margarita* was observed. Siqueira *et al*. (1982) reported that range of organic substrates such as sucrose, fructose and pyruvic acid affected germination and germ tube growth in *Gigaspora albida*. Requena *et al.* (2003) using a molecular approach, analyzed the impact of Sucrose and P on the expression of two genes (*GmPMA1* and *GmHA5*) of the plasma membrane H^+-ATPase from *G. mosseae*. They found that *GmPMA1* was highly expressed during fungal pre-symbiotic development, whereas the *GmHA5* transcript was down-regulated by sucrose. The different methods and techniques used to date in *in vitro* germination studies involving AM fungi have usually been carried out by using MSR medium with sucrose. However, the present study, recommends the use of MSR medium minus sucrose to enable faster germination.

The present study suggests a method of using vesicles as inoculum in *in vitro* studies. Likewise in earlier studies involving *Glomus* species (Sturmer and Morton 1997), in the present study the germ tube emerges from the subtending hypha. The evidence presented here demonstrates that the *G. clarum* vesicles can be successfully used as additional propagules for *in vitro* studies. De Souza and Berbara (1999)

reported germination after seven days in *G. clarum* using spore as inoculums. However, in the present study, an early initiation of germination using vesicles as propagule was observed. Strullu and Romand (1986) suggested that vesicles act as a source of reserves, with higher inoculation potential than other AM propagules such as spores and hyphae. Being juvenile stage, the vesicle is rich in energy sources, nuclei and fewer wall laminations (Mosse 1988) thus resulting in more rapid germination than the spores.

Selection and efficiency of sterilization process is the key to success of axenic or monoxenic AM cultures. In the present study, we did not allow the inoculum (vesicles) to come in direct contact with soil, as the isolation was done by separating the vesicles from the root fragment thus minimizing the chances of contamination. It is observed that in earlier studies (Declerck *et al*. 1998; David and Douds 2002) the Ri-TDNA roots were placed in close proximity to germinating spores. However in our study germinated vesicles were transferred into Petri plates containing actively growing roots (15 days) to maximize germ tube growth due to specific compounds present in root exudates (Chabot *et al*. 1992). It is suggested these compounds may be important factors in the improvement of mass-inoculum production and therefore need to be tested in root-organ culture systems. Contact between fungus and transformed roots occurred on the fourth day after transferring the germinating vesicles in the MSR medium. After initial root colonization, extensive development of extra-matrical hyphae was observed. The mycelial growth pattern of *G. clarum* consisted of long non-septate hyphae growing on the medium. Strullu and Plenchette (1991) hypothesized that the growth-promoting substances derived from host roots are accumulated in intra-radical structures like vesicles which allows some degree of independent growth. The ability of vesicles to initiate the complete fungal life cycle *in*

vitro allows the long-term maintenance of single-isolate cultures, enhancing fungal biomass production. Chabot *et al*. (1992) and St-Arnaud *et al*. (1996) used intra-radical forms as source of inocula and reported sporulation after three months. However, in the present study involving vesicles as the source of inoculum, sporulation was observed after six weeks and continued until roots were harvested at senescence (10 weeks). Results in our study demonstrate that the ontogeny of *G. clarum* spores occurs along the sporogenic hypha as suggested previously for the genus *Glomus* (Morton 1990, Morton and Benny 1990). New spores were seen extending from the sporulating hyphae and appeared mostly in terminal or intercalary position. The intercalary formation of *Glomus* spores was also reported for *G. versiforme* in association with Ri T-DNA transformed roots (Bonfante and Bianciotto 1995; Declerck *et al.* 1996) and for *G. intraradices* in root organ culture (Bago *et al*. 1998). This suggest that patterns of spore formation (terminally and intercalary), may differ depending on AM species.

Considering the high production of vesicles in some *Acaulospora* species and their richness in energy sources and nuclei (Smith and Read 1996), these propagules could serve as an important inoculum to establish *in vitro* cultures in future studies. Our study confirms that the vesicles, isolated from roots or within root pieces readily germinated on the MSR medium, demonstrating their role as effective propagule. Furthermore they can be easily cultured and sub-cultured *in vitro* and thus aiding in the long-term maintenance of the species.

Numerous methods have been developed for decades for the large-scale production of AM fungi. It is tempting to extrapolate by saying that there are almost as many methods as there are laboratories working with AM fungi, since production is a pre-requisite to fundamental research as well as for application purpose. The sectors

of utilization widely vary from lab scale to large field, with production methods (and thus costs) and factors (*e.g.* host plant, AM fungi, substrate, nutrition) specifically custom-made. *In vitro* culture systems offer enormous potential for application in many fields of AM fungal research. In the near future, the coupling of powerful emerging research fields such as genomics and proteomics with cutting-edge technologies (*in vivo* microscopy, *in situ* molecular biology, four-dimensional determinations), with the increasing number of AM fungal isolates available *in vitro*, and with the unique and necessary inventive force of mycorrhizologists, will allow to translate basic research into a respectful, integrated and wide use of AM fungi. The different methods and techniques used to date in *in vitro* studies involving AM fungi have usually relied on spores as propagule. However, in the present study, the use of vesicles has been confirmed as an additional propagule for *in vitro* multiplication involving transformed roots. Furthermore, this technique can be exploited for the genus *Acaulospora* as to date there are fewer *in vitro* studies because of their poor germination and sporulation. Although the *in vitro* system is artificial it allows non-destructive, morphological and physiological investigations of the AM symbiosis.

Sporogenesis was observed which offers the possibility for future physiological and anatomical studies on the biogenesis of spores, and perhaps the potential for aseptic, large-scale production of inoculum. The success achieved by using this technique in cultivation of AM fungi *in vitro* is not only restricted to the study of the symbiotic interactions, but also permits the increase of knowledge in the morphology, taxonomy, phylogeny and biochemistry fields together with some aspects of their ecology. We are at the beginning of an era where the utilization of beneficial microbes among which AM fungi will take more and more importance. The continued development of high quality and low-cost inoculum methods can therefore

be expected, which could lead to more new and advanced methods for AM fungal large-scale inoculum production.

7.0: Introduction

Mangroves are woody plants inhabiting intertidal zones (Parida and Jha 2010) that tolerate wide range of salinity (Suarez *et al.* 1998). In tropical regions they are under intense pressure from development but are extremely important for sustainability of coastlines and coastal populations (Alongi 2002; Walters *et al.* 2008). Nutrient availability is an important driving variable influencing community structure in mangroves (Lovelock and Feller 2003). Most mangrove plant species that have been studied are found to be highly sensitive to variation in nutrients availability (Feller *et al.* 2003; Naidoo 2006; Lovelock *et al.* 2007). Enhancement in nutrient availability have mostly led to faster growth rates which are associated with an increase in allocation of leaf area relative to the roots, along with suite of physiological changes that include hydraulic conductivity and photosynthesis rates (Mckee 1996). The responses to enhancement in the levels of limiting nutrients are similar in mangroves as those observed in other species (Chapin 1980). However, in some settings where high salinity, extreme aridity, or shade limits growth, nutrient additions have not enhanced growth (McKee 1995). Many mangrove species have large propagules and the reserves contained within them support growth for an extended period of time (Tomlinson 1986). Ball (2002) observed that for seven species of the Rhizophoraceae, those with the largest propagule mass were greater after one year of growth compared to those species with smaller mass, although propagule mass did not influence survival. Lin and Sternberg (1995) reported similar importance of propagule size on intra-specific seedling vigour in *R. mangle*.

Arbuscular mycorrhizal (AM) fungi are important partners in natural plant communities (Karagiannidis and Nikolaou 1999). Mycorrhizal colonization of roots results in increased root surface area for nutrient acquisition. The extra-metrical

fungal hyphae can extend several centimeters into the soil and absorb large amounts of nutrients for the host root (Khan *et al.* 2000). While the effects of AMF on plant physiology (Auge 2001), soil stability and nutrient cycling (Rillig and Mummey 2006) in terrestrial environments are well known, their importance in mangroves has received little attention. Mangroves have been observed to have AM fungal associations at low salinity (<25 ppt) (Sengupta and Chaudhuri 2002). However, the effects of AM fungi on mangrove seedling growth are poorly understood and growth benefits of these associations are yet to be determined. The seedling stage is the most sensitive stage in mangrove (Yan *et al.* 2007) and responses to flooding at the seedling stage are considered one of the most important determinants of species composition in mangroves (Bedinger 1978). Consequently, several questions remain regarding the role of AM fungi in promoting seedling growth and the conditions required for AM colonization in mangroves.

Ceriops tagal is the true mangrove species with a distinct stem that grows up to 20 m in height, short basal buttresses, and pneumatophores that sometime develop as looped surface roots (Tomlinson 1986). The general perception among researchers is that the AM fungi are obligate aerobes and therefore their ability to form successful symbiotic associations with wetland and aquatic plants would be limited. Previous studies in mangrove growth response to mycorrhizal inoculation include an increase in root/shoot mass ratio (Komiyama *et al.* 2000) and response to water-logging (Ye *et al.* 2003). However, studies regarding the functional role of AM fungi on growth of mangrove plants species and their ecological significance are scarce. Azcȯn-Aguilar and Barea (1997) have reported that application of AM fungal species as bio-fertilizers in the initial stage play an important role in growth and establishment of different type of plant species during transplantation in field conditions. If mangrove

nursery techniques are to be successfully applied it is essential to know which AM fungal species are most favoured by the host mangrove plant species. A better understanding of mechanisms pertaining to action, nutrition and ecology of AM might help us in exploiting the benefits of AM fungi and thus aid in future reforestation programme of mangroves. The objective of the present study was to determine the role of dominant and native AM fungal species in promoting growth and establishment of *Ceriops tagal*.

7.2: Materials and Methods

Ceriops tagal (Perr.) C.B. Robinson a mangrove plant species belonging to family Rhizophoraceae and three dominant native AM fungal species *viz.*, *Glomus intraradices*, *Glomus clarum* and *Acaulospora laevis* were selected for the study.

7.2.1: Collection of planting material (pods) and transplanting - Uniform sized pods (15cm) of *C. tagal* were collected, surface sterilized with 0.2% Sodium hypochloride and then rinsed several times in sterile deionized water and sown in pots (one pod pot^{-1}) containing sand.

7.2.2: AM fungal inoculum preparation - Three AM fungal fungal species *viz.*, *Glomus intraradices*, *Glomus clarum* and *Acaulospora laevis* were used for inoculation in the present study. Pure cultures of all the three AM fungal species used in the present study were prepared using *Solenostemon scutellaroids* (L.) Codd. as host. Inoculum consisted of spores and colonized root fragments. Inoculum (10g) consisting of spores and colonized root fragments was added per pot. The pots were flooded with water from mangroves site. The experiment was conducted in a

glasshouse, day/night temperatures of 32/25°C and relative humidity 65-95%. Besides the three AM treatments, uninoculated sterilized and unsterilized controls were also maintained.

7.2.3: Experimental design, measurement and data analysis - Completely randomized block design with 10 replicates was employed for the five treatments *viz*., three AM fungal isolates and two controls comprising of uninoculated sterilized and unsterilized sand as given in **(Table 21)**. Five of the ten replicates were used for biomass (root and shoot) study, and the remaining were used to study the colonization levels.

7.2.4: Root colonization of AM fungi - For processing of root samples, Phillips and Hayman (1970) staining technique was employed. Estimation of root colonization of AM fungi was carried out using the Slide method (Giovannetti and Mosse 1980). Total root colonization by AM fungi was calculated using the following formula.

Root colonization (%) = Number of mycorrhizal root segments/ Total number of root segments x 100

Mycorrhizal Growth Responsiveness (MGR) was calculated by using the formula of Hetrick *et al.* (1992) as given below

MGR (%) = AM - NM /NM x 100

AM = biomass of mycorrhizal plants; NM= biomass of non-mycorrhizal plants

7.2.5: Statistical analysis - The data were statistically analyzed by using WASP 1.0 (Web Based Agricultural package: www.icar.goa.res.in/wasp/). ANOVA was used to test the significant differences among the treatments and their interactions for all

responsive growth parameters. To determine differences in effects among the AM fungal assemblages, a separate statistical analysis was performed that excluded the non-mycorrhizal controls. For analysis, difference was considered significant when $P \leq 0.05$.

7.3: Results

7.3.1: AM Colonization in roots - Except for sterilized control (SC), mycorrhizal colonization was recorded in all the other treatments. Hyphal, arbuscular and vesicular colonization was observed. Maximum colonization was recorded in plants inoculated with *G. clarum* (47%) followed by *G. intraradices* (39%) and least in *A. laevis* (38%) **(Fig. 27).** Variation in AM colonization was observed among the treatments. *Glomus clarum* inoculated plants showed significant variation in AM colonization compared to the other two AM treatments. In the present study, plants inoculated with *G. clarum* recorded maximum colonization (47%) while plants growing in unsterile control (UC) recorded least colonization (8%).

7.3.2: AM Efficiency - AM inoculated plants showed increase in growth compared to both un-inoculated (sterilized and unsterilized) controls. The study revealed that *G. clarum* was the most efficient AM species exhibiting maximum influence on growth and biomass followed by *G. intraradices* and *A. laevis*. Inoculation with *G. clarum* significantly increased plant height, leaf area and number of leaves compared to other AM treatments. Similarly fresh and dry weight biomass in *G. clarum* inoculated plants exhibited significant increase compared to other AM treatments **(Table 22)**. Mycorrhizal plant responsiveness (MGR) was greater in AM inoculated plants compared to both controls. Among AM fungal treatments, *G. clarum* inoculated

plants recorded significant increased in mycorrhizal growth response compared to other two AM treatments **(Fig. 28) (Plate XIII a & b)**. In the present study, a distinct relationship between extent of colonization and mycorrhizal efficiency was observed.

7.4: Discussion

The present study revealed a distinct variation in AM colonization among the different treatments. This suggests that not all combinations of host and AM fungal species have similar growth effects. Such observations were recorded earlier by Janos (1980) suggest a functional host preference (Bever 2002). The presence of arbuscules known as site for nutrient exchange between host plant and the fungus in mangroves probably indicates the existence of active AM symbiosis. At the end of 24 weeks, AM fungal inoculation significantly increased plant height and biomass. White and Charvat (1999) however recorded non-significant increase in growth and biomass in the wetland plant species *Lythrum salicaria*. Lack of growth benefit in plants has been attributed to factors such as the carbohydrate cost of supporting the fungus (Son and Smith 1988). Sanders and Fitter (1992) suggested that mycorrhizal benefit occur only during certain stages of the plant life cycle which might be other factor resulting in lack of growth benefits in AM inoculated plants.

Plants growing in unsterilized sand (UC) recorded least colonization compared to AM inoculated plants. This may be due to the presence of less efficient AM fungal isolates in unsterile sand. Furthermore, the effect of mycoparasites in unsterile sand limit AM colonization and therefore affects the plant growth (Linderman 1992).

Growth efficiency in *G. clarum* inoculated seedling of *C. tagal* was maximum followed by seedlings inoculated with *G. intraradices* and *A. laevis*. These inter-specific variations in growth promoting abilities of AM fungal species observed may

be attributed to mechanism of mycorrhizal development (Hart and Reader 2002), physiological difference in rate of nutrient uptake, translocation and release (George 2000). Colonization with *G. clarum* resulted in significant increase in dry weight of *C. tagal* plants. This could be attributed to longer and more-branched root system leading to exploration of larger soil volumes for water and nutrients.

An increased root length was recorded in seedlings inoculated with AM fungi. This is in accordance with earlier studies (Berta *et al.* 1995; Fidelibus *et al.* 2001) who reported increase in root growth in AM inoculated plants. Arbuscular Mycorrhizal fungi are known to affect root plasticity, but the exact mechanism is not clear (Berta *et al.* 1995). Berta *et al.* (2002) suggested enhancement of mineral nutrition, hormone synthesis, hyper polarization of cortical cell membrane potential and proton extrusion possibly play a role in root plasticity. Total root surface area and volume, number of tips and degree of root branching was maximum in *G. clarum* inoculated plants. Allen *et al.* (1981) reported that mycorrhizal roots have greater surface absorbing area because of greater root length and increase branching. Such changes in root morphology are known to change hydraulic conductivity and water flows rates (Fidelibus *et al.* 2001). Longer and more-branched root systems could be considered more efficient both in soil exploration and in nutrient uptake and transport, favouring the successful establishment of many plant species. This suggests the use of AM fungi can benefit host plants by contributing to plant growth promotion and by positively affecting root system architecture.

Effect of *G. clarum* on dry weight was more pronounced in aerial biomass (shoot) than root biomass. This may be attributed to the proportionally greater allocation of carbohydrates to the shoot than root tissues caused due to AM colonization (Shokri and Maadi 2009). Larger leaf area in *G. clarum* inoculated plants

was observed in present study. This might be due to fact that larger leaf area is known to support sufficient amount of photosynthate to the symbiont (Feng *et al*. 2002). Similar observation was reported earlier by Krishna *et al.* (1995) who reported noticeable anatomical modification like increase in leaf thickness, size of midrib vein, mesophyll cells and number of plastid in the leaves following mycorrhizal colonization.

The present study reveals a definite relationship between extent of root colonization and mycorrhizal efficiency in *C. tagal*. Menge (1983) observed that rapid AM fungal colonization is an essential criterion for good host growth response. This was confirmed by Miranda *et al*. (2011), who reported AM fungi which are more effective in increasing plant growth, colonize the plant more rapidly and extensively.

The present study suggests that AM fungal colonization provides nutritional benefit in *C. tagal*. Differences in growth parameters between AM treatments and both controls (UC and C) may be linked to the presence and function of mycorrhiza. The beneficial activity of AM fungi is most likely to be favoured due to level of colonization. Therefore, further studies on mangrove-AM fungal symbiosis under varying salinity and water level along with different type of stress are needed to elucidate the extent of nutritional benefits and to examine the influence of environmental conditions and habitat type on the association. Furthermore, large-scale methods for direct inoculation with AM have not yet been devised, but in small trials such as in seedling stages have proved effective. The potential for employing AM fungi on a wide scale in afforestation programme of degraded mangrove areas is dependent on mass multiplication of superior, dominating and growth-promoting AM fungal species isolated from mangrove habitat. Results from the present study revealed the differences in the symbiotic physiology of different host-endophyte

associations. Therefore selection of more adapted AM fungal species for introduction into mangrove environments is needed in maintaining and restoring the plant-soil equilibrium in natural ecosystem.

Mangroves are projected as powerful bioshields, in coastal ecosystem engineering and offers protection against geo- and eco-hazards in the age of global warming climate change and impending sea level rise. A few studies have demonstrated the association of AM fungi with mangrove plant species but the efficiency of AM fungi in promoting growth of mangrove plant species has received little attention. The present study was undertaken to evaluate the AM fungal diversity in mangrove plant species, to study AM dynamics in response to mangrove plant phenology, development of techniques for mass inoculum production and to test the efficiency of AM fungi in promoting growth of mangrove plant species. The work carried out in the present study can be summarized as follows:

A survey of AM fungal status in mangrove plant species of Goa was undertaken. Studies on soil characteristics at the study sites revealed differences in soil properties. Soil pH was acidic nature and was deficient in available P at all the sites. The micronutrients also exhibited variation at all the sites.

Mycorrhizal colonization was recorded in 16 out of 17 mangrove plant species selected for the study. The AM colonization was characterized by arbuscules and/or vesicles and intra-radical hyphae. Both *Arum*- and *Paris*-type of morphologies were observed. *Paris*-type was more dominant, and was observed in 74% of the plant species. Higher root colonization levels were recorded during pre-monsoon and least in post-monsoon season.

Studies on AM fungal diversity revealed 28 AM fungal species belonging to five genera *viz.*, *Glomus, Acaulospora, Gigaspora, Scutellospora* and *Entrophospora. Glomus* was most dominant genus and *G. intraradices* was the most dominant species. Three sporocarpic forms *i.e. G. rubiforme, G.aggregatum* and *G. taiwanense* were encountered. No significant correlation was observed between percent root

colonization and spore density. Maximum species richness was recorded in Zuari site where 16 AM fungal species were recovered. Diversity indices showed less variation indicating stable AM fungal community. In seasonal studies higher spore density was recorded during monsoon and least in post-monsoon season.

Studies on variation in AM colonization in different growth stages in selected mangrove plant species revealed higher AM colonization in flowering stage and least in vegetative stage. The study indicates that the spore density is mainly influenced by growth stage and showed variation. Mean spore density decreased from vegetative to flowering stage and recorded increase in the fruiting stage. Fifteen AM fungal species belonging to four genera *viz., Glomus*, *Acaulospora*, *Scutellospora*, and *Gigaspora* were recovered from the rhizosphere soils. *Acaulospora* (6) and *Glomus* (6) were dominant genera followed by *Scutellospora* (2) and *Gigaspora* (1) with species number given in parenthesis. *Acaulospora scrobiculata* was dominant species and recorded most frequently in all the growth stages. Maximum species richness was recorded in the fruiting stage with 14 AM species, whereas in the vegetative and flowering stages 8 AM fungal species each were recorded.

Four out of 28 species were successfully multiplied using pot culture with *Solenostemon scutellarioides* (L.) Codd as host plant include *G. intraradices, G. clarum, A. scrobiculata* and *A. laevis* which were further used for preparation of monospecific cultures by using *in vivo* and *in vitro* studies.

In present study effect of sucrose in MSR medium on spore germination and germ tube growth of *Glomus intraradices* was investigated. Germination in MSR medium without sucrose was earlier and occurred within 38 h after inoculation, with highest germination rate (90%). Similarly, hyphal length and width was significantly greater in spores grown on MSR medium devoid of sucrose. The study revealed that

MSR medium without sucrose initiated early germination and hence strongly recommended for *in vitro* germination of AM fungi.

In vitro sporulation was successfully recorded in *G. intraradices* and *G. clarum* using intraradical spores and vesicles as inocula. The study reported the establishment of *in vitro* culture of *Glomus clarum* and *G. intraradices* by using mature vesicles grown monoxenically with Ri T-DNA transformed *Cichorium intybus* L. (chicory) roots. Upon inoculation, 90% germination was recorded in vesicles after 36 h in MSR medium. Sporulation was observed after five weeks of inoculation. The study confirms that isolated vesicles and intraradical forms constitute an excellent source of inocula for successful *in vitro* culture. AM fungal *in vitro* sporulation was initiated after a period of five weeks. This technique can be exploited for the genus *Acaulospora* as to date only one species *viz., A. rehmii* (4) has been successfully cultured using transformed roots due to their poor germination and sporulation ability. Using vesicles as source of inoculum has obvious advantages over traditional systems involving pot cultures and permits production of pure, viable and contamination free inocula.

Studies on the effect of different AM fungal species on growth biomass in *Ceriops tagal* revealed significant increase in growth in all three AM treatments (*G. intraradices, G. clarum* and *A. laevis*) compared to both the controls. Growth efficiency in *G. clarum* inoculated seedling of *C. tagal* significantly increased all growth parameters followed by *G. intraradices* and *A. laevis*. Total root biomass was maximum in *G. clarum* inoculated plants. These results indicate a definite relationship between extent of colonization and mycorrhizal efficiency in host growth. The study showed that AM fungi have the potential to provide nutritional benefits to selected mangrove plant species. Differences in growth parameters between AM fungal

treatments and both controls (unsterilized and sterilized control) are reasonably linked to the presence and function of mycorrhiza. The beneficial activity of AM fungi is most likely to be favoured due to level of colonization. Furthermore, large-scale methods for direct inoculation with AM fungi have not yet been devised, but in small trials such as in seedling stages have proved effective. The potential for employing AM fungi on a wide scale in afforestation programmes of degraded mangrove areas is dependent on mass multiplication of superior, dominating and growth-promoting AM fungal species isolated from mangrove forest. Results from the present study revealed the differences in the symbiotic physiology of different host-endophyte associations. Therefore selection of more adapted AM fungal species for introduction into mangrove environments is needed in maintain and restore the plant-soil equilibrium in natural ecosystem.

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Introduction

Mangroves are facultative halophytes, characterized by regular tidal inundation and fluctuating salinity (Gopal and Chauhan 2006). Mangrove plant species are highly adapted to coastal environment and thrive in intertidal zones of tropical and sub-tropical regions (Ball 1996; Naidoo *et al.* 2002). They exhibit exposed breathing roots, extensive support roots and buttresses, salt-excreting leaves and viviparous water dispersed propagules. These adaptations vary among taxa and with physico-chemical variations of habitat (Duke 1990). Distribution is governed by topography, tidal height, substratum and salinity. The species display extreme variations in plant composition, forest structure and growth rate. Mangrove forests can vary from a narrow fringe along the banks of an estuary to dense stands covering many square kilometres. Total mangrove area in India is 6740 km^2 and 80% of that are found along the east coast, 20% on the west coast. Deltaic environments on India`s east coast support extensive mangrove forest formations due to intertidal slope and heavy impact of siltation. The western coastline has narrow intertidal belts which support only fringe mangroves (MOEF 1994). All the estuaries in Goa are classified as micro-tidal estuary as tidal level is below two meters (Ahmad 1972). Mangroves have become the centre of many conservation and environmental issues because of loss of beneficial effects on the coastal environment. Anthropogenic pressure is constantly increasing and immediate protection and conservation of the ecosystem is necessary. Reforestation of mangrove is a promising solution to restoration. Mangroves are known to protect environment from the harmful effects of strong cyclone and other natural calamities (Badola and Hussain 2005; Danielsen *et al*. 2005).

Ecological functions attributable to arbuscular mycorrhizal (AM) fungi include helping to increase plant tolerance of adverse soil conditions, influencing response to severe climatic conditions and increasing plant productivity in natural plant communities (Brundrett and Kendrick 1996). Arbuscular mycorrhizal fungi enhanced availability of nutrients is described as a primary factor affecting abundance and composition of plant species communities (Klironomos 2003). The major nutrients, phosphorus (P) and nitrogen (N) are deficient in mangrove ecosystems (Carr and Chambers 1998) and likely to limit the growth of mangrove plant species.

Microorganisms such as phosphate solubilizers, N fixers and AM fungi are known to interact in the rhizosphere soils and can solubilise the bound P into available form. Arbuscular mycorrhizal fungal hyphae aid in transport of nutrients by extending beyond the depletion zone (Ciu and Cladwell 1996). These fungi play significant role in physiological processes such as water use efficiency (Ruiz-Lozano *et al.* 1996) modify the structure and function of plant communities and are useful indicators of ecosystem change (Miller and Bever 1999). Burke *et al.* (2003) demonstrated that inoculation with AM fungi improves growth of plants under salinity stress. Previous studies have shown that these fungi are either absent (Mohankumar and Mahadevan 1986), rare (Kothamasi *et al.* 2006) or ubiquitous (Sengupta and Chaudhuri 2002; Kumar and Ghose 2008) in mangrove ecosystem. In India, most studies on mangroves and AM fungi are carried out along the east coast while studies on the west coast are scarce. Besides, any attempts for afforestation, established AM fungal plant species may serve as important sources of inocula for initially non-mycorrhizal conspecifics, which may affect their regeneration and to contribute patchy distribution of species within community (Koide *et al.* 2000). Hence, it is important to study the diversity of AM fungal species and identify their potential in native plant species to be used for

afforestation of mangrove habitats. The aim of the present study was to determine the AM fungal diversity in selected mangrove sites from Goa and to identify the dominant AM fungal species found therein.

Aims and Objectives

- ✓ To study AM fungal root colonization in mangrove plant species of Goa.
- ✓ To isolate and identify spores of AM fungi from the rhizosphere soils of mangrove of Goa.
- ✓ To assess the AM fungal spore density in the rhizosphere soils of mangrove of Goa.
- ✓ To study the mycorrhizal status of selected plant species as influenced by phenology.
- ✓ To produce monospecific cultures of dominant AM fungal species and their mass multiplication.
- ✓ To evaluate the effect of dominant AM fungal species on growth of selected mangrove plant species.

Methodology

1. Root and rhizosphere soil samples of selected mangrove plant species were collected from the seven major sites from Goa.
2. Association of AM fungal colonization was carried out in root of selected plant species by trypan blue staining method (Koske and Gemma 1989).
3. Quantification of AM fungal colonization in roots was carried out using Slide method (Giovannnetti and Mosse 1980).
4. Arbuscular mycorrhizal fungal spores were isolated by wet sieving and decanting technique (Gerdemann and Nicolson 1963) and quantification of spore density was carried as described by Gaur and Adholea (1994).

5. Trap and monospecific cultures of isolated AM fungal species were prepared by using open pot culture method (Gilmore 1968) using *Solenostemon scutellarioides* (L.) Codd as trap (host) plant.
6. Taxonomic identification of intact and unparasitized spores of AM fungi was carried out by using various bibliographies (Schenck and Perez 1990; Rodrigues and Muthukumar 2009) and INVAM (International culture collection of vesicular arbuscular fungi (http.invam.cafu.edu).
7. Diversity studies were carried out using Shanon-Weiner index (Shannon and Weaver 1949) and Simpson`s index (Simpson 1949).
8. To study effect of sucrose on *in vitro* germination of *Glomus intraradices*, MSR medium with and without sucrose was used as substrate.
9. To study *in vitro* sporulation, vesicles of *Glomus clarum* were used as propagules and Ri T-DNA transformed *Cichorium intybus* L. (chicory) roots as host.
10. Mycorrhizal growth response (MGR) was calculated by using formula of Hetrick *et al*. (1992).

Observations

The first chapter deals with status of AM fungal colonization and diversity in the selected mangrove sites from Goa. A survey of AM fungal status indicated that 16 out of 17 mangrove plant species selected for the study showed AM colonization. The AM colonization was characterized by presence of arbuscules and/or vesicles and intra-radical hyphae. Both *Arum*- and *Paris*-type of morphologies were observed, the latter being dominant was observed in 74% of the plant species. Studies on AM fungal diversity revealed 28 AM fungal species belonging to five genera *viz*., *Glomus, Acaulospora, Gigaspora, Scutellospora* and *Entrophospora. Glomus* was most

dominant genus and *G. intraradices* being the dominant species. In the present study three sporocarpic forms *i.e. G. rubiforme, G. taiwanense* and *G. aggregatum* were recorded. No significant correlation was observed between percent root colonization and spore density. Spore density varied from 8 spores (*Avicennia marina*) to 324 spores (*Acanthus ilicifolius*) $100g^{-1}$ of soil. Maximum species richness was recorded in Zuari site where 16 AM fungal species were recovered. In seasonal studies, higher spore density was recorded during monsoon and least in post-monsoon. Mangrove plant species exhibited higher root colonization during pre-monsoon and least in post-monsoon season in both the study sites. Diversity indices showed less variation indicating a stable AM fungal community.

The second chapter deals with AM fungal status of mangrove plant species as influenced by its phenology. Studies on variation in AM colonization of different growth stages in selected mangrove plant species revealed higher AM colonization in flowering stage and least in vegetative stage. The study indicated that the spore density is mainly influenced by growth stage and showed variation. Mean spore density decreased from vegetative to flowering stage and recorded increase in the fruiting stage. Fifteen AM fungal species belonging to four genera *viz., Glomus*, *Acaulospora*, *Scutellospora* and *Gigaspora* were recovered from the rhizosphere soils. *Acaulospora* (6) and *Glomus* (6) were dominant genera followed by *Scutellospora* (2) and *Gigaspora* (1) with species number given in parenthesis. Two sporocarpic forms *i.e. G. taiwanense* and *G. aggregatum* were recovered. *Acaulospora scrobiculata* was the dominant species and recorded most frequently in all the growth stages. It was common species despite differences in the plant composition. Maximum species richness was recorded in the fruiting stage with 14

AM species, whereas in the vegetative and flowering stages 8 AM fungal species each were recorded.

The third chapter deals with taxonomy and mass multiplication of dominant AM fungal species using substrate based (trap and monospecific cultures) and *in vitro* culturing. The study revealed that *Glomus* was most dominant genus and *G. intraradices*, *A. laevis* and *A. scrobiculta* were dominant AM fungal species. Trap cultures of dominant AM fungal species were prepared by using *S. scutellarioides* (L.) Codd as host plant. From a total of 174 trap cultures, 28 AM fungal species were recovered, of which four monospecific cultures *viz.*, *G. intraradices, G. clarum, A. scrobiculata* and *A. laevis* were recovered which were later used for *in vitro* studies.

To study *in vitro* spore germination and germ tube growth of *G. intraradices* MSR medium with and without sucrose was used. Germination in MSR medium without sucrose commenced earlier and occurred within 38 h after inoculation, with highest germination rate (90%). Similarly, hyphal length and width was significantly greater in spores grown on MSR medium devoid of sucrose. The study revealed that MSR medium without sucrose initiated early germination and hence is strongly recommended for *in vitro* germination of AM fungi. *In vitro* sporulation was successfully recorded in *G. intraradices* and *G. clarum* using intraradical spores and vesicles as inocula. The study reported the establishment of *in vitro* culture of *G. clarum* and *G. intraradices* by using mature vesicles grown monoxenically with Ri T-DNA transformed *Cichorium intybus* L. (chicory) roots. Upon inoculation, 90% germination was recorded in vesicles after 36 h in MSR medium. Sporulation was observed after five weeks of inoculation. The study confirms that isolated vesicles constitute an excellent source of inoculum for *in vitro* culture system. This technique can be exploited for the genus *Acaulospora* as to date only one species *viz., A. rehmii*

has been successfully cultured using transformed roots due to their poor germination and sporulation ability. The use of vesicles as a source of inoculum has obvious advantages over traditional systems involving pot cultures and permits production of pure, viable and contamination free inocula.

The fourth chapter deals with the role of dominant AM fungal species on growth and biomass in *Ceriops tagal* (Perr.) C.B. Rob. Studies on the effect of different AM fungal species on growth and biomass in *C. tagal* revealed a significant increase in all three AM treatments (*G. intraradices, G. clarum and A. laevis*) compared to both the controls. Growth efficiency in *G. clarum* inoculated seedling of *C. tagal* significantly increased all growth parameters followed by *G. intraradices* and *A. laevis*. Total root biomass was maximum in *G. clarum* inoculated plants. These results indicate a definite relationship between extent of colonization and mycorrhizal efficiency in host growth. The study showed that AM fungi have the potential to provide nutritional benefit to mangrove plant species. Differences in growth parameters between AM fungal treatments and both controls (unsterilized and sterilized control) are reasonably linked to the presence and function of AM fungal species. The beneficial activity of AM fungi is most likely to be favoured due to level of colonization. Furthermore, large-scale methods for direct inoculation with AM have not yet been devised, but in small trials such as in seedling stages have proved effective. The potential for employing AM fungi on a wide scale in afforestation programmes of degraded mangrove areas is dependent on mass multiplication of superior, dominating and growth-promoting AM fungal species isolated from mangrove forest. Results from the present study revealed the differences in the symbiotic physiology of different host-endophyte associations. Therefore selection of

more adapted AM fungal species for introduction into mangrove environments is needed to maintain and restore the plant-soil equilibrium in natural ecosystem.

Conclusion

A survey of AM fungal status indicated that 16 out of 17 mangrove plants species were mycorrhizal. Both *Arum*- and *Paris*-type of morphologies were observed, the latter being dominant was observed in 74% of the plant species. The study revealed stable AM fungal community in mangrove plant species of Goa. A total of 28 AM fungal species belonging to five genera was recorded. *Glomus* was most dominant genus and *G. intraradices* being the dominant species. Arbuscular mycorrhizal colonization and spore density were mainly influenced by various growth stages. Colonization and mean spore density recorded variation depending on growth stages of selected mangrove plant species, showing maximum in flowering and fruiting stage respectively. Four monospecific cultures *viz., G. intraradices, G. clarum, A. scrobiculata* and *A. laevis* were recovered which were used for *in vitro* studies. *In vitro* germination in MSR medium without sucrose commenced earlier and occurred within 38 h after inoculation, with highest germination rate (90%). *In vitro* sporulation was observed after five weeks in *G. clarum* using vesicles as inoculum. This confirms that isolated vesicles constitute an excellent source of inoculum for successful *in vitro* culture. This technique can be exploited for the genus *Acaulospora* to their poor germination and sporulation ability.

Studies on the effect of different AM fungal species on growth and biomass in *C. tagal* revealed significant increase in all the three AM treatments (*G. intraradices, G. clarum and A. laevis*) compared to both the controls. Maximum growth efficiency was recorded in *G. clarum* inoculated seedling, increasing all the growth parameters.

Definite relationship between extent of colonization and mycorrhizal efficiency in host plants growth (*Ceriops tagal*) was recorded. The study showed that AM fungi have the potential to provide nutritional benefit to selected mangrove plant species (*Ceriops tagal*).

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Signature of Student **Signature of Guide**

Table 2: Geographic location and physico-chemical analysis of the study sites.

Sites	pH	EC (dSm^{-1})	OC (%)	P (g/Kg)	K (g/Kg)	N (g/Kg)	Zn (g/Kg)	Mn (g/Kg)	Cu (g/Kg)	Fe (g/Kg)
Terekhol (28 Km) 15° 72’ 28’’N & 73° 72’ 99’’ E	6.7	3.30	5.21	0.13	7.34	0.61	0.011	0.043	0.052	0.143
Chapora (31 Km) 15o 63’ 98’’N & 73° 73’ 61’’ E	6.0	3.86	0.92	0.44	67.43	0.69	0.014	0.038	0.062	0.234
Mandovi (81 Km) 15° 48’ 64’’N & 73° 86’ 52’’ E	5.5	4.59	1.30	0.24	62.72	0.29	0.022	0.011	0.042	0.425
Zuari (67 Km) 15° 32’ 56’’N & 73° 89’ 71’’ E	5.9	8.49	2.0	traces	85.12	0.81	0.016	0.032	0.040	0.563
Sal (35 Km) 15° 15’ 52’’N & 73° 95’ 30’’E	6.0	5.25	1.80	0.40	56.04	0.62	0.013	0.015	0.022	0.192
Talpona (9 Km) 14° 98’ 75’’N & 74° 06’ 15’’ E	6.4	2.40	4.20	0.41	65.37	1.21	0.025	0.044	0.037	0.242
Galgibagh (16 Km) 14° 95’ 83’’N & 74° 04’ 95’’ E	6.4	2.19	3.35	0.32	64.99	0.21	0.032	0.072	0.015	0.283

Legend: Data presented is mean of three readings (n=3) Km represent length of each river in Kilometres.

Table 3: Mangrove plant species distribution and root colonization from all the study sites.

Mangrove plant species	Family	Habit	Type of Colonization (%)	Root colonization (%)
I- Terekhol				
Avicennia marina (Frosk.) Vierh	Acanthaceae	TM	H, V	6.21 ± 0.35
Acanthus ilicifolius L.	Acanthaceae	TM	H, V, A	60.23 ± 3.23
Rhizophora apiculata Blume.	Rhizophoraceae	TM	H, V	13.18 ± 1.10
Ceriops tagal (Perr.) C.B. Robinson	Rhizophoraceae	TM	H, V, A	32.42 ± 2.34
Excoecaria agallocha L.	Euphorbiaceae	TM	H, V, A	46.04 ± 2.78
				31.41
II- Chapora				
Rhizophora mucronata Poir.	Rhizophoraceae	TM	H, V, A	12.32 ± 1.26
Acanthus ilicifolius L.	Acanthaceae	TM	H, V, A	42.21 ± 3.42
Avicennia officinalis L	Acanthaceae	TM	H, V, A	21.28 ± 1.12
Avicennia marina (Frosk.) Vierh	Acanthaceae	TM	H, V	6.02 ± 0.56
Excoecaria agallocha L.	Euphorbiaceae	TM	H, V, A	58.09 ± 4.24
Aegiceras corniculatum (L) Blanco	Myrsinaceae	TM	H, V, A	12.14 ± 1.04
Salvadora persica L.	Salvadoraceae	MA	-	-
				25.1
III- Mandovi				
Sonneratia alba (L.) Smith.	Sonneratiaceae	TM	H, V, A	23.45 ± 2.45
Sonneratia caseolaris (L.) Engler	Sonneratiaceae	TM	H, V, A	38.45 ± 4.23
Acanthus ilicifolius L.	Acanthaceae	TM	H, V, A	69.48 ± 6.25
Avicennia officinalis L	Acanthaceae	TM	H, V, A	12.36 ± 0.89
Acrostichum aureum L.	Ceratopteridaceae	MA	H, V, A	72.23 ± 5.28
Derris heterophylla Willd.	Fabaceae	MA	H, V	14.86 ± 1.24
Kandelia candel (L.) Druce.	Rhizophoraceae	TM	H, V	15.52 ± 1.42
Rhizophora apiculata Blume.	Rhizophoraceae	TM	H, V	28.42 ± 3.78
Rhizophora mucronata Poir.	Rhizophoraceae	TM	H, V, A	41.26 ± 3.89
Sonneratia alba (L.) Smith.	Sonneratiaceae	TM	H, V, A	23.14 ± 3.12

Excoecaria agallocha L.	Euphorbiaceae	TM	H, V, A	77.29 ± 6.48
Salvadora persica L.	Salvadoraceae	MA	-	-
Aegiceras corniculatum (L) Blanco	Myrsinaceae	TM	H, V, A	42.74 ± 4.02
				35.3
IV- Zuari				
Acrostichum aureum L.	Ceratopteridaceae	MA	H, V, A	17.45 ± 1.42
Derris heterophylla Willd**.**	Fabaceae	MA	H, V	7.24 ± 0.75
Bruguiera cylindrica (L.) Bl.	Rhizophoraceae	TM	H, V, A	9.45 ± 1.14
Bruguiera gymnorrhiza (L.) Lam.	Rhizophoraceae	TM	H, V	19.23 ± 3.21
Rhizophora apiculata Blume.	Rhizophoraceae	TM	H, V	19.12 ± 2.89
Rhizophora mucronata Poir.	Rhizophoraceae	TM	H, V, A	26.49 ± 4.29
Acanthus ilicifolius L.	Acanthaceae	TM	H, V, A	53.24 ± 5.48
Avicennia officinalis L	Acanthaceae	TM	H, V, A	13.45 ± 1.29
Avicennia marina (Frosk.) Vierh	Acanthaceae	TM	H, V	18.27 ± 2.03
Salvadora persica L.	Salvadoraceae	MA	-	-
Excoecaria agallocha L.	Euphorbiaceae	TM	H, V, A	42.46 ± 4.19
Sonneratia caseolaris (L.) Engler	Sonneratiaceae	TM	H, V, A	27.32 ± 3.45
Sonneratia alba (L.) Smith.	Sonneratiaceae	TM	H, V, A	29.19 ± 3.21
				33.6
V-Sal				
Acanthus ilicifolius L.	Acanthaceae	TM	H, V, A	36.23 ± 5.23
Avicennia officinalis L.	Acanthaceae	TM	H, V, A	7.02 ± 1.23
Rhizophora mucronata Poir.	Rhizophoraceae	TM	H, V, A	28.28 ± 3.56
Sonneratia alba (L.) Smith.	Sonneratiaceae	TM	H, V, A	40.31 ± 4.59
				27.7
VI- Talpona				
Acanthus ilicifolius L.	Acanthaceae	TM	H, V, A	65.26 ± 7.26
Avicennia officinalis L	Acanthaceae	TM	H, V, A	19.28 ± 3.24
Avicennia alba Blume.	Acanthaceae	TM	H, V, A	48.45 ± 6.45
Bruguiera cylindrica (L.) Bl.	Rhizophoraceae	TM	H, V, A	14.75 ± 1.28

Bruguiera gymnorrhiza (L.) Lam.	Rhizophoraceae	TM	H, V	17.12 ± 1.89
Rhizophora mucronata Poir.	Rhizophoraceae	TM	H, V, A	42.24 ± 6.56
				34.1
VII- Galgibagh				
Excoecaria agallocha L.	Euphorbiaceae	TM	H, V, A	34.23 ± 4.23
Acanthus ilicifolius L.	Acanthaceae	TM	H, V, A	64.45 ± 8.23
Avicennia officinalis L.	Acanthaceae	TM	H, V, A	27.25 ± 4.63
Bruguiera cylindrica (L.) Bl.	Rhizophoraceae	TM	H, V, A	60.78 ± 6.84
Rhizophora apiculata Blume.	Rhizophoraceae	TM	H, V	29.42 ± 3.46
				42.8

Legend: Total root samples = 51, TM = True mangrove; MA = Mangrove associate.

Table 4: Arbuscular mycorrhizal spore density and species richness in mangrove species from all the study sites.

Mangrove plant species	Habit	Family	AMF species	Spore density*	Species Richness
I- Terekhol			**3, 1, 12, 14, 15, 19, 27**		
Avicennia marina (Frosk.) Vierh	TM	Acanthaceae	1	27.21 ± 2.31	1
Acanthus ilicifolius L.	TM	Acanthaceae	3, 27, 14	148.00 ± 5.23	3
Rhizophora apiculata Blume.	TM	Rhizophoraceae	1, 15	42.36 ± 4.56	2
Ceriops tagal (Perr.) C.B. Robinson	TM	Rhizophoraceae	1, 3, 12, 19	192.45 ± 8.89	4
Exoecaria agallocha L.	TM	Euphorbiaceae	14	32.00 ± 4.12	1
				88.2	
II- Chapora			**1, 6, 19, 22, 23, 25**		
Rhizophora mucronata Poir.	TM	Rhizophoraceae	1, 25	40.00 ± 4.56	2
Acanthus ilicifolius L.	TM	Acanthaceae	1, 19, 25	49.00 ± 7.45	3
Avicennia officinalis L	TM	Acanthaceae	6, 19	172.28 ± 10.29	2
Avicennia marina (Frosk.) Vierh	TM	Acanthaceae	25	19.00 ± 1.28	1
Exoecaria agallocha L.	TM	Euphorbiaceae	1, 6, 22, 23	240.00 ± 11.23	4
Aegiceras corniculatum (L) Blanco	TM	Myrsinaceae	19	34.00 ± 3.29	1
Salvadora persica L.	MA	Salvadoraceae	-	**-**	-
				99.3	
III- Mandovi			**1, 4, 5, 6, 9, 13, 14, 17, 18, 19, 21, 26**		
Sonneratia alba (L.) Smith.	TM	Sonneratiaceae	5	13.00 ±1.29	1
Sonneratia caseolaris (L.) Engler	TM	Sonneratiaceae	1, 5, 17, 21	132.00 ±8.29	4
Acanthus ilicifolius L.	TM	Acanthaceae	1, 5, 14, 19, 21,	245.13 ± 4.59	5
Avicennia officinalis L	TM	Acanthaceae	14	14.45 ± 2.48	1
Acrostichum aureum L.	MA	Ceratopteridaceae	17, 19,	57.13 ± 7.27	2
Derris heterophylla Willd**.**	MA	Fabaceae	13	8.00 ± 1.03	1
Kandelia candel (L.) Druce.	TM	Rhizophoraceae	4	17.00 ± 1.45	1
Rhizophora apiculata Blume.	TM	Rhizophoraceae	13, 14, 19, 21	187.00 ± 12.39	4
Rhizophora mucronata Poir.	TM	Rhizophoraceae	1, 26	58.12 ± 6.23	2

Exoecaria agallocha L.	TM	Euphorbiaceae	1, 4, 13, 18	176.00 ± 13.25	4
Salvadora persica L.	MA	Salvadoraceae	-	-	-
Aegiceras corniculatum (L) Blanco	TM	Myrsinaceae	14, 21	42.00 ± 4.12	2
				86.2	
IV- Zuari			**1, 2, 3, 5, 7, 8, 10, 11, 12, 13, 16, 17, 19, 24, 27, 28**		
Acrostichum aureum L.	MA	Ceratopteridaceae	2, 17, 19	89.23 ± 8.45	3
Derris heterophylla Willd.	MA	Fabaceae	12	13.00 ± 1.02	1
Bruguiera cylindrica (L.) Bl.	TM	Rhizophoraceae	1, 8, 10, 11, 13	234.00 ± 9.23	5
Bruguiera gymnorrhiza (L.) Lam.	TM	Rhizophoraceae	8	24.00 ±1.26	1
Rhizophora apiculata Blume.	TM	Rhizophoraceae	1, 27	136.00 ±7.56	2
Rhizophora mucronata Poir.	TM	Rhizophoraceae	1, 19, 27	76.23 ± 5.69	3
Acanthus ilicifolius L.	TM	Acanthaceae	1, 7, 13, 16 19, 24, 28	284.12 ±13.45	7
Avicennia officinalis L	TM	Acanthaceae	5, 7, 12	136.08 ±11.23	3
Avicennia marina (Frosk.) Vierh	TM	Acanthaceae	-	-	-
Salvadora persica L.	MA	Salvadoraceae	-	-	
Excoecaria agallocha L.	TM	Euphorbiaceae	1, 3, 7, 11	98.56 ± 8.56	4
Sonneratia caseolaris (L.) Engler	TM	Sonneratiaceae	7, 13	33.45 ± 5.23	2
Sonneratia alba (L.) Smith.	TM	Sonneratiaceae	-	**-**	-
				122.1	
V-Sal			**1, 4, 7, 13, 14, 19, 20, 26**		
Acanthus ilicifolius L.	TM	Acanthaceae	1, 13, 19, 26	324.23 ±14.23	4
Avicennia officinalis L.	TM	Acanthaceae	7, 14,19	180.00 ±8.28	3
Excoecaria agallocha L.	TM	Euphorbiaceae	4, 19, 20	145.78 ± 7.56	3
Sonneratia alba (L.) Smith.	TM	Sonneratiaceae	1,19,	86.23 ±4.56	2
				183.7	
VI- Talpona			**1, 2, 19, 21, 27**		
Acanthus ilicifolius L.	TM	Acanthaceae	1, 21	53.23 ± 4.25	2
Avicennia officinalis L	TM	Acanthaceae	19	14.00 ± 0.89	1

Avicennia alba Blume.	TM	Acanthaceae	2	11.00 ± 0.56	1
Bruguiera cylindrica (L.) Bl.	TM	Rhizophoraceae	2, 19, 27	172.23 ± 8.58	3
Bruguiera gymnorrhiza (L.) Lam.	TM	Rhizophoraceae	21	10.00 ± 0.78	1
Excoecaria agallocha L.	TM	Euphorbiaceae	1, 2, 19	65.45 ± 5.23	3
				54.1	
VII- Galgibagh			**1, 2, 6, 9, 19, 24**		
Excoecaria agallocha L.	TM	Euphorbiaceae	1, 2, 19	164.42 ±6.56	3
Acanthus ilicifolius L.	TM	Acanthaceae	2, 19, 24	228.45 ±10.23	3
Avicennia officinalis L.	TM	Acanthaceae	9	17.00 ± 1.03	1
Bruguiera cylindrica (L.) Bl.	TM	Rhizophoraceae	1, 6	49.04 ±4.23	2
Rhizophora apiculata Blume.	TM	Rhizophoraceae	19	12.00 ±2.30	1
				94.0	

Legend: Total Soil samples = 51, average spore density = 103.94 ± 40.5; average species richness = 8.5 ± 3.9.TM = True mangrove; MA = Mangrove associate;

*= spores 100g-1 of soil; Number listed in column labelled AM fungal species correspond to **Table 4.**

Table 5: Relative abundance (RA) and Isolation Frequency (IF) of AM fungi in selected study sites.

Sr. no.	AM fungal species	Terekhol		Chapora		Mandovi		Zuari		Sal		Talpona		Galgibagh	
		IF	RA	IF	RA	IF	RA	IF	RA	IF	RA	IF	RA	IF	RA
1	*Glomus intraradices* Schenck & Smith	60.0	49.4	42.8	48.6	33.3	14.7	71.4	25.0	80.0	51.9	50.0	29.5	60.0	43.9
2	*Glomus clarum* Nicolson & Smith	-	-	-	-	-	-	7.1	25.9		-	33.3	24.61	40.0	22.70
3	*Glomus multicaule* Gerdemann & Bakshi	40.0	10.4	-	-	-	-	31.4	3.30		-	-	-	-	-
4	*Glomus aggregatum* Schenck & Smith	-	-	-	-	16.6	1.38		-	28.5	3.09	-	-	-	-
5	*Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe	-	-		-	25.4	2.02	14.2	0.89	-	-	-	-	-	-
6	*Glomus fasciculatum* (Thaxter) Almeida & Schenck	-	-	28.5	8.45	8.3	17.1	-	-	-	-	-	-	20.0	20.90
7	*Glomus geosporum* (Nicol. & Gerd.) Walker	-	-	-	-	-	-	36.2	11.27	72.7	14.80	-	-	-	-
8	*Glomus hyderabadensis* Swarupa, Kunwar, Prasad, & Manohar	-	-	-	-	-	-	21.4	1.52	-	-	-	-	-	-
9	*Glomus formosanum* Wu & Chen	-	-	-	-	8.3	1.70	-	-	-	-	-	-	20.0	2.88
10	*Glomus nanolumen* Koske & Gemma	-	-	-	-	-	-	7.1	5.99	-	-	-	-	-	-
11	*Glomus constrictum* Trappe	-	-	-	-	-	-	14.2	3.84	-	-	-	-	-	-
12	*Glomus taiwanense* Wu & Chen	20.0	7.93	-	-	-	-	21.4	2.14	-	-	-	-	-	-

13	*Glomus rubiforme* Gerdemann & Trappe	-	-	-	-	8.3	12.5	7.1	3.13	60.0	7.28	-	-	-	-
14	*Glomus etunicatum* Becker & Gerd.	40.0	9.52	-	-	33.3	19.6	-	-	-	-	-	-	-	-
15	*Glomus maculosum* Miller & Walker	20.0	0.90	-	-	-	-	-	-	-	-	-	-	-	-
16	*Acaulospora foveata* Trappe & Janos	-	-	-	-	-	-	7.1	1.07	-	-	-	-	-	-
17	*Acaulospora bireticulata* Rothwell & Trappe	-	-	-	-	16.6	18.5	42.8	10.91	-	-	-	-	-	-
18	*Acaulospora delicata* Walker, Pfeffer & Bloss	-	-	-	-	8.3	0.74	-	-	-	-	-	-	-	-
19	*Acaulospora laevis* Gerdemann & Trappe	20.0	18.82	42.8	26.39	41.7	27.2	21.4	1.25	28.5	3.15	50.0	8.0	20.0	8.46
20	*Acaulospora mellea* Spain & Schenck	-	-	-	-	-	-	-	-	20.0	5.21	-	-	-	-
21	*Acaulospora scrobiculata* Trappe	-	-		-	58.3	2.98	-	-	-	-	33.3	34.76	-	-
22	*Acaulospora spinosa* Walker & Trappe	-	-	14.2	2.47	-	-	-	-	-	-	-	-	-	-
23	*Scutellospora gregaria* (Shenck & Nicolson) Walker & Sanders	-	-	14.2	3.91	-	-	-	-	-	-	-	-	-	-
24	*Scuetellospora weresubiae* Koske & Walker	-	-		-	-	-	7.1	2.32	-	-	-	-	20.0	1.08
25	*Scutellospora dipurpurescens* Morton & Koske	-	-	14.2	10.10	-	-	-	-	-	-	-	-	-	-

26	*Scutellospora calospora* (Nicolson & Gerdemann) Walker & Sanders	-	-	-	-	8.3	0.42	-	-	-	-	-	-	-	-
27	*Gigaspora albida* Schenck & Smith	20.0	2.94	-	-	-	-	14.2	0.35	-	-	16.6	3.07	-	-
28	*Entrophospora infrequens* (Hall) Ames & Schneider		-	-	-	-	-	7.1	0.98	-	-	-	-	-	-

Legend: Total AMF = 28 species.

Table 6: Diversity measurements of AMF communities in selected study sites.

Sr. no.	Ecological parameters	I	II	III	IV	V	VI	VII
1	Shannon- Wiener index of diversity (H)	0.66	0.60	0.44	0.40	0.44	0.78	0.55
2	Simpson`s index of dominance (D)	0.98	0.98	0.99	0.99	0.99	0.97	0.99
3	AMF species evenness(E)	0.34	0.31	0.22	0.21	0.22	0.41	0.28
4	AMF species richness (SR)	7	6	12	16	8	5	6

Legend: I-Terekhol; II- Chapora; III- Mandovi; IV-Zuari; V-Sal; VI-Talpona; VII- Galgibagh.

Table 7: Pearson correlation coefficient (r value) between spore density (SD) v/s root colonization (RC); and relative abundance (RA) and isolation frequency (IF) and spore density (SD) v/s species richness (SR) in selected study sites.

Sr. no.	Ecological parameters	I	II	III	IV	V	VI	VII
1	SD v/s RC	0.670	0.62	0.597	0.376	0.117	-0.242	0.552
2	RA v/s IF	*0.869	*0.894	*0.675	*0.723	*0.718	0.834	*0.817
3	SD v/s SR	*0.965	0.720	*0.938	*0.925	*0.932	*0.836	*0.937

Legend: I-Terekhol; II- Chapora; III- Mandovi ; IV-Zuari; V-Sal; VI-Talpona; VII- Galgibagh;* = significant; ($P \leq 0.05$)

Table 8: Geographic location and physico-chemical analysis of the study sites.

Soil characteristics	Pre-monsoon		Monsoon		Post-monsoon	
	Terekhol	Zuari	Terekhol	Zuari	Terekhol	Zuari
pH	6.5 ± 0.12	5.9 ± 0.08	6.8 ± 0.07	6.2 ± 0.02	6.7 ± 0.19	5.5 ± 0.10
EC (d Sm^{-1})	4.12 ± 1.02	8.19 ± 1.64	4.03± 1. 79	8.11 ± 1.24	4.30 ± 1.12	8.49 ± 1.24
OC (%)	4.79 ± 1.12	3.61 ± 1.29	4.01 ± 1.37	3.01 ± 1.02	4.45 ± 1.41	3.12 ± 1.06
P (g/kg)	0.17 ± 0.02	0.13 ± 0.01	0.16 ± 0.04	0.14 ± 0.06	0.13 ± 0.01	0.11 ± 0.01
K (g/kg)	63.23 ± 2.12	87.96 ± 4.12	68.14 ± 1.96	81.28 ± 2.12	70.34 ± 3.16	85.12 ± 2.12
N (g/kg)	0.54 ± 0.06	0.73 ± 0.03	0.57 ± 0.04	0.79 ± 0.03	0.61 ± 0.02	0.81 ± 0.05
Zn (g/kg)	0.049 ± 0.01	0.030 ± 0.01	0.031 ± 0.01	0.029 ± 0.04	0. 043 ± 0.01	0.032 ± 0.01
Mn (g/kg)	0.042 ± 0.04	0.029 ± 0.04	0.033 ± 0.04	0.021 ± 0.04	0.037 ± 0.04	0.026 ± 0.04
Cu (g/kg)	0.069 ± 0.02	0.051 ± 0.01	0.041 ± 0.01	0.032 ± 0.01	0.052 ± 0.01	0.040 ± 0.01
Fe (g/kg)	0.189 ± 0.06	0.621 ± 0.02	0.112 ± 0.04	0.511 ± 0.07	0.143 ± 0.08	0.563 ± 0.03

Legend: Data presented are means of three readings at each season.

Table 9: Arbuscular mycorrhizal root colonization in selected mangrove plant species at the two study sites.

Sr. no.	Plant species	AM Colonization (%)	
		Terekhol	Zuari
1.	*Acanthus ilicifolius* L.		
	Pre-monsoon	80.12 ± 10.21	64.42 ± 9.65
	Monsoon	43.11 ± 3.41	39.18 ± 3.52
	Post- monsoon	36.51 ± 5.16	13.20 ± 2.15
2.	*Rhizophora mucronata* Poir.		
	Pre-monsoon	26.23 ± 7.24	23.40 ± 2.98
	Monsoon	17.12 ± 2.20	14.10 ± 1.37
	Post- monsoon	14.45 ± 2.56	13.11 ± 1.28
3.	*Excoecaria agallocha* L.		
	Pre-monsoon	86.29 ± 9.17	78.41 ± 8.22
	Monsoon	69.13 ± 8.34	58.10 ± 2.41
	Post- monsoon	27.15 ± 3.20	39.14 ± 6.12
4.	*Avicennia marina* (Frosk.) Vierh		
	Pre-monsoon	42.25 ± 5.23	60.42 ± 7.40
	Monsoon	29.28 ± 1.89	40.24 ± 3.21
	Post- monsoon	24.21 ± 1.30	11.10 ± 1.74
5.	*Sonneratia alba* (L.) Smith		
	Pre-monsoon	39.37 ± 4.20	32.15 ± 3.81
	Monsoon	36.15 ± 6.60	34.21 ± 4.24
	Post- monsoon	22.26 ± 4.71	21.40 ± 1.14

Legend: All values are means of 3 replicates; n=15 in each season, n= 90 overall.

Table 10: Arbuscular mycorrhizal spore density in selected mangrove plant species at the two study sites.

Sr. no.	Plant species	Spore density *	
		Terekhol	Zuari
1	*Acanthus ilicifolius* L.		
	Pre-monsoon	76.00 ± 4.21	46.00 ± 5.61
	Monsoon	230.00 ± 9.41	149.00 ± 3.52
	Post- monsoon	40.00 ± 3.56	28.00 ± 5.45
2	*Rhizophora mucronata* Poir.		
	Pre-monsoon	38.00 ± 5.24	57. 00 ± 2.98
	Monsoon	17.00 ± 1.20	24.00 ± 7.37
	Post- monsoon	7.45 ± 2.56	13.00 ± 8.23
3	*Excoecaria agallocha* L.		
	Pre-monsoon	128.0 ± 8.57	186.00 ± 9.32
	Monsoon	49.00 ± 4.35	56.00 ± 2.41
	Post- monsoon	21.00 ± 2.25	26.00 ± 3.24
4	*Avicennia marina* (Frosk.) Vierh		
	Pre-monsoon	42.15 ± 5.23	32.22 ± 8.43
	Monsoon	39.28 ± 1.89	42.14 ± 4.23
	Post- monsoon	24.21 ± 1.30	13.10 ± 1.34
5	*Sonneratia alba* (L.) Smith		
	Pre-monsoon	46.36 ± 4.20	47.45 ± 4.31
	Monsoon	27.25 ± 6.60	36.11 ± 3.22
	Post- monsoon	12.12 ± 4.71	12.30 ± 2.24

Legend: All values are means of 3 replicates; n=15 in each season, n= 90 overall; *= spores 100g-1 of soil.

Table 11: Seasonal variation in relative abundance (RA) of AM fungi in the selected study sites.

Sr. no.	AM fungal species	Pre-monsoon		Monsoon		Post-monsoon	
		Terekhol	Zuari	Terekhol	Zuari	Terekhol	Zuari
1	*Glomus intraradices* Schenck & Smith	-	-	18.68	15.71	58.82	42.16
2	*Glomus multicaule* Gerdemann & Bakshi	-	-	14.14	-	-	-
3	**Glomus aggregatum* Schenck & Smith	-	15.64	-	10.72	10.29	-
4	*Glomus fasciculatum* (Thaxter) Almeida & Schenck	20.42	-	-	-	-	-
5	*Glomus geosporum* (Nicol. & Gerd.) Walker	7.04	16.32	-	-	-	-
6	*Glomus nanolumen* Koske & Gemma	-	-	9.84	-	-	-
7	**Glomus rubiforme* Gerdemann & Trappe	-	-	-	13.24	-	25.30
8	*Acaulospora bireticulata* Rothwell & Trappe	-	-	8.83	31.67	-	-
9	*Acaulospora laevis* Gerdemann & Trappe	23.94	25.85	9.84	33.41	30.88	32.53
10	*Acaulospora scrobiculata* Trappe	35.91	48.97	33.8	-	-	-
11	*Scutellospora gregaria* (Shenck & Nicolson) Walker & Sanders	12.67	8.84	4.79	5.23	-	-

Legend: * = Sporocarpic forms

Table 12: Pearson correlation coefficient (r value) between spore density (SD) and species richness (SR) at the two study sites.

Ecological parameters	**Terekhol site**			**Zuari site**		
	Pre-monsoon	Monsoon	Post-monsoon	Pre-monsoon	Monsoon	Post-monsoon
SD v/s SR	*0.843	0.712	0.632	*0.821	0.743	0.596

Legend: SD – Spore density; SR – Species richness; * = significant at $p \leq 0.05$

Table 13: Diversity measurements of AM fungal communities in different seasons at the two study sites.

Study sites & ecological parameters	Pre-monsoon	Monsoon	Post-monsoon
Terekhol site			
Shannon-Weiner Index (H)	0.795	0.358	0.852
Simpsons Index of Dominance (D)	0.93	0.79	0.97
Evenness (E)	0.40	0.32	0.52
Zuari site			
Shannon-Weiner Index (H)	0.927	0.361	0.774
Simpsons Index of Dominance (D)	0.99	0.784	0.99
Evenness (E)	0.48	0.32	0.51

Table 14: **Mangrove plant species selected for the study and period of their flowering and fruiting.**

Sr. no.	Plant species	Family	Chapora	Mandovi	Periods of flowering and fruiting
1	*Rhizophora apiculata* Blume.	Rhizophoraceae	-	+	Feb -July
2	*Acanthus ilicifolius* L.	Acanthaceae	+	+	Feb - May
3	*Rhizophora mucronata* Poir.	Rhizophoraceae	+	-	Feb - May
4	*Excoecaria agallocha* L.	Euphorbiaceae	+	+	June - July
5	*Avicennia marina* (Frosk.) Vierh	Acanthaceae	+	-	May - August
6	*Aegiceras corniculatum* (L) Blanco	Myrsinaceae	+	+	May - Aug
7	*Salvadora persica* L.	Salvadoraceae	-	+	Jan -May

Legend: + = present, - = absent

Table 15: Mycorrhizal root colonization and spore density in selected mangrove plant species at Chapora (Agarwada) site.

Sr. no.	Plant species	Colonization (%)	Type of Colonization			Spore density (Spore100g $^{-1}$ of soil)
			H	V	A	
1	*Acanthus ilicifolius* L.					
	Vegetative stage	42.12 ± 4.21	+	+	-	38.00 ± 5.61
	Flowering stage	58.21 ± 4.41	+	+	+	43.00 ± 3.52
	Fruiting stage	47.50 ± 3.56	+	-	+	238.00± 12.45
2	*Rhizophora mucronata* Poir.					
	Vegetative stage	16.23 ± 7.24	+	+	-	27.00 ± 2.98
	Flowering stage	39.12 ± 2.20	+	+	+	32.00 ± 7.37
	Fruiting stage	17.45 ± 2.56	+	+	+	78.00 ± 8.23
3	*Excoecaria agallocha* L.					
	Vegetative stage	68.19 ± 3.57	+	+	-	84.00 ± 16.32
	Flowering stage	90.23 ± 9.35	+	+	+	24.00 ± 2.41
	Fruiting stage	38.45 ± 4.25	+	-	+	298.00 ± 17.25
4	*Avicennia marina* (Frosk.) Vierh					
	Vegetative stage	16.45 ± 1.23	+	+	-	15.00 ± 2.40
	Flowering stage	34.23 ± 1.89	+	+	+	19.00 ± 3.21
	Fruiting stage	12.45 ± 2.34	+	-	+	27.00 ± 2.74
5	*Aegiceras corniculatum* (L) Blanco					
	Vegetative stage	22.73 ± 3.20	+	+	-	38.00 ± 4.89
	Flowering stage	66.00 ± 5.60	+	+	+	33.00 ± 6.24
	Fruiting stage	34.22 ± 4.21	+	-	+	149.00 ± 3.24

Legend: All values are means of 3 replicates. Legend; H = hyphal; V= vesiclulars and A= arbuscular colonization; + = present; - = absent.

Table 16: Mycorrhizal root colonization and spore density in selected mangrove plant species at Mandovi (Chorao).

Sr no.	Plant species	Colonization (%)	Type of Colonization			Spore density (Spore $100g^{-1}$ of soil)
			H	V	A	
1	*Rhizophora apiculata* Blume.					
	Vegetative stage	24.21 ± 2.35	+	+	-	7.00 ± 1.89
	Flowering stage	46.12 ± 1.26	+	+	+	21.26± 2.34
	Fruiting stage	13.00 ± 1.48	+	+	+	25.00 ± 1.52
2	*Acanthus ilicifolius* L.					
	Vegetative stage	29.02 ± 3.47	+	+	+	53.00 ± 6.89
	Flowering stage	79.01 ± 4.62	+	+	+	27.00 ± 4.23
	Fruiting stage	20.22 ± 10.53	+	-	-	257.00± 12.34
3	*Excoecaria agallocha* L.					
	Vegetative stage	53.26 ±7.2	+	-	+	29.00 ± 1.89
	Flowering stage	61.42 ± 2.6	+	+	+	49.00 ± 4.23
	Fruiting stage	32.49 ± 4.56	+	+	+	329.00 ±14.23
4	*Salvadora persica* L.					
	Vegetative stage	6.12 ± 1.23	+	+	-	19.00 ± 1.24
	Flowering stage	24.19 ± 4.25	+	+	+	14.00 ± 1.08
	Fruiting stage	14.00 ± 2.34	+	-	+	29.00 ± 2.19
5	*Aegiceras corniculatum* (L) Blanco					
	Vegetative stage	17.18 ± 2.34	+	+	-	41.00 ± 3.24
	Flowering stage	31.01 ±2.19	+	+	+	30.00 ± 3.87
	Fruiting stage	14.13 ± 1.89	+	-	+	126.00 ± 9.25

Legend: All values are means of 3 replicates. Legend; H = hyphal; V= vesiclulars and A= arbuscular colonization; + = present; - = absent.

Table 17: List of AM fungal species isolated during different growth stages of selected mangrove plant species at study sites.

	AMF species	Chapora			Mandovi		
		V	Fl	Fr	V	Fl	Fr
	Glomus						
1	*Glomus intraradices* Schenck & Smith	-	-	+	+	-	+
2	*Glomus maculosum* Miller & Walker	-	-	+	-	-	-
3	*Glomus aggregatum* Schenck & Smith	+	-	+	-	-	+
4	*Glomus taiwanense* Wu & Chen	+	-	-	+	+	-
5	*Glomus rubiforme* Gerdemann & Trappe	+	-	-	+	-	+
	Acaulospora						
6	*Acaulospora morrowiae* Spain & Schenck	-	-	-	-	-	+
7	*Acaulospora laevis* Gerdemann & Trappe	+	-	-	+	+	-
8	*Acaulospora mellea* Spain & Schenck	-	+	+	-	-	-
9	*Acaulospora scrobiculata* Trappe	+	+	+	+	+	+
10	*Acaulospora spinosa* Walker & Trappe	+	+	-	-	-	+
11	*Acaulospora rugosa* Gerdemann & Trappe	-	-	+	-	-	-
	Scutellospora						
12	*Scutellospora dipurpurescens* Morton & Koske	-	-	+	-	-	-
13	*Scutellospora calospora* (Nicolson & Gerdemann) Walker & Sanders	-	+	-	-	-	+
	Gigaspora						
14	*Gigaspora albida* Schenck & Smith	-	-	-	-	-	+

Legend: Total AM fungal species = 15, V= Vegetative stage, Fl= flowering stage, Fr = fruiting stage, + = present, - = absent

Table 18: Pearson correlation coefficient (r value) between spore density (SD) v/s root colonization (RC), and Relative abundance (RA) and Isolation frequency (IF) in selected study sites.

Sr. no.	**Ecological parameters**	**Chapora site**			**Mandovi site**		
		Vegetative	Flowering	Fruiting	Vegetative	Flowering	Fruiting
1	SD v/s RC	0.935	0.071	* 0.893	0.070	0.475	* 0.926
2	RA v/s IF	*0.726	0.675	*0.813	0.718	0.326	*0.842

Legend: SD – Spore density; RC - Root colonization; RA - Relative abundance; IF- Isolation frequency; * = significant at $P \leq 0.05$

Table 20: Germination of spores, length and width of germ tube and number of hyphal branches in MSR media with or without after 26 days

Substrate	Germination (%)	Germ tube growth		
		Length (µm)*	**Width (µm)***	**No. of branches***
MSR medium (+ Sucrose)	75.0 b	354.2b	9.8b	3b
MSR medium (+ Sucrose)	90.0 a	658.4a	10.0a	1.7a

Legend: * Average number length width and number of branches in germinated spores. Substrate followed by the same letter do not differ significantly ($P \leq 0.05$).

Table 19: Composition of modified Strullu-Romand (MSR) media.

	Component	Concentration (µM)
1	$N(NO_3^-)$	3800
2	$N(NH_4^+)$	180
3	P	30
4	K	1650
5	Ca	1520
6	Mg	3000
7	S	3013
8	Cl	870
9	Na	20
10	Fe	20
11	Mn	11
12	Zn	1
13	B	30
14	Mo	0.22
15	Cu	0.96
16	Panthotenate	1.88
17	Biotin	0.004
18	Pyridine	4.38
19	Thiamine	2.96
20	Cyanocabalamine	0.29
21	Nicotinic acid	0.10
22	Sucrose (g/L)	10
23	pH(before autoclave)	5.5
24	Clarigel (g/L)	5

Table 21: Details of various treatments, AM fungal species and host plant.

Treatments	AM fungal species	Host plant
SC	Uninoculated control (sterilized sand)	*Ceriops tagal*
UC	Uninoculated control (unsterilized sand)	*Ceriops tagal*
AL	*Acaulospora laevis* Gerdemann & Trappe	*Ceriops tagal*
GC	*Glomus clarum* Nicolson & Smith	*Ceriops tagal*
GI	*Glomus intraradices* Schenck & Smith	*Ceriops tagal*

Table 22: Effect of AM inoculation on growth and biomass in *Ceriops tagal*.

Treatment	Plant height (cm)	Total leaf area $(cm)^2$	Number of leaves plant$^-$	Total fresh weight (g)	Total dry weight (g)
SC	29.6 ± 0.29 ^{e}	5.73 ± 0.64 ^{e}	2 ± 0.80 ^{d}	7.56 ± 0.46 ^{e}	5.90 ± 0.26 ^{e}
UC	35.8 ± 0.37 ^{d}	14.25 ± 1.43 ^{d}	4 ± 0.80 ^{c}	9.54 ± 1.20 ^{d}	8.10 ± 0.80 ^{d}
AL	42.2 ± 1.27 ^{c}	30.15 ± 0.91 ^{c}	8 ± 0.80 ^{b}	12.44 ± 1.72 ^{c}	11.20 ± 1.50 ^{c}
GC	51.2 ± 1.68 ^{a}	43.16 ± 2.13 ^{a}	10 ± 0.80 ^{a}	15.14 ± 2.41 ^{a}	13.44 ± 1.80 ^{a}
GI	44.4 ± 1.37 ^{b}	31.77 ± 0.88 ^{b}	8 ± 0.80 ^{b}	13.76 ± 1.62 ^{b}	12.33 ± 1.40 ^{b}

Legend: All values presented are mean of five readings. Means followed by different letters in column are significantly different at $P \leq 0.05$.

Fig.1: Map of Goa showing major mangrove sites undertaken for the study.

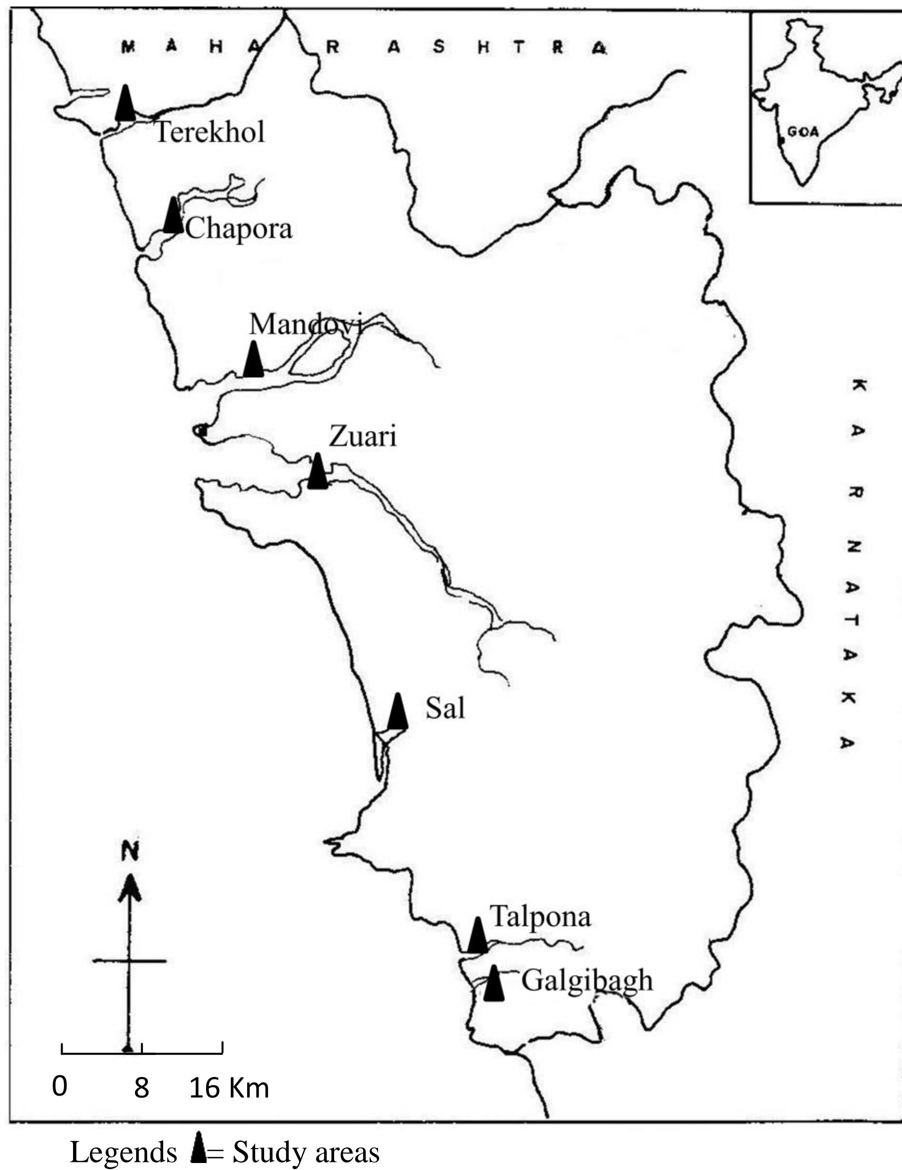


Fig. 2: Average AM root colonization in mangrove plant species in selected study sites.

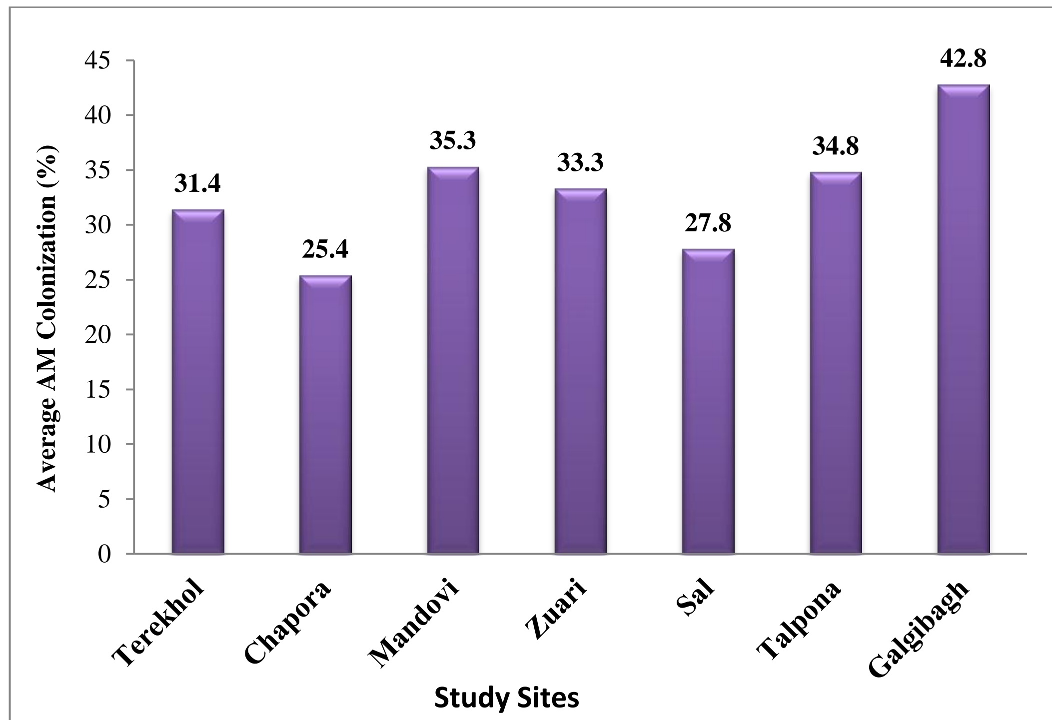


Fig. 3: Mean spore density in mangrove plant species from selected study sites.

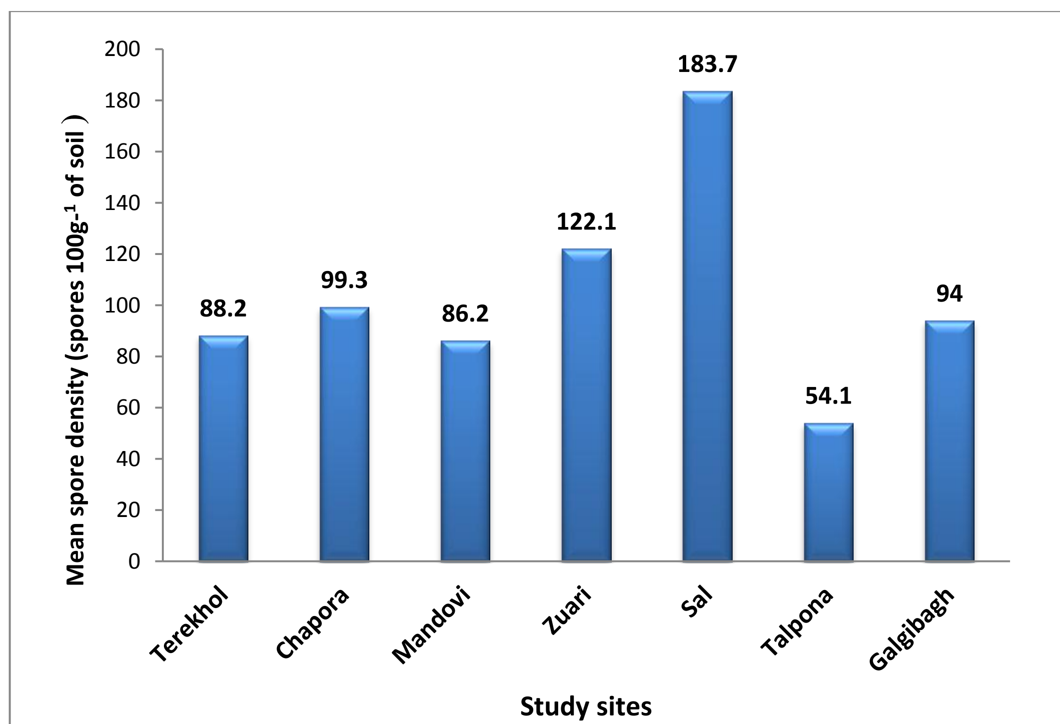


Fig. 4: AM fungal species richness in all the selected study sites.

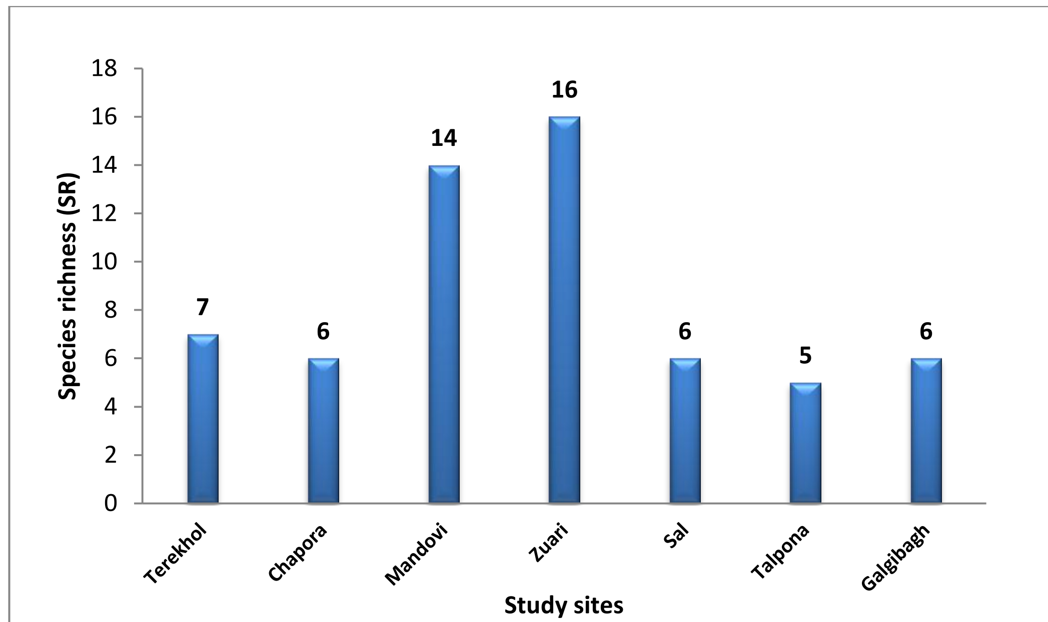


Fig. 5: Relative abundance of *Glomus intraradices* and *Acaulospora laevis*.

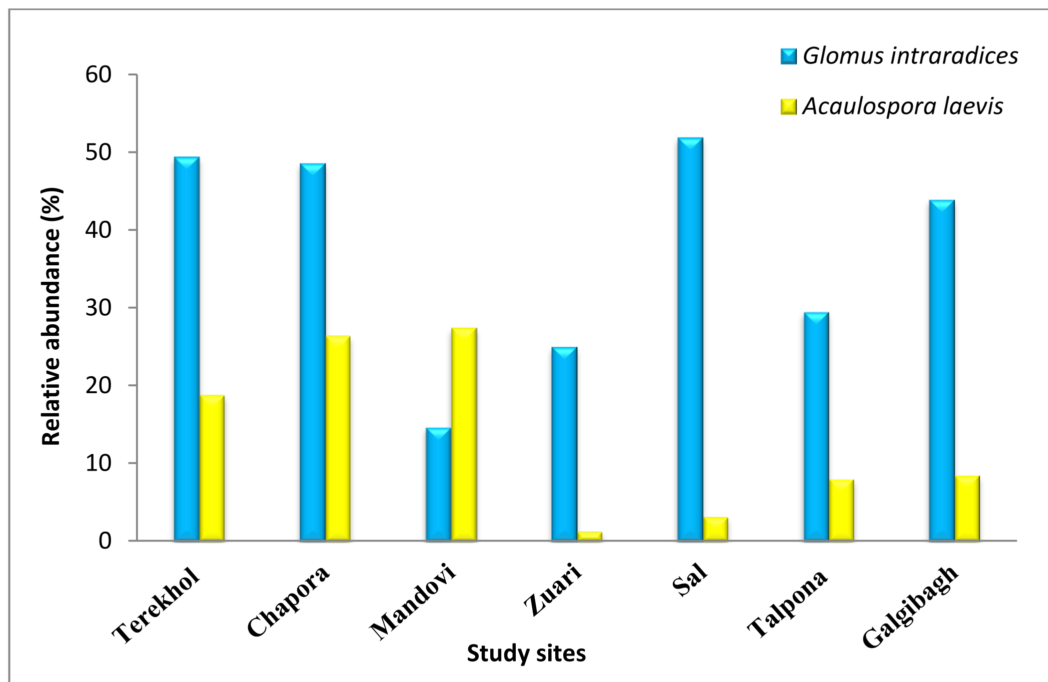


Fig. 6: Average AM root colonization in mangrove plant species at the two study sites.

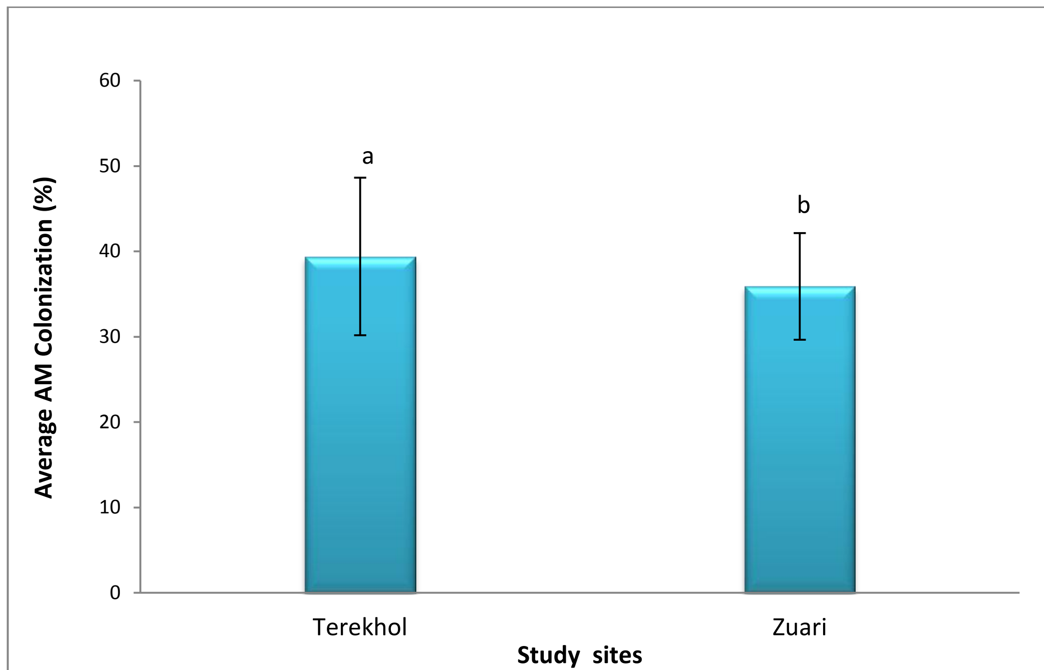


Fig. 7: Average AM root colonization in different seasons at the two study sites.

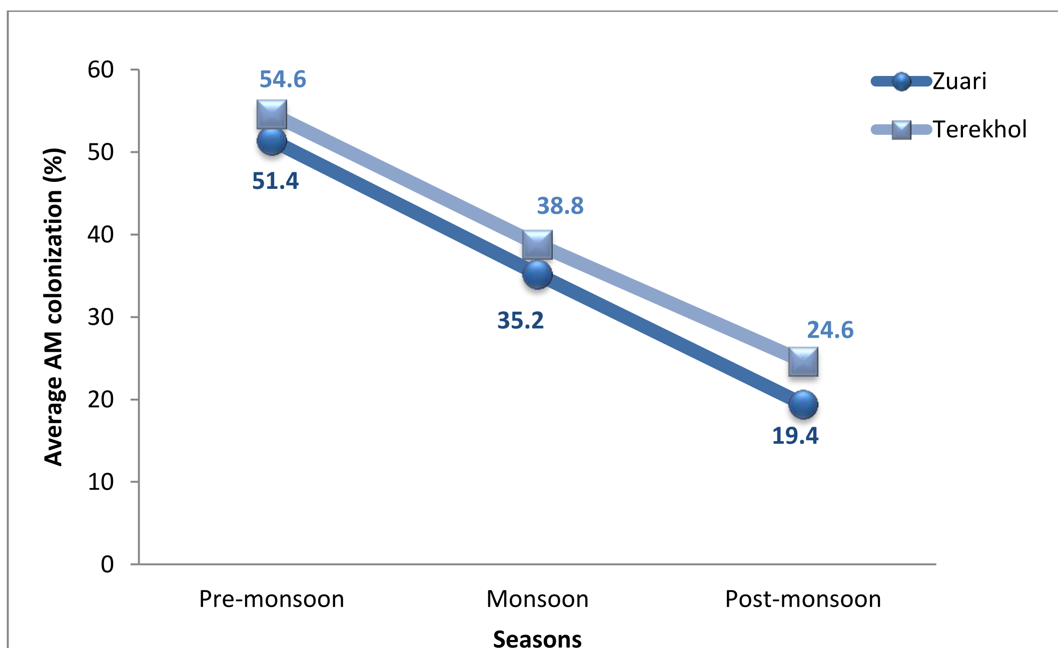


Fig. 8: Mean AM fungal spore density at the two study sites.

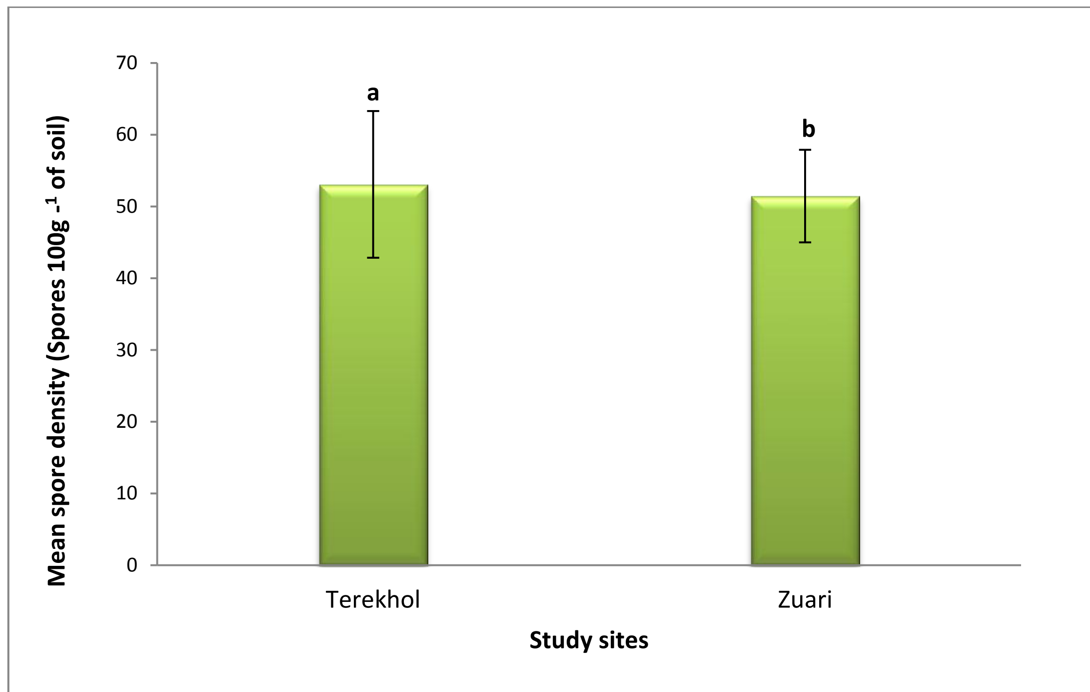


Fig. 9: Mean AM fungal spore density in different seasons at the two study sites.

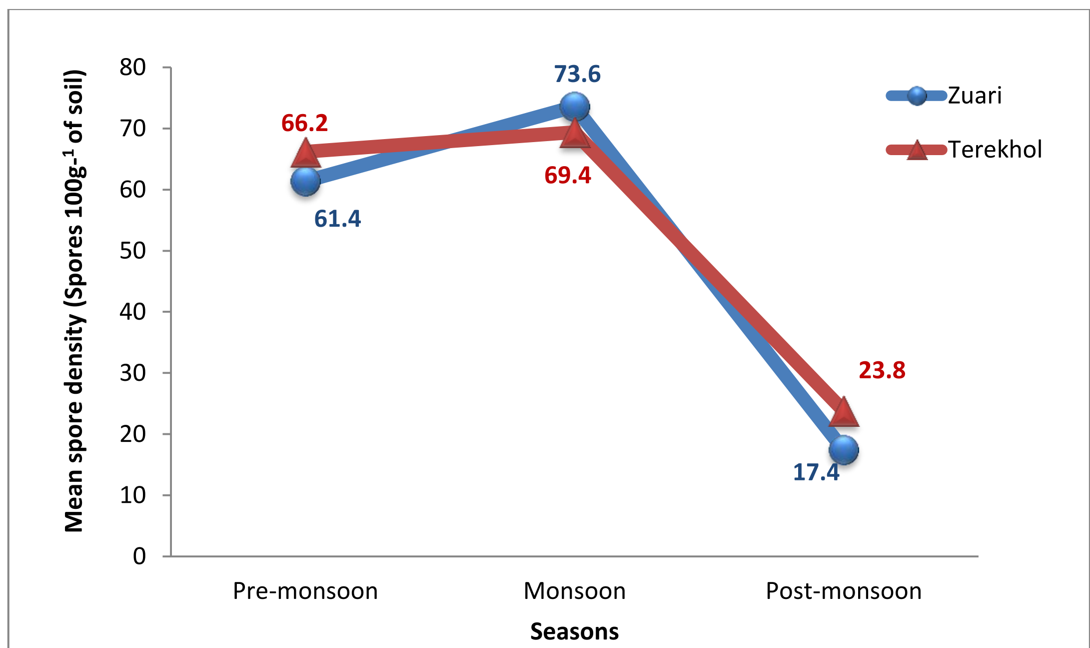


Fig. 10: Arbuscular mycorrhizal species richness (SR) in various seasons at the two sites.

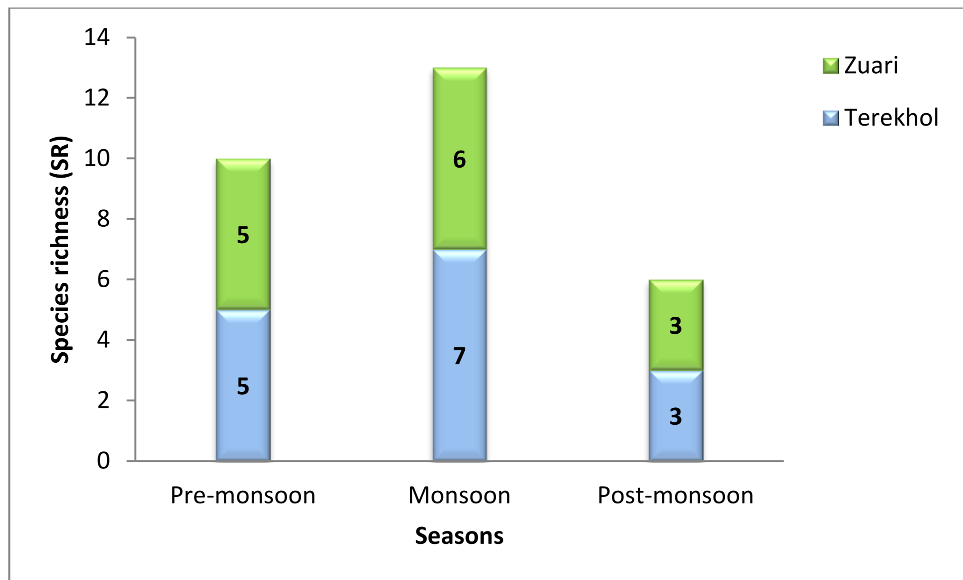


Fig. 11: Average AM root colonization in mangrove plant species at the two study sites.

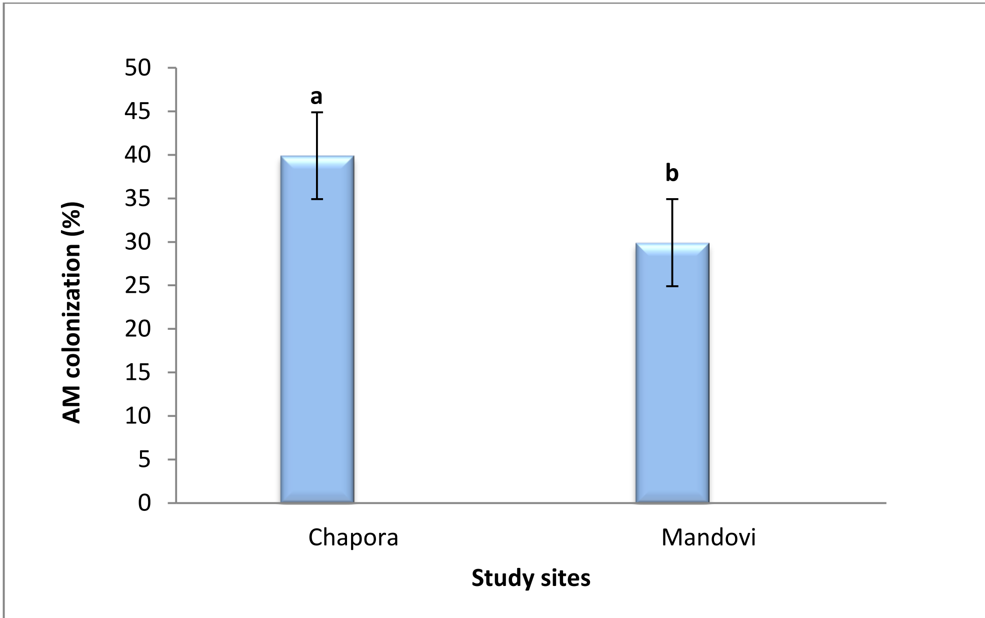


Fig. 12: Average AM root colonization in different growth stages of mangrove plant species at the two study sites.

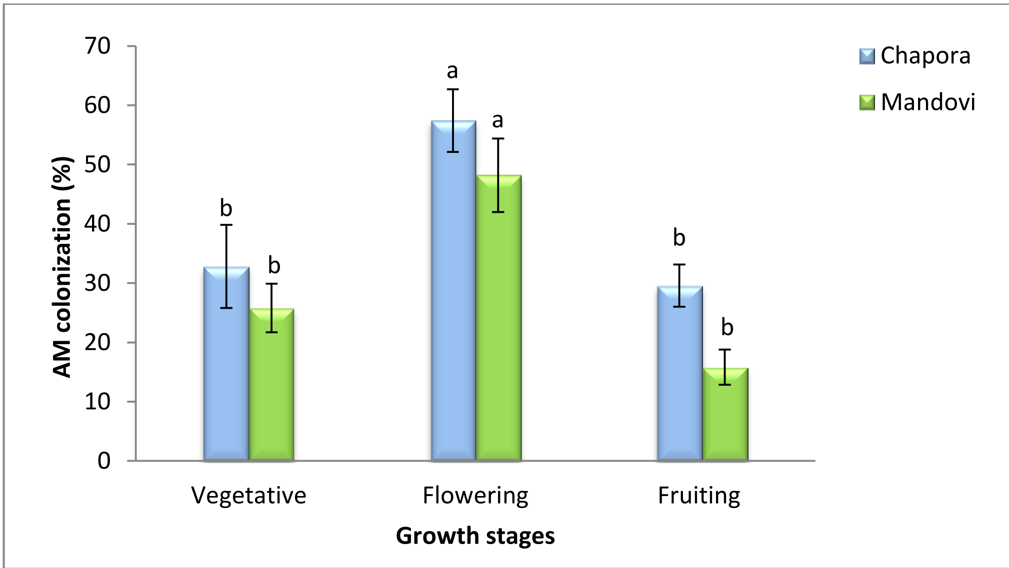


Fig. 13: Mean AM spore density at the two study sites.

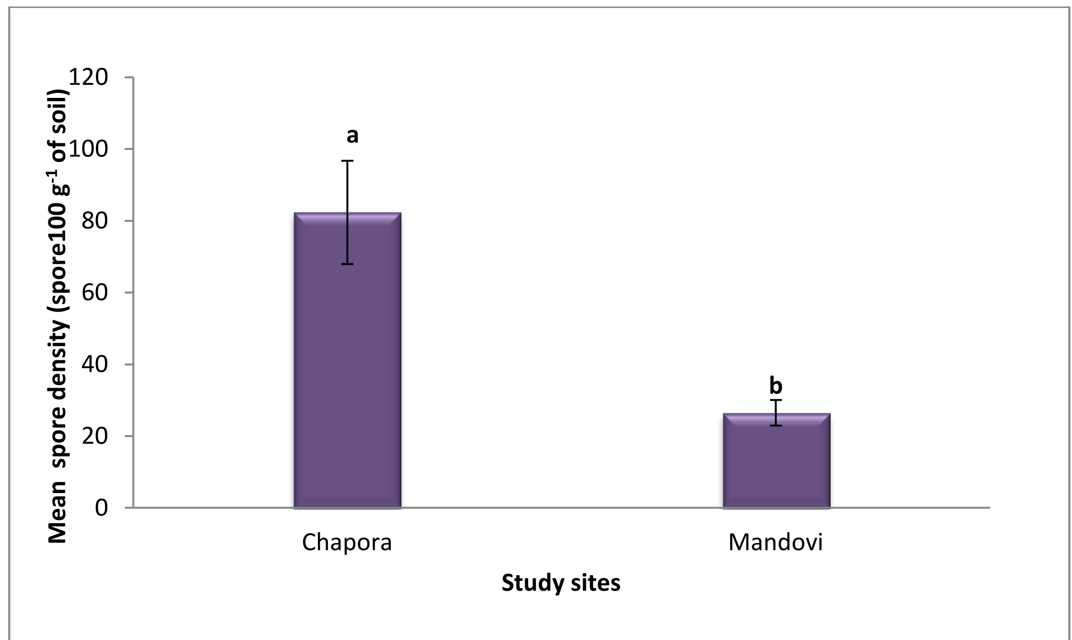


Fig. 14: Mean spore density in different growth stages of mangrove plant species at the two study sites.

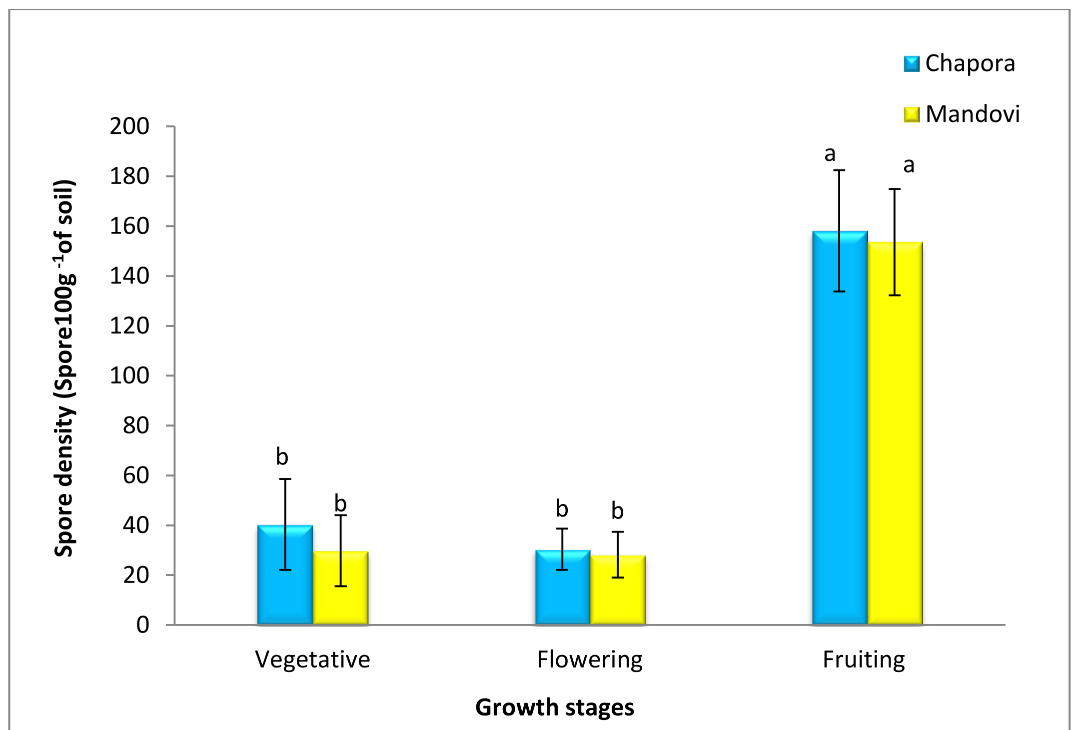


Fig. 15: Relative Abundance of AM species in the vegetative stage at the two study sites.

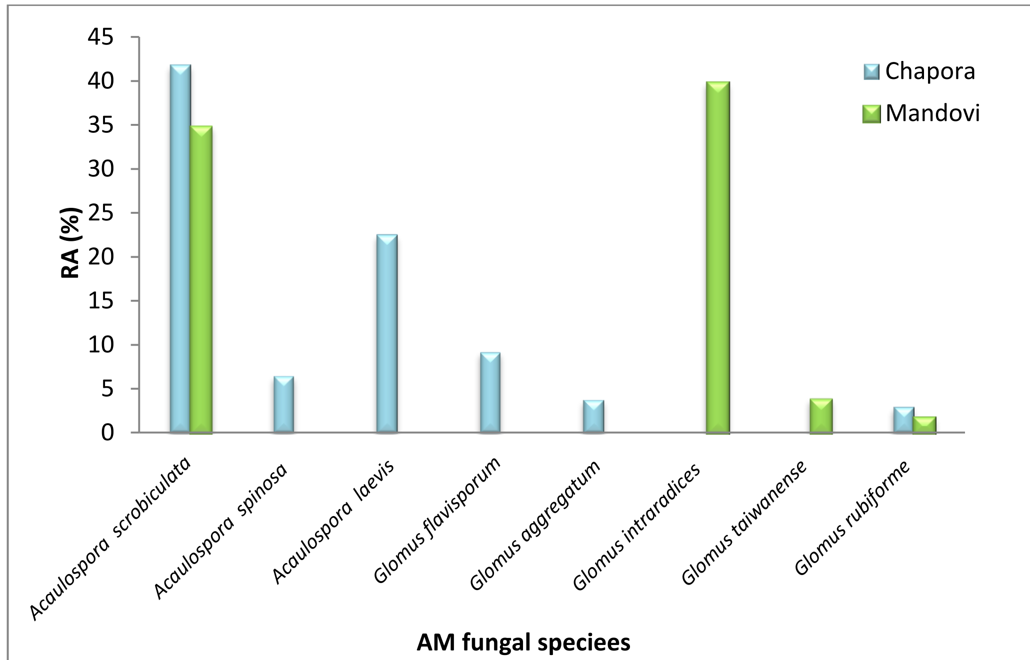


Fig. 16: Isolation Frequency (IF) of AM species in the vegetative stage at the two study sites.

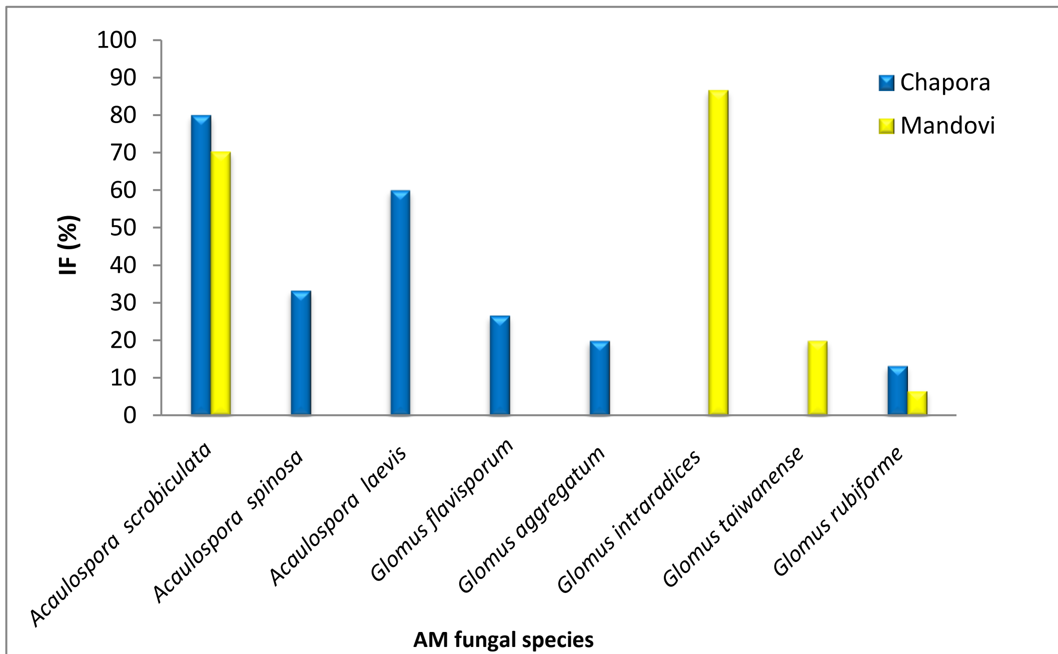


Fig. 17: Relative Abundance of AM species in the flowering stage at the two study sites.

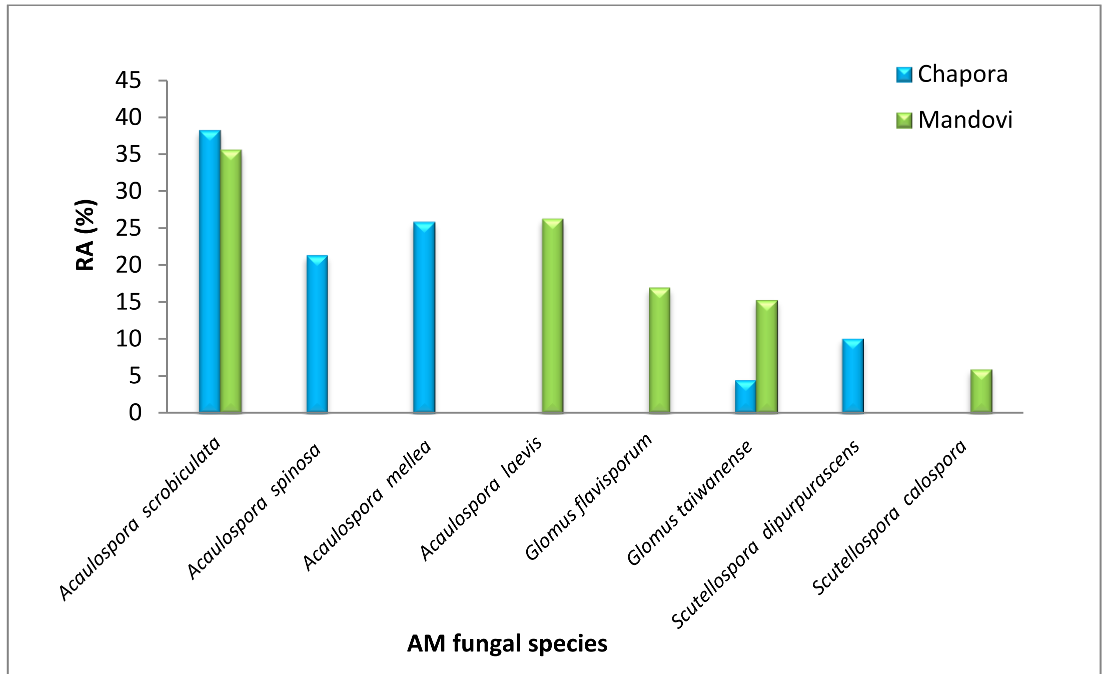


Fig. 18: Isolation Frequency (IF) of AM species in the flowering stage at the two study sites.

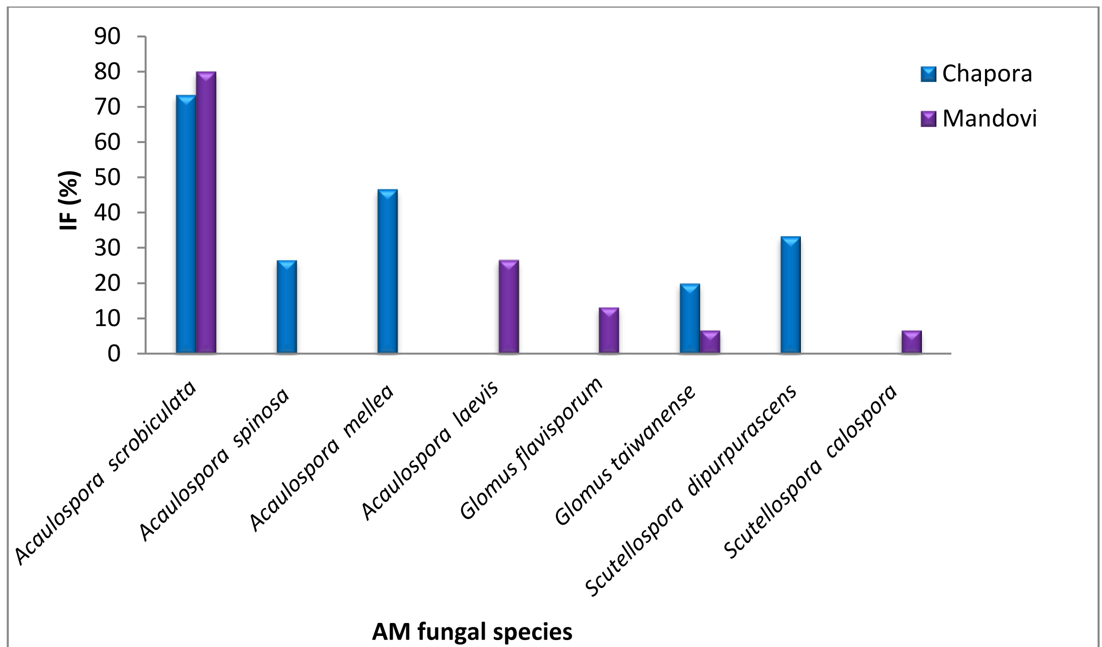


Fig. 19: Relative Abundance of AM species in the fruiting stage at the two study sites.

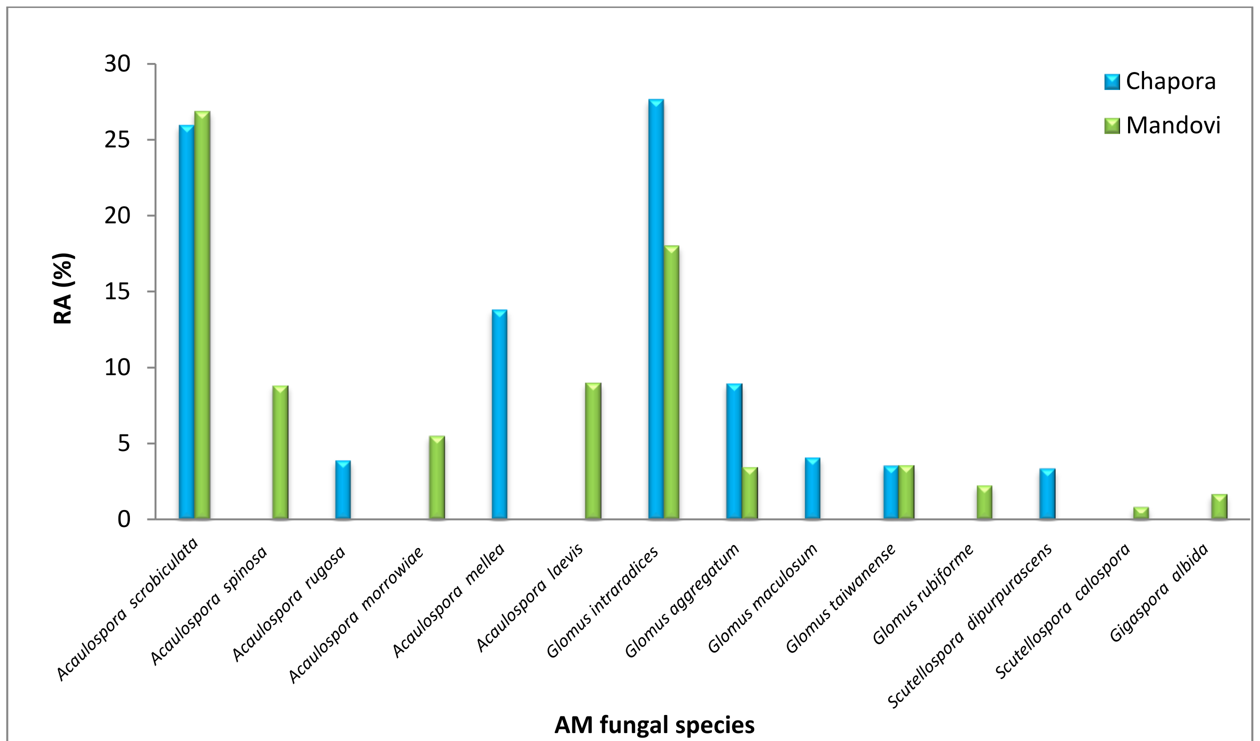


Fig. 20: Isolation Frequency (IF) of AM species in the fruiting stage at the two study sites.

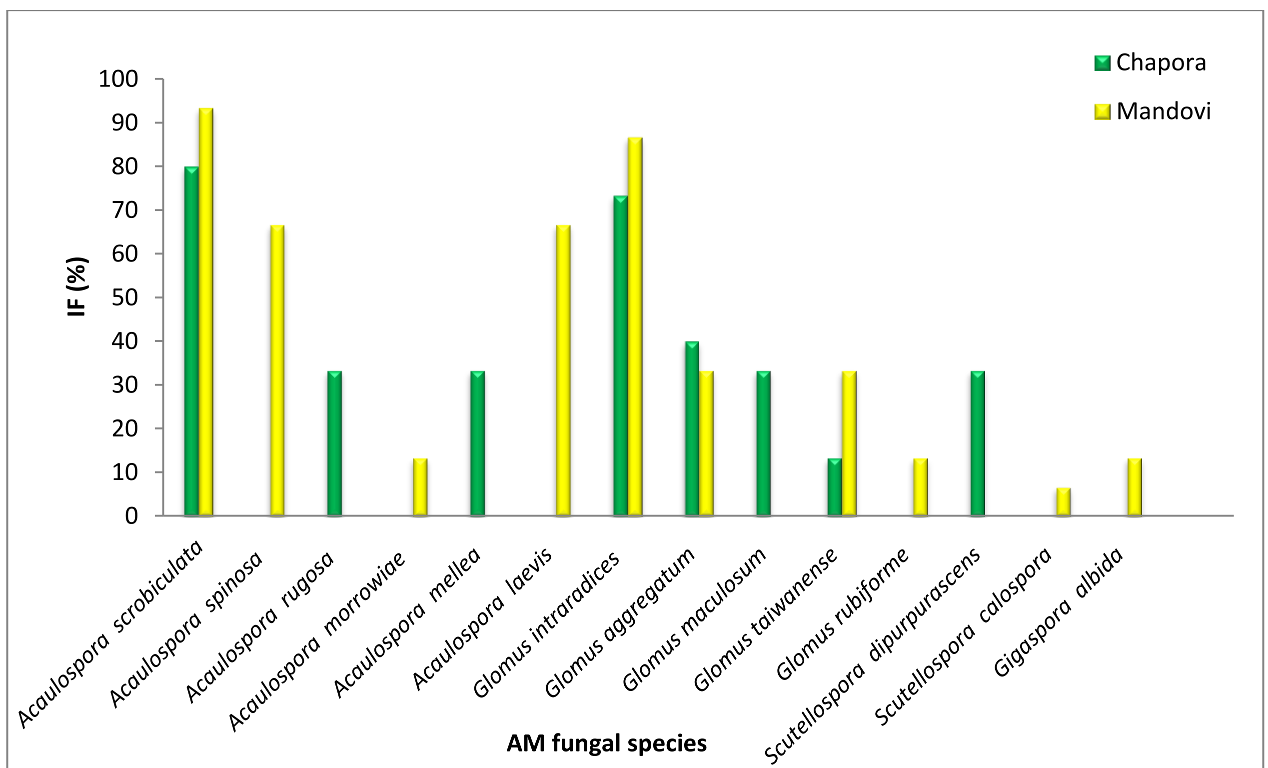


Fig. 21: AM fungal Species Richness (SR) in various seasons at the two sites.

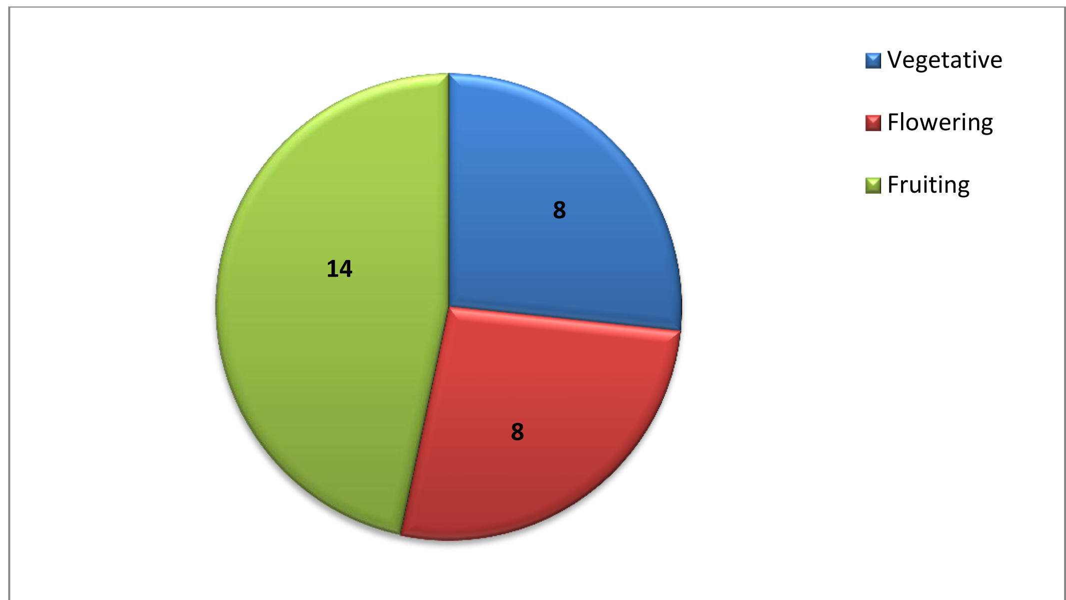


Fig. 22: Arbuscular mycorrhizal Species Evenness (SE) in various seasons at the two sites.

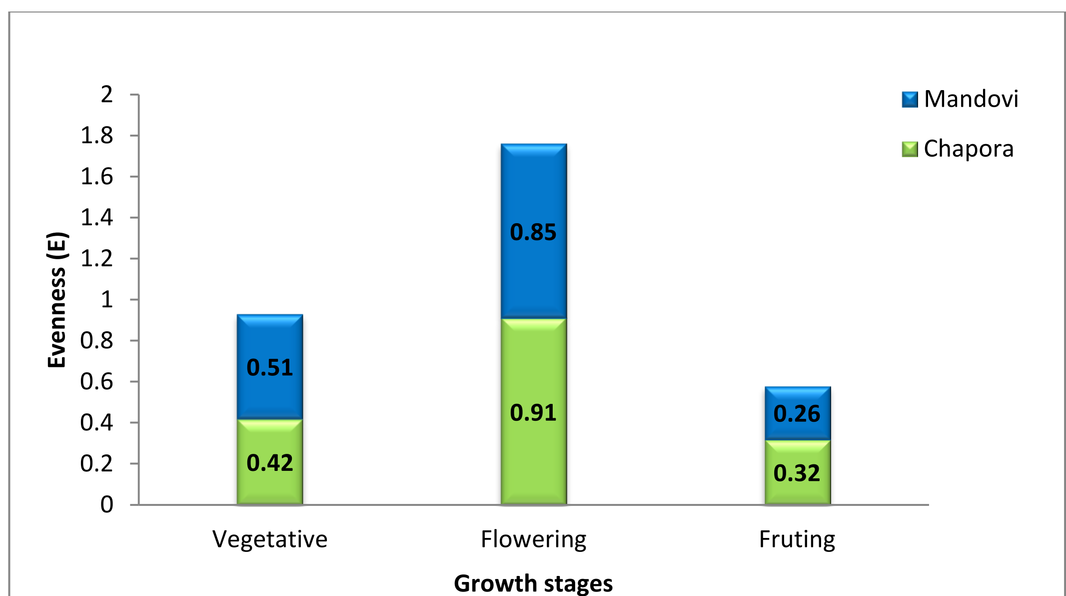


Fig. 23: Shannon–Weiner index (H').

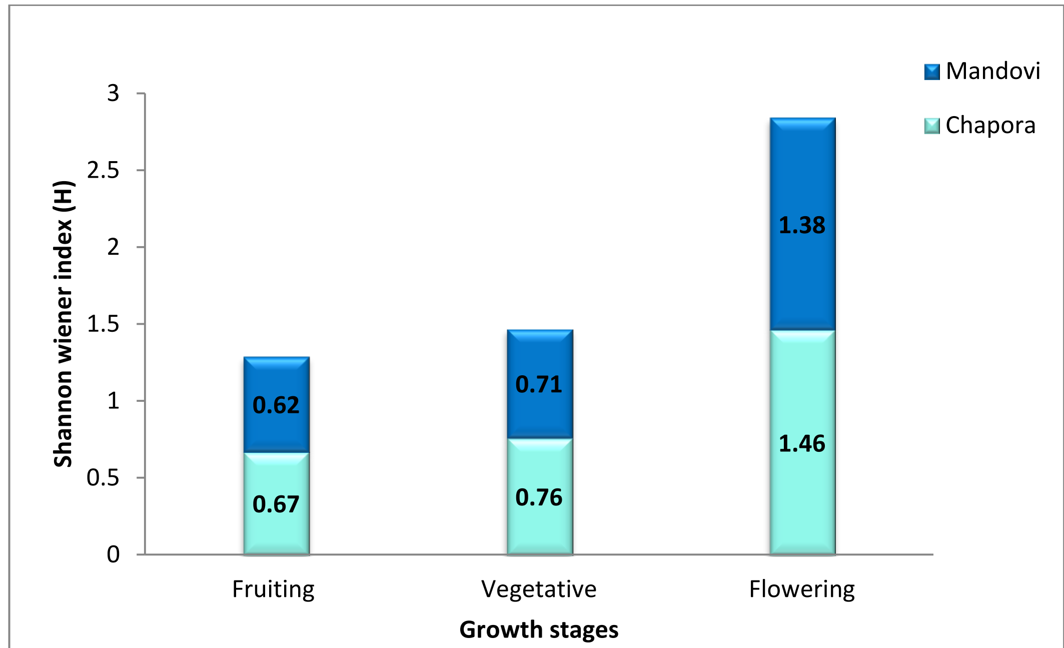


Fig. 24: Simpson's dominance index (D) in various growth stages.

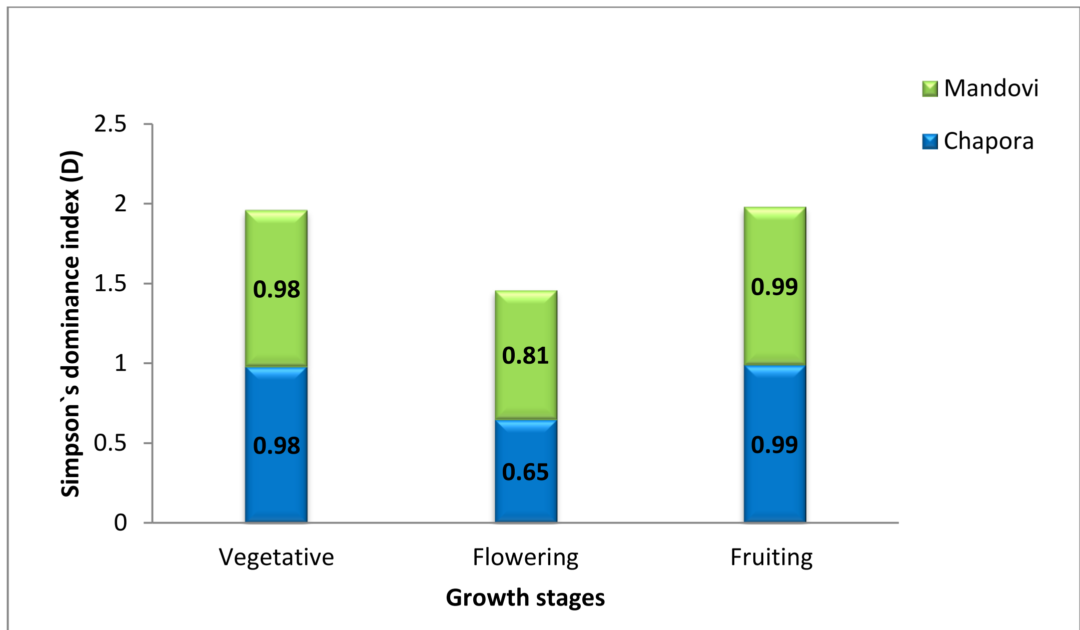


Fig. 25: Percent germination of spores in MSR media with and without sucrose after 26 days.

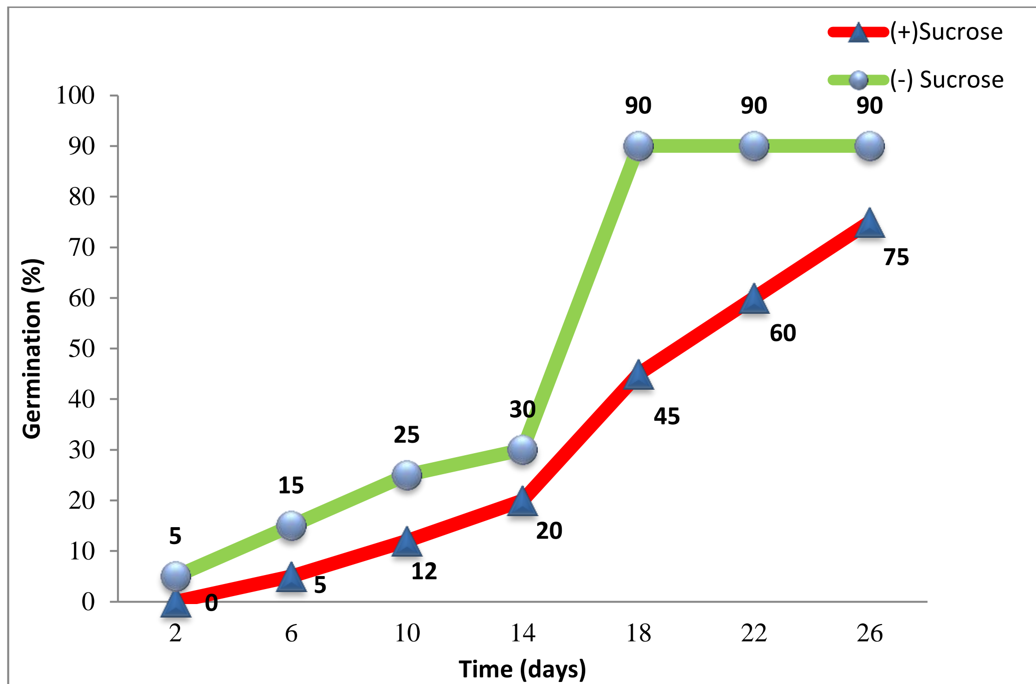


Fig. 26: Length of germ tube of germinating spore in MSR media with and without sucrose and after 26 days.

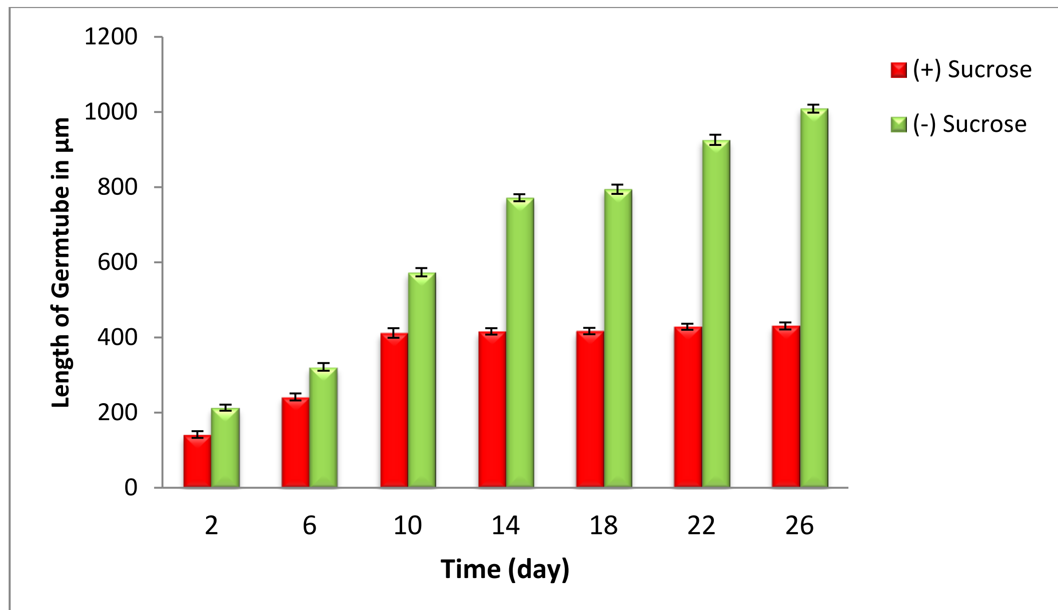
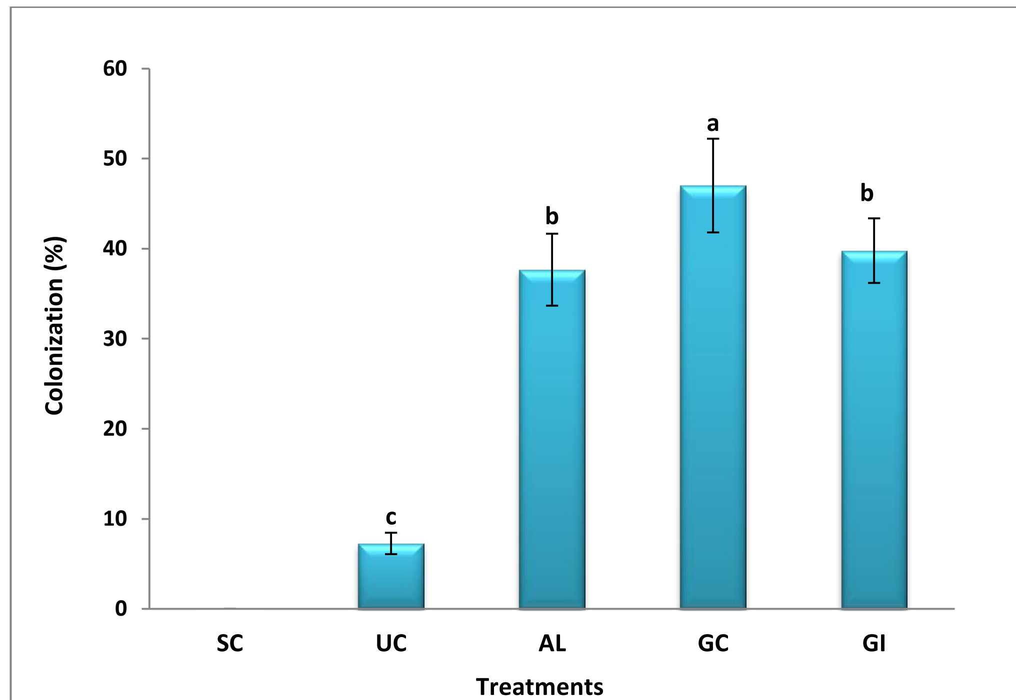
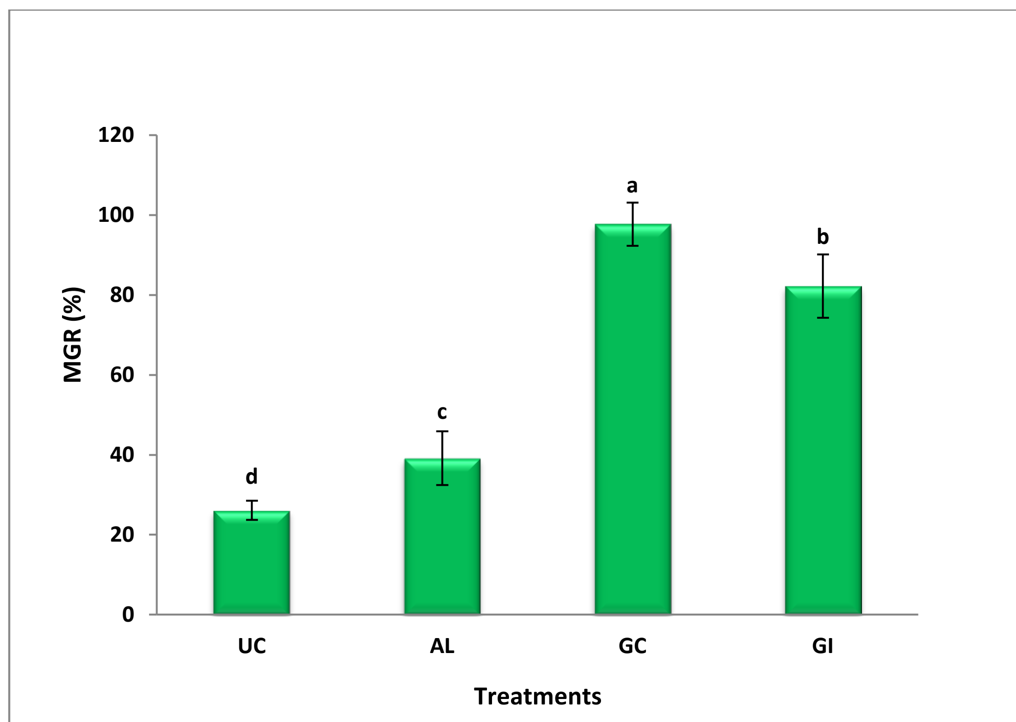


Fig. 27: Effect of AM fungal inoculation on root colonization in *Ceriops tagal* plants.



Legend: Bars followed by the different letters are significantly different at P ≤ 0.05 (n=5).

Fig. 28: Mycorrhizal Growth Responsiveness (%) in *Ceriops tagal* plants inoculated with different treatments.



Legend: Bars followed by the different letters are significantly different at P ≤ 0.05 (n=5)

APPENDIX

RESEARCH PAPERS PUBLISHED

- **D'Souza J** and Rodrigues BF. 2013. Biodiversity of Arbuscular mycorrhizal (AM) fungi in mangroves of Goa in West India. ***Journal of Forestry Research*** 24(3), 515-523. doi:10.1007/s11676-013-0342-0.
- **D'Souza J**, Rodrigues KM and Rodrigues BF. 2013. Modified Strullu and Romand (MSR) Medium Devoid of Sucrose Promotes Higher *in vitro* Germination in *Rhizophagus irregularis*. ***Journal of Mycology & Plant Pathology****,* 43(2), 240-242.
- **D'Souza J** and Rodrigues BF. 2013. Seasonal diversity of arbuscular mycorrhizal (AM) fungi in mangroves of Goa, India. ***International Journal of Biodiversity*** (Accepted, In Press) doi:10.1155/2013/196527.

- Radhika KP, **D'Souza J**, Rodrigues BF. 2012. Arbuscular Mycorrhizae in aquatic plants, India. ***In*****: Mycorrhiza: Occurrence in Natural and Restored Environments.** (*Ed.* Marcela Pagano) Nova Science, Inc, USA *pp*. 265-274. **(USA).**
- Muthukumar T, Radhika KP, Vaingankar J, **D'Souza J,** Dessai S, Rodrigues BF. 2009. Taxonomy of AM fungi - an update. ***In*****: Arbuscular Mycorrhizae of Goa – A Manual of Identification Protocols** (*Eds.* B. F. Rodrigues and T. Muthukumar). Goa University Publication. *pp.* 79- 115.

RESEARCH PAPERS PRESENTED:

- Presented a poster entitled **“Studies on Arbuscular mycorrhizal (AM) fungal in mangroves of Goa”** at XVII Southern Regional Conference on “Microbial Inoculants”, February 2010 at NIO, Dona Paula, Goa.
- Presented a paper entitled **“Establishment of *in vitro* culture of arbuscular mycorrhizal (AM) fungus *Glomus Clarum* using Ri T-DNA transformed *Cichorium intybus* L. (chicory) roots and vesicles as inoculums**” at the National Conference on “Mycodiversity with its Sustainable Exploration and Biotechnological Applications” and 38th Annual meeting of the Mycological Society of India, February 2012 at Shri Shivaji Science College, Amaravati, Maharashtra.