Diversity of Arbuscular Mycorrhizal fungi in selected grass species in the coastal sand dunes of Goa

A dissertation
Course code and Title: BOO- Diss: Dissertation
Credits-08
Submitted in partial fulfillment of Master's Degree

in Botany

by

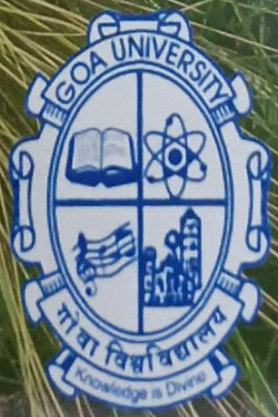
MS. NIVEDITA BABUSO PARWAR

21P048024

Under the Supervision of

PROF. B. F. RODRIGUES

School of Biological Sciences and Biotechnology
Botany Discipline



GOA UNIVERSITY
APRIL 2023

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DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation entitled, **"Diversity of Arbuscular Mycorrhizal (AM) fungi in selected grass species from the dunes of Goa"** is based on the results of investigations carried out by me in the Botany Discipline at the School of Biological Sciences and Biotechnology, Goa University under the Supervision of Prof. B.F. Rodrigues and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will not be responsible for the correctness of observations / experimental or other findings given in the dissertation. I hereby authorize the University authorities to upload this dissertation on the dissertation repository or anywhere else as the UGC regulations demand and make it available to any one as needed.

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Place: Goa University

COMPLETION CERTIFICATE

This is to certify that the dissertation **"Diversity of Arbuscular Mycorrhizal fungi in selected grass species from dunes of Goa"** is a bonafide work carried out by **Ms. Nivedita Babuso Parwar** under my supervision in partial fulfillment of the requirements for the award of the degree of Master of Science in the Discipline of Botany at the School of Biological Sciences and Biotechnology, Goa University.

Prof. B. F. Rodrigues
Botany Discipline

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DEDICATED TO MY PARENTS

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Nivedita Babuso Parwar

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INTRODUCTION

Coastlands are highly organized natural and ecologically sensitive marine habitats connecting terrestrial and marine ecosystems. This ecosystem undergoes a continuous transformation due to the geomorphological differences (Rodrigues and Rodrigues, 2022) and involves various microenvironments due to substrate mobility and physiological processes (Arun *et al.*, 1999). Ranwell (1972) considered the coastal dunes as a low nutrient stressful environment, making it unstable for economic and ecological importance. This extreme habitat involves various plant species, and microorganisms adapted to high salinity, low fertility, temperature fluctuation, drought, and an unstable sandy substrate (Sridhar *et al.*, 2001; Yamato *et al.*, 2012; Cui *et al.*, 2016).

Nicolson (1959) reported many different surveys conducted in temperate, sub-tropical, and tropical coastal dunes of the world. According to Sridhar (2009), coastal ecosystems show characteristic vegetation cover in tropical and temperate dunes. He reported that the members of Poaceae predominantly stabilize the temperate regions, whereas the members of Asteraceae, Convolvulaceae, Poaceae, and Leguminosae are dominant in tropical coastal dunes. Read (1989) suggested an interaction between the biotic and physiological properties of the sand controls the successional dune chronosequence of the plant community. The characteristic vegetation covers susceptible to such stressful habitat is exposed to various environmental factors such as temperature, desiccation, low moisture, retention, soil erosion, sand accretion, soil salinity, salt spray, variation in pH, and organic matter (Maun 1994). Woodhouse (1982) considered the natural dunes buffers protecting against storm tides, waves, and wind. The vegetation on the dunes plays a vital role in trapping and holding the sand grains blown up by the wind, thus leading to the formation and preservation of dunes and protecting the coastline (Desai and Untawale, 2002).

According to Gadgil (1993), few plant species would survive in stressful habitats when some salinity, shelter, and rudimentary organic matter are available. Besides, developing specialized anatomical and physiological characteristics in dune plants allows them to tolerate several edaphic and climatic challenges. Koske and Gemma (1997) reported not only the age and seasonal variation in the dune systems can cause a change in the plant species composition, but also an increase in organic matter, improved substrate stability, and nutrient enrichment can change the association of soil microorganisms with the succession. The loss of dune vegetation can naturally cause dune erosion. A large volume of sand is shifted by wave attack with a higher velocity, leading to a larger depression in the dunes. Additionally, the currents and the waves provide a balance in preventing dune erosion by supplying the sand from near the continental shelf to the beaches (Rodrigues and Rodrigues, 2022).

Sridhar *et al.* (2001) reported tropical dune vegetation such as sedges, shrubs, climbers, creepers, and tree species provide an opportunity to initiate revegetation, restoration, and stabilization of the coastline. Thus, mutualistic interaction and adaptation of biota to environmental stress can be better understood. *Ammophila arenaria* known as Marram grass, is native to the European coastal dune ecosystem (Sutton and Sheppard, 1976). Cockayne (1911) considered *A. arenaria* the most effective sand binder as it grows rapidly even under local conditions and is inexpensive to grow on a large scale. To prevent dune erosion, the grass species *Ammophila breviligulata* has been cultivated on the coast of Massachusetts since 1985. Also, he described that *Spinifex hirsutus*, *Scirpus frondorus*, and *Euphorbia glauca* are the major sand-binding species native to New Zealand which can effectively help in the wind-blown sand colonization and stabilizing process.

The survival rate of different plant species in an extreme coastal habitat mainly depends upon the mutualistic association of the plants with soil microorganisms such as AM fungi, rhizobia, and endophytes (Rodrigues and Rodrigues, 2022). The phylum Glomeromycota consists of a ubiquitous, obligatory soil-borne fungus known as Arbuscular Mycorrhizal (AM) Fungi (Redecker *et al.*, 2000) that form a symbiotic association with the majority of land plants (Smith and Read, 2008). Rodrigues and Rodrigues (2022) emphasized that these fungi develop two types of structural networks, *viz.*, intra-radical and extra-radical mycelial networks. The intra-radical structure (hyphae, arbuscles, and vesicles) is found in cortical cells whereas the extra-radical structures (hyphae and spores) of AM fungi are in the soil.

AM fungal associations are widely distributed biota among habitats such as aquatic, desert, coastal dunes, tropical rain forests, and canopy epiphytes (Sahay *et al.*,1998). In addition, the coastal dunes favour the occurrence of AM fungi mainly because of low phosphorus (P) content (Ranwell, 1972). Gemma and Koske (1989) suggested the AM fungal association in the dune greatly benefits the dune vegetation in the uptake of nutrients, mainly P, tolerance to salinity, reduction in abiotic stress, and formation of wind-resistant soil aggregates. It also helps the host plant to improve soil stability, binding, and water retention (Rillig and Mummey, 2006; Bedini *et al.,2009),* thereby contributing to the plant succession and stabilization process (Koske *et al.*, 1984). In addition, it increases the shelf life of feeder roots and improves the soil texture by increasing soil particle aggregation (Nasim, 2005). (Abe *et al.*, 1994; Yamato *et al.*, 2012) suggested that the gradients in plant community and soil chemical properties towards the sea play an important role in structuring the AM fungal communities.

There are several edaphic climatic factors susceptible to coastal dunes that may indirectly affect dune vegetation and AM fungal diversity. The major drawback faced by dune vegetation is the

deficiency of N, P, K, organic matter, and water availability (Maun, 1994), whereas Ca and Mg availability provides an adequate growth of dune vegetation (Vander Valk (1974). John *et al.* (1983) observed the associations of AM fungi with decaying organic matter since it serves as a major energy source. The increase in the level of organic matter is known to increase the hyphal growth of AM fungi in soil (Joner and Jakobson, 1995). In contrast, the activity of AM fungi in soil decreases with the decline in organic matter. In addition, it also facilitates the supply of P as a substrate for the acid and alkaline phosphatase of AM fungi (Sridhar, 2009).

The complexity increases N and P availability with mycorrhizal response to different plants on the dunes (Hoeksema *et al.,* 2010). Also, AM fungi have a significant role in N acquisition (Hodge *et al.,* 2010; Smith *et al.,* 2011) and Ca uptake, thereby helping in P and water uptake by the plants (Pai *et al.,* 1994). Usually, the dunes provide a low level of P to dune plants, so when a narrow depletion zone is created, available P is extracted by absorbing surface roots. The extra radical hyphae of AM fungi have the potential to cross this depletion zone and make P available to the plant (Koske, 1984).

Koske and Tews (1987) conducted comparative studies of AM flora on different dunes. Although AM fungi have a broader host range, maximum similarity in AM diversity was observed between Rhode Island and New Jersey to Virginia. It was concluded that the differences in the similarity might be because of responses of perennial beach grasses, AM fungi, and various other edaphic factors. According to Druva-lusite and Ievisnsh (2010), there is variability in the degree of root colonization depending on the plant species associated symbiotically with AM fungi. Further, the seasonal variation and stage of development of the host plant also show a greater impact on AM colonization and spore density. It was reported that AM colonization was lower in summer, whereas in autumn, they remained relatively constant

and escalated slowly from January to June during flowering. The highest AM fungal root colonization was reported in the plant species belonging to families Asteraceae, Papilionaceae, and Poaceae on the Italian dunes (Giovannetti, 1985).

Sutton and Sheppard (1976), reported abundant AM colonization in *Ammophilia arenaria,* a native grass species of Europe. Whereas, *Uniola paniculata* and *Panicum sp.* grown in a replenished area of Northern Florida showed a greater decline in root colonization and sporulation (Giovannetti, 1985). Nicolson *et al.* (1979) reported that the dune vegetation showed two peaks of root colonization from July and October to November in the fixed dunes of Scotland. Additionally, a greater count of arbuscles and vesicles was reported in moist and dry soils, respectively (Sridhar *et al.,* 2001).

AM fungal genera such as *Acaulospora, Gigaspora, Glomus,* and *Scutellospora* are most common worldwide in the coastal dune system (Maun, 2009). Mohankumar *et al.* (1988) surveyed 56 plant species along the coast of Chennai and reported the presence of *Entrophospora* and *Glomus sp.* Koske and Walker (1984) reported *Scutellospora erythropa* in the Bahamas, *Acaulospora scrobiculata,* and *Gigaspora albida* in North America. Also, *Glomus* species in Japanese dunes formed the most dominant AM fungal species in dune systems (Abe *et al.,* 1994). According to Maun (2009), different factors, *viz.,* season, host genotype, phenology, and environment, showed a greater variation in the spore densities. In Japan, coastal dune plant *Elymus mollis* showed AM spores up to a depth of 90-130 cm and found *Glomus* species to be most dominant whereas, in *Zoysia macrostachya* spores of *Acaulospora* sp. 1, *G. tortuosum, Glomus* sp. 1, *Glomus* sp. 2, *S. gregaria* and *Scutellospora* sp.1 were recovered (Abe *et al.,* 1994). The study also reported that the spore abundance increased from July and peaked in

December, with *Gigaspora gigantea* as the dominant AM species in the Atlantic coastal dune (Lee and Koske, 1994).

Rodríguez-Echeverría and Freitas (2006) demonstrated that the AM fungi associating with *A. arenaria were* more diverse in the well-preserved dunes than in the degraded dunes. Further, AM fungal diversity reported to be higher in the early plant successional dunes than in the intermediate and late successional dunes (Sikes et al. 2012). Sea oats planted on Florida beaches showed high AM fungal colonization, fungi, and bacteria than in vacant or recently planted beach sites (Sylvia and Will, 1988). Rhizomatous perennial grass, *Spartina ciliata,* in fixed CSDs of Brazil, dominated due to high AM fungal diversity (Sturmer and Bellei, 1994). The successful growth of dune plant species depends on AM fungi in soils (Gemma and Koske, 1997). Artificial inoculation of AM fungi (e.g., *Gigaspora gigantea*) enhanced the transplanting success of *Ammophila* sp. and elevated AM fungal species diversity and spore density (Koske and Halvorson, 1981). Inoculation of *Glomus macrocarpum* and *G. fasciculatum* stimulated beach grass growth in Scotland's unstable foredunes (Sylvia, 1986). The Association of *Uniola paniculata* (sea oats) with AM fungi decreased the environmental stresses and resulted in dune stability (Sridhar 2006).

The seasonal variation and the stage of development of the host plant are two main factors in determining the mycorrhizal colonization and differences in the spore density (Bouamri 2014). Several studies have been emphasized by (Koske and Halvorson, 1981) on the variability in spore densities in the soil and the presence of an endophyte species associated with particular host plants. Several other reports emphasize based upon the interactions between individual fungal species on the same host plant (Koske 1981). Usually, comparative studies of the spore density from different study sites become easy to understand based upon the variation in the

season than that of locality, host plant, soil characteristics, and other edaphic factors. Koske (1981) reported applying different sampling methods to understand AM fungal diversity that provided a close relationship when samples were collected from the same plant species over a year from the same study sites. On the other hand, the findings did not show any correlation when sampled from different plant species in different study sites. According to Giovannetti (1985), *Ammophilia arenaria* showed the highest root colonization and spore density in May-June, whereas it showed lower root colonization and spore density the rest of the year.

Nicolson and Johnston (1979) reported increased AM fungal colonization in September-October and June-July. Read *et.al* (1976) reported higher colonization during the winter season. The drastic decline in root colonization was observed during summer due to dry edaphic conditions that affect the vegetative growth of the host plant (Giovannetti 1985). Relatively the spore number may increase due to the accumulation of organic matter, dead insects, or nematodes (Nicolson and Johnston, 1979; Koske, Sutton, and Sheppard, 1975; Nicolson, 1960).

Contrarily, it is difficult to determine and distinguish the influence of the host plant's seasonal variation and developmental stages on mycorrhizal colonization and spore density if the plant species have a simultaneous growth stage (Bouamri *et.al* 2014).Therefore, in the current scenario, it creates a problem to compare the seasonal effects from effects related to host developmental stages and intermittent growth of roots (Moose, 1973).

Coastal regions of India lie between 7^0 to 24^0 N latitude and 70^0 to 94^0 E longitude, bounded by the Arabian Sea on the west and the Bay of Bengal on the east. The west is characterized by sandy beaches backed by dunes, cliffs, promontories, and drowned estuaries whereas the east coast exhibits a sequence of delta formation (Mascarenhas, 1998). The littoral areas of India constitute the states *viz.*, Gujarat, Maharashtra, Goa, Karnataka, Kerala, Tamil Nadu, Andhra

Pradesh, Orissa, and West Bengal, the group of Islands of Lakshadweep (Arabian Sea), and Andaman and Nicobar (Bay of Bengal).

Goa is a small state on the southern coast of India within the Konkan region and geographically separated from the Deccan highlands by the Western Ghats. It encompasses an area of 3,702 km^2 and lies between 14^0 53'54''N and 15^0 40'00''N and longitude 73^0 40'33''E and 74^0 20'13''(*en.m.wikipedia.org*).

This study aims to understand the effect of seasonal variation on the diversity of AM fungi in different grass species from the selected coastal dunes of Goa.

The main objectives of this study are:

1. To conduct a preliminary survey at the study site and to analyze different grass species.
2. To determine AM fungal root colonization, spore density, species richness, and relative abundance in different grass species at the study sites.
3. To isolate and prepare the trap cultures.
4. To identify AM fungal species from the rhizosphere of dune soils.
5. To study the effect of seasonal variation on AM fungal diversity.

REVIEW OF LITERATURE

Nicolson (1960) reported that dune grasses show more complexity with respect to AM colonization in their dune system with the rapid increase in AM activity from the fore dunes to recently fixed dunes.

Koske and Gemma (1997) emphasized with improvement in substrate stability, nutrient enrichment, and an increase in organic matter, the composition of plant species greatly differs with seasonal variation and age of the dune system. Also, the association with soil microorganisms changes with succession.

Yamato *et al.* (2012) considered that environmental factors such as variations in salinity and temperature and low infertility make the coastal land more stressful. Therefore, coastal vegetation also gets adapted to such an environment. A transition in coastal plant species was observed in response to environment gradient with the distance from the sea; thereby, the more close the dune vegetation to the sea side more will be stress condition.

Beena *et.al* (2000 a), carried out two-year seasonal study in *Ipomea pes-caprae* on the coastal sand dunes of West coast of India. It was reported that the percent AM root colonization significantly decreased during Monsoon and increase greatly during Post monsoon with least mean of spore density whereas , the summer showed highest mean of spore density. *Glomus* was found to be most common genera with mean species richness being highest in *Ipomea pes-caprea*.

(https://www.ehp.qld.gov.au/coastal/ecology/beaches-dunes/coastal_dunes .html), reported because of presence of specialized structures like waxy coating on the stem and leaves and well developed spreading root system in pioneer plants such as *Spinifex littoreus*, *Ipomea pes-caprae*

etc. provide greater capacity to withstand extreme condition colonized areas exposed to salt spray, sandblast, strong wind and flooded by sea.

Desai (1995), surveyed on the Coastal sand dunes of Goa and found *Ipomea pes-caprae* and *Spinifex littoreus* to be the most dominant plant species growing on the Coastal sand dunes of Goa. It was also reported that *Spinifex littoreus* to most successful trapping plant as it has an ability to grow through accumulation of wind blow plant and also the presence of spiny and rigid leaves that does not permit grazing.

Sridhar (2009), emphasizes greatly on the coastal sand dune vegetation with AM fungi association as the rate of equilibrium of both plant and productivity adversely decreases with the elimination of AM fungi. It was reported that plant species belonging to poaceae were dominant in temperate dune whereas the plant species belonging to Asteraceae, Convolvulaceae, Fabacea and Poaceae were dominant in tropical dunes.

Koske and Tews (1987), carried out comparable studies of AM flora on different sand dunes. Although, AM fungi have a broader host range but maximum similarity among AM diversity were observed between the Rhode Island and New Jersey to Virginia, it was concluded that the differences in the similarity may be because responses of perennial beach grasses, AM fungi and various other edaphic factors.

Koske and Gemma (1997) reported not only age and seasonal variation in the dune systems can cause a change in the plant species composition but also increase in organic matter, improved substrate stability, and nutrient enrichment can also change the association with soil microorganisms with the succession.

MATERIALS AND METHODS

Study Sites

The study was conducted along the coastline of Goa from north to south in the months of June to March 2022 to assess the AM fungal diversity in grass species growing on the dunes. In all, ten coastal sites were selected, five each from north and south Goa. The study sites selected for the study from north Goa include Mandrem, Ashvem, Morjim, Candolim, and Siridao, while the study sites selected from south Goa include Colva, Arossim, Cavelossim, Galgibag, and Rajbag. The grass species selected from the various dune sites were collected and identified using relevant floras. The selected grass species were photographed at the sites.

Sample Collection

The roots samples of selected grass species from the different study sites were collected to assess the AM diversity. The rhizosphere soil samples were collected to quantity the AM fungal spore density and species richness, and also to carry out the soil analysis. The roots and rhizosphere soil were placed in *ziploc* bags, labeled and brought to the laboratory.

Soil Chemical Analysis

The soil sample was air-dried and later sieved to remove larger soil particles and other debris. soil analysis was carried out at Directorate of Agriculture, Soil Testing Laboratory (Ela Farm), Old Goa. Various physio-chemical properties such as soil pH, EC, OC, N, P, K, B, and S content in soil were determined.

Soil pH was analyzed using a pH meter and Electrical Conductivity (EC) using a Conductivity meter. The organic Carbon (OC) was determined by Walkley and Black Titration Method (Walkley and Black, 1934). Nitrogen (N) was analyzed by Aerobic Incubation Method (Keeney

and Bremner, 1966). Phosphorus (P) content was estimated using Bray and Kurtz method (Bray and Kurtz, 1945) and available Potassium (K) content by using Ammonium acetate method (Hanway and Heidal, 1952). The available micro-nutrients *viz.*, Boron (B) was estimated by using Azomethine-H method (Gupta ,1979) and Sulphur (S) by Turbidimetric method (Chesnin and Yien, 1950).

Estimation of AM Fungal Root Colonization

Quantification of AM fungi colonization was carried out using Trypan blue staining technique (Phillips and Hayman, 1970). The roots were gently washed with tap water several times to remove the attached soil particles and then cut the roots into 1 cm segments. The cleaned root segments were then cleared in 10% KOH by heating at 90^0C for one hour in the oven. Later, the roots were rinsed thoroughly in water, acidified with 5N HCl for 5 minutes, and kept overnight in 0.05% Trypan blue stain. The root segments were then mounted in PVLG and observed under a Nikon microscope.

Presence or absence of AM hyphae, vesicles, arbuscles, hyphal coils and hyphal swelling were recorded. Microscopic photographs were taken by using Olympus DP 12-2 and Nikon Eclipse E200 digital camera.

Estimation of Root Colonization

The estimation of AM root colonization was determined using Root Slide Method (Read *et al.*,1976). The root segments stained in Trypan blue were mounted in PVLG (Polyvinyl- lacto-glycerol) and scored for the presence or absence of AM structures.

The percent root colonization was calculated using the formula:

$$\textbf{Percent Colonization} = \frac{\textbf{No. of root segments colonized}}{\textbf{Total no. of root segments observed}} \times 100$$

Extraction of the AM fungal spores

Isolation of AM fungal spore was done using the Wet Sieving and Decanting technique (Gerdemann and Nicolson, 1963). 100g of rhizosphere soil was taken in a beaker and 1000 ml of water was added to make a suspension. This suspension was then stirred with a glass rod for 1 min and then allowed to settle. Without disturbing the sediment layer settled at the bottom, the supernatant was slowly decanted through a number of sieves arranged in the descending order (200µm, 140µm, 120µm, 100µm, 60µm). The process was repeated twice. The residue from each sieve was collected in separate beakers, and aliquot was filtered separately using Whatman No.1 filter paper. The filter paper was then placed in a Petri plate and then examined for AM fungal spores using a stereomicroscope (*Olympus* SZ16).

Estimation of AM Fungal Spore Density

AM fungal spore density was estimated by using the modified method of Gaur and Adholeya, (1994). The spore density was assessed per 100g of the soil sample.

Taxonomic Identification of Spores

Morphological characters play a key role in understanding the taxonomical relationship between the AM fungal species. The intact, living spores were mounted on slide with PVLG. Depending upon the morphological characters such as colour, shape, size, ornamentation, wall layers,

auxiliary cells and shape of germination shield, AM spores were identified at the species level. Taxonomical identification of AM fungal species was carried by using the relevant bibliographies (Rodrigues and Muthukumar, 2009, INVAM).

Estimation of AM species Richness, Spore Density and Relative Abundance

The AM fungal spore density was estimated by using the method of Gaur and Adholeya, (1994). While, the Relative abundance of AM fungi was estimated using the formula described by Beena *et al*., (2000).

Species Richness: Species no. per 100g soil sample.

Spore abundance: Number of spores of a particular species per 100g of soil sample.

Spore density: Total no. of spores per 100g soil sample.

$$\textbf{Relative abundance (RA \%)} = \frac{\text{No. of spores of a particular species}}{\text{Total no. of spores}} \times 100$$

Sterilization of Pots and Sand

The pots used for trap culture were washed with soap and rinsed thoroughly under tap water. Then, the pots were wiped using absorbent cotton dipped in absolute alcohol. The soil used for potting was sterilized in a hot air oven at 180° C for two consecutive days.

Preparation of Trap cultures

Sterilized pots were filled with a mixture of rhizosphere soil collected from the host plant (containing viable AM propagules) and sterilized sand in a 1:1 ratio. The seeds of Ragi (*Eleusine*

coracana) were sowed in the pots. The pots were maintained at 28^0C for 90 days in the polyhouse. The pots were watered at regular intervals. After 90 days watering was stopped and the plants were left for drying. AM spores were then examined by isolating them using wet sieving and decanting method.

RESULTS AND DISCUSSION

The present study was carried out to study the AM fungal diversity in the different grass species growing on the dunes at various sites in south and north Goa. The study revealed the presence of 11 grass species belonging to 6 genera and 2 families at the selected sites of Goa.

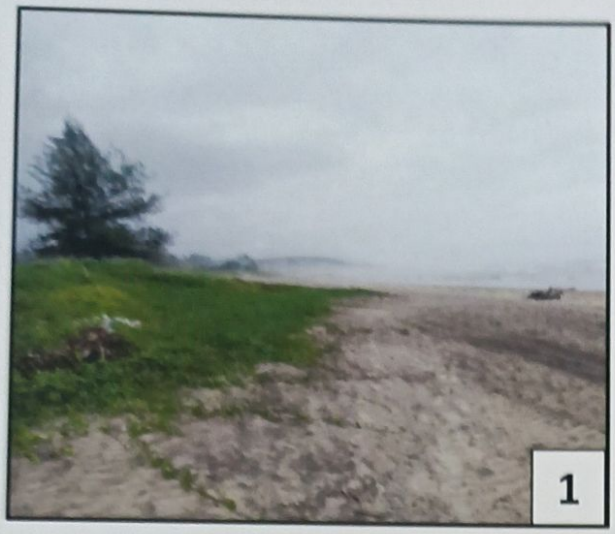
Both Poaceae and Cyperaceae family found to be most common on the coastal regions **(Plate 1 and 2).** Of these two families, members of Cyperaceae were dominant in north Goa whereas the members of family Poaceae were found in both the study areas **(Plate 3,4, and 5) (Table 1 & 2).**

Table 1: Diversity of grass species recorded in north Goa dune sites.

Sr. No	Family	Grass species	Common Name	Sites
1.	Cyperaceae	*Cyperus capitatus*	Sedge	II, IV
		Cyperus rotundus	Nutgrass	III, IV
		Cyperus sp.	Sedge	IV
		Cyperus esculentus	Yellow nutsedge	V
2.	Poaceae	*Spinifex littoreus*	Ravan's Moustache	I
		Paspalum sp.	Bahia grass	I
		Digitaria longifera	Crab grass	II
		Sporobolus virgincus	Seashore dropseed	II,III, V
		Cynodon dactylon	Bermuda grass	V

Table 2: Diversity of grass species recorded in south Goa dune sites.

Sr. No	Family	Grass species	Common Name	Sites
1.	Cyperaceae	*Cyperus esculentus*	Yellow nutsedge	I, III, IV, V
		Cyperus capitatus	Sedge	II
2.	Poaceae	*Cynodon dactylon*	Bermuda grass	I, III, IV, V
		Paspalum sp.	Bahia grass	I, II
		Sporobolus virgincus	Seashore dropseed	III, IV
		Paspalum dilatatum	Dallis grass	V



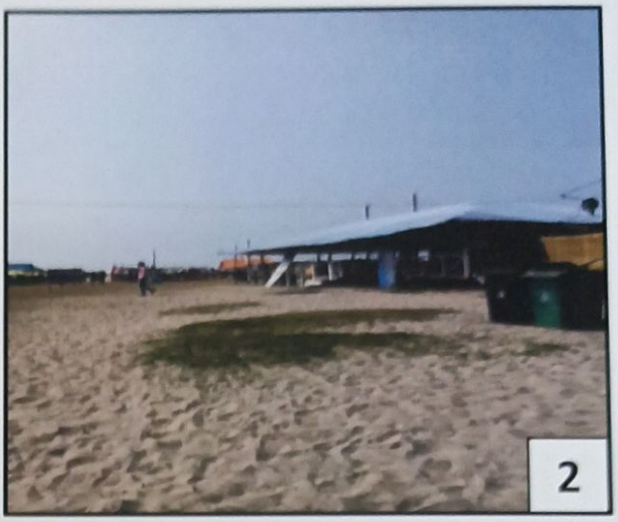
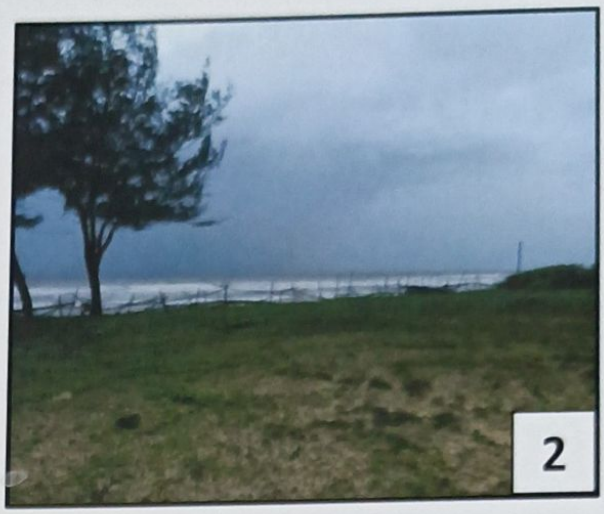
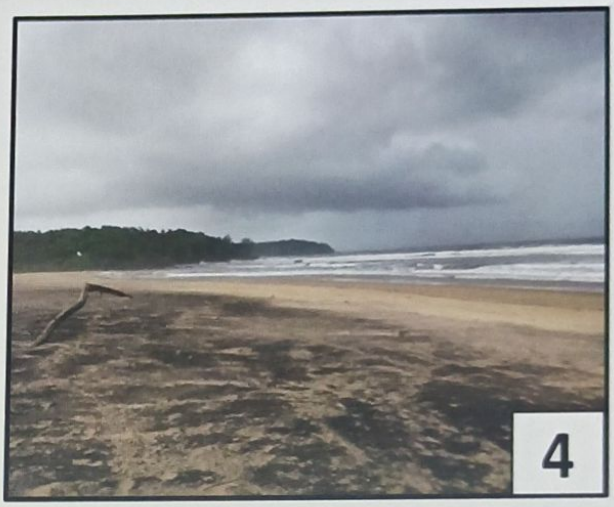


Plate 1: Study sites in north Goa: 1. Mandrem, 2. Morjim, 3. Ashvem, 4. Candolim, 5. Siridao.





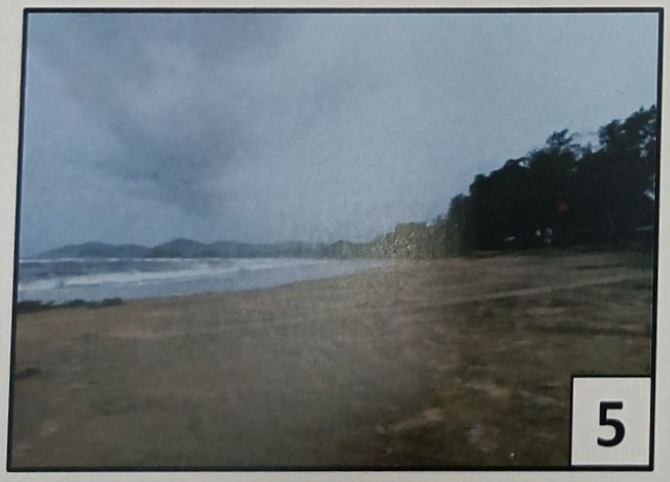


PLATE 2: Study sites in south Goa.

1.Colva, 2. Cavelossim, 3. Arossim, 4. Galgibag, 5. Rajbag

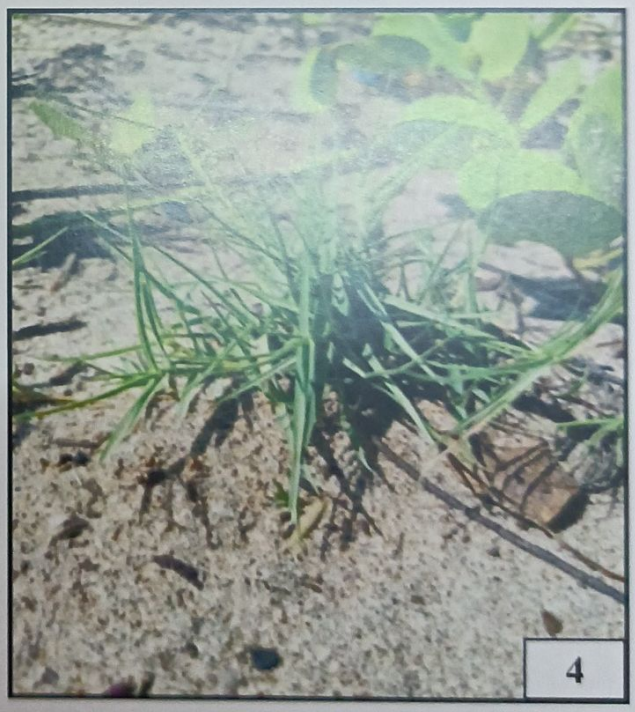
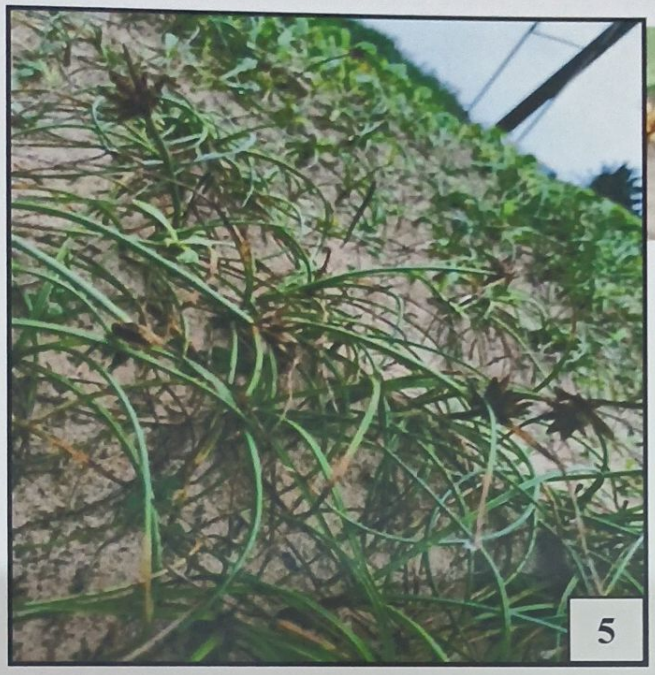


PLATE 3: Grass species at the study sites: 1. *Spinifex littoreus,* 2. *Paspalum* sp., 3.*Digitaria longifera,* 4. *Sporobolus virgincus.*



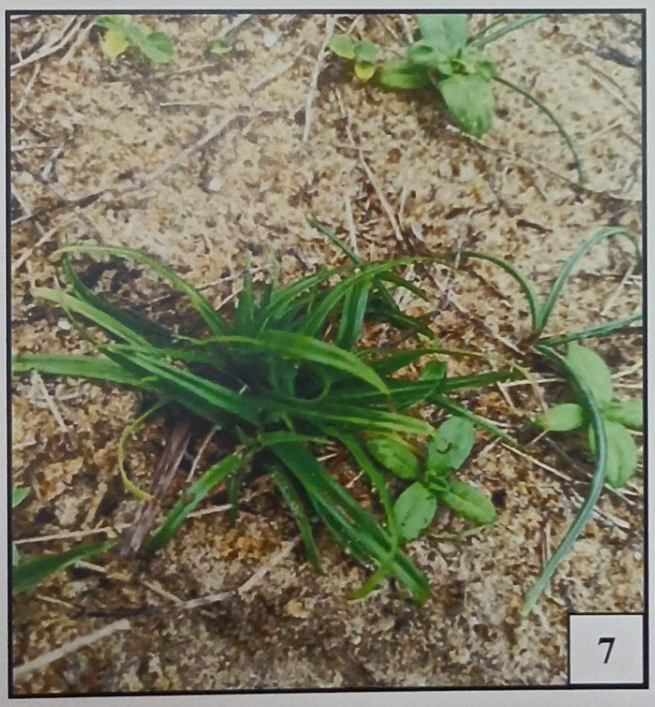


PLATE 4:
5. *Cyperus capitatus* 6,7. *Cyperus rotundus* 8.*Cyperus sp.*

PLATE 5:
9.*Cynodon dactylon* , 10. *Cyperus esculentus* , 11. *Paspalum dilatatum*

Soil Analysis:

The physio-chemical properties play a crucial role in understanding complications related to several edaphic factors for the establishment of perennial grasses and other vegetation on the stressful ecosystem (Read 1989).

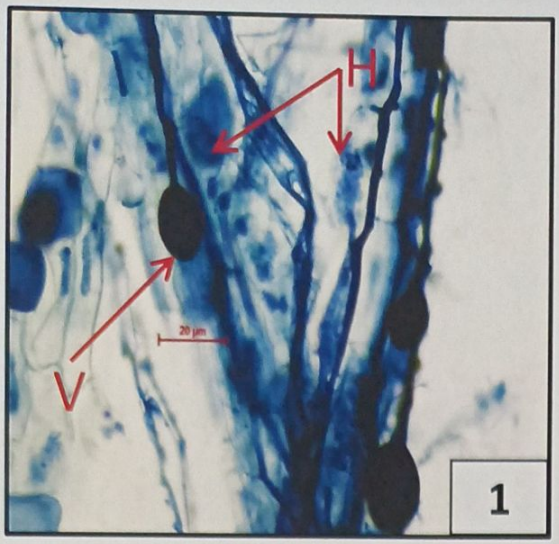
The analysis of soil revealed that pH of the soil samples was alkaline. The pH of sand ranged from 8.0 to 8.8 in north Goa while it varied from 7.7 to 8.7 in south Goa. The Electrical Conductivity (EC) and Organic carbon of soils was less except the site Galgibag recorded higher salinity. The macro- and micro-nutrient levels varied at the different study sites (**Table 3 and 4).**

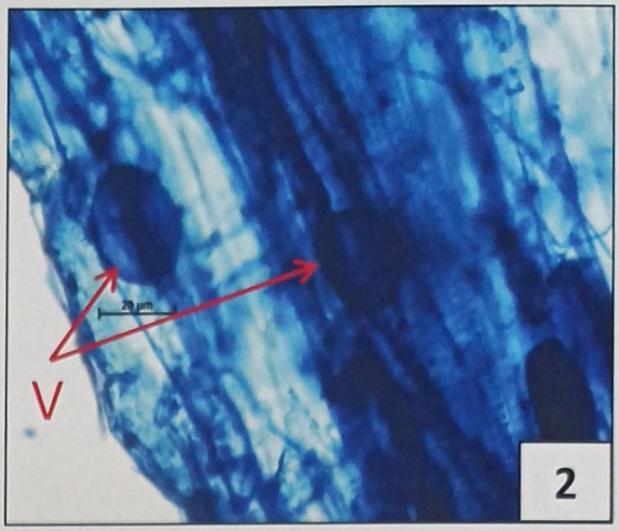
Table 3: Physio-chemical analysis of dune soils at north Goa.

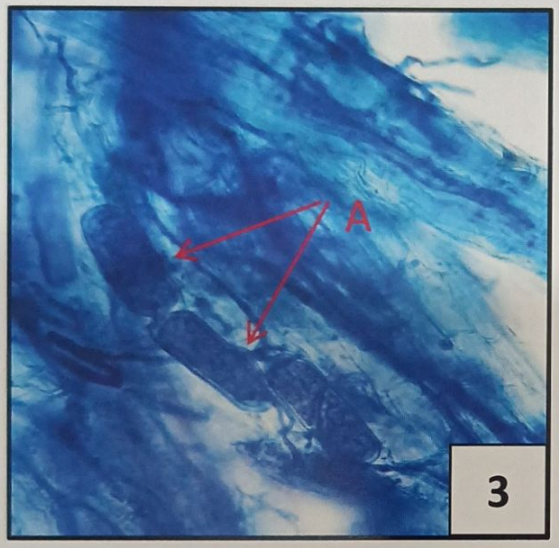
Study Sites	North Goa						
	Parameters						
	pH	EC (m. mhos/ cm)	OC (%)	P (Kg/ha)	K (Kg/ha)	B (ppm)	S (ppm)
I	8.8±0.20	0.17±0.01	0.19±0.02	20.60±0.20	160.80±0.21	0.57±0.01	3±0.0
II	8.6±0.10	0.07±0.00	0.07±0.00	27.50±0.08	134.40±0.11	0.57±0.00	2±0.0
III	8.7±0.03	0.18±0.00	0.19±0.01	23.70±0.120	282.20±0.05	0.43±0.00	17±0.33
IV	8.3±0.12	0.17±0.01	0.31±0.04	224.60±20.3	107.50±62.00	0.42±0.04	Traces
V	8.8±0.03	0.22±0.01	0.29±0.03	18.70±0.57	147.80±44.80	0.66±0.02	Traces

Legend: All the values are mean of 3 readings, ±: Standard deviations; E.C: Electrical Conductivity; OC= Organic carbon, P=Phosphorus, K= Potassium, B=Boron, S=Sulphur.

AM fungal colonization during the different seasons was recorded in the roots of all the selected grass species **(Table 5 and Table 6)**. Root colonization recorded in the presence of hyphae, vesicles, arbuscules, hyphal coils and hyphal swellings **(Plate 6).**







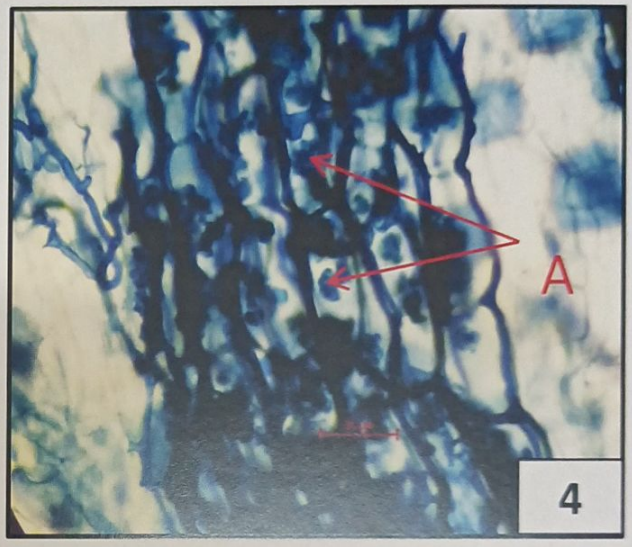


PLATE 6: AM fungal colonization in roots of different grass species : 1.Hyphae (H) and vesicles (V), 2. Vesicles (V) 3 & 4. Arbuscules (A), 5. Hyphal coils (HC) 6. Hyphal swelling (HS).

Table 4: Physio-chemical analysis of dune soils at north Goa.

Study Sites	South Goa						
	Parameters						
	pH	EC (m. mhos/ cm)	OC (%)	P (Kg/ha)	K (kg/ha)	B (ppm)	S (ppm)
VI	7.80±2.40	0.19±0.15	0.28±0.20	143.80±4.20	228.40±1.20	0.49±0.50	8.00±0.00
VII	8.60±0.05	0.11±0.00	0.13±0.01	81.80±0.24	120.90±0.23	0.54±0.00	1.00±0.00
VIII	8.20±0.17	0.12±0.10	0.15±0.12	78.20±11.00	115.60±1.50	0.44±0.10	1.00±0.00
IX	7.70±0.03	2.24±0.00	0.77±0.01	40.92±0.12	120.90±0.12	0.36±0.00	30.00±0.30
X	8.70±0.11	0.17±0.00	0.13±0.00	16.70±0.14	120.90±0.12	0.65±0.00	0.50±0.030

Legend: All the values are mean of 3 readings, ±: Standard deviations; E.C: Electrical Conductivity; OC= Organic carbon, P=Phosphorus, K= Potassium, B=Boron, S=Sulphur.

Seasonal variation recorded 100% root colonization during monsoon (June - August) and post-monsoon (October-December) in north Goa. Whereas, maximum root colonization was recorded during post-monsoon followed by pre-monsoon (January - March) and monsoon seasons in south Goa.

During the monsoon season, maximum (100%) root colonization was recorded in *Cyperus rotundus* and *Cynodon dactylon* in north Goa. While in south Goa maximum root colonization was recorded in *Paspalum sp.* (85%). Minimum root colonization was recorded in *Sporobolus virgincus* (60%) in north Goa. In south Goa, minimum root colonization was recorded in *C. esculentus* (20%).

During the post-monsoon season, 100% root colonization was recorded in *Spinifex littoreus* and *S. virgincus* in north Goa, while in south Goa, *Paspalum* sp., *C. capitatus*, and *C. esculentus*

Table 5: AM fungal root colonization and spore density in grass species from coastal dunes of North Goa.

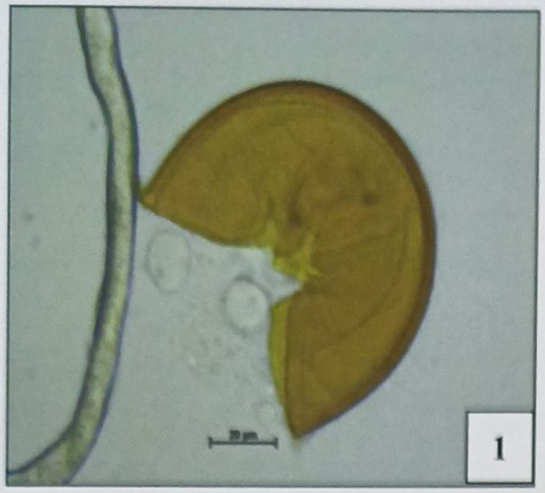
Sr. No.	Site	Plant species	Monsoon		Post monsoon		Pre monsoon	
			RC	SD	RC	SD	RC	SD
1	I	*Spinifex littoreus*	95.00±0.57	63.00±2.08	100.00±1.53	15.00±2.88	90.00±1.15	56.00±2.90
		Paspalum sp.	ND	ND	ND	ND	70.00±1.73	56.00±2.60
2	II	*Digiteria longifera*	75.00±2.00	64.00±4.04	ND	ND	ND	ND
		Cyperus capitatus	80.00±1.52	90.00±2.90	70.00±0.57	83.00±3.28	70.00±0.57	30.00±2.80
		Sporobolus virgincus	ND	ND	100.00±1.73	52.00±0.0	95.00±2.08	40.00±3.52
3	III	*Sporobolus Virgincus*	60.00±2.52	90.00±3.28	40.00±1.00	0.0±0.0	30.00±1.53	3.00±0.88
		Cyperus rotundus	100.00±2.64	80.00±4.70	25.00±1.15	0.0±0.0	60.00±1.15	3.00±0.33
4	IV	*Cyperus capitatus*	65.00±2.08	184.00±14.5	70.00±1.15	30.00±1.45	75.00±2.51	11.00±1.76
		Cyperus rotundus	60.00±2.00	96.00±4.81	80.00±1.53	59.00±0.20	70.00±1.52	9±0.50
		Cyperus sp.	90.00±1.53	9.00±0.88	ND	ND	ND	ND
5	V	*Cynodon dactylon*	100.00±1.00	0.00±0.0	90.00±0.00	77.00±3.48	ND	ND
		Cyperus esculentus	80.00±0.57	79.00±4.51	75.00±2.31	28.00±1.20	85.00±0.0	60.00±7.67
		Sporobolus virgincus	ND	ND	100±0.57	3.00±1.85	ND	ND

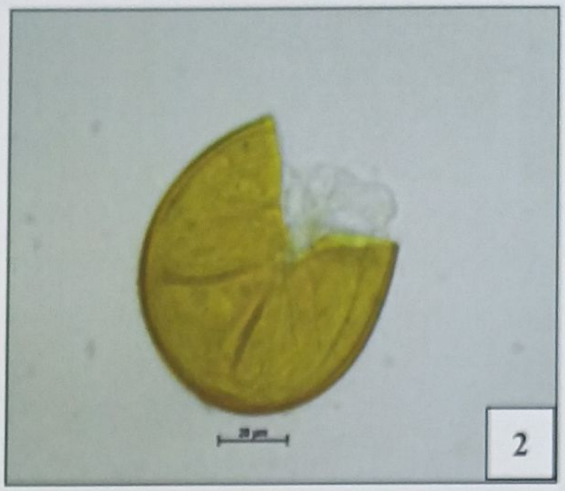
Legend: RC = Root colonization; SD = Spore density; all values are mean of three reading; ± = Standard deviation; **Study sites:** I – Mandrem; II – Morjim, III – Ashvem; IV – Candolim; V - Siridao.

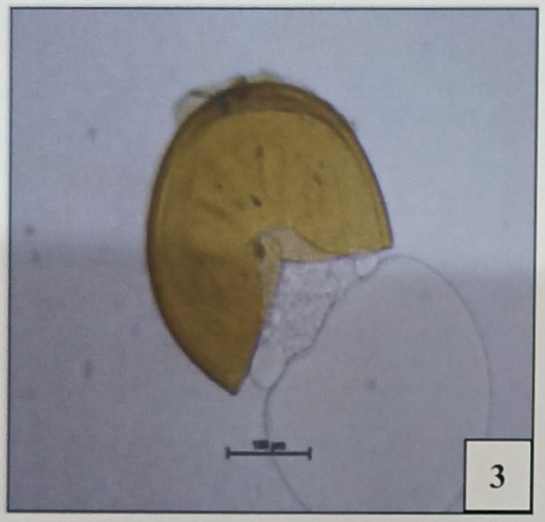
Table 6: AM fungal root colonization and spore density in grass species from dunes of South Goa.

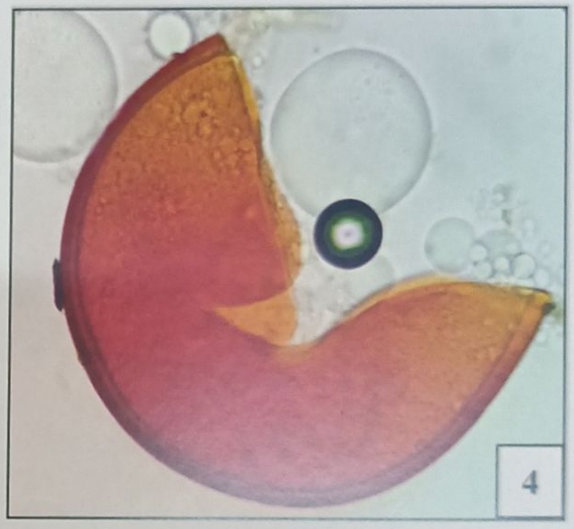
Sr. No.	Site	Plant species	Monsoon		Post monsoon		Pre monsoon	
			RC	SD	RC	SD	RC	SD
1.	VI	*Cyperus esculentus*	55.00±0.58	135.00±10.14	ND	ND	ND	ND
		Cynodon dactylon	40.00±1.52	50.00±4.45	70.00±2.52	39.00±3.60	80.00±1.53	40.00±3.79
		Paspalum sp.	ND	ND	ND	ND	70.00±1.00	30.00±4.41
2.	VII	*Cyperus capitatus*	75.00±1.73	27.00±2.96	100.00±0.57	61.00±2.31	75.00±1.52	85.00±2.33
		Paspalum sp.	85.00±1.00	125.00±9.27	100.00±1.00	46.00±1.33	95.00±2.51	50.00±2.89
3.	VIII	*Cynodon dactylon*	75.00±2.52	64.00±4.16	ND	ND	ND	ND
		Sporobolus virgincus	ND	ND	85.1.53	64.00±4.10	60.00±3.00	20.00±2.79
		Cyperus esculentus	ND	ND	ND	ND	35.00±2.64	3.00±0.58
4.	IX	*Cyperus esculentus*	20.00±1.00	9.00±1.15	70.00±1.53	36.00±2.60	75.00±1.53	40.00±2.87
		Cynodon dactylon	35.00±2.00	10.00±1.53	ND	ND	ND	ND
		Sporobolus virgincus	ND	ND	35.00±0.57	7.00±1.45	35.00±3.21	3.00±0.33
5.	X	*Cyperus esculentus*	80.00±1.15	62.00±3.71	90.00±0.58	36.00±2.60	ND	ND
		Paspalum dilatatum	25.00±2.00	72.00±2.96	75.00±1.15	30.00±6.00	80.00±1.00	20.00±2.08
		Cynodon dactylon	30.00±2.64	22.00±1.45	75.00±1.79	70.00±2.8	75.00±2.08	50.00±1.45

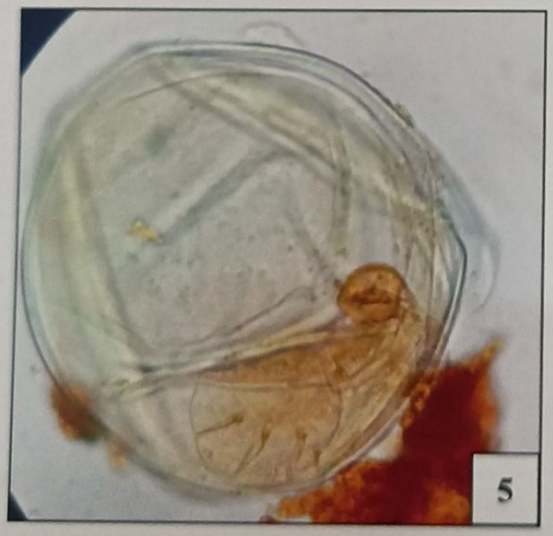
Legend: RC = Root colonization; SD = Spore density; all values are mean of three reading; ± = Standard deviation; **Study sites:** VI – Colva; VII – Cavelossim, VIII – Arossim, IX – Galgibag, X - Rajbag.











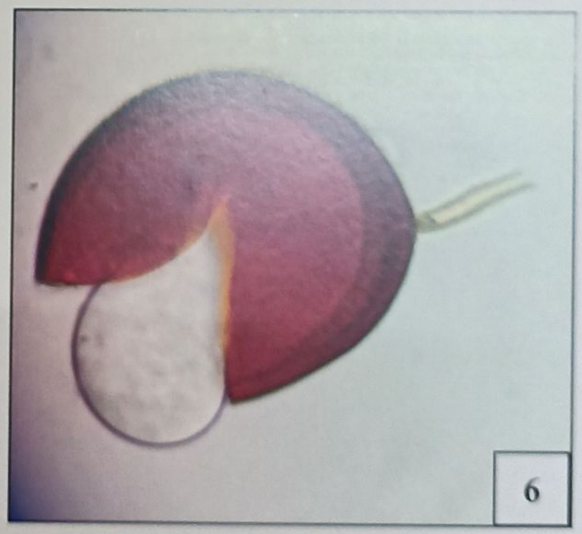
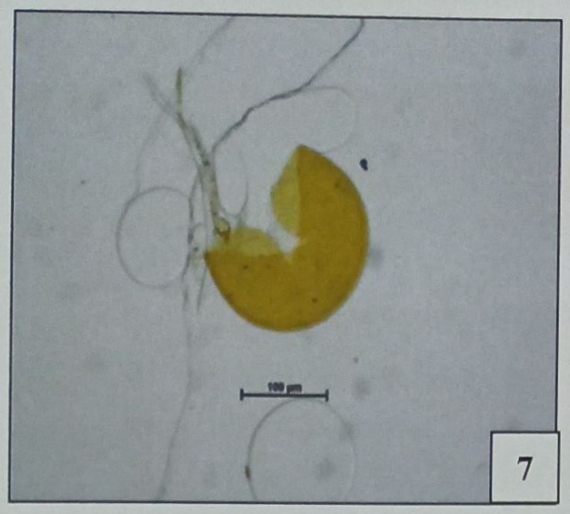


PLATE 7: 1 & 2. *Acaulospora spinosa,* 3. *Acaulospora scrobiculata,* 4. *Acaulospora* sp.1, 5. *Dentiscutata heterogama,* 6. *Glomus geosporum.*



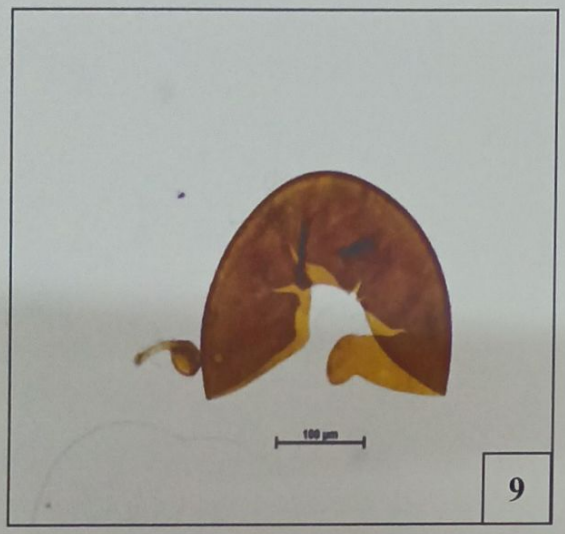


PLATE 8:
7. *Gigaspora decipiens,* 8. *Gigaspora* sp.3, 9&10. *Gigaspora* sp.1
11. Spore in spore syndrome

PLATE 9:

12. Sporocarp of *Glomus* sp.
13. Sporocarp of *Glomus* sp. showing subtending hyphae
14. Spore of *Glomus* sp.

recorded maximum root colonization ranging from 90-100%. Minimum root colonization (25-40%) was recorded in *S. virgincus* and *C. rotundus* in north Goa, while minimum root colonization was recorded in *S. virgincus* (35%) in south Goa.

In the pre-monsoon season, maximum root colonization ranging from 85-95% was recorded in *S. littoreus, C. esculentus, and S. virgincus* in north Goa, it was maximum (80-95%) in *P. diltatum, C. dactylon and Paspalum sp.* in south Goa. Minimum root colonization was recorded in *S. virgincus* (30%) in north Goa and 35% *in S. virgincus* and *C. esculentus* in south Goa.

14 AM fungal species belonging to four genera *viz., Acaulospora , Dentiscutata, Gigaspora,* and *Glomus* were recovered from the rhizosphere soils of the selected study sites **(Plate 7,8 and 9)**. The present study revealed that the genus *Gigaspora* (3 spp.) was dominant due to alkaline nature of the soil followed by *Acaulospora* (5 spp.), *Glomus* (3 spp.), and *Dentiscutata* (2 spp.). The seasonal variation in spore density at the different study sites is depicted in **Table 5 and 6.** Maximum spore density was recorded during the monsoon season in both north (Site I-V) and south Goa (VI-X), while minimum spore density was recorded in the pre-monsoon season in north Goa and post monsoon season in south Goa.

Maximum spore density in north Goa was observed in *C. capitatus* (184 spores/100g soil) at site IV followed by *C. rotundus* (96 spores/100g soil) at site IV, *C. capitatus* (90 spores/100g soil) at site II, and *S. virgincus* (90 spores/100g soil) at site III. Maximum spore density in south Goa was observed in *C. esculentus* (135.00±10.14 spores/100g soil) at site VI and in *Paspalum* sp. (125.00±9.27 spores/100g soil) at site VII. Minimum spore density was recorded in *Cyperus* sp. (9.00±0.88 spores/100g soil) at site IV in north Goa while *C. esculentus* (9.00±1.15 spores/100g

soil) and *C. dactylon* (10.00±1.53 spores/100g soil) at site IX in south Goa recorded least spore density.

During the post-monsoon season, maximum spore density in north Goa was observed in *C. capitatus* (83.00±3.28 spores/100g soil) at site II and *C. dactylon* (77.00±3.48 spores/100g soil) at site V. While in south Goa maximum spore density was recorded in *C. dactylon* (70.00±2.8 spores/100g soil) at site X and in *S. virgincus* (64.00±4.10 spores/100g soil) at site VIII. Minimum spore density was recorded in *S. virgincus* in both the study areas i.e. north (3.00±1.85 spores/100g soil) at site V and south (7.00±1.45 spores/100g soil) Goa at site IX.

In the pre-monsoon season, maximum spore density in south Goa was observed in *C. capitatus* (85.00±2.33 spores/100g soil) at site VII while in North Goa, *Cyperus esculentus* recorded maximum spore density (60.00±7.67 spores/100g soil) at site V. Minimum spore density was recorded in *C. rotundus* (3.00±0.33 spores/100g soil) and *Sporobolus virgincus* at site III (3.00±0.88 spores/100g soil) in north Goa while, *S. virgincus* and *C. esculentus* at site IX and site VII revealed 3.00±0.33 spores/100g soil and 3.00 ±0.58 spores/100g soil in south Goa, respectively.

Table 9: Relative abundance (%) of AM fungal species in dunes of study sites in north and south Goa.

Sr. No.	AM fungal species	Relative abundance (%)					
		North Goa			South Goa		
		M	PM	PRM	M	PM	PRM
1.	*Gigaspora decipiens*	6.27	6.94	19.45	10.47	36.36	13.00
2.	*Gigaspora* sp. 2	13.03	7.34	13.12	22.23	34.13	14.70
3.	*Gigaspora* sp. 3	25.51	27.38	25.79	24.20	11.32	43.34
4.	*Glomus* sp.1	4.82	17.65	6.33	5.16	2.06	5.26
5.	*Glomus* sp. 2	16.46	1.38	0.00	15.78	1.89	0.00
6.	*Glomus geosporum*	0.00	0.59	0.00	0.00	0.00	0.00
7.	*Acaulospora* sp.1	7.72	0.79	7.69	12.06	0.34	0.00
8.	*Acaulospora* sp.2	6.87	0.00	2.71	0.00	1.54	1.08
9.	*A.spinosa*	0.00	0.00	0.00	0.30	1.03	0.62
10.	*A.scrobiculata*	0.00	0.00	0.00	0.45	0.69	0.46
11.	Unidentified sp.	19.30	37.5	24.8	0.00	0.51	10.06
12.	*Dentiscutata heterogama*	0.00	0.39	0.00	0.00	1.03	0.00
13.	*D. biornata*	0.00	0.00	0.00	7.28	0.00	0.00

Legend: All the values are mean of 3 readings, ±: Standard deviations; E.C: Electrical Conductivity; OC= Organic carbon, P=Phosphorus, K= Potassium, B=Boron, S=Sulphur.

AM fungal species richness and species diversity is depicted in **Table 7& 8.** Based upon the seasonal variation, monsoon season showed maximum RA in *Gigaspora* sp 3. in both north Goa (25.51%) and south Goa (24.20%). Post monsoon season revealed maximum RA in unidentified sp. (37.5%) in north Goa whereas, south Goa showed highest RA in *Gigaspora decipiens* (36.6%). While in the pre monsoon season highest RA was recorded in *Gigaspora* sp. 3 in both north Goa (25.79%) and south Goa (43.34%).

Table 7: AM fungal species richness and species diversity in grass species from dune of North Goa.

Sr. no	Site	Grass species	Monsoon		Post monsoon		Pre monsoon	
			AM species	SR	AM species	SR	AM species	SR
1.	I.	*Spinifex littoreus*	*Gigaspora* sp.1, *Glomus* sp.1	2	*Gigaspora* sp.1,*Glomus* sp.1, *Glomus geosporum* *Acaulospora* sp1.	4	*Gigaspora* sp.1,*Glomus* sp.1	2
		Paspalum sp.	ND	ND	ND	ND	*Gigaspor*a sp.2., *Glomus* sp.1, *Acaulospora* sp.1, *Acaulospora* sp.2	4
2.	II.	*Digitaria longifera*	*Gigaspora* sp.2, *Glomus* sp.1, *Acaulospora* sp.1	3	ND	ND	ND	ND
		Cyperus capitatus	*Gigaspora* sp.3, *Glomus* sp.1, *Glomus* sp.2	3	*Gigaspora* sp.3, *Glomus* sp.1. *Glomus* sp.2, *Acaulospora spinosa*	4	*Gigaspora* sp.3, *Glomus* sp.1, *Acaulospora* sp.2	2
		Sporobolus virgincus	ND	ND	*Gigaspora* sp.3, *Glomus* sp.1, *Glomus* sp.2. *Dentiscuta heterogama*	4	*Gigaspora* sp.3, *Glomus* sp.1,	2
3.	III.	*Sporobolus virgincus*	Gigaspora sp.3, *Gigaspora* sp.2	2	ND	ND	*Gigaspora* sp.2, *Gigaspora* sp.3	2
		Cyperus rotundus	Unidentified sp. 1	1	ND	ND	Unidentified sp. 1	1
4.	IV.	*Cyperus capitatus*	*Gigaspora* sp.2, *Gigaspora* sp.3, *Acaulospora* sp.2	3	*Gigaspora* sp.3	2	*Gigaspora* sp.3	2
		Cyperus rotundus	*Gigasporasp*.3, *Gigaspora* sp.2, *Glomus* sp.2	3	*Gigaspora* sp.3, *Gigaspora* sp.2, *Glomus* sp.2	3	*Gigaspora* sp.2, *Gigaspora* sp.3	2
		Cyperus sp.	*Gigasporas*p.2, *Gigaspora* sp.3	3	ND	ND	ND	ND
5.	V.	*Cynodon dactylon*	ND	ND	Unidentified sp.	1	ND	ND
		Cyperus esculentus	*Glomus geosporum*, *Gigaspora* sp.3	2	*Glomus* sp.2,*Acaulospora* sp.2	2	*Glomus geosporum*, *Gigaspora* sp.3	2
		Sporobolus virgincus	ND	ND	*Glomus geosporum*, *Gigaspora* sp.3,*Gigaspora* sp.1,*Glomus* sp.2	4	Unidentified sp.	1

Table 8: AM fungal species richness and species diversity in grass species from dune of South Goa.

Sr. no	Site	Grass species	Monsoon		Post-monsoon		Pre-monsoon	
			AM species	SR	AM species	SR	AM species	SR
1.	VI	*Cyperus esculentus*	*Gigaspora* sp.2,*Gigaspora* sp.3,*Glomus* sp.2	3	ND	ND	ND	ND
		Cynodon dactylon	*Gigaspora* sp.2, *Gigaspora* sp.3,*Acaulospora* sp.1	3	*Gigaspora* sp.2,*Glomus* sp.2, *Acaulospora* sp.2	3	*Gigaspora* sp.2,*Gigaspora* sp.3	2
		Paspalum sp.	ND	ND	ND	ND	*Gigaspora* sp.2, *Acaulospora* sp.2, unidentified sp.	3
2.	VII	*Cyperus capitatus*	*Gigaspora* sp.1,*Acaulospora spinosa*,*Glomus* sp.1, *A.scrobiculata*	4	*Gigaspora* sp.1,*Acaulospora scrobiculata* ,*A.laevis*,*Dentiscutata heterogama*, *A.spinosa*	5	Gigaspora sp.3, *A.scrobiculata*, *A.laevis*,*A.spinosa*	4
		Paspalum sp.	*Dentiscutata bior nata*,*Gigaspora* sp.2,,*Acaulospora* sp.1	3	*Gigaspora* sp.2,*Glomus* sp.1,*Gigaspora* sp.3,*A.scrobiculata*,*A.spinosa*,*Glomus* sp.2	6	*Gigaspora* sp.2, *Gigaspora* sp.3,*Glomus* sp.1	3
3.	VIII	*Cynodon dactylon*	*Gigaspora* sp.2,*Gigaspora* sp.3	2	ND	ND	ND	ND
		Sporobolus virgincus	ND	ND	*Gigaspora* sp.2	1	*Gigaspora* sp. 2	1
		Cyperus esculentus	ND	ND	ND	ND	*Gigaspora* sp.2, *Gigaspora* sp.3	2
4.	IX	*Cyperus esculentus*	Sporocarp of *Glomus* sp.,*Glomus* sp.1	2	Sporocarp of *Glomus* sp.,*Glomus* sp.1	1	Scorocarp of *Glomus* sp.	1
		Cynodon dactylon	*Gigaspora* sp.3		ND	ND	ND	ND
		Sporobolus virgincus	ND	ND	*Gigaspora* sp.1, *Gigaspora* sp.2, Sporocarp of *glomus* sp.	3	*Gigaspora* sp.3, Sporocarp of Glomus sp.	2
5.	X	*Cyperus esculentus*	*Glomus* sp.2	1	*Acaulospora* sp.1, *Acaulospora* sp.2,*Dentiscutata heterogama*	3	ND	ND
		Paspalum dilatatum	*Glomus* sp.1,*Acaulospora* sp.2, *Gigaspora* sp.3	3	*Gigaspora* sp.3	1	*Gigaspora* sp.3	1
		Cynodon dactylon	*Gigaspora* sp.1, *Glomus* sp.2, *Gigaspora* sp.3	2	*Gigaspora* sp.1,*Glomus* sp.1,*Acaulospora spinosa*, *Acaulospora* sp.1	4	*Gigaspora* sp.1, *Glomus* sp.2	2

PLATE 10: A & B. Trap cultures of isolated AM fungal spores.

CONCLUSION

The present study was carried out to study the AM fungal diversity in the different grass species growing on the dunes at various sites in south and north Goa. The study revealed variations in root colonization level, mean spore density and species richness in different plant species.

It was observed that the family Cyperaceae was more dominant in north Goa. Study on the seasonal variation revealed maximum root colonization during the monsoon season (June - September) followed by post-monsoon season (October - December) in north Goa. While, south Goa recorded maximum root colonization during the post-monsoon season, followed by pre-monsoon in the grass species studied.

The grass species viz., *Cyperus rotundus*, and *Cynodon dactylon* recorded higher percent root colonization in the monsoon season, while *Spinifex littoreus,* and *Sporobolus virgincus* recorded higher percent root colonization in the post-monsoon season. The study recorded least AM colonization in *Sporobolus virgincus*, and *Cyperus rotundus* during post monsoon in north Goa. Similarly *Sporobolus virgincus* recorded least AM colonization in south Goa. In the pre-monsoon season, *Sporobolus virgincus* recorded least root colonization in both the study areas.

The AM fungal root colonization was strongly influenced by several edaphic factors and plant phenological events, while the spore density is known to depend on the water availability (Bouamri and Serrhini 2014). Fourteen AM fungal species belonging to four genera *viz., Acaulospora, Dentiscutata, Gigaspora, Glomus* were recovered from the study sites. Highest spore density was recorded during the monsoon and post-monsoon season in north Goa. The grass species *Cyperus capitatus* at site IV, *Sporobolus virgincus* at site II, and *Spinifex littoreus* at site I recorded higher spore density in north Goa. Whereas, in south Goa, *Cyperus esculentus* at site VI and *Paspalum* sp. at Site VII recorded higher spore density. Least spore density was recorded in summer in both the study areas. *Gigaspora* was the most dominant genus. *Gigaspora*

sp. 3 revealed maximum population up to 43.34% and found to be abundant during pre-monsoon in south Goa. Whereas, post-monsoon season revealed the abundance of *Gigaspora* sp. 3 up to 27.38% in north Goa study area.

The root colonization and diversity of AM fungal species associated with different grass species suggests the applicability of AM symbiosis in the management and stabilization of the coastal dunes. Further, practical knowledge help in understanding the ecology and behavioural patterns of AM symbiosis with different vegetation. Also, several hypothesis are known with respect to correlation between AM fungal root colonization and spore density that synchronize AM fungal development with the functional requirements by the plants.

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