EVALUATION OF ARBUSCULAR MYCORRHIZAL DIVERSITY ON POST-CYCLONIC FORMED SAND SPIT OF GALGIBAGAA RIVER, GOA

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BY

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DECLARATION

I hereby declare that the matter provided in this thesis entitled, **"Evaluation of Arbuscular Mycorrhizal Diversity on Post-cyclonic formed Sand spit of Galgibagaa River, Goa"** is the result of investigations carried out by me, under the supervision of Prof. B. F. Rodrigues and it has not previously formed the basis for the award of any degree, diploma, associate ship, fellowship or other such similar title.

Goa University **Ms. Lenora Vas**

May 2022 (Candidate)

CERTIFICATE

This is to certify that the work incorporated in this thesis entitled, **"Evaluation of Arbuscular Mycorrhizal Diversity on Post-cyclonic formed Sand spit of Galgibaga River, Goa"** submitted by Ms. Lenora Vas, constitutes her independent work and the same has not been previously submitted for the award of any degree, diploma, associate ship, fellowship or other such similar title.

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INTRODUCTION

Among the various coastal systems, sand spit is described as an accretion of sediments connecting to the mainland at one end and ceasing in the open water at the other, and being relatively younger to the mainland, which it is linked to. Sand spits defend the mainland coast from the harsh wave forces of the ocean during storms (Evans, 1942). The main components that determine the formation of sand spits are the wave-generated processes. Other components that influence spit formation and morphological transformations are geographic framework, sea-level rise, available sediment, wind and tides, and possible human impact (Allard *et al.,* 2008). Development or washing of sand spits at river mouths arises based on control of wave action or affluence of river accordingly. The benefit of sand spits development is the prevention of salinity and wave encroachment. Contrastingly, it interferes with effluent flood flow into the sea, encouraging unwanted floods and destroying structures (Tanaka and Samad, 1970).

Coastal ecological communities are notably susceptible to sea-level rise and the uncertain prevalence of tropical storms and hurricanes, which are likely to develop with global climatic variations (Duran and Moore, 2013). These climatic effects are perhaps chiefly vital because dunes have a high ecosystem value as a natural environment for endemic plants and animals while protecting bay habitats such as seagrass, oyster beds, and saltmarsh, as well as inland wetlands (Martinez and Psuty, 2004; Gutierrez *et al.*, 2011). These plants are considered to regulate sand motion and judge the form and location of the dunes (Moreno-Casasola, 1986). The structure of the dune can single out the abiotic factors such as soil moisture and nutrients that regulate the plants' foundation, development, and reproduction (Ehrenfeld, 1990). The different types of dunes showed the presence of various plant species. Miller *et al.* (2010) observed that the interdunes were subdued by species corresponding to the moist areas like *Juncus* spp., *Phyla nodiflora,* and *Paspalum*

distichum. In contrast, the foredunes and backdunes have species linked with dry areas like *Uniola paniculata, Schizachyrium maritima,* and *Ipomoea imperata*. Previous studies based on coastal dune-type vegetation detected severe substantial factors like salt spray, soil moisture, and sand motion as the prime factors accountable for the zonation forms parallel to the beach (Oosting and Billings, 1942; Miller *et al.*, 2010).

Arbuscular mycorrhizal (AM) fungi are ubiquitous, obligate symbiotic fungi that colonize more than 80% of the terrestrial plants (Wang and Qui, 2006). This mutualistic relationship benefits both; the host plant that provides and fulfills the carbon requirements of the fungus, and in turn, the plant is supplied with mineral nutrients, especially P, and an increase in root surface area, which helps in the absorption of water (Willis *et al.*, 2013). AM fungi have also been shown to increase disease tolerance by reducing the invasion of soil- microbial plant pathogens (Newsham *et al*., 1995), improving the water balance in host plants when there is drought or abundant water (Augé, 2001), to reduce the uptake of phytotoxic heavy metals (Göhre and Paszkowski, 2006), their role in plant secondary succession (Janos, 1980).

AM fungi belong to Phylum Glomeromycota (Berruti *et al.*, 2016). The unique features of these mycorrhizae are the formation of arbuscules and vesicles in the root cortex of plants. The hyphae penetrate the external cell wall of the roots and spread across the inter- and intra-cellular regions towards the outer cortex with hyphal coiling. As it passes through the inner cortex, it forms arbuscules (branch-like structures involved in the material exchange between the host and fungus). In the middle and outer cortex layers, swollen terminal ends appear either inside or in the cells, known as vesicles (globose to ellipsoid structures that function as temporary storage organs). These two structures are interlinked to the external hyphae by a network of internal hyphae system that appears like pipe structures (Nicholson, 1967).

Various surveys of AM fungi present on the coastal sand dunes were carried out in temperate, subtropical, and tropical regions (Koske and Halvorson, 1981; Sturmer and Bellei, 1994). AM fungi are of pivotal significance to the foundation, development, and the existence of dominating plant species that inhabit dunes (Koske and Polson, 1984). Any type of disturbance to a flourishing ecological community that involves variation in Physico-chemical properties of the soil and eradication of plants will have a significant influence on the symbiotic association between plants and AM fungi (Sylvia and Will, 1988; Beena *et al.,* 2000). Due to this, there is a difference noted in plant species with response to AM fungi in the soil, and the presence or absence of AM fungi has been associated with the structure of plant populations that have been established in dune sites (Koske and Gemma, 1992; Francis and Read, 1995).

There have been many reports regarding the role and benefits of AM fungi in association with plants. Improvements were observed in the vigour and yield response of the host plant, which was inoculated with AM fungi when they were grown in P-deficient soils (Koske and Polson, 1984). AM fungi help improvise the plant growth parameters and nutrient uptake of important elements such as nitrogen and phosphorus in stress conditions. This is due to their ability to extend the fungal hyphae, which are much thinner in diameter than the host plant roots and which can penetrate smaller pores, beyond the nutrition depletion zone of the rhizosphere, for the nutrient uptake. This function helps the host plant access the nutrients that have poor mobility or those present in very low concentrations in the soil (Diagne *et al.,* 2020). The role of AM fungi and salt stress exhibited various processes wherein AM symbiosis ameliorates salt stress in host plants. In plants with no symbiotic association, their development suffer an obstruction due to spending their energy neutralizing the toxic effects of NaCl and deficient nutrient availability in their surroundings. (Zuccarini and Okurowska, 2008). AM fungi perform a vital role in the

plant's survival on toxic metal soils by serving as purification barriers to the distribution of heavy metals to shoots (Gaur and Adholeya, 2004).

Goa, a small state situated along the Central West Coast of India, is bounded by Maharashtra in the North, Karnataka in the East and South, and the Arabian Sea in the West. This state hosts a diversity of ecosystems, which comprises of the Coastal, Mangrove, Estuarine, Grassland, Wetland, and Western Ghat Habitats (http://ces.iisc.ernet.in/hpg/envis/envis_centres/homepages/goa.htm). Goa has a coastal region of 105 km consisting of both sandy beaches and rocky shorelines. Nine main rivers flow through Goa, of which Mandovi and Zuari are the two chief rivers, and the remaining are Terekhol, Colvale or Chapora, Sal, Talpona, Saleri, Canacona, and Galgibagaa. Goa witnesses a tropical, warm, humid climate with temperatures ranging from 21 °C to 36 °C, with an average being 27 °C, and has three seasons: a monsoon (June to September or mid-October), accompanied by a short winter (November to February) and summer (March to May). There is a variation in rainfall from the coastal area, about 2,500 mm to about 4,500 mm within the Western Ghats (Sonak *et al.*, 2014).

The soils of Goa are grouped into three main types: Lateritic, Alluvial, and Sandy. Sandy soil covers about 9 km of the coastal belt. These soils are acidic, sandy to sandy loams, relatively rich in organic matter but limited in phosphate and potash. Mangrove forests and dunes are essential coastal ecosystems of Goa. Several species of mangrove plants were recorded in Goa, to name a few, *Rhizophora mucronata, Rhizophora apiculata, Avicennia officinalis, Avicennia alba, Avicennia marina, Sonneratia alba,* of which, *R. mucronata, S. alba,* and *A. officinalis* are the dominant ones (Sonak *et al.,* 2014). Dune vegetation constitutes about 156 species of plants recorded, of which *Spinifex, Ipomoea, Acanthus, Clerodendrum, Vitex, Spermacosea, Urginea, Dioscorea, Pandnus, Crotalaria, Duranta, Leucus, Cyprus, etc.,* are the noticeable ones. Mangrove forests and dunes protect the

coastal environment against natural hazards. (Desai and Untawale, 2002)

Galgibaga River is the sustenance of Canacona, situated in the south of Goa, and is recognized for its rich mangroves and other coastal vegetation. For ages now, this River has been supplying a source of livelihood for many coastal communities. Galgibaga beach isknown for its sand bar established near the mouth of River Galgibaga years ago, and due tothis, the course of the River deviated and submerged into the Arabian by taking an 'S-like'turn. Consequently, various coastal dynamics caused the sand bar to develop quicklythrough the South end, threatening the existent mouth of the River. (https://www.heraldgoa.in/Goa/Cyclones-changing-course-of-Galgibagaa-River-experts/153318). Due to the recent cyclone (Cyclone Tauktae), which hit the coast of Galgibaga on May 2021, the Galgibaga River changed its course while detaching from theMaxem River, and leading the flow directly into the Arabian Sea at present. The cyclonepartly fragmented this diversion created by the development of a sand spit that acted as anatural barrier. It was observed by experts that Galgibaga was exposed to erosion for a timeperiod due to storm surges and roughsea conditions.(https://timesofindia.indiatimes.com/city/goa/Galgibagaa-river-changes-course-locals- worried-about-flooding-in-low-lying-areas/articleshow/84360530.cms). The shape of thepresent coastal landscape is the result of continuous coastal processes.

The main objectives of this study are as follows:

1. To conduct a preliminary survey of the study site and its plant diversity.
2. To analyse the post-cyclonic effects on the study site using basic remote sensing techniques and ground-truthing.
3. To isolate AM fungal species from the rhizosphere soil and prepare trap cultures.
4. To study the AM diversity in plant species from the study site.

LITERATURE OF REVIEW

Saxena *et al.,* (2017), reported on the role of AM fungi and tolerance of salt stress in the plant. They stated that soil salinity was the most severe abiotic stress that affected the plants' establishment, growth, and development, and that it altered the major metabolic processes of plants. They found that AM fungi under salinity stress were able to regulate several mechanisms like photosynthetic processes, accumulation of osmolytes, control of water and ionic homeostasis, reduction in oxidative damage, and control over ultrastructure alterations in plants. Though many of the physiological and biochemical mechanisms still remain unknown at the molecular level.

Zhu *et al.,* (2017), addressed the various possible mechanisms of AM symbiosis that improved the tolerance to temperature stress, efficiency and photosynthetic ability, protection against oxidative damage, accumulation of osmolytes, and water and nutrient uptake in plants. They noted that although AM fungi are diverse and not specific to their host plant, their effectiveness is different in adverse temperature-stressed conditions. However, the underlying physiological mechanisms of AM-plant symbiosis involved in protection against temperature stress are yet to be further elucidated.

Bukhari and Rodrigues (2009), recorded 31 AM fungal species belonging to four genera *viz., Acaulospora, Glomus, Gigaspora,* and *Scutellospora,* in 10 plant species from Bimbol Iron Ore mine of Goa. The most common AM fungal species were *Glomus macrocarpum* and *Acaulospora spinosa*. They suggested that extreme environmental conditions with large AM fungal diversity indicated to be the center of evolution and conservation of biodiversity gene pool.

D'Souza and Rodrigues (2013), reported that 16 out of 17 mangrove species were mycorrhizal and that there was a stable AM fungal community in mangrove species of Goa. They recorded 28 AM fungal species belonging to five genera *viz., Entrophospora*, *Acaulospora, Glomus, Gigaspora,* and *Scutellospora*, with genus '*Glomus*' and species '*G. intraradices'* being the most dominant.

Yamato *et al.,* (2012), considered the coastlands to be stressful environment due to high salinity, temperature changes, low infertility, intermittent drought, unstable sandy substrate, *etc.,* and the coastal plants are specifically adapted to these extreme environmental conditions. They observed a transition of coastal plant species in response to the environmental gradient with the distance of the sea and the vegetation which experienced the most stressful conditions was the closest to the seaside. Likewise, their study also indicate that some of the AM fungi may be specifically adapted to such environments.

Studies by Sharifi *et al.,* (2007) and Yamato *et al.,* (2008), indicate that AM symbiosis reduced the concentration of Na^+ translocation in shoots of plants growing in saline conditions. They suggested that the adaptation of the AM fungi to salt-stressed conditions may be an important factor that helps coastal plants to grow in saline conditions.

Sridhar (2009), reported that the members of *Poaceae* were dominant in temperate dunes, whereas members of *Convolvulaceae, Fabaceae, Asteraceae,* and *Poaceae* were dominant on the tropical dunes. He also stated that the diversity of AM fungi is one of the major factors that govern the function and plant diversity of the dune ecosystems.

Desai (1995) carried out a survey of dune vegetation along the coasts of Goa, with *Ipomeapes-caprae* and *Spinifex littoreus* being the most dominant flora in the pioneer zone, along with other dominant plants like *Cyperus arenarius, Spermacoce stricta,*

Launea pinnatifida, Justicia simplex, Lactuca remotiflora, Sporobolus virginicus, Clerodendron inerme, and other plants.

Sutton and Sheppard (1976), studied the factors of aggregation of sand particles in the host plant (*Phaseolus vulgaris*) in association with, AM fungi *Glomus* sp., or with *Glomus* and soil extract inoculum. They observed that the weight of the sand that adhered to the roots of the host plant was three times more in mycorrhizal roots than in non-mycorrhizal roots. On further microscopic analysis, they observed that the major mechanism linking sand grains in aggregates was the binding of sand to extensive *Glomus* mycelia.

Beena *et al.*, (2001), reported the AM fungal status in 28 plant species present on coastal sand dunes of the southwest coast of India. They recorded 23 plant species colonized by AM fungi, with highest colonization in *Canavalia cathartica*, and 20 plant species withAM fungal spore present in the rhizosphere, with highest number of spores present in *Borreria articularis* rhizosphere. They recorded the highest mean species richness in *Ipomoea pes-caprae*, the highest AM fungal diversity in *Alysicarpus rugosus*. Among the AM fungi they recovered, *Scutellospora erythropa* showed a wide host range, while *Scutellospora gregaria* showed high spore abundance per plant species.

Fitter *et al.*, (2011), reported the nutritional exchanges in the arbuscular mycorrhizal symbiosis. After many interactions with other researchers, an argument was put forward stating that AM fungi transfer to the host plant whatever ions would elicit the local sugar flux which could be spared, which could be either phosphate or ammonium ions. This came as a counter-argument to what Fitter and Hodge (2010) reported that AM fungi were likely to transfer a significant amount of nitrogen (N) to the plants because the AM fungi themselves have high N demand and would do so after their demands are satisfied.

Druva-Lusite and Ievinsh (2010), reported the diversity of mycorrhizal symbiosis in plants on coastal habitats. They observed that, despite the sometimes low intensity of mycorrhizal colonization, the roots of the coastal plants studied, possessed functional structures of AM symbiosis. They suggested that the fluctuation in mycorrhizal colonization could be due to unfavorable environmental conditions during the vegetative season, also in salt marsh plants, the high salinity may be one of the main factors for low-intensity mycorrhizal colonization in them.

Kulkarni *et al.*, (1997), reported mycorrhization and spore density of AM fungi in 12 dune plant species on the west coast of India, post-monsoon. They recorded *Borreria articularis, Ipomoea pes-caprae and Launaea sarmentosa* as the most dominant plant species. They observed that AM fungal spores were present in the rhizosphere of all plants except in *Ipomoea pes-caprae*. Out of 16 AM fungi species they recorded, nine species belonged to the genus '*Glomus*', and they recorded *Gigaspora ramisporophora, Glomus albidum, Glomus clarum,* and *Scutellospora gregaria* as dominant ones among the other spores. They suggested astrong positive correlation between spore density and the number of species present, and therelative abundance and frequency of occurrence of AM spores in the rhizosphere.

Koske and Gemma (1997), conducted a survey of AM fungi and hyphal networks of AM fungi of sand dune sites in different successional stages of American Beachgrass (*Ammophila breviligulata*). They recovered 17 species of AM fungi spores and observed an increase in richness and population of spores in the AM fungi community present, the extent of mycorrhization in*A. breviligulata* roots, and the mycorrhizal inoculum potential of the soil. They also observed that the unvegetated site which lacked AMF propagules, planted with colonized culms of *A. breviligulata* became mycorrhizal later. Based on these observations and records, they stated that the rate of invasion of later successional plant

species in the areas planted to the American beachgrass may depend upon the establishment of a vigorous network of hyphae of AMF in the site.

MATERIALS AND METHODS

Study Site

The study area (**Fig. 1**) is a newly formed island on a coastal dune situated at the mouth of the Galgibaga-Maxem river (14°57'28.64" N, 74°03'09.30" E), in Canacona taluka, located in the South District of the State of Goa, India. This island has not been very consistent and has been changing its shape based on the course of the flowing river and the post-cyclonic effect. It has very little dune-type vegetation.

Remote sensing data

The mapping of the study area was done by using basic techniques of remote sensing with the help of Google Earth software. It helps in the analysis of the gradual changes that took place during the last 20 years (2002 to 2022) in a given area. This can help us predict the visible frequency of variation through images. The area and perimeter can also be measured through remote sensing. Ground truthing mainly refers to relating the actual features and resources on the ground to the images acquired through remote sensing. A field survey, analysis of aerial photographs, and high spatial resolution data were done (**Plates 1 and 2**).

Sample Collection

The sampling of plant species at the site was carried out to assess the arbuscular mycorrhizal (AM) diversity. Six soil samples were collected (from a depth of 15-20cm) from different parts of the island and mixed to form a composite sample used to analyze the Physico-chemical properties. Root and rhizosphere soil samples were placed in *Ziploc*

bags, labeled and brought to the laboratory. The roots were used to determine the root colonization, while the soil samples were stored at 4°C for further analysis.

Soil Chemical Analysis

The soil sample was sun-dried, sieved to remove larger soil particles and debris, and was sent for analysis at the Directorate of Agriculture, Soil Testing Laboratory (Ela Farm), Old Goa. The soil was analyzed for various physical parameters, *viz.*, soil pH by 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), Available Phosphorus (P) content was determined by Bray and Kurtz method (Bray and Kurtz, 1945), Available Potassium (K) content was determined using the Ammonium Acetate Method (Hanway and Heidal, 1952) and Available Nitrogen (N) content was determined by Aerobic Incubation Method (Keeney and Bremner, 1966). The available micro-nutrients like Boron (B) were estimated by using Azomethine-H Method (Gupta, 1979) and Sulphur (S) content by Turbidimetric Method (Chesnin and Yien, 1950) were carried out.

Estimation of AM Fungal Root Colonization

Assessment of AM colonization in roots was carried out using Trypan blue staining technique (Phillips and Hayman, 1970). The roots were gently washed with tap water to remove the attached soil particles and then were cut into 1cm segments. The cleaned root segments were then cleared in 10% KOH by heating at 90°C for one hour in the oven.

These root segments were thoroughly rinsed in water, acidified with 5N HCl for 5 minutes, and kept overnight in a 0.05 % Trypan blue stain.

The presence or absence of AM hyphae, arbuscules, vesicles, and hyphal coils in the root segments wasobserved using Olympus BX14 and the data recorded. Microscopic photographs were taken by Olympus DP 12-2 and Nikon Eclipse E200 digital camera.

Estimation of Percent Root Colonization

The estimation of AM root colonization was carried out using the Root Slide Method (Read *et al.,* 1976). The Trypan blue stained roots were mounted in polyvinyl-lacto-glycerol (PVLG) on slides, and the presence or absence of AM colonization was scored. The percent root colonization was calculated using the formula:

Percent Colonization = No. of root segments colonized ÷ Total number of root segments observed × 100

Extraction of the AM Fungal Spores

AM spore extraction was done using the Wet Sieving and Decanting Technique (Gerdemann and Nicolson, 1963). Rhizosphere soil (100g) was added to a beaker. To this, water was added to create a suspension. This suspension was stirred using a glass rod, and the sediment was allowed to settle down for 1 minute. Then, without disturbing the sediment layer at the bottom, the suspension mixture was slowly decanted through several sieves arranged in the descending order **(**50µm, 150µm, 250µm, 750µm). This procedure was repeated twice. The residue from each sieve was collected in separate beakers, and the aliquot was filtered separately using Whatman No. 1 filter paper. The filter paper was placed onto a petriplate, ensuring that it remained moist. The filter paper was then

examined for the presence or absence of AM spores and sporocarps using a stereomicroscope (Olympus SZ16).

Estimation of AM Fungal Spore density

The estimation of AM fungal spore density was carried out by following the modified method of Gaur and Adholeya, (1994). The AM diversity and dominant species found inthe study site were also recorded. The procedure was carried out using Whatman No. 1 filter paper folded twice in two halves resulting in four equal quadrants. Once the filter paper was re-opened, vertical lines were drawn on the one-half side of the filter paper divided into ten columns, each being 0.5cm apart from the other. The direction of counting and numbering of columns was marked. Then the filter paper was folded in such a way that the aliquot containing the spores was poured onto the marked portion and the unmarked portion was free of spores. This filter paper was then placed in a petriplate and counted under a stereomicroscope (OLYMPUS SZ16). Intact living spores were isolated using a needle. The percent spore density was assessed by the total number of spores recorded per 100g of the soil sample.

Taxonomic Identification of Spores

Intact or crushed, clean spores were mounted in PVLG on a slide and used for taxonomic identification. Various morphological characteristics play an important role in confirming the taxonomic identity and the relationships of AM species. Some characteristics like colour, shape, size, ornamentation patterns, number of wall layers, mycorrhizal anatomy, pattern and shape of germination shield, *etc.,* assist in identifying AM at the species level. Taxonomic identification of AM fungal spores was done using morphological criteria

as basis, reference to the original species was made as described by Rodrigues and Muthukumar (2009), and online species description were provided by INVAM (International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi) at the West Virginia University, USA (https://invam.wvu.edu/).

Estimation of AM Species Richness, Spore Density, and Relative Abundance

The estimation of the diversity of the AM fungal spores was carried out by evaluating the spore abundance and calculating the spore density (Gaur and Adholeya, 1994), and the relative abundance (RA%) of AM fungi using the following formulae (Beena *et al.*, 2000c, 2001).

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

Relative Abundance (RA %): RA% = Number of spores of a particular species ÷ total number of spores × 100

Statistical Analysis

Pierson's Coefficient of Correlation (*r*) was calculated to compare the relationship between spore density and root colonization using IBM SPSS Statistic 22 software.

Sterilization of Pots and Sand

The pots to be used for trap culture were soaked in soap water and thoroughly rinsed with water.Then, the pots were wiped using absorbent cotton dipped in absolute alcohol, and the holes at the base of the pot were blocked with non-absorbent cotton.

The soil used for potting was sterilized prior to using a hot air oven at 180°C for three hours for a period of three 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. Ragi (*Eleusine coracana*) seeds were sowed in the pots and maintained in the polyhouse at 28°C for 90 days. The pots were watered at regular intervals and provided with proper growth conditions. After 90 days, watering was stopped, and the plant was left to dry and was checked for AM spores using the wet sieving and decanting technique.

RESULTS AND DISCUSSION

Remote Sensing Analysis

With the aid of Basic Remote Sensing Techniques, using Google Earth Software, analysis of the post cyclonic effect was studied. It was observed that there were distinct variations over the years that led to the formation of the sand spit at the mouth of Galgibagaa River. The geomorphological attributes, *viz.*, position of the sand spit, position of the river and the shoreline status of the area in comparison with the study site during 2002, 2005, 2011, 2014, 2015, 2017, 2018, 2019, 2020 and 2021 are depicted in **(Fig. 2, 3, 4 and 5)**. The Google Earth images of the years 2002 and 2005 **(Fig. 2A and 2B)** showed the gradual formation of the sand spit. During the year 2009, Canacona was affected by fresh floods due to a massive cloudburst. This resulted in accumulation of eroded beach sand towards the mouth of the river. The Google Earth image of the year 2011 **(Fig. 2C),** depicted the after-effect of the floods and how the accumulated sand led to the further development and variation of the sand spit in the years 2014 and 2015 **(Fig. 3D & 3E)**. According to Begum *et al.*, (2014), the spit establishment at the river mouth area is associated with the transport of sediments from the long shore and the chief effect of the monsoons. In the year 2017, it was observed that part of the sand spit that was attached to the main shoreline, fragmented and deposited at the Maxem river outlet, while attaching itself to the mainland. In 2018, the sand spit was further divided creating small third sand spit toward the mangrove area. Due to coastal dynamics like tidal levels, river discharge, wind patterns and storm surges, there is constant variation in the appearance and size of the spits formed **(Fig. 4G)**. As seen in 2019, the sand spit attached to the shoreline started increasing in length **(Fig. 4H)** and by 2020, the sand spit grew larger

forming a s-shaped curve **(Fig. 4I)**. There was a significant change in the perimeter of the sand spit in May 2021. This was due to the effect of a strong cyclonic surge by Tauktae that divided the sand spit into three fragments, and subsequently changing the course of the River. The perimeter of the sand spit was 0.86km during 2020 (pre-Tauktae). The perimeter of the first fragment was 0.66km while the second fragment was 0.30km, in 2022 (post-cyclone) **(Fig. 5J and 5K) (Table 1)**. Thus, there was an increase seen in the perimeters of the two fragments in the last two years mainly due to deposition of sediments. Due to fragmentation of the sand spit, the course of the Galgibaga River changed, and thereby affected the ecosystem.

Table 1: The variations in the perimeter of the sand pit Pre- and Post-Cylone

	Year	**Perimeter**
Pre-Cyclone Spit	2020	0.86km
Post-Cylone Sand spit 1	2022	0.66km
Sand spit 2		0.30 km

Survey of Plant Diversity

The survey of the plant diversity was studied using ground truthing. The vegetation present at the site mostly consists of the coastal dune-type along with few mangrove and flowering-plants (**Table 2)**. The members of various families *viz.,* Cyperaceae, Aizoaceae, Convolvulaceae, Fabaceae, Casuarinaceae, Lecythidaceae, Asteraceae, Poaceae, Dioscoreaceae, Amaranthaceae, Arecaceae, Phrymaceae, Apocynaceae, Acanthaceae, and Lamiaceae were found occurring at the study site. As shown in (**Plate 3, 4, 5 and 6**).

Table 2: List of Plant Diversity recorded at the study site.

Sr. No.	Plant species	Family	Common name
1	*Cyperus* sp. L.	Cyperaceae	Nutgrass, FlatSedge
2	*Sesuvium portulaca* Crantz	Aizoaceae	Sea Purslane
3	*Ipomoea pes-caprae* (L.) R.Br.	Convolvulaceae	Beach Morning Glory, Sea Morning Glory
4	*Derris trifoliata* Lour.	Leguminosae	Common Derris
5	*Remirea maritima* Aubl.	Cyperaceae	Beach star
6	*Casuarina equisetifolia* L.	Casuarinaceae	Casuarina, Beach She-Oak, Horsetail Tree
7	*Caesalpinia pulcherrima* (L.)	Leguminosae	Peacock Flower, Gold Mohur, Flower Fence
8	*Pongamia pinnata* (L.)	Leguminosae	Indian Beech Tree, Pongam, Seashore Mempari
9	*Barringtonia racemosa* (L.) Spreng.	Lecythidaceae	Sea Poison Tree, Powder-Puff Tree
10	*Chromolaena odorata* (L.) R.M. King & H.Rob.	Asteraceae	Jack in the bush, Siamweed
11	*Canavalia rosea* (Sw.) DC.	Leguminosae	Bay Bean, Seaside Bean, Coastal Jackbean
12	*Sporobolus virginicus* (L.) Kunth	Poaceae	Seashore Dropseed
13	*Tamarindus indica* L.	Leguminosae	Wild Tamarind
14	*Dioscorea bulbifera* L.	Dioscoreaceae	Air Potato, Air Yam, Bitter Yam
15	*Alternanthera sessilis* R.Br.	Amaranthaceae	Carpet weed, Sessile Joyweed
16	*Launaea sarmentosa* (Willd.) Kuntze	Asteraceae	Beach Launaea
17	*Rhizophora mucronata*	Rhizophoraceae	Asiatic Mangrove
18	*Leucas lavandulifolia* Sm.	Lamiaceae	Common Leucas, Thumba
19	*Avicennia officinalis* L.	Acanthaceae	Black Mangrove, Api Api Ludat
20	*Schizachyrium maritimum* (Chapm.) Nash	Poaceae	Gulf Bluestem, Gulf False Bluestem
21	*Sphagneticola trilobata* (L.) Pruski	Asteraceae	Yellow Creeping Daisy, Creeping Ox-eye
22	*Calotropis gigantea* (Linn.) Aiton f.	Apocynaceae	Giant Indian Milkweed, Crown Flower, Swallow-wort
23	*Peplidium maritimum* (L.f.) Asch.	Phrymaceae	Marsh Peplidium
24	*Cocos nucifera* L.	Arecaceae	Coconut Palm

Soil Analysis

Table 3: Physico-chemical analysis of soil at the study site.

Parameter	Value
pH	7.7 ± 0.15
Electrical Conductivity (EC) (m.mhos/cm)	2.24 ± 0.01
Nitrogen (Kg/ha)	263 ± 1.52
Organic carbon (%)	0.77 ± 0.01
Phosphorous (Kg/ha)	40.92 ± 0.01
Potassium (Kg/ha)	120.9 ± 0.15
Boron (ppm)	0.358 ± 0.001
Sulphur (ppm)	30 ± 1.52
All values are mean of 3 readings; ± : Standard deviation; E.C. : Electrical Conductivity	

The results of the soil analysis indicate that the pH of the soil was neutral, while the EC revealed high salinity at the study site. The macro-nutrient status revealed that the concentration of available nitrogen (N) and phosphorus (P) was present in moderate levels compared to Potassium (K) which was low. The organic carbon percentage was high. The micro-nutrients, *viz.,* Boron (Bo) and Sulphur (S) were in moderate levels.

Assessment of Arbuscular Mycorrhizal Diversity

Root colonization showed the presence of hyphae, vesicles, and arbuscules as shown in **Plate 7** and **Plate 8**. Along with AM colonization, endophytes were also found associated with the roots of the plant species. Highest root colonization (100%) was recorded in four different

plant species, *viz.*, *Canavalia rosea, Dioscorea bulbifera, Sphagneticola trilobata, and Calotropis gigantea*. Least root colonization was recorded in *Caesalpinia pulcherrima* (30%). Rauf *et al.,* (2016) reported mycorrhization to be more than grand mean value in *Cyperus* sp. and less than grand mean value in *Ipomoea pes-caprae*. Whereas in the present study, *Cyperus* sp. was found to have least colonization and *Ipomoea pes-caprae* had a moderate colonization. While other plant species in (**Table 4**) showed moderate to high AM colonization, it was observed that the roots of *Remirea maritima* was found to be associated with endophytes and did not show AM colonization. No AM colonization was observed in *Launaea sarmentosa*. Kulkarni *et al.,* (1997), reported root colonization below 50% in *Alysicarpus rugosus, Canavalia rosea,* and *Launaea sarmentosa*, while in the present study *Canavalia rosea* recorded the highest colonization while *Launaea sarmentosa* showed no AM colonization.

The species richness in (**Table 4**) recorded the highest in seven plant species, *viz.*, *Cyperus* sp., *Sesuvium portulaca, Derris trifoliata, Remirea maritima, Tamarindus indica, Launaea sarmentosa,* and *Sphagneticola trilobata,*where as the least was observed in *Leucas lavandulifolia.*

Table 4: AM fungal root colonization and species richness in the plant species at the study site.

Sr No.	Plants species	Root Colonization (%)	Species Richness
1	*Cyperus* sp.	35 ± 2.12	4
2	*Sesuvium portulaca*	95 ± 0.70	4
3	*Ipomoea pes-caprae*	60 ± 1.14	2
4	*Derris trifoliata*	95 ± 0.70	4
5	*Remirea maritima*	00 ± 0.00	4
6	*Casuarina equisetifolia*	45 ± 0.70	2
7	*Caesalpinia pulcherrima*	30 ± 4.24	2
8	*Pongamia pinnata*	80 ± 1.41	3
9	*Barringtonia racemosa*	70 ± 1.41	3
10	*Chromolaena odorata*	90 ± 0.00	3
11	*Canavalia rosea*	100 ± 0.00	2
12	*Sporobolus virginicus*	50 ± 1.41	3
13	*Tamarindus indica*	50 ± 0.00	4
14	*Dioscorea bulbifera*	100 ± 0.00	2
15	*Alternanthera sessilis*	50 ± 1.41	2
16	*Launaea sarmentosa*	00 ± 0.00	4
17	*Rhizophora mucronata*	00 ± 0.00	2
18	*Leucas lavandulifolia*	75 ± 2.12	1
19	*Avicennia officinalis*	55 ± 3.53	3
20	*Schizachyrium maritimum*	80 ± 1.41	2
21	*Sphagneticola trilobata*	100 ± 0.00	4
22	*Calotropis gigantea*	100 ± 0.00	3
23	*Peplidium maritimum*	100 ± 0.00	3
24	*Cocos nucifera*	45 ± 0.70	3

Table 5: Am fungal spore density in the plant species.

Sr No.	Plants species	SPORE DENSITY
1	*Cyperus* sp.	51.5±6.36
2	*Sesuvium portulaca*	11.5±3.53
3	*Ipomoea pes-caprae*	37.5±10.60
4	*Derris trifoliata*	64.5±6.36
5	*Remirea maritima*	14.5±0.07
6	*Casuarina equisetifolia*	13.5±3.53
7	*Caesalpinia pulcherrima*	8±2.82
8	*Pongamia pinnata*	52.5±6.36
9	*Barringtonia racemosa*	23±4.24
10	*Chromolaena odorata*	25±7.07
11	*Canavalia rosea*	21.5±4.94
12	*Sporobolus virginicus*	18±2.82
13	*Tamarindus indica*	25.5±7.77
14	*Dioscorea bulbifera*	10.5±3.53
15	*Alternanthera sessilis*	13.5±3.53
16	*Launaea sarmentosa*	14.5±4.94
17	*Rhizophora mucronata*	12.5±3.53
18	*Leucas lavandulifolia*	8±1.41
19	*Avicennia officinalis*	18.5±3.53
20	*Schizachyrium maritimum*	18±2.82
21	*Sphagneticola trilobata*	30±7.07
22	*Calotropis gigantea*	66.5±9.19
23	*Peplidium maritimum*	20±5.65
24	*Cocos nucifera*	35.5±7.77

Ten AM fungal species belonging to four genera, *viz., Acaulospora, Glomus, Gigaspora* and *Scutellospora* were recovered from the rhizosphere soil of the study site **(Fig. 1)**. *Glomus* (with 5 spp.) was the dominant genus followed by *Acaulospora* (4 spp.), *Gigaspora* (1 sp.) and *Scutellospora* (1 sp.). The diversity of AM Fungal species is shown in **Plate 9** and **Plate 10**. Variation in spore density was observed in different plant species as shown in **Table 5**.

Among the species, *Calotropis gigantea* recorded highest spore density, while *Leucas lavandulifolia* and *Caesalpinia pulcherrima* recorded the least. The maximum Relative Abundance (**Table 6**), was observed in *Acaulospora scrobiculata* (23.47%) and the least was noted in *Acaulospora* sp. 1 (0.63%). Pierson's correlation coefficient analysis showed that there was no correlation observed between spore density and root colonization (r =0.205). Trap cultures were prepared and maintained (**Plate 11**).

Table 6: Relative abundance (RA%) in each AM fungal species

SR NO.	AM FUNGAL SPECIES	RELATIVE ABUNDANCE (RA%)
1	*Glomus* sp 1	15.96
2	*Glomus* sp 2	7.98
3	*Glomus* sp. 3	9.08
4	*Rhizoglomus clarus*	2.97
5	*Funneliformis geosporum*	3.29
6	*Acaulospora spinosa*	8.29
7	*Acaulospora scrobiculata*	23.47
8	*Acaulospora* sp. 1	0.63
9	*Acaulospora* sp. 2	4.38
10	*Gigaspora albida*	23
11	*Scutellospora* sp.	0.94

CONCLUSION

The present study was carried out to evaluate the AM fungal diversity in plant species growing on a post-cyclonic formed sand spit, in the Galgibaga River. Also, the study assessed the AM mycorrhizal colonization, spore density and relative abundance in plant species found on the site.

Results obtained in the present study revealed good AM fungal association with the roots of plant species found at this study site, indicating the dependence of the initial establishment of vegetation on AM fungi. Variations were observed in the spore density, AM fungal root colonization, spore abundance and species richness, suggesting the influence of various ecological factors which could play an important role in forming the AM fungal communities and in stabilization of coastal vegetation in an ecosystem.

This study can be further elucidated to check the AM fungal species that can survive on these temporarily formed sand spits and how it affects their distribution across various seasons in this particular ecosystem.

REFERENCES

- Allard, J., Bertin, X., Chaumillon, E., & Pouget, F. (2008). Sand spit rhythmic development: A potential record of wave climate variations? Arçay Spit, western coast of France. *Marine Geology*, *253*(3-4), 107-131.
- Augé, R. M. 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza*, 11: 3–42.
- Beena, K. R., Arun, A. B., Raviraja, N. S., & Sridhar, K. R. (2001). Association of arbuscular mycorrhizal fungi with plants of coastal sand dunes of west coast of India. *Tropical Ecology*, *42*(2), 213-222.
- Beena, K. R., Raviraja, N. S., Arun, A. B., & Sridhar, K. R. (2000). Diversity of arbuscular mycorrhizal fungi on the coastal sand dunes of the west coast of India. *Current Science*, *79*(10), 1459–1466.
- BEGUM, S. N., RAVIKUMAR, K., & VED, D. (2014). Consequence of cyclonic storm Phailin on coastal morphology of Rushikulya estuary: an arribada site of vulnerable Olive Ridley sea turtles along the east coast of India. *Current Science*, *107*(1), 28.
- Berruti, A., Lumini, E., Balestrini, R., & Bianciotto, V. (2016). Arbuscular mycorrhizal fungi as natural biofertilizers: let's benefit from past successes. *Frontiers in microbiology*, *6*, 1559.
- Bray RH, Kurtz LT (1945) Determination of total, organic and available forms of phosphorus in soils. Soil Sci. 59: 39-45.
- Bukhari, M. J., & Rodrigues, B. F. (2009). Occurrence of Arbuscular Mycorrhizal (AM) fungi in selected plant species from Bimbol iron ore mine of Goa. Broadway Book Centre, Panaji, Goa.

- Chesnin L. and C. H. C. Yein. 1950. Turbidimetric determination of available sulphates. Soil Sci. Am. Proc. 15: 149-151
- D'Souza, J., & Rodrigues, B. F. (2013). Biodiversity of Arbuscular Mycorrhizal (AM) fungi in mangroves of Goa in West India. *Journal of Forestry Research*, *24*(3), 515-523.
- Dessai KN (1995) The structure and function of the sand dune vegetation along the Goa coast. PhD thesis, Goa University, Goa, pp. 218.
- Dessai KN, Untawale AG (2002) Sand dune vegetation of Goa: Conservation and management, Botanical Society of Goa.
- Diagne, N., Ngom, M., Djighaly, P. I., Fall, D., Hocher, V., & Svistoonoff, S. (2020). Roles of arbuscular mycorrhizal fungi on plant growth and performance: Importance in biotic and abiotic stressed regulation. *Diversity*, *12*(10), 370.
- Druva-Lusite I, Ievinsh G (2010) Diversity of arbuscular mycorrhizal symbiosis in plants from coastal habitats. Environ. Exp. Biol. 8: 17-34
- Duran O, Moore LJ. 2013. Vegetation controls on the maximum size of coastal dunes. Proceedings of the National Academy of Sciences of the USA 110:17217–17222.
- Ehrenfeld JG. 1990. Dynamics and processes of barrier island vege- tation. Reviews in Aquatic Sciences 2:437–480.
- Evans, O. F. (1942). The origin of spits, bars, and related structures. *The Journal of Geology*, *50*(7), 846-865.
- Fitter, A. H., Helgason, T., & Hodge, A. (2011). Nutritional exchanges in the arbuscular mycorrhizal symbiosis: implications for sustainable agriculture. *Fungal Biology Reviews*, *25*(1), 68-72.

- Francis R and Read DJ. 1995. Mutualism and antagonism in the mycorrhizal symbiosis with special reference to impacts on plant community structure. Can J Bot 73 (Suppl.): S1301-S130.

- Gaur A, Adholeya A. 2004. Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. *Current Science*, 86: 528- 534.

- Gerdemann JW, Nicolson TH (1963) Spores of mycorrhizal endogenous species extracted from soil by wet sieving and decanting. Trans. Brit. Mycol. Soc. 46: 235-244.

- Göhre, V., & Paszkowski, U. (2006). Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. *Planta*, *223*(6), 1115-1122.

- Gupta UC (1967) A simplified method for determining hot-water soluble boron in podzol soils. Soil Sci. 103: 424-428.

- Gutierrez JL, Jones CG, Byers JE, Arkema KK, Berkenbusch K, Commito JA, Duarte CM, Hacker SD, Lambrinos JG, Hendriks IE, Palomo MG, Wild C. 2011. Physical ecosystem engineers and the functioning of estuaries and coasts. In: Heip CH, Middelburg JJ, Philippart CJ, eds. Treatise on estuarine and coastal science, vol. 7: functioning of ecosystems at the land-ocean interface. Amsterdam: Elsevier, 53–81.

- Hanway JJ, Heidel H (1952) Soil analysis method as used in Iowa State College soil testing laboratory. Iowa State College of Agriculture. 57: 1-31.

- http://ces.iisc.ernet.in/hpg/envis/envis_centres/homepages/goa.htm

- https://powo.science.kew.org/

- https://timesofindia.indiatimes.com/city/goa/Galgibagaa-river-changes-course-locals-worried-about-flooding-in-low-lying-areas/articleshow/84360530.cms
- https://www.heraldgoa.in/Goa/Cyclones-changing-course-of-Galgibagaa-River-experts/153318
- https://www.itis.gov/
- https://www.nparks.gov.sg/florafaunaweb/flora
- invam.wvu.edu
- Janos, D. P. (1980). Mycorrhizae influence tropical succession. *Biotropica*, 56-64.
- Keeney, D. R., & Bremner, J. M. (1966). Comparison and evaluation of laboratory methods of obtaining an index of soil nitrogen availability 1. *Agronomy journal*, *58*(5), 498-503.
- Koske RE and Halvorson WL. 1981. Ecological studies of vesicular-arbuscular mycorrhizae in a barrier sand dune. *Can J Bot* 59:1413-1422.
- Koske RE and Polson WR. 1984. Are VA mycorrhizae required for sand dune stabilization? Bioscience 34: 420-424.
- Koske, R. E., & Gemma, J. N. (1997). Mycorrhizae and succession in plantings of beachgrass in sand dunes. *American Journal of Botany*, *84*(1), 118-130.
- Koske, R. E., Gemma, J. N., & Flynn, T. (1992). Mycorrhizae in Hawaiian angiosperms: a survey with implications for the origin of the native flora. *American Journal of Botany*, *79*(8), 853-862.
- Kulkarni, S. S., Raviraja, N. S., & Sridhar, K. R. (1997). Arbuscular mycorrhizal fungi of tropical sand dunes of west coast of India. *Journal of Coastal Research*, 931-936.

- Martinez ML, Psuty NP. 2004. Coastal dunes: ecology and conserva- tion. Berlin: Springer.

- Miller TE, Gornish ES, Buckley HL. 2010. Climate and coastal dune vegetation: disturbance, recovery, and succession. Plant Ecology 206:97 – 104.

- Moreno-Casasola P. 1986. Sand movement as a factor in the distribu- tion of plant communities in a coastal dune system. Vegetatio 65: 67–76.

- Newsham, K. K., Fitter, A. H., & Watkinson, A. R. (1995). Arbuscular Mycorrhiza Protect an Annual Grass from Root Pathogenic Fungi in the Field. *Journal of Ecology*, *83*(6), 991–1000.
- Nicolson, T. H. (1967). Vesicular-arbuscular mycorrhiza—a universal plant symbiosis. *Science Progress (1933-)*, 561-581.
- Oosting HJ, Billings WD. 1942. Factors affecting vegetational zonation on coastal dunes. Ecology 23:131–142.

- Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit. Mycol. Soc. 55: 158-161.
- Rauf, A., Khan, D., & Zaki, M. J. (2016). Arbuscular mycorrhizal fungi from some Coastal Plants of Karachi (Pakistan). *International Journal Biology Biotechnology*, *13*(2), 317-327.
- Read DJ, Koucheki HK, Hodgson J (1976) Vesicular-arbuscular mycorrhiza in natural vegetation systems. I. The occurrence of infection. New Phytol. 77: 641-653.
- Rodrigues BF, Muthukumar T (2009) Arbuscular mycorrhizae of Goa - a manual of identification protocols, Goa University, Goa, pp. 109-135.

- Saxena, B., Shukla, K., & Giri, B. (2017). Arbuscular mycorrhizal fungi and tolerance of salt stress in plants. *Arbuscular mycorrhizas and stress tolerance of plants*, 67-97.
- Sharifi M, Ghorbanli M, Ebrahimzadeh H. 2007. Improved growth of salinity-stressed soybean after inoculation with salt pre-treated mycorrhizal fungi. *Journal of Plant Physiology*, 164: 1144 -1151.
- Sonak, S. M. (2014). An Introduction to Goa and Khazan Ecosystems. In *Khazan Ecosystems of Goa* (pp. 15-31). Springer, Dordrecht.
- Sridhar KR (2009) Bioresources of coastal sand dunes - are they neglected? Jayappa KS, Narayana AC (Eds.) In: Coastal environments: problems and perspectives, pp: 53-76. IK International Publishing House, New Delhi.
- Sturmer SL and Bellei MM. 1994. Composition and seasonal variation of spore populations of arbuscular mycorrhizal fungi in dune soils on the island of Santa Catarina, Brazil. *Can J Bot* 72:359-363.
- Sutton, J. C., & Sheppard, B. (1976). Aggregation of sand-dune soil by endomycorrhizal fungi. *Canadian Journal of Botany*, *54*(3-4), 326-333.
- Sylvia DM and Will ME. 1988. Establishment of vesicular arbuscular mycorrhizal fungi and other microorganisms on beach replenishment site in Florida. *App Environ Microbiol* 54:348-352.
- Tanaka, H., & Samad, M. A. (1970). Influence of river mouth sand spit level on the time variation of river stage and bed topography. *WIT Transactions on The Built Environment*, *43*.

- Walkley A, Black JA (1934) An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic titration method. Soil Sci. 37: 29-38.
- Wang, B. and Qui, Y-L. 2006. Phylogenetic distribution and evolution of mycorrhizae in land plants. *Mycorrhiza*, 16: 299–363.
- Willis, A., Rodrigues, B. F., & Harris, P. J. (2013). The ecology of arbuscular mycorrhizal fungi. *Critical Reviews in Plant Sciences, 32*(1), 1-20.
- Yamato M, Ikeda S, Iwase K (2008) Community of arbuscular mycorrhizal fungi in coastal vegetation on Okinawa Island and effect of the isolated fungi on growth of sorghum under salt-treated conditions. Mycorrhiza 18:241–249
- Yamato M, Yagame T, Yoshimura Y, Iwase K (2012) Effect of environmental gradient in coastal vegetation on communities of arbuscular mycorrhizal fungi associated with *Ixeris repens* (Asteraceae). Mycorrhiza 22: 623-630.
- Zhu, X., Song, F., & Liu, F. (2017). Arbuscular mycorrhizal fungi and tolerance of temperature stress in plants. In *Arbuscular mycorrhizas and stress tolerance of plants* (pp. 163-194). Springer, Singapore.
- Zuccarini P, Okurowska P. 2008. Effects of mycorrhizal colonization and fertilization on growth and photosynthesis of sweet basil under salt stress. *Journal of Plant* Nutrition, 31: 497-513.