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Studies on Arbuscular Mycorrhizal (AM) profiles of coastal soils in Karaikal district, U.T of Puducherry, India

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Abstract

Arbuscular mycorrhizal (AM) fungal type of symbiosis is found in vast taxonomic range of both herbaceous and woody plants that are found in diverse habitats ranging from arctic to the tropics, arid to aquatic environments and stable plant communities to highly disturbed ecosystems. However the AM fungal community and AM colonization of plant roots may vary greatly in different soil types. The AM fungi differ widely in the level of colonization in the root system and in their impact on nutrient uptake and plant growth. Soil salinity is a problem of grave concern because it adversely affects growth and development of plants especially in arid and semi-arid regions. Mycorrhizal plants are of great interest in bioremediation since the management of mycorrhizal systems is necessary for the success of saline soil reclamation programs. In this study, coastal soils of Karaikal district, U.T of Puducherry was selected as a study area. The present work was investigated the AM mycorrhizal status in coastal soils of Karaikal District. A total of 21 species of vascular plants screened for mycorrhizal colonization, among them, 13 plants are mycorrhizal dependent. Ten species of AM mycorrhizal fungal species were recorded in the rhizosphere soils based on seasonal field surveys. Climatic seasons are strongly influence the percentage of colonization and AM spore population in the present study. Physio-chemical analyses of rhizosphere soils showed that all the study sites had alkaline, high Electrical Conductivity and ESP levels. Among the AM mycorrhizal genus, Glomus and Scutellospora populations were dominant in this ecosystem. The potential adaptation of this dominant indigenous AM fungi and their ability to colonize saline tolerant plant communities in the coastal regions indicate that mycorrhizal biofertilizers will help to remediate the coastal soils with suitable host plants.

Keywords: *Arbuscular mycorrhizal fungi, Coastal soils, Karaikal District, Biofertilizers.*

1. Introduction

Microorganisms are present in great number near the fine feeder roots of most of the plant species, and they play vital role in numerous physiological processes. These dynamic microbial processes include saprophytism, pathogenecity and symbiosis. The most wide spread symbiosis of plants is the mycorrhizal association between root-inhabiting fungi and the feeder roots [1, 2]. Mycorrhiza refers to an association or symbiosis between plants and fungi that colonize the cortical tissue of roots during periods of active plant growth. These symbioses are characterized by bi-directional movement of nutrients where carbon flows to the fungus and inorganic nutrients move to the plant, thereby providing a critical linkage between the plant root and soil.

Mycorrhizal fungi usually proliferate both in the root and in the soil. The soil borne or extramatrical hyphae take up nutrients from the soil solution and transport them to the root. By this mechanism, mycorrhizae increase the effective absorptive surface area of the plant roots. In nutrient-poor or moisture-deficient soils, nutrients taken up by the extramatrical hyphae can lead to improved plant growth and reproduction [3]. As a result, mycorrhizal plants are often more competitive and are able better to tolerate environmental stresses than non-mycorrhizal plants.

Arbuscular Mycorrhizal (AM) fungal type of symbiosis is found in vast taxonomic range of both herbaceous and woody plants. AM fungi are ubiquitous and are found in diverse habitats ranging from arctic to the tropics, arid to aquatic environments and stable plant communities to highly disturbed ecosystems. However the AM fungal community and AM colonization of plant roots may vary greatly in different soil types [4]. The AM fungi differ widely in the level of colonization they produce in a root system and in their impact on nutrient uptake and plant growth [5]. The diversity of these root-fungal associations provides plants with a range of strategies for efficient functioning in an array of plant-soil systems.

Arbuscular mycorrhizae, acting as a bio-fertilizer and produced with little energy with no polluting effect, can

play a key role in future as a partial substitute to chemicals. AM fungi are known to form mutualistic symbiotic association with many plants of economic importance and found to improve plant growth mainly through increased uptake of diffusion, limited plant nutrients particularly phosphorous and by producing growth promoting substances [6-8]. Some of the early reports also exhibited changes of the quality and quantity of some secondary metabolites due to the influence of AM fungi in stressed conditions [9].

Soil salinity is a problem of grave concern around the world because it adversely affects growth and development of plants [10]. In India alone, salinity affects seven million hectares of land, which is mainly attributed to irrigation with ground water of high salt content, sodic and alkaline parent material. Additionally, arid lands exhibits high evaporation rate and insufficient leaching of ions due to low precipitation result in a supra-optimal level of accumulation of salts, which render soils unproductive [11-14].

Excess amount of salt in the soils adversely affects plant growth and development [15]. High salt concentrations decrease the osmotic potential of soil solution creating a water stress in plants. Secondly they cause severe ion toxicity, since Na+ is not sequestered into vacuoles as in halophytes. Finally, the interactions of salts with mineral nutrition may result in the growth arrest and molecular damage [12, 14].

The exploitation of soil microbes for utilizing salt stressed coastal lands is of great importance. Symbiotic association of a plant with AM fungi makes them able to access immobile nutrients in nutrient-poor soils [16]. Besides improving nutrition, AM fungi improve physiological processes like water absorption capacity of plants by increasing root hydraulic conductivity and favorably adjusting the osmotic balance and composition of carbohydrates [17-18]. Thus, they mitigate the adverse effects of excess salt accumulated in the root [19].

Mycorrhizal plants are of great interest in bioremediation since the management of mycorrhizal systems is a prerequisite for the success of saline soil reclamation programs. The role of mycorrhizal fungi in salt stress conditions, particularly coastal soils are completely unknown. Baseline studies of the relationship between plants and mycorrhizal fungi in the coastal saline environments are urgently required for future reclamation programs. Therefore, the present study is investigated the mycorrhizal profile of native plants of coastal saline soil ecosystem coastal belt of Karaikal District located in Puducherry, India.

2. Materials and Methods

2.1. *Study site*

Karaikal (Union Territory of Puducherry) with long coastal borders of Bay of Bengal ia an ideal site for the study of arbuscular mycorrhizal-plant interactions. Karaikal district is one of four erstwhile French establishments of the Union Territory Puducherry. Karaikal district is embedded in the Nagappattinam and Tiruvarur District of Tamil Nadu State. Latitude lies between 10^{o} 49' and 11^{o} 01' N and Longitude lies between 79^{o} 43' and 79^{o}52' E. Area of Karaikal district is around 161 sq. km. and has a population of 200,222 as per the 2011 census.

2.2. *Sampling of roots and rhizosphere soils*

Representative sites of coastal soils were selected around Karaikal coast. Selected Plants are examined for arbuscular mycorrhizal association. Periodic surveys for the year 2012-2013 are undertaken to study the seasonal variation of mycorrhizal fungi. Karaikal district is very warm and dry throughout the year. Based on the Climate, three seasons were recognized.

Winter (December, January, February and March)
Summer (April, May, June and July)
Rainy (August, September, October, November)

The average annual rainfall varies from 827 mm to 2100 mm (during last ten years) and it is received both from Southwest monsoon (June to September) and Northeast monsoon (October to December). Throughout the year, the coastal areas of Karaikal show higher relative humidity (average value 78%).

Fine feeder roots of plants growing in coastal saline soils were collected and cut into 1 cm fragments and fixed in FAA, Root zone soil samples are collected up to 30 cm depth [20], sealed and brought to the laboratory. Soil samples are air died and kept 5^{o}C to 10^{o}C [21] for further analysis. Part of the soil sample was used to analyze the physicochemical properties and other part was used for isolation of AM spore extraction.

2.3. *Preparation of soil for analysis*

Each soil sample was spread on a flat wooden or plastic tray and was allowed to dry in air under shade. Stones and pieces of macro organic matter were removed. Large lumps were broken by hand and the soil was ground by rolling gently with a wooden roller. After grinding, the soil screened through a 2mm sieve and the fine soil was used for further analyses. Elico P^{H} meter was to measure the soil P^{H}. Soil organic matter was determined by rapid titration method of Walkey and Black [22]. Available nitrogen (N), Potassium (P) [23], available phosphorus (P) [24] and available micronutrients [25] were estimated.

2.4. *Evaluation and Arbuscular mycorrhizal colonization*

The root samples were cleared and stained in tryphan blue employing modified version of Phillips and Hayman's [26] method. Roots were cut into 1-2 cm pieces, heated at 90 c in 10% KOH for about 1 h. For thicker and older roots, the duration was increased. The

root segments were stained with 0.05% tryphan blue in lactophenol for 5 min and the excess stain was removed with clear lactophenol.

The pigmented roots were heated at 90°C in 10 % KOH for 2 h, washed with fresh 10%KOH and immersed in a an alkaline solution of H_20_2 for 30 min at 25°C until bleached. They were rinsed thoroughly with water to remove the H_20_2 acidified in dilute HCL and stained as described earlier. In some cases the modified method of Merryweather and Fitter [27] was followed where autoclaving and bleaching with H_20_2 were omitted. In a few cases, direct observation of unstained, fresh and intact fine feeder roots [28] was made.

AM colonization in the roots was assessed following the gridline-intersect method of Giovennetti and Mosse [29]. The stained root pieces were spread out evenly on a square plastic Petri dish (10.2x10.2cm). A grid of lines was marked on the bottom of the dish to form 1 cm inch squares. Vertical and horizontal gridlines were scanned under a dissecting microscopes and the presence of colonization was recorded at each point where the roots intersected a line. The percentage of AM infection was calculated using the formula:

$$\text{Per cent Colonization} = \frac{\text{No. of root segments infected}}{\text{Total no. of root segments observed}} \times 100$$

When sufficient root pieces are not available, the slide method [29] was followed. Root pieces, 1cm long were selected at random form a stained sample and mounted on microscope slides in groups of 10. Presence of colonization was recorded in each of the 10 pieces and per cent colonization was calculated. To observe hyphae, vesicles and arbuscules under light microscope, the root pieces were mounted on glass slides either temporarily in lactophenol or permanently in polyvinyl alcohol resin lactophenol. The cover slip was pressed gently to make the roots flattened and sealed with DPX.

2.5. *Isolation of arbuscular mycorrhizal spores from soil samples*

Spores were recovered from soil samples by the wet sieving and decanting method [30]. Form each soil sample, l00g soil was taken and mixed with 1L of luke-warm water in large beaker until all the aggregates dispersed to leave a uniform suspension. Heavier particles were allowed to settle down. To remove organic matter and roots, the suspension was decanted through a 710 urn sieve. The suspension sieves were collected in petri dishes with about 10-20 mL water and were observed under a dissection microscope for AM fungal spores.

Sucrose centrifugation method [31] was also followed to isolate spores. Soil of l00 g was mixed with 1 L of water in a large beaker. Heavier particles were allowed to settle down. The suspension was decanted through 710 um and 45 um sieve was transferred into two or four 50 mL centrifuge tubes and centrifuged for 5 min at 1750 RPM on a horizontal rotor. The supernatant liquid was carefully decanted and the pellet was re suspended in a 48% (w/v) sucrose solution (227g sucrose in 500mL water). Prior to re centrifugation, the suspension was thoroughly and centrifuged at 1750 rpm for 15 sec. The supernatant containing the spores was quickly poured through a 45 µ*m* sieve and rinsed with water to remove the sugar in order to reduce the osmotic pressure. The spore sample was transferred to a small petri dish by washing with above 10-20 mL of water and observed under a dissecting microscope.

Total spore count was calculated by counting the spores by MPN method [32]. These the spores were separated using a glass pipette and segregated. The spores were mounted on clean glass slides using lactophenol or polyvinyl alcohol lactophenol (PVL) covered with cover slips and sealed with DPX. Spores were stored in distilled water after disinfection with traces of surfactant in small tubes for further studies.

2.6. *Identification of arbuscular mycorrhizal Fungi*

Based upon microscopic character, the AM spores were identified. For identification and nomenclature, synoptic keys of the following authors were used: Hall and Fish [33]; Trappe [34]; Walker [35-36]; Hall [37]; Walker and Sanders [38]; Walker and Koske [39]; Raman and Mohan Kumar [40]; Morton and Benny [41]; Schenck and Perez [42]; Walker and Trappe [43] and Redecker et al. [44]. Classification was based on colour, size, shape, surface structure, general nature of the spore contents and hyphal attachments. Photomicrographs were taken with the help of a Ziess Jena microscope.

3. Results & Discussion

In the present study, five sites were selected based on the vegetation availability in the coastal soils. Plant root samples and their rhizosphere soils of five study sites of Karaikal coastal region were collected every month intervals (Table-1). Physio-chemical analyses of rhizosphere soils showed that all the study sites were alkaline and high Electrical Conductivity levels were more than 4 dsm^{-1}. Exchangeable Sodium Percentage (ESP) of all the study sites coastal soils exhibited moderately sodic category. Organic matter and macronutrients were found to be very low, especially soil phosphorus (2.9 to 4.8 kg per acre) in all the study sites (Table-2).

A total of twenty one species of vascular plants were screened for mycorrhizal colonization and ten species of AM mycorrhizal fungal species were recorded in the rhizosphere soils based on seasonal field surveys. Twenty-one plant species in five study sites were analyzed for mycorrhizal colonization. Among them, thirteen plant species belonging to nine families were found to be mycorrhizal (Table-3). Mycorrhizal fungi in coastal soils colonized maximum number of herbaceous plants than

Table -1. Study sites of coastal soils of Karaikal District

S.No.	**Sites**	**Nearest places**
1.	**Site -1**	Kalikuppam coastal region
2.	**Site-2**	Kottucheri Medu coastal region
3.	**Site-3**	Karaikal Medu coastal region
4.	**Site-4**	Neravy coastal region
5.	**Site-5**	T.R.Pattinum coastal region

Table –2. Physio-chemical characteristics of coastal soils of Karaikal District

Sites	**pH**	**EC (dsm^{-1})**	**ESP**	**OC (%)**	**N (kg/ha)**	**P (kg/ha)**	**K (kg/ha)**
Site-1	7.6	4.5	12.1	0.57	91	4.0	120
Site-2	7.9	4.7	12.6	0.91	85	4.8	132
Site-3	8.1	5.6	13.1	0.87	54	2.9	156
Site-4	7.9	4.9	14.3	0.39	99	3.7	129
Site-5	8.2	5.3	14.4	0.61	52	4.6	101

Table – 3. Arbuscular mycorrhizal status of plant species in coastal soils of Karaikal District

S.No.	**Name of the Plant species**	**Family**	**Habit**	**Mycorrhizal colonization**
1.	*Calophyllum inophyllum* L.	Clusiaceae	Tree	--
2.	*Thespesia populnea* Cav.	Malvaceae	Tree	+
3.	*Azadirachta Indica* A.Juss.	Meliaceae	Tree	+
4.	*Pongamia pinnata* (L.) Pierre.	Faboideae	Tree	+
5.	*Prosopis juliflora* (Sw.) DC.	Faboideae	Herb	+
6.	*Samanea saman (Jacq.)Merr.*	Faboideae	Tree	+
7.	*Tephrosia purpurea*(L.) Pers.	Faboideae	Herb	+
8.	*Rhizophora apiculata* Blume	Rhizophoraceae	Tree	-
9.	*Sesuvium portulacastrum* L.	Aizoaceae	Herb	+
10.	*Catharanthus roseus* (L.) Don.	Apocynaceae	Herb	+
11.	*Calotropis gigantea* R.Br.	Asclepiadaceae	Shrub	+
12.	*Ipomaea per-caprae* (L.) R.Br.	Convoluvaceae	Herb.	+
13.	*Datura metel* L.	Solanaceae	Herb	+
14.	*Solanum trilobatumL.*	Solanaceae	Herb	+
15.	*Acanthus ilicifolius* L.	Acanthaceae	Shrub	-
16.	*Avicennia marina* (Forsskal) Vierh.	Avicenniaceae	Shrub	-
17.	*Excoecaria agallocha* L.	Euphorbiaceae	Shrub	-
18.	*Casuarina equisetifolia Forst. & Forst.*	Casuarinaceae	Tree	+
19.	*Pandanus tectorius* Park.	Pandanaceae	Shrub	-
20.	*Cyperus rodentus* L.	Cyperaceae	Herb	-
21.	*Spinifix littoreus* (Brum.f) Merr.	Poaceae	Herb	-

Table-4: Seasonal variation of colonization percentage of mycorrhizal fungi in various plant species growing in coastal soils of Karaikal district

S.No.	**Plant's name**	**Percentage of colonization of mycorrhizal fungi**		
		Summer	**Rainy**	**Winter**
1.	*Azadirchta indica*	60	20	35
2.	*Pongamia pinnata*	54	16	24
3.	*Tephrosia purpurea*	50	28	39
4.	*Prosopis juliflora*	53	21	40
5.	*Samanea saman*	51	18	34
6.	*Catharanthus roseus*	53	30	39
7.	*Calotropis gigantea*	41	29	32
8.	*Datura metel*	43	28	38
9.	*Solanum trilobatum*	51	33	41
10.	*Casuarina equisetifolia*	73	38	58

Table- 5: List of arbuscular fungal species isolated from Coastal soils of Karaikal district

Genus	Species
1. *Glomus*	1. *Glomus fasciculatum* 2. *Glomus mosseae* 3. *Glomus geosporum* 4. *Glomus sp.*
2. *Gigaspora*	1. *Gigaspora margarita*
3. *Scutellospora*	1. *Scutellospora calospora* 2. *Scutellospora nigra* 3. *Scutellospora* sp.
4. Sclerocytis	1. *Sclerocytis dussii* 2. *Sclerocytis pachycaulis*

Table-6: Seasonal variation of arbuscular mycorrhizal fungal spores in coastal soils of Karaikal district

S.No.	Plant's name	Arbuscular mycorrhizal fungal spore population (per 100 g of rhizosphere soil)		
		Summer	Rainy	Winter
1.	*Azadirchta indica*	139	100	208
2.	*Pongamia pinnata*	140	098	203
3.	*Tephrosia purpurea*	138	080	180
4.	*Prosopis juliflora*	130	074	185
5.	*Samanea saman*	125	085	173
6.	*Catharanthus roseus*	100	060	161
7.	*Calotropis gigantea*	090	051	118
8.	*Datura metel*	093	058	105
9.	*Solanum trilobatum*	109	071	190
10.	*Casuarina equisetifolia*	145	110	258

tree plants whereas higher percentage of colonization found only in tree plants.

All the study sites revealed that AM colonization was found in all the months. AM colonization was indicated by the presence of hyphal networks, arbuscules, vesicles and endospores. Roots segments of AM colonization percentage were ranging from 0 - 73%. The highest colonization (73%) found in roots of *Casuarina equisetifolia* during summer season (Table-4). Time of sampling was compared with AM colonization levels, and seasonal trend of AM colonization was similar for all the six study sites. Highest levels of AM colonization were observed in summer season and lowest levels of colonization in rainy season. In summer, arbuscular number found to be high in colonized roots. Vesicles were present in all the seasons of the year.

A total of 10 species of AM fungi belonged to 4 genera, including one species which belongs to *Glomus,* three species to *Scutellospora,* two species to *Sclerocystis* and one to *Gigaspora* were isolated from coastal soils of Karaikal (Table-5). AM spore densities were also surveyed in rhizosphere soils of mycorrhizal plants growing in study sites of Karaikal. There had significant differences in AM spore density between different Soil depths in the rhizosphere. Maximum AM spore density recorded in 0 to 40 cm soil depth. Number of AM spores decreased with increasing soil depth in coastal study sites. Among the mycorrhizal plants, *Casuarina equisetifolia* had maximum AM spore density (258 AM spores/100g) followed by *Azadirachta indica* (208) and *Pongamia pinnata* (203). Lowest AM spore density (105) was recorded in rhizosphere soils of *Datura metel* (Table -6). In winter season AM spore density was maximum and rainy season it was very low. In the present work, *Glomus* and *Scutellospora* species were dominant in coastal soils of Karaikal District.

Coastal saline soils are subjected to large temporal and spatial variation of soil properties. Arbuscular mycorrhizal fungi (AMF) occur in a wide variety of ecosystems, such as farmland to forestland as well as many stressful environments. The distribution of AMF in different soil regions and their relations to soil properties and native plants have been investigated by several workers [45-47]. High levels of salinity and flooding in soils were observed to significantly reduce the extrametrical mycelium length of host plants from the Pancas salt marsh [48]. In this present work, proportion of active spores in the coastal soils indicate that the pool of AM spores is likely sufficient to maintain the usual level of AM colonization in coastal saline soils. Further studies on the effect of flooding, infectivity of each type of propagule are necessary to determine whether spores as resistant structures, function as survival units of AMF in coastal saline soils.Spore density and root colonization showed significant correlation with plant species and soil properties. High soil salinity, poor plant diversity and low

vegetation cover in saline-alkaline soils of the yellow River Delta may severely restrict colonization and diversity of AMF [49]. AMF may not only benefit plant growth and development, but also increase the resistance of plants to stresses such as extremes of pH and salinity [17, 50]. The present study found evidences for potential adaptation of indigenous AM fungi in salt affected soils and their ability to colonize saline tolerant plant communities in the coastal soils. Results of the present work suggest that the dominat AM fungi in coastal soils may be used as potential biofertilizers for reclamation of this ecosystem.

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