IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 12, Issue 6 Ver. III (Nov. – Dec. 2017), PP 53-59
www.iosrjournals.org

Morphological Type, Host Specificity And Molecular Characterization Of Arbuscular Mycorrhiza Colonizing Roots In Salt Marsh Plant Of Mallipattinam And Manora Estuaries Southern Coastal Area Tamil Nadu, India.

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Abstract: *In this investigation, the morphological types of AM fungi in 12 plant species belonging to 10 genera from 8 families of salt marsh plants were examined. Among them eleven salt marshes in nature establishes AM fungal symbiosis of the Arum-type except in Sesuvium portulacastrum which is Paris-type of AM and can be positively colonized by AM fungi viz., Glomus aggregatum and G. geosporum in among 24 species were recovered. Modification of the diversity of AMF in two salt marsh plants, Salicornia brachiata and Sesuvium portulacastrum, were evaluated by analysis of partial sequences in the large subunit (LSU) ribosomal DNA (rDNA) genes. General primers for AMF were designed for PCR amplification of partial sequences using DNA extract from root tissues of S. brachiata and S. portulacastrum.PCR products were used to construct LSU rDNA libraries, sequencing of randomly selected clones indicated that plant roots were colonized by AMF belonging to the genera Glomus particularly G. aggregatum and G. geosporum.*

Keywords: *AM Fungi, Salt marsh plants, Paris and Arum, LSU rDNA, PCR.*

Date of Submission: 04-10-2017 Date of acceptance: 30-11-2017

I. Introduction

1.1Morphological type and Host specificity

Salt marsh plants are being detritus based ecosystem, Substantial AM Fungal population are involved in detritus processing. The accumulation and infection of AM fungi provide nutrients and energy to the host plant. Root morphology of AM fungi is divided into two types, Arum type and Paris type. In Arum-type associations, the hyphae grow intercellularly in the root cortex and penetrate with short side branches into cortical cells to form arbuscules within. In Paris-type associations, intracellular hyphal coils frequently having intercalary arbuscules spread cell to cell in the cortex. Until, recently, it has been believed that the Arum-type of AM is more common than the Paris-type, since most cultivated herbaceous plants that have been used in studies form the Arum-type [1,2] indicated in reviews that the Paris-type of AM is found in a rather wide range of plant taxa such as ***Paris, Parnassia, Colchicum*** [3]. ***Gentiana*** [4], ***Erythronium, Trillilum, Asarum*** [5] and Acer [6]. Brundrett and Kendrick(1990) discussed that slower colonization of Paris-type AM might be beneficial for the host plants to keep the energy supply to the fungi reduced and might be desirable for plants growing slowly in a woodland environment. Yamato and Iwasaki (2002) [7] found that morphological types of AMF in roots of understory plants were examined in three different Japanese deciduous broad leaved forests. In total, 43 species belonging to 33 genera from 27 families were examined for the morphological types of AMF. The number of flowering plant species having Paris-type AM was greater than those having Arum-type AM in each plot. This tendency was more prominent in herbaceous plants than plants with nine species having Paris-type associations among ten herbaceous plant species examined. It is well known that the AMF are not host specific. But the degree of AM infection and its effect can differ with different host endophyte combinations [8] Several recent studies support the notion of host preference by AM fungi[9,10] and forage legumes with AM, exhibited considerable host-mycorrhizal specificity [11, 12]. Different levels of host mycorrhizal affinity existed when plants were inoculated with a number of AM species [13]. There is little evidence of host specificity in most types of mycorrhizal associations [14, 15].

1.2 Molecular Characterization:

There is restrictions make the identification of AMF colonizing certain plant hosts difficult to achieve with field-collected material [16, 17]. There is increasing evidence that plant biodiversity and productivity in ecosystems are significantly influenced by AMF diversity [18]. It is also known that different AMF can

simultaneously colonize a single root segment [17]. In an attempt to determine the identity and diversity of AMF within roots, there is increasing in the use of molecular-based tools [19, 20, 21, 22, 23, 24]. Recent studies have used polymerase chain reaction (PCR) technique have used polymerase chain reaction (PCR) techniques coupled with isolate – of group-specific primers [16,25,26,27,28,29,30]. PCR procedures with species-specific primers have been successfully used on material from pot cultures of known fungal species [25,31,32,28] and also on field-collected material [22,26,27,33,34,35,36]. Hence the present study was to know the morphological type and molecular characterization of Arbuscular Mycorrhiza colonizing roots in salt marsh plant.

II. Materials And Methods

2.1 Sampling

Twelve salt marsh plant species, from eight families, soil and root sample were collected at two different study localities viz., Manora and Mallipattinam coast of Thanjavur District, TamilNadu. AM Fungi spore and sporocarps were isolated [37] and identified (38, 39, 40]. Root samples were cut into one cm bits and then cleared with trypan blue in lactophenol was performed using the method [41] and root colonization was calculated [42].The morphological types of AM were examined [2].

2.2 Molecular characterization of AMF in the roots of salt marsh plants

Modification of the diversity of AMF in salt marsh plants were evaluated by analysis of partial sequences in the large subunit (LSU) ribosomal DNA (rDNA) genes. General primers for AMF were designed for the PCR amplification of partial sequences using DNA extracted from root tissues of *Salicornia brachiata* (Arum-type) and *Sesuvium portulacastrum* (Paris-type) only with different type of infection. There was a difference in the diversity of AM fungal colonizing roots of both the plants that was confirmed by PCR using primers specific for each sequence group

2.3DNA extraction from roots

Aliquots (1 g) of fresh roots samples in each plot were homogenized in liquid nitrogen and then in 1 ml of extraction buffer (0.2 M Tris-HCl pH 8.0, 0.25 M NaCl, 0.025 M EDTA, 0.5%, SDS, 1% PVP, 29 mM β-mercaptoethanol). The supernatant obtained after a 10 min centrifugation at 12,000 rpm at 4°C was recovered and mixed with an equal volume of phenol. After centrifugation, one volume of chloroform was added to the aqueous phase and the samples were centrifuged again. DNA was then precipitated from the resulting aqueous phase by adding one volume of isopropanol. The pellet was rinsed with 100 µl 75% ethanol, dried and resuspended in 50 µl sterile distilled water. The quality and quantity of DNA in the samples were checked on a 0.8% agarose gel and a 1/1000 dilution was used for PCR. Initially, aliquots of DNA extracted from roots of ***S. brachiata*** and ***S. portulacastrum*** were pooled for the construction of LSU rDNA libraries, each representing a total of 12 cores. Afterwards, PCR using cluster-specific primers was performed on each of the five replicated DNA extracts per plant.

2.4 Design of general PCR primers for AM fungi

The 5' end of LSU rDNA sequences from different AM fungi previously published in Genbank was aligned using ClustalW 1.8.1[43]. This sequence information was used to design new primers: FLR3 (5'-TTG AAA GGG AAA CGA TTG AAG T-3') and FLR4 (5'-TAC GTC AAC ATC CTT AAC GAA-3') using Amplify [44] for the PCR amplification of AM fungal sequences from root tissues without recognistion of plant DNA. FLR3 is situated between the D1 and D2 domains of LSU rDNA whilst FLR4 is in the D2 domain.

2.5 PCR amplification of a partial LSU rDNA region

The primers LR1 and FLR2 [45, 30] were used for the amplification of the 5' end of LSU rDNA sequences in fungi in general. A 20-µl reaction mix contained 2 µl 10 x PCR buffer (Appligene), 20 mM dNTPs, 500 nM each primer and 0.4 U Taq polymerase (Appligene). An aliquot (1 µl) of diluted root DNA extract was added to 19 µl PCR mix. The PCR program was as follows: 93°C for 1 min. 58°C for 1 min and 72°C for 1 min (35 cycles), followed by 10 min at 72°C. PCR products were diluted 1/100 and used as templates for the second PCR with the primers FLR3 and FLR4 under the same PCR conditions, PCR products were run on a 2% agarose gel in TAE buffer and visualized under UV light after staining with ethidium bromide.

2.6 Construction of LSU rDNA libraries and sequencing

The PCR products generated from roots of ***S. portulacastrum*** and ***S. brachiata*** using the primers FLR3 and FLR4 were cloned into the PCR 2.1 vector (Invitrogen). Inserts from 50 randomly selected clones in each LSU rDNA library were sequenced using the M13 forward (-20) primer on an ABI automated sequencer

(Department of Medical Microbiology, University of Aberdeen, UK). Sequences were compared to known sequences using BLASTIN[46].

III. Results

Tamil Nadu State; India	
District	**Study localities**
Mallipattinam – Thanjavur district	S1-Puduppattinam
	S2-Kollukkadu
	S3-Chinnamanai
	S4-Karanguda
Manora – Thanjavur district	S5-Pillaiyar Thidal
	S6-Sethubhava Chattram
	S7-Senthanai
	S8-Sembaippattinam

3.1 Morphological types of AM fungi in salt marsh plants

From the study locality (Table 1) of 12 salt marsh plants, 1-24 AM fungal species belonging to 5 genera were recovered (Table 2). In root study some new records on the morphological types of AM in some non-mycorrhizal plant families were obtained including the first report of a typical Arum-type AM and Paris-type of AM in salt marsh angiosperms (Plates I-III; Table 3). All the twelve salt marsh plants found at each study site of both estuaries were colonized by some kinds of mycorrhizal fungi. No plants having both or intermediate types of AM were found in this study, thus all of the examined plant species colonized by AMF can be divided into two groups with Arum-type or Paris-type AM within the survey. In both the study localities, the Arum-type was more frequently found than the Paris-type in each level of plant taxonomy, from species to family. Only one species (***Sesuvium postulacastrum***) showed the Paris-type among 12 plant species examined.

3.2 Molecular characterization of AM colonizing roots of salt marsh plants

Modification of the diversity of AMF in two salt marsh plants were evaluated by analysis of partial sequences in the large subunit (LSU) ribosomal DNA (rRNA) genes. General primers for AMF were designed for PCR amplification of partial sequences using DNA extract from root tissues of ***S. brachiata*** and ***S. portulacastrum***. PCR products were used to construct LSU rDNA clones indicated that plant roots were colonized by AMF belonging to the genera ***Glomus*** particularly ***G. aggregatum*** and ***G. geosporum***. There was a difference in the diversity of AM fungal colonizing roots of ***S. brachiata*** and ***S. portulacastrum*** that was confirmed by PCR using primers molecular data suggest the existence of a selection pressure of plants on AM fungal communities (Plate IV-V).

IV. Figures And Tables

Table 1: Sampling Area

Table 2: AMF in the rhizosphere soil of 12 salt marsh plants of Mallipattinam and Manora estuaries.

AMF SPECIES	UNIQUE CODE
ACAULOSPORA	
Acaulospora delegata (Walker & Sanders)	ADLA
A. denticulata (Sieverding & Toro)	ADCT
A. lacunose (Trappe)	ALNA
A. marrowae (Becker & Hall)	AMWE
A. scrobiculata (Trappe)	ASCB
GIGASPORA	
Gigaspora decipiens (Hall & Abbott)	GDCP
G. margarita (Becker & Hall)	GMRG
GLOMUS	
Glomus aggregatum (Schenck & Smith)	LAGR
G. deserticola (Trappe, Bloss & Menge)	LDST
G. dimorphicum (Bcyetchko & Tewari)	LDPM
G. fasciculatum (Thaxter Sensu Gerd & Trappe)	LFSC
G. intraradices (Schenck & Smith)	LGSP
G. mosseae (Nicol & Gerd)	LMSS
G. maculosum (Thaxtar) Trappe & Gerd.	LMTM
G. reticulatum (Bhattacharjee & Mukerji)	LRTC
SCLEROCYSTIS	
Sclerocystis pakistanika (Iqbal & Bushra)	SPKS

S. pachycaulis (Iqbal & Bushra)	SSNS
SCUTELLOSPORA	
Scutellospora calospora (Nicol. Gerd.) Walker & Sanders	CCLS
S. erythropa (Koske & Walker) Walker & Sanders	CEPA
S. gregaria (Schencki & Nicol) Walker & Sanders	CHTG
S. heterogama (Nicol. & Gerd.) Walker & Sanders	CHTG
S. pellucida (Nicol. & Schenck) Walker & Sanders	CPLC
S. persica (Red head) Walker & Sanders	CPSA
S. verrucosa (Koske & Walker) Walker & Sanders	CVRC
* Unique code – Schenck and Perez (1990).	

Table 3: Morphological types and positive for AM fungi in the roots of salt marsh plants of Mallipattinam (S1-S4) and Manora (S5-S8) estuary study localities (Mean of five replicates)

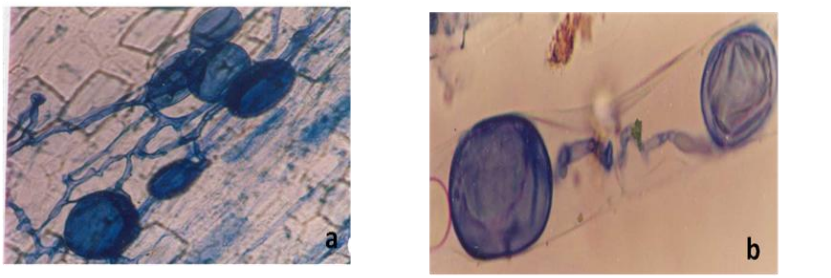
Plant family	Plant species	Positive for AM fungi in the roots	Morphological types of AM fungi
Acanthaceae	*Acanthus ilicifolius* (L.)	*Glomus aggregatum*	Arum-type
Aizoaceae	*Sesuvium portulacastrum* (L.)	*Glomus geosporum*	Paris-type
Chenopodiaceae	*Arthrocenemum indicum* Moq	*Glomus* spp.	Arum-type
	Salicornia brachiata Roxb.	*Glomus aggregatum*	Arum-type
	Suaeda maritima (L.) Dumat	*Glomus aggregatum*	Arum-type
	Suaeda monoica Fersk.	*Glomus aggregatum*	Arum-type
	Suaeda nudiflora Moq.	*Glomus* spp.	Arum-type
Euphorbiaceae	*Excoecaria agalocha* L.	*Glomus* spp.	Arum-type
Lamiaceae	*Leucas plukenetti* (Roth)	*Glomus aggregatum*	Arum-type
Poaceae	*Alerupus lagopoides* (L.) Trin. ex. Thw.	*Glomus aggregatum*	Arum-type
Solanaceae	*Solanum xanthocarpum* L.	*Glomus aggregatum*	Arum-type
Verbinaceae	*Phyla nodiflora* (L.) Greene.	*Glomus aggregatum*	Arum-type

***S1-S8 – Study Sites.**

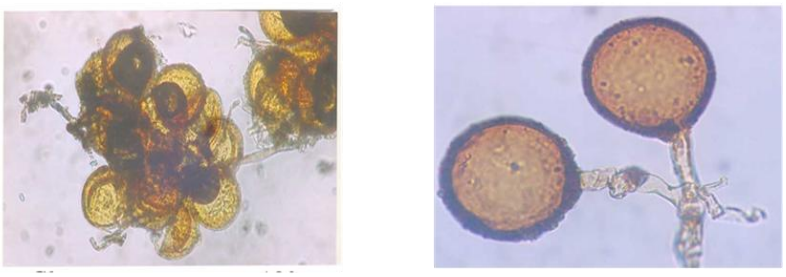
Fig I-III

Arum and Paris type of AM fungi in root cortical cells.

a. Arum-type *salicornia brachiata* b.Paris-type *sesuvium portulacastrum*



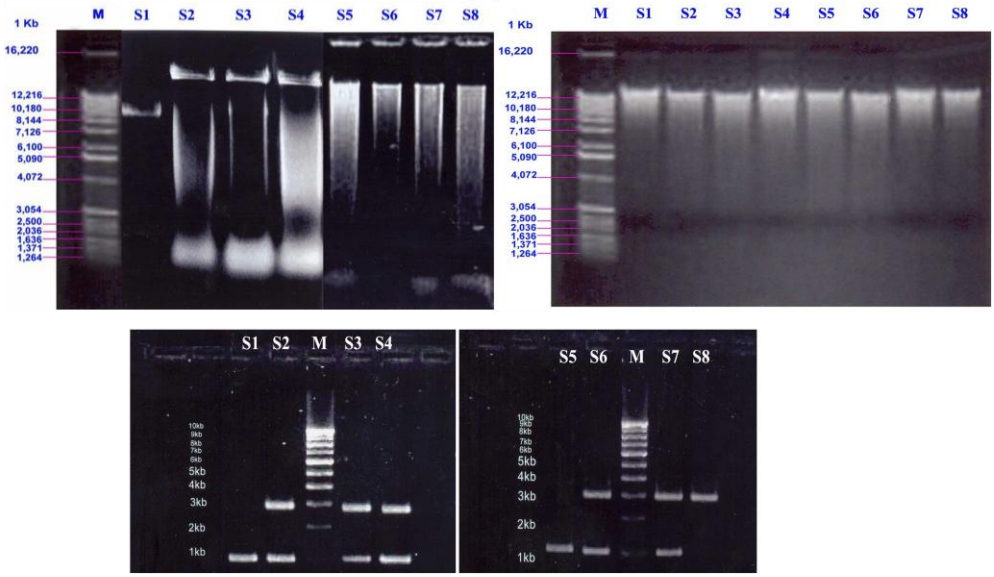
Chlamydospores of Glomus species isolated from root-zone soils of marsh plants at Manora and Mallipattinum esturies.



Glomus aggregatumx180 *Glomus geosporum x240*

Fig: IV

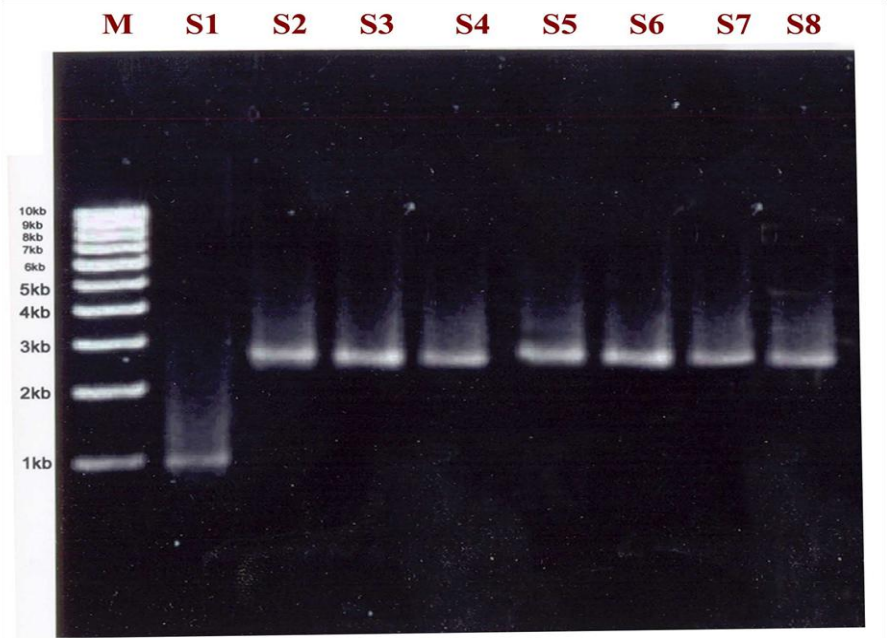
Genomic DNA separated from AMF colonized roots 2 species.
a.*Salicornia brachiata.* *b.sesuvium portulacastrum.*



General primers for AM fungi designed for the PCR amplification of partial sequences using DNA extracted from root tissues of *salicornia brachita.*

Mycorrhizal colonized roots of salicornia brachita collected from different study sits (S1-S8)
Study sites: Abbreviations as per tables 1.

Fig- V



General primers for AM fungi designed for the PCR amplification of partial sequences using DNA extracted from root tissues of *sesuvium portulacastrum.*

*Study sites: Abbreviations as per tables 1.

IV. Discussion And Conclusion:

It is well known that the AMF are not host specific. Any AM plant species can be infected by any AM fungal species but the degree and type of AM infection and its effect can differ with different host endophyte combinations. Out of nineteen plant species; Graw *et al.* (1979) found that ***Glomus gerdemanni*** infected only one plant species. Mc Gonigle and Fitter (1990) reported in a study of two native grasses and forbs in England that arbuscular mycorrhizas in the field demonstrated a degree of ecological specificity. An interesting feature observed in this study was the AM spore and sporocorps isolation and colonization pattern in salt marshes which showed exclusively of ***Glomus aggregatum*** and ***Glomus geosporum.*** A similar report of AM colonization in specific host plants were also appeared [11,42,47,48,49,50,51,52].

There is increasing evidence that their diversity has a significant impact on plant biodiversity, productivity and ecosystem stability[18]. A number of molecular techniques for identification of AMF have been employed to study their diversity and identity. In this study, the modification of the diversity of AMF in salt marsh plants was evaluated by analysis of partial sequences in the large subunit (LSU) ribosomal DNA (rDNA) genes. General primers for AMF were designed for the PCR amplification of partial sequences using DNA extracted from root tissues of ***S. brachiata*** and ***S. portulacastrum***. PCR products were used to construct LSU rDNA libraries. Sequencing of randomly selected clones indicated that plant roots were colonized by AMF belonging to the genera, ***Glomus, Acaulospora*** and ***Scutellospora***. There was a difference in the diversity of AMF colonizing roots of ***S. brachiata*** and ***S. portulacastrum*** that was confirmed by PCR using primers molecular data suggest the existence of a selection pressure of plants on AM fungal communities.

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IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) is UGC approved Journal with Sl. No. 5012, Journal no. 49063.

Asokan.S Morphological Type, Host Specificity And Molecular Characterization Of Arbuscular Mycorrhiza Colonizing Roots In Salt Marsh Plant Of Mallipattinam And Manora Estuaries Southern Coastal Area Tamil Nadu, India." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) , vol. 12, no. 6, 2017, pp. 53-59.