# ARBUSCULAR MYCORRHIZAL FUNGI FROM SOME COASTAL PLANTS OF KARACHI (PAKISTAN)

## Abdul Rauf, D. Khan and M. Javed Zaki

Department of Botany, University of Karachi, Karachi – 75270, Pakistan.

#### **ABSTRACT**

Fourteen species of angiosperms belonging to 11 genera and 8 families were tested for their association with AM fungi in their roots and rhizospheres in Hawkes Bay coastal environment of Karachi, Pakistan. Thirteen species of AM fungi belonging to four genera were found to associate with the angiosperms. Among these fungi, Genus Glomus was the most dominant genus - represented by nine species. The maximum number of AM fungi (8) associated with a legume, Lotus garcinii, followed by a composite Launaea resedifolia (7 AM fungi) and a grass Aeluropus lagopoides (7 AM fungi). The lowest number of AM fungi (2) associated with Atriplex griffithii and Avicennia marina. Each of the 14 species tested for AMF occurrence had at least association with one Glomus species. The number of Glomus species associated with some angiospermic species was quite substantial. The frequency of occurrence of AM fungi with the angiospermic species, Glomus macrocarpum was the most frequent fungi (57.14%), Glomus epigaenum and Gigaspora geosporum associate with 7 angiospermic species each. (Freq: 50%). Glomus leptotichum and Gigaspora margarita associated with 6 species each (Freq: 42.86%) and Glomus mossaee, Acaulospora laevis and A. birecticulata had frequency of 35.71% (association of each AM fungi with five angiospermic species. The number of AMF spores per 100 g rhizospheric soil of angiospermic species varied substantially (CV: 48.7%) amongst the species tested and averaged to a grand mean of  $400.31 \pm 52.09$  spore. The number of AMF spores were lesser than the grand mean in case of Cyperus longus, Indigofera argentea, Ipomoea pes-caprae, Heliotropium subulatum, Heliotropium sp. and Suaeda sp. whereas the number of AMF spores were near equal to grand mean value in case of species viz. Aeluropus lagopoides, Atriplex griffithii and Suaeda fruticosa. The number spores were considerably larger than the grand mean value in A. marina, C. cretica, L. resedifolia, L. garcinii, and Cyperus sp.

Root colonization (%) also varied substantially amongst the angiospermic species (CV: 49.59%) and averaged to a grand mean of 22.40 ± 2.97. Root colonization happened to be lesser than the grand mean value in *Atriplex griffithii*, *Cyperus longus, Indigofera argentea, Ipomoea pes-caprae, Heliotropium* sp., *Launaea resedifolia* and *Suaeda* sp. and substantially larger than the grand mean value in *Aeluropus lagopoides, Avicennia marina, Cressa cretica, Heliotropium subulatum, Lotus garcinii, Suaeda fruticosa*, and *Cyperus* sp. The mean number of AMF spores per 100 g rhizospheric soil was quite larger in magnitude in Families Leguminosae and Asteraceae and somewhat equally moderate in Families such as Convolvulaceae, Chenopodiaceae, Cyperaceae and Poaceae.

AMF clusters were recognized by Ward method of Hierarchical clustering – each cluster was a collection of highly similar species.

Cluster A: Glomus clariodeum + Glomus geosporium; Cluster B: Gigaspora margarita; Cluster C: Glomus epigaenum + Acaulospora birecticulata + Scutellospora dipapillosa; Cluster D: Glomus scaledonium + Glomus leptotichum; Cluster E: Glomus mosseae + Glomus intraradices; Cluster F: Glomus clariodeum + Glomus macrocarpum + Acaulospora laevis

The following seven clusters of angiospermic species were recognized on the basis of presence or absence of AMF species. Each cluster was a collection of closely similar species.

Cluster A: A. marina + S. fruticosa; Cluster B: Ipomoea pes-caprae + Cyperus longus; Cluster C: Cressa cretica + Heliotropium sp.; Cluster D: Lotus garcinii; Cluster E: Indigofera argentea + Suaeda sp., Cluster F: Launaea resedifolia + Cyperus sp.; Cluster G: Atriplex griffithii + Heliotropium subulatum + Aeluropus lagopoides.

Key-words: Arbuscular mycorrhizal (AM) fungi, coastal plants, Hawkes Bay, Karachi, Pakistan.

#### INTRODUCTION

Arbuscular mycorrhizal (AM) fungi are very important and almost essential components of soil rhizosphere and play important role in sustainable plant soil environment by forming symbiotic association with roots of plants (Sharma *et al.*, 2009;Vogelsand and Bever, 2010). AM fungi form association with roots of plant belonging to ecologically different habitats. AM fungi are widely distributed and have been found to show mutual relationship with almost 80-85% of all vascular plants (Brundrett, 2002; Wang and Qiu, 2006) from all continents including Asia (Ganesan *et al.*, 1991), Europe (Jansa *et al.*, 2002; Land and Schonbeck, 1991), South America (Caproni *et al.*, 2003; Vestberg, 1999), North America (Dalpe and Aiken, 1998), Antarctica (Cabello *et al.*, 1994), Africa (Redhead, 1977) and from Oceania (Australia) (Hall, 1977) as well. AM fungal colonization have been found to almost all woody plant groups including flowering families like angiosperm and gymnosperm as well as some other non-

318 ABDUL RAUF ETAL.

flowering group including some members of pteridophytes (Mishra *et al.*, 1980; Muthukumar and Udaiyan, 2000), gametophytes of bryophytes (Schüβler 2000) and some aquatic plants (Nielsen *et al.*, 2004) are also known to show AM colonization within roots. In some families like Chenopodiaceae, Cruciferae and Cyperaceae AM association is rare (Hudson, 1986) while no association has so far been reported in Pinaceae, Betulaceae, and Urticaceae (Bagyaraj, 1986). Their density has been reported to vary in wide range of environmental conditions in soil like salinity (Gerdemann, 1968), soil phosphorus level (Jeffries *et al.*, 1988) and soil pH (Read *et al.*, 1976).

AM fungi are universal in occurrence – throughout the World in most terrestrial ecosystems (Vogelsang and Bever (2010). Khan (1974) investigated 52 xerophytes, 21 halophytes and 16 hydrophytes from Pakistan for incidence of mycorrhiza. Since then, a number of researchers in Pakistan have described status of AM fungal spore in different areas and around different crops (Saif and Khan, 1975; Jalal-ud-din and Anwar, 1991). Khan (1972) in Lahore, Anwar and Jalaluddin (2011) in different localities of Sindh, Burni *et al.* (2007, 2011), Burni and Ilahi (2004), Zainab and Burni (2005), Nasrullah *et al.* (2010) in KPK, Sharief *et al.* (2005) in D.I Khan described the distribution of AM fungal spores.

In this paper, 14 plant species of Hawkes Bay (Karachi coast) have been investigated for their association with AM fungi. Isolation of AMF spores from the rhizospheric soil of the studied species and the root colonization of these species by AMF have also been studied.

## MATERIALS AND METHODS

# Isolation and extraction of arbuscular mycorrhizal fungi Soil and root sample collection

To study the population and composition of AM fungal spore in soil, samples of soil along with plant root were collected from five different locations on sand ridge running along the shore line of Hawkes Bay (Karachi Coastal area), Karachi. Roots of plants also collected along with soil sample but they were separated from soil sample in the laboratory. Soil and root samples were kept separately in plastic bags. EC of the saturated extract of the soil was determined as described by Estefan *et al* (2013).

## Isolation of arbuscular mycorrhizal fungi

Spores of Arbuscular mycorrhizal fungi were isolated from soil by wet sieving and decanting technique (Gerdemann and Nicolson, 1963) followed by sucrose centrifugation technique (Jenkins, 1964).

# Study of AM fungal Spores

Fungal spore were quantified in 100ml suspension after sucrose centrifugation technique. Spores of AM fungi were identified morphologically as described by Schenek and Perez (1990).

# Root colonization of AM fungi

To determine AM fungal infection and colonization within root, a combined method of Philip and Haymen (1970) and Koske and Gemma (1989) were used. While percent root colonization were determined as described by Giovannetti and Mosse (1980), McGonigle *et al.* (1990).

## Tissue clearing

Roots of plants were rinsed with tap water, cut into pieces of 1cm and placed in petri plates containing 10% (w/v) KOH. Petri plates containing 10% KOH were heated in autoclaved for 10 min at 15 psi. After heating root segments were taken out from 10% KOH solution and rinse with sterilized water.

## **Bleaching**

Root segments rinse with sterilized water then in alkaline  $H_2O_2$ solution for 10 to 20 minutes until root became bleached. Alkaline  $H_2O_2$  solution was prepared by adding 3mL of NH<sub>4</sub>OH to 30 mL of 10%  $H_2O_2$  and adds 567mL distilled water in it. After treating with  $H_2O_2$  solution, root segments were washed with sterilized water.

#### Acidification

Roots segments were soaked in 1% HCl solution for about 10 minutes for acidification purpose to bind the stain to study AM structure within root, after 10 minutes separate out roots from solution and poured off the solution.

## **Staining**

Roots after acidification with 1% HCl were placed in beaker containing lacto phenol along with 0.05% trypan blue. Roots in lacto phenol solution were heated in autoclaved for 10 min at 15 PSI. Poured off the staining solution and roots were dipped in destaining solution.

## AM fungal root colonization

Ten root segments from each sample were examined under stereoscopic microscope and percent AM root colonization calculated.

#### Statistical analysis

The data were statistically analyzed for descriptive statistical parameters using computer package SPSS v. 12. The dendrograms for AMF and angiospermic species were prepared by cluster analysis on the basis of the presence or absence of species and Ward (1963) method was used for joining species in form of a dendrogram.

#### RESULTS

## Occurrence of AM fungi

Fourteen species of angiosperms belonging to 11 genera and 8 families were tested for their association with AM fungi in their roots and rhizospheres (Table 1) in Hawkes Bay coastal environment of Karachi, Pakistan. Many of these species (9 in number) were the well known halophytic species and associated with soils of differential salinity status (Table 1). Thirteen species of AM fungi belonging to four genera were found to associate with the angiosperms. Among these fungi, Genus Glomus was the most dominant genus - represented by nine species. The occurrence of AM fungi in respect of the angiosperms tested is presented in Fig.1. The maximum number of AM fungi (8) associated with a legume, Lotus garcinii, followed by a composite Launaea resedifolia (7 AM fungi) and a grass Aeluropus lagopoides (7 AM fungi). Cressa cretica, Heliotropium subulatum, Indigofera argentea, Cyperus sp. and Suaeda sp. associated with 5 AM fungi each. Four AM fungi were found to be in the rhizosphere of Cyperus longus and Ipomoea pes-caprae each. Suaeda fruticosa had three AM fungi. The lowest number of AM fungi (2) associated with Atriplex griffithii and Avicennia marina. Each of the 14 species tested for AMF occurrence had at least association with one Glomus species. The number of Glomus species associated with some angiospermic species was quite substantial e.g. six Glomus spp. occurred with A. lagopoides, five Glomus spp. with I. argentea and L. garcinii each, four Glomus spp. occurred with L. resedifolia, Heliotropium subulatum, C. cretica and Ipomoea pes-caprae each. Cyperus longus, Cyperus sp. Heliotropium subulatum and Suaeda sp. had three Glomus spp. each. A. griffithii and S. fruticosa associated with Glomus each.

Table 1. Occurrence of angiospermic species in sites sampled and salinity associated with them.

S.			site	EC: dS.m <sup>-1</sup>
No.	Species	Family	Number	
1.	Aeluropus lagopoides (L.) Trin. Ex Thw.*	Poaceae	1,3	10.10 ± 1.20 (8.9-11.30)
2.	Atriplex griffithii Moq. *	Chenopodiaceae	3,4	13.42 ± 5.59 (7.83-19.0)
3.	Avicennia marina *	Acanthaceae	3	32.70
4.	Cyperus longus	Cyperaceae	3,5	6.69 ±0.31 (6.38-7.0)
5.	Cyperus sp.	Cyperaceae	1,4	$6.66 \pm 1.22 (5.44-7.88)$
6.	Cressa cretica L. *	Convolvulaceae	2,4	14.76 ± 4.24 (10.52-19.0)
7.	Indigofera argentea Burm. F.	Leguminosae	3,5	19.12 ± 10.0 (9.12-29.12)
8.	Ipomoea pes-caprae (L.) R.Br. *	Convolvulaceae	1,5	$9.82 \pm 1.24  (8.58\text{-}11.06)$
9.	Heliotropium subulatum (Hochst) Vatke	Boraginaceae	2	15.05
10.	Heliotropium sp.	Boraginaceae	1,5	17.50 ± 5.50 (12.0-23.0)
11.	Launaea resedifolia (L.) O.K. *	Asteraceae	1,3	13.38 ± 2.40 (8.58-15.79)
12.	Lotus garcinii DC. *	Leguminosae	2,3,4	$7.24 \pm 5.24 \ (1.60 - 12.89)$
13.	Suaeda fruticosa (L.) Forsk. *	Chenopodiaceae	2,5	11.35 ± 1.48 (9.87-12.83)
14.	Suaeda sp. *	Chenopodiaceae	1	15.7

<sup>\*,</sup> well known coastal halophytic species.

320 ABDUL RAUF ETAL.

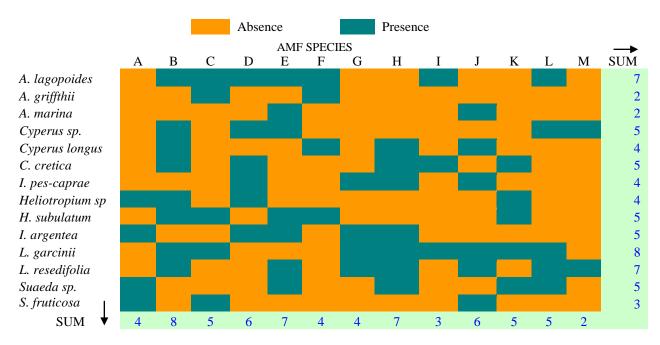


Fig. 1. Representation of association of AMF with various coastal angiospermic species. Acronyms for AMF species: A, *Glomus scaledonium*; B, G. macrocarpum; C, G. mossaee; D, G. leptotichum; E, G. epigaenum; F, G. intraradices; G, G. clarum; H, G. geosporum; I, G. claroideum; J, Gigaspora margarita; K, Acaulospora laevis; L, A. birecticulata; M, Scutellospora dipapillosa.

As regard to the frequency of occurrence of AM fungi with the angiospermic species, Glomus macrocarpum was the most frequent fungi (57.14%), Glomus epigaenum and Gigaspora geosporum associate with 7 angiospermic species each. (Freq: 50%). Glomus leptotichum and Gigaspora margarita associated with 6 species each (Freq: 42.86%) and Glomus mossaee, Acaulospora laevis and A. birecticulata had frequency of 35.71% (association of each AM fungi with five angiospermic species. Three AM fungi viz. Glomus scaledonium, Glomus intraradices and Glomus clarum associated with four angiospermic species. Glomus claroideum and Scutellospora dipapillosa associated with 3 and 2 species respectively. It follows from the results that G. macrocarpum was the most frequent AM fungus in salinity-affected Hawkes Bay ecosystem and S. dipapillosa, the least frequent species.

## Isolation of AM fungal spores from the rhizospheric soil

Spores of AM fungi, in these studies, were identified morphologically as described by Schenek and Perez (1990). The spores of some important AM fungi are shown in Fig. 2 and 3 and their numerical abundance along with root colonization data are presented in Table 2. The number of AMF spores per 100 g rhizospheric soil of angiospermic species varied substantially (CV: 48.7%) amongst the species tested and averaged to a grand mean of 400.31 ± 52.09 spore. The number of AMF spores were lesser than the grand mean in case of *Cyperus longus*, *Indigofera argentea*, *Ipomoea pes-caprae*, *Heliotropium subulatum*, *Heliotropium* sp. and *Suaeda* sp. whereas the number of AMF spores were near equal to grand mean value in case of species viz. *Aeluropus lagopoides*, *Atriplex griffithii* and *Suaeda fruticosa*. The number spores were considerably larger than the grand mean value in *A. marina*, *C. cretica*, *L. resedifolia*, *L. garcinii*, and *Cyperus* sp.

#### **Root Colonization (%)**

Root colonization (%) also varied substantially amongst the angiospermic species (CV: 49.59%) and averaged to a grand mean of 22.40 ± 2.97. Root colonization happened to be lesser than the grand mean value in *Atriplex griffithii*, *Cyperus longus*, *Indigofera argentea*, *Ipomoea pes-caprae*, *Heliotropium* sp., *Launaea resedifolia* and *Suaeda* sp. and substantially larger than the grand mean value in *Aeluropus lagopoides*, *Avicennia marina*, *Cressa cretica*, *Heliotropium subulatum*, *Lotus garcinii*, *Suaeda fruticosa*, and *Cyperus* sp. (Table 2).

It is obvious from the Table 3 that mean number of AMF spores per 100 g rhizospheric soil was quite larger in magnitude in Families Leguminosae and Asteraceae and somewhat equally moderate in Families such as Convolvulaceae, Chenopodiaceae, Cyperaceae and Poaceae. The spores were lesser in Family Boraginaceae.

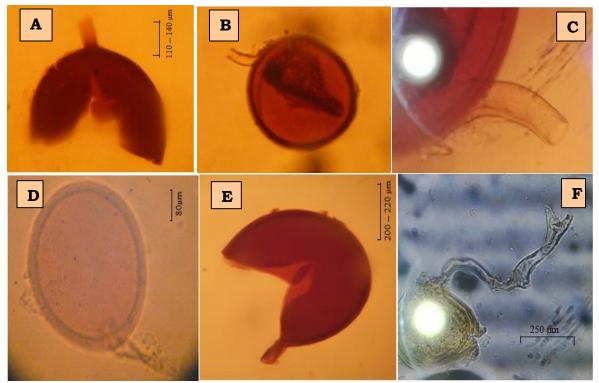


Fig. 2.Spores of *Glomus* species isolated from rhizospheric soil of different plant species. **A-C**) *Glomus intraradices* D) *Glomus claroideum* E) *Glomus geosporum*, F, G. clarum

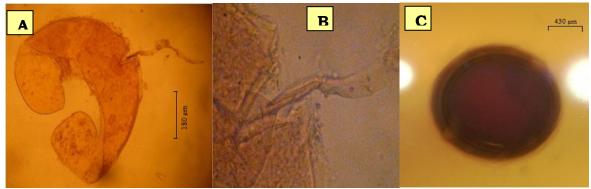


Fig. 3. Spores of *Gigaspora* and *Scetullospora* and *Glomus* isolated from rhizospheric soil from different plant species. A and B) Scetullospora dipapillosa; C) Gigaspora margarita.

Root colonization was more or leas comparable in Families Poaceae, Leguminosae, Convolvulaceae (25-27%), relatively lesser in Boraginaceae, Chenopodiaceae, and Cyperaceae (17-19%) and substantially high (40%) in Acanthaceae. Root colonization was the minimum in Asteraceae (8%).

#### **Agglomerative Clustering**

Dendrograms based on agglomerative clustering of AM fungi (when angiospermic species have been the attributes for the presence or absence of AMF) and the angiospermic species (when AM fungi have been the attributes for the presence or absence with the angiospermic species) are presented in Fig. in 4 and 5.

From Fig. 4, following AMF clusters were recognized – each cluster was a collection of highly similar species in the given context of their occurrence amongst the angiospermic species. Each cluster is a composite of two or more AMF species in connection with the angiospermic flora in sandy salinity-affected environment of Hawkes Bay.

Cluster A: Glomus clariodeum + Glomus geosporium,

Cluster B: Gigaspora margarita,

322 ABDUL RAUF ETAL,

Cluster C: Glomus epigaenum + Acaulospora birecticulata + Scutellospora dipapillosa,

Cluster D: Glomus scaledonium + Glomus leptotichum,

Cluster E: Glomus mosseae + Glomus intraradices,

Cluster F: Glomus clariodeum + Glomus macrocarpum + Acaulospora laevis

Table 2. Number of AMF spores per 100g of the rhizospheric soil and the root colonization (%) of various angiospermic species of Karachi coast, Pakistan.

inic species of Karacin Coast, I akistan.							
S.	g .	Spores per 100g	Root				
No.	Species	soil	colonization				
			(%)				
1.	Aeluropus lagopoides (L.) Trin. Ex Thw.*	398.00 ± 99.96	$27.0 \pm 4.0$				
2.	Atriplex griffithii Moq. *	$392.50 \pm 94.51$	$14.25 \pm 10.3$				
3.	Avicennia marina *	858.00	40.0				
4.	Cyperus longus	$288.00 \pm 52.01$	$12.80 \pm 1.80$				
5.	Cyperus sp.	$443.0 \pm 57.01$	$26.50 \pm 1.50$				
6.	Cressa cretica L. *	$466.050 \pm 233.5$	$38.0 \pm 15.0$				
7.	Indigofera argentea Burm. F.	$288.50 \pm 78.50$	$14.0 \pm 1.0$				
8.	Ipomoea pes-caprae (L.) R.Br. *	$288.50 \pm 78.50$	$15.0 \pm 2.0$				
9.	Heliotropium subulatum (Hochst) Vatke	302.00	34.1				
10.	Heliotropium sp.	$163.00 \pm 51.01$	$9.0 \pm 0$				
11.	Launaea resedifolia (L.) O.K. *	$495.0 \pm 120.02$	$13.95 \pm 9.05$				
12.	Lotus garcinii DC. *	$703.33 \pm 71.23$	$31.62 \pm 3.93$				
13.	Suaeda fruticosa (L.) Forsk. *	$390.33 \pm 123.02$	$29.40 \pm 7.4$				
14.	Suaeda sp. *	128.0	8.0				
	Grand mean	$400.31 \pm 52.09$	$22.40 \pm 2.97$				
	CV (%)	48.7	49.59				

Table 3. Average number of spores, number of AMF species and mean root colonization (%) for various plant families of the site studied in Karachi coast.

11105 01	nes of the stee studied in Karaem coast.							
			Mean Root	Mean Number of				
S.	Plant Families	Mean Number of spores	Colonization	AMF Species				
No.		per 100g Soil	(%)					
1	Acanthaceae	858.0	$40.0 \pm 0$	2				
2	Asteraceae	$495.0 \pm 120.02$	8.0	7				
3	Boraginaceae	$209.33 \pm 54.90$	$17.37 \pm 8.37$	$4.5 \pm 0.5$				
4	Convolvulaceae	377.50 ± 112.94	$27.75 \pm 8.99$	$4.5 \pm 0.5$				
5	Chenopodiaceae	$338.60 \pm 71.96$	$19.06 \pm 6.01$	$3.33 \pm 0.88$				
6	Cyperaceae	$365.50 \pm 54.72$	$19.65 \pm 4.07$	$4.5 \pm 0.5$				
7	Leguminosae	540.20 ± 106.21	$24.60 \pm 4.84$	$6.5 \pm 1.5$				
8	Poaceae	$398.0 \pm 99.96$	$27.0 \pm 4.0$	7				

From Fig. 5, following seven clusters of angiospermic species were recognized on the basis of presence or absence of AMF species. Each cluster was a collection of closely similar species in the context of the intensity of the association of AM fungi.

Cluster A: A. marina + S. fruticosa,

Cluster B: Ipomoea pes-caprae + Cyperus longus,

Cluster C: Cressa cretica + Heliotropium sp.

Cluster D: Lotus garcinii,

 $Cluster \ E: \textit{Indigofera argentea} + \textit{Suaeda sp.}$ 

Cluster F: Launaea resedifolia + Cyperus sp,

Cluster G: Atriplex griffithii + Heliotropium subulatum + Aeluropus lagopoides

## HIERARCHICAL CLUSTER ANALYSIS

ASSOCIATED ANGIOSPERMIC SPECIES ARE THE ATTRIBUTES FOR SIMILARITY AMONG AMF

#### Dendrogram using Ward Method Rescaled Distance Cluster Combine 20 0 5 10 15 25 AMF GCLARIO GGEOS В GIGAMA ABIRECT SDIPAP C GEPIGA GSCA D GLEPTO GMOSS E GINTRA GCLARIO ALAEVIS

Fig.4. Cluster dendrograms for AMFs. Acronyms: GMOSS, Glonus mossaee; GCLARIO, G claroideum; GMACRO, G macrocarpum; GINTRA, G intraradices; G. LEPTO, G. leptotichum, GEPIGA, G. epigaenum; ABIRECT, Acaulospora birecticulata; SDIPAP, Scutellospora dipapillosa; GSCA, Glonus scaledonium; ALAEVIS, A laevis; GCLARIUM, Glonus clarum; GGEOS, Glonus geosporum; CIGAMA, Gigaspora margarita.

## HIERARCHICAL CLUSTER ANALYSIS

ASSOCIATED AMF ARE THE ATTRIBUTES FOR SIMILARITY AMONG ANGIOS PERMIC SPECIES

Dendrogram using Ward Method

Rescaled Distance Cluster Combine

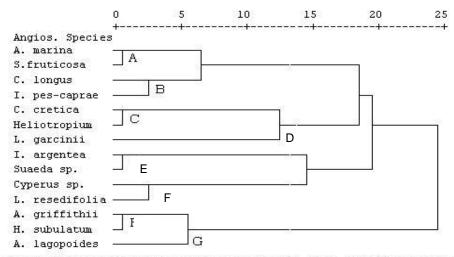


Fig. 5. Cluster dendrogram for angiospermic species. Actonyms: A. marina, Avicennia marina; L. resedi., Launaea resedifolia; I. pescap., Ipomoea pes-caprae; A. griffi., Atriplex griffthii; S. fruti., Suaeda finticosa, C. longus, Cyperus longus, C. cretica, Cressa cretica; L. garcinii, Lotus garcinii, Helio. Sp., Heliotropium sp.; I. argent. Indigofera argentea; A. lagopo., Aelwopus lagopoides; H. Sabula. Heliotropium sabulatum.

## DISCUSSION

**GMACRO** 

Mycorrhizal fungi are one of the important components of microflora around rhizosphere of plants (Mukerji *et al.*, 2012). Because of their symbiotic efficiency, they play an important role in association between plant and soil.

ABDUL RAUF ET AL.,

These fungi produces spores and sporocarps in soil and these spores are the key structures helpful in identification of fungal species. Study was carried out to obtain AM fungal spore dispersion, their distribution and composition in a salinity-affected coastal sand ridge of Hawkes Bay, Karachi. AM fungi were identified by the identification manual of Schenck and Perez (1990). During study variation in composition of AM species were found in which species belonging to four genera (*Glomus*, *Gigaspora*, *Acaulospora* and *Scutellospora*) isolated showed different dispersion pattern amongst the angiospermic species. All tested species associated with  $\geq 2$  AM fungi. Beena *et al.* (2001) tested 28 species of West coast of India for mycorrhizal fungi and found 23 plants (71.43%) colonized by AM fungi.

Each of the 14 species tested for AMF occurrence had at least association with one *Glomus* species. The number of *Glomus* species associated with some angiospermic species was quite high e.g. six *Glomus* spp. occurred with *A. lagopoides*, five *Glomus* spp. with *I. argentea* and *L. garcinii* each, four *Glomus* spp. occurred with *L. resedifolia*, *Heliotropium subulatum*, *C. cretica* and *Ipomoea pes-caprae each*. With other species three or less than three AM fungi associated. *In Ipomoea pes-caprae* association of moderately and severely disturbed dunes, three *Glomus* species (*G. mosseae*, *G. dimorphicum*, and *G. fasciculatum* associated with *Ipomoea* roots besides other genera like *Gigaspora* and *Acaulospora*. In both sites, spore density strongly correlated with nitrogen content of the rhizospheric soil in the West coast of India (Beena *et al.*, 2000).

All the angiospermic species tested by us for their AMF relations were found to associate with differential degree of salinity. In salt -affected areas Occurrence of vesicular and arbuscular mycorrhiza provide added protection to the plant against salinity effects (Rozema et al., 1986). In our studies, The number of AMF spores isolated from the rhizospheres of the subject species, however, showed no correlation with the salinity of the soil (r = 0.326, F = 1.427, NS) i.e. the salinity effects within the given range of salinity were not significantly detected There are, however, reports that occurrence and abundance of AM fungi related with physico-chemical properties of the soil. Schalamuk et al., (2006) and Pagano et al. (2010) also isolated Glomus, Gigaspora, Acaulospora and Scutellospora from spore population. Number of abiotic factors influences the composition of AM fungal species. Physio-chemical properties of soil including pH, electrical conductivity (EC), and total dissolve solvent (TDS) play important role in fungal spore composition and distribution. Asghari et al. (2008) investigated the effects of soil salinity on AM fungi colonization of halophytes. The colonization of halophytes by AM fungi was inhibited by very high salinity (45 dS.m<sup>-1</sup>) that may be due to inability of AM fungi to survive under very high salinity which may limit the beneficial effects of AM fungi to the halophytes under moderate and lower salinity level (Aggarwal et al., 2012; Al-Garni, 2006; Sevraj and Kim, 2004). Other factors besides salinity such as soil moisture and soil chemical and physical properties and plant species themselves control AM colonization (Wang et al., 2004; Asghari et al., 2008) A number of scientists have published similar reports of variation in spore composition due to physical and chemical nature of soil (Abbot and Robson, 1985; Porter et al., 1987). Variation of AM spore in wide range of soil pH (6.19 to 10) was recorded. Abbott and Robson (1985) also described range of soil pH (5.3 to 7.5) in spore variation. Joshi and Singh (1995) and Duponnois et al. (2001) also correlated soil properties with AM fungal population. These results concluded that link between physical and chemical properties of soil and abundance of AM fungal spore vary among different species of AM fungi. Out of 05 different localities, greater number of spore were recorded from Thatta (2794.8/100g soil) along with 85 % AM fungal occurrence, while soil collected from coastal area showed least number of spore (409/100g soil) but with 100% occurrence. Ross and Ruttencutter (1977) also correlated particular plant species with AM fungal spore population in good soil condition (physiochemical properties of soil) and the favorable soil condition favors AM fungal population to increase in Thatta region. Anwar and Jalaluddin (2011) described the distribution of AM fungal spore in different localities of Sindh, according to them maximum spore were recorded in Nawabshah region while rhizospheric soil sample from Thatta showed relatively low result. Various studies showed the occurrence and distribution of AM fungal spore in different part of Pakistan. Burni et al. (2011) in KPK, Nasrullah et al. (2010) in North West frontier province, Sharief et al. (2005) in D.I Khan and Anwar and Jalaluddin, (2011) in Sindh. In present study, Glomus was found to be most dominant genus which is parallel with the finding of Morton (1988) that Glomus species are most distributed genera in soil all over globe followed by Acaulospora and Gigaspora, Glomus is the most frequently occurring AM fungi. Hamayani and Thippeswamy (2013) investigated 10 species of Asteraceae for AM fungi association and found ten species of Glomus associated with these species in addition to Acaulospora and Archeospora. The results of the present studies are also in agreement with some studies conducted recently (Zang et al., 2003; Tao et al., 2004; Panwar and Tarafdar, 2006; Wang and Zao, 2008; Sharma et al., 2009; Burni et al., 2011). This dominance of Glomus species in different soil conditions is presumably because of their ability to adopt various soil condition and survival in different pH soil (Pande and Tarafdar, 2004). Glomus and Acaulospora, because of their smaller spore morphology take minimum time to produce spore as compared to Gigaspora (Hepper, 1984). Among Glomus species, Glomus mosseae was found to be most occurring species. Previous work of Anwar and Jalaluddin (2011) is in agreement with previous studies. They showed the highest number of Glomus mosseae in soil collected from Sindh. Out of 11

plant families, family Malvaceae and Poaceae was showed to have maximum number of AM fungal species. Silva *et al.* (2001) also described the family Poaceae as second best among other monocot after Cyperaceae showed highest AM fungal occurrence. Harley and Harley (1987) reported 60% of 173 Poaceae species which showed AM fungal association. The variation in root colonization of AM fungi is observed to mainly depend on water content of soil. The availability of and availability of phosphorus (Wang *et al.*, 2010), and physiology and growth of roots (Lugo *et al.*, 2003) are other controlling factors.. In present study maximum root colonization were observed in *Avicennia marina* (40%). D'Souza and Rodrigues (2013) also reported presence of AM fungal structure inside root of *Avicennia marina*.

## REFERENCES

- Abbott, L. K. and A. D. Robson (1985). The effect of soil pH on the formation of VA mycorrhizas by two species of *Glomus. Australian Journal of Soil Science*, 23 (2): 253-261.
- Aggarwal, A., N. Kadian, Karishma, Neetu, A. Talwar and K.K. Gupta (2012). Arbuscular mycorrhizal symbiosis and alleviation of salinity stress. *J. Appl. & Nat. Sci.* 4(1): 144-155.
- Al-Garni, S.M.S. (2006). Increasing NaCl-salt tolerance of a halophytic plant *Phragmites australis* by mycorrhizal symbiosis. *Am.-Eur. J. Agric. & Environ. Sci.* 1(2): 119-126.
- Anwar, Q. M. K. and M. Jalaluddin (2011). The species composition and dispersion of arbuscular mycorrhizal fungal (AM FUNGI) spores in wheat fields of eight districts of Sindh, Pakistan. *International Journal of Biology and Biotechnology*. 8 (4):567-573.
- Asghari, H.R., M.R. Amerian and H. Gorbani (2008). Soil salinity affects arbuscular mycorrhizal colonization of halophytes. *Pak. J. Biol. Sci.* 11 (15): 1909 1915.
- Bagyaraj, D. J. (1986). Mycorrhizal association in crop plants and their utilization in agriculture. *In: Beneficial fungi and their utilization*. (Eds. Nair, M.C. and S. Balakrishnan). Scientific Publishers, Jodhpur, India.
- Beena, K.R., N.S. Raviraja, A.B. Arun and K.R. Sridhar (2000). Diversity of arbuscular mycorrhizal fungi on the West coast of India. *Curr. Sci.* 79 (10): 1459-1466.
- Beena, K.R., A.B. Arun, N.S. Raviraja and K.R. Sridhar (2001). Association of arbuscular mycorrhizal fungi with plants of coastal sand dunes of West coast of India. *Tropical Ecology* 42(2): 213-222.
- Brundrett, M. C. (2002). Co-evolution of roots and mycorrhizas of land plants. New Phytopathol, 154: 275-304.
- Burni, T. and I. Illahi. 2004. Quantification and correlation of VAM spores with the soil characteristics of Wheat fields of NWFP. *Pak. J. Pl. Sci.*, 10: 139-144.
- Burni, T., F. Hussain and M. Sharief (2011). Arbuscular mycorrhizal fungi (amf) associated with the rhizosphere of *Mentha arvensis* 1., and *M. longifolia* huds. *Pakistan Journal of Botany*, 43(6): 3013-3019.
- Burni, T., M. Shah and F. Hussain. 2007. Occurrence and characterization of VAM infections in *Mentha longifolia* (L.) and *Nepeta cataria* L. *Pak. J. Pl. Sci.*, 13(2): 147-150.
- Cabello, M.L., L. Gaspar and R. Pollero (1994). *Glomus antarcticum sp.* Nov., a vesicular arbuscular mycorrhizal fungus from *Antarctica. Mycotaxon*, 51: 123-128.
- Caproni, A.L., A. A. Franco, R. L. L. Berbara, S. B. Trufem, J. R. D. Granha, and A. B. Monteiro (2003). Arbuscular mycorrhizal fungi occurrence in revegetated areas after bauxite mining at Porto Trombetas, Para state, Brazil. *Pesquisa-Agropecuária-Brasileira*, 38: 1409-1418.
- D'souza, J and F.B. Rodrigues (2013). Biodiversity of Arbuscular Mycorrhizal (AM) fungi in mangroves of Goa in West India. *Journal of Forestry Research*, 24(3): 515-523.
- Dalpe, Y. and S. G. Aiken (1998). Arbuscular mycorrhizal fungi associated with *Festuca* species in Canadian High Arctic. *Canadian Journal of Botany*, 76: 1930-1938.
- Duponnois, R., C. Plenchette, J. Thioulouse and P. Cadet (2001). The mycorrhizal soil infectivity and Arbuscular mycorrhizal fungal spore communities in soils of different aged fallows in Senegal. *Applied Soil Ecology*, 17(3): 239-251.
- Estefan, G., R. Sommer and J. Ryan (2013). *Methods of soil, plant, and water analysis: A manual for the west, Asia and North Africa region. ICARDA, Beirut, Lebanon.*
- Ganesan, V., S. Ragupathy, B. Parthipan, D. B. Rajini-Rani and A. Mahadevan (1991). Distribution of vesicular-arbuscular mycorrhizal fungi in coal, lignite and calcite mine spoils of India. *Biology and Fertility of Soils*, 12: 131-136.
- Gerdemann, J. W and T. H. Nicolson (1963). Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. *Transactions of the British Mycological society*, 46(2): 235-244.

326 ABDUL RAUF ETAL.

Gerdemann, J.W. (1968). Vesicular-arbuscular mycorrhizae and plant growth. *Annual Review of Phytopathology*, 6: 397-418.

- Giovannetti, M and B. Mosse (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist*, 84(3): 489-500
- Hall, I.R. (1977). Species and mycorrhizal infections of New Zealand Endogonaceae. *Transaction of the British Mycological Society*, 68: 341-356.
- Hamavani, C. and B. Thippeswamy (2013). Arbuscular mycorrhizal fungi associated with some plants of Asteraceae in Bhadra Wild Life Sanctuary. *Int. J. Pl. Anim. & Environ. Sci.* 3(2): 106-110.
- Harley, J. I. and F. I. Harley (1987). A Checklist of mycorrhiza in the British flora. *New Phytol.*, 105 (2 supplements): 1-102.
- Hepper C. M. (1984). Isolation and culture of VA mycorrhizal (VAM) fungi. In: C.L. Powell and D.J. Bagyaraj (eds), *VA mycorrhizae*. Florida: CRC Press, pp. 95–112.
- Hudson, J. H. (1986). Fungal Biology. Edward Arnold Baltimore Maryland, USA.
- Jalaluddin, M. and Q.M. Anwar (1991). VAM fungi in wheat and rice fields. Pak. J. Bot., 22(1): 115-122.
- Jansa, J., A. Mozafar, T. Anken, R. Ruh, I.R.Sanders and E. Frossard (2002). Diversity and structure of AMF communities as affected by tillage in a temperate soil. *Mycorrhiza*, 12: 225-234.
- Jeffries, P., T. Spyropoulos, and E. Vardavarkis (1988). Vesicular-arbuscular mycorrhizal status of various crops in different agricultural soils of northern Greece. *Biology and Fertility of Soils*, 5: 333-337.
- Jenkins, W. (1964). A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant disease reporter*, 48(9): 692.
- Joshi K C and H P. Singh (1995). Interrelationships among vesicular arbuscular mycorrhizae, population, soil properties and root colonization capacity of soil. *Journal of the Indian Society of Soil Science* 43: 204–207.
- Khan, A. G. (1972). The effect of Vesicular Arbuscular Mycorrhizal associations on growth of Cereals. *New Phytologist*, 71(4), 613-619.
- Khan, A.G. (1974). The occurrence of mycorrhizas in halophytes, hydrophytes and xerophytes, and Endogone spores in adjacent soils. *J. Gen. Microbiol.* 81: 7-14.
- Koske, R. E. and J. N. Gemma (1989). A modified procedure for staining roots to detect VA mycorrhizas. *Mycological research*, 92(4): 486-488.
- Land, S., F. Schonbeck (1991). Influence of different soil types on abundance and seasonal dynamics of vesicular arbuscular mycorrhizal fungi in arable soils of North Germany. *Mycorrhiza*, 1: 39-44.
- Lugo M, M. E. Gonzalez and M. N. Cabello (2003). Arbuscular mycorrhizal fungi in a mountain grassland, Seasonal variation of colonization studied, along with its relation to grazing and metabolic host type. *Mycologia*, 95: 407–415.
- McGonigle, T. P., M. H. Miller, D. G. Evans, G. L. Fairchild and J. A. Swan (1990). A new method which gives an objective measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. *New Phytologist*, 115(3): 495-501.
- Mishra, R. R., G. D. Sharma, and A. R. Gatphoh (1980). Mycorrhizas in the ferns of North Eastern India. In *Proc. Indian Academy of Science*, Vol. 46, pp. 546-551.
- Morton, J. B. 1988. Taxonomy of VAM fungi, classification, nomenclature and identification. *Mycotaxon*. 32: 267-324.
- Mukerji, K.G., B.P. Chamola, and J.S. Singh (2012). *Mycorrhizal Biology*. Springer Sci. and Business Media. 336 Pp.
- Muthukumar, T., K. Udaiyan (2000). Influence of organic manures on arbuscular mycorrhizal fungi associated with *Vigna unguiculata* (L.) Walp. In relation to tissue nutrients and soluble carbohydrate in roots under field conditions. *Biology Fertilizer and Soil*, 31:114-120
- Nasrullah, M. Sharief, K. Robina and T. Burni. (2010). Occurrence and distribution of AM FUNGI in wheat and Maize crops of Malakand Division of North west Frontier Province. *Pak. J. Bot.*, 42(2): 1301 -1312.
- Nasrullah, M. Sharief, K. Robina and T. Burni. 2010. Occurrence and distribution of AMF in wheat and Maize crops of Malakand Division of North west Frontier Province. *Pak. J. Bot.*, 42(2): 1301-1312.
- Nielsen, K.B., R.Kjoller, P.A. Olsson, P.F.Schweiger, F. O. Andersen and S. Rosendahl (2004). Colonization and molecular diversity of arbuscular mycorrhizal fungi in the aquatic plants *Littorella uniflora* and *Lobelia dortmanna* in southern Sweden. *Mycological Research*, 108 (6):616-25.
- Pagano, M. C., A. I. C. Persiano1, M. N. Cabello and M. R. Scotti (2010). Elements sequestered by Arbuscular mycorrhizal spores in riverine soils. *Journal of Biophysics and Structural Biology*, 2(2): 16-21.
- Pande, M. and J. F. Tarafdar (2004). Arbuscular Mycorrhizal fungal diversity in Neem based Agroforestry Systems in Rajistan. *Applied Soil Ecology*, 26: 233-241.

- Panwar, J. and J.C. Tarafdar. 2006. Distribution of three endangered medicinal plant species and their colonization with arbuscular mycorrhizal fungi. Journal of Arid Environments, 65: 337-350.
- Phillip, J. M., &D. S. Hayman (1970). Improved procedures for clearing roots and staining parasitic and VAM fungi for rapid assessment of infection. *Transactions of the British Mycological Society*, 55: 158-161.
- Porter, W. M, A. D. Robson and L. K. Abbott (1987). Factor affecting controlling the distribution of vesicular arbuscular mycorrhizal fungi in relation to soil pH. *Journal of Applied Ecology*, 24: 663-672.
- Read, D.J., H. K. Koucheki and J.Hodgson (1976). Vesicular-arbuscular mycorrhiza in natural vegetation systems I; The occurrence of infection. *New Phytologist*, 77: 641-653.
- Redhead, J. F. (1977). Endotrophic mycorrhizas in Nigeria: species of the Endogonaceae and their distribution. *Transactions of the British Mycological Society*, 69(2): 275-280.
- Ross, J. P. and R. Ruttencutter (1977). Population dynamics of two vesicular arbuscular endomycorrhizal fungi and the role of hyperparasitic fungi. *Phytopathology*, 67: 490-496.
- Rozema, J., W. Aspi, J. Van Diggelen, M. Van Esbrock and R. Brockman (1986). Occurrence and significance of vesicular arbuscular mycorrhizal in the salt marsh environment. *Acta Bot. Nether.* 35: 457-467.
- Saif, S.R. and A.G. Khan (1975). The influence of season and stage of development of plants on Endogone, mycorrhiza of field grown wheat. *Canadian Jour. Microbiol.*, 21: 1020-1024.
- Schalamuk, S., S. Velazquez, Chidichimo and M. Cabello (2006). Fungal spore diversity of arbuscular mycorrhizal fungi associated with spring wheat: effects of tillage. *Mycologia*, 98 (1): 16-148.
- Schenck, N. C. and Y. Perez (1990). *Manual for the Identification of VA mycorrhizal fungi*. Synergistic Publication, Gainesville, USA.
- Schuβler, A. (2000). *Glomus claroideum* forms and arbuscular mycorrhiza-like symbiosis with the hornwort *Anthoceros punctatus. Mycorrhiza*, 10: 15–21.
- Sevraj, T. and H. Kim (2004). Ecology of Vesicular-Arbuscular mycorrhizal (VAM) fungi in coastal areas of India. *Agric. Chem. Biotechnol.* 47(2): 71-76.
- Sharief, M., T. Burni and Saima (2005). Arbuscular mycorrhizal incidence and infectivity in wheat and Maize crops of Bannu and D.I. Khan Areas, Pakistan. *Pakistan Journal of Plant Science*, 11(1): 67-77.
- Sharma, D., R. Kapoor and A.K. Bhatnagar (2009). Differential growth response of *Curculigo orchioides* to native arbuscular mycorrhizal fungal (AMF) communities varying in number and fungal components. *European Journal of Soil Biology*, 45(4): 328-333.
- Silva, G. A. D., B. A. D. Santos, M. V. Alves, and L. C. Maia (2001). Arbuscular mycorrhiza in species of Commelinidae (Liliopsida) in the state of Pernambuco (Brazil). *Acta Botanica Brasilica*, 15(2): 155-165.
- Tao, L., L. Jianping and Z. Zhiwei. (2004). Arbuscular mycorrhiza in valley type Savanna in South West China. *Mycorrhiza*, 14: 323-327.
- Vestberg, M. (1999).Occurrence of arbuscular mycorrhizal fungi in different cropping systems at Cochabamba, Bolivia. *Agricultural and Food Science in Finland*, 8: 309-318.
- Vogelsand, K.M. and J.D. Bever (2010). *The use of native plant and mycorrhizal fungi for slope stabilization and top soil management.* (Indiana Univ. Dept. Biol. Bloomington, Indiana). Rep. for Calif. Dept. Transport, Roadside Research Innovation, Landscape Architecture. Program, Sacramento. Pp. 98.
- Wang, B., and Y.L. Qiu (2006). Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza*, 16: 299–363.
- Wang, F.Y. and Y. S. Zao (2008). Biodiversity of Arbuscular fungi in China. A review. *Advances in Environmental Biology*, 2: 31-39.
- Wang, F.Y., R.J. Liu, X.G. Lin and J.M. Zhou (2004). Arbuscular mycorrhizal status of wild plants in saline-alkaline soils of the yellow River Delta. *Mycorrhiza*, 14: 133-137.
- Wang, Y.T., Q. Qiu, Z.Y. Yang, Z.J. Hu, F.Y.T. Nora and G.R. Xin (2010). Arbuscular mycorrhizal fungi in two mangroves in South China. *Plant Soil*, 331: 181-191.
- Ward, J.H. Jr. (1063). Hierarchical grouping to optimize and objective function. J. Am. Stat. Assoc. 58: 236 244.
- Zainab, S.B. and T. Burni (2005). Vesicular arbuscular mycorrhizal studies in weeds of wheat fields of Peshawar, Pakistan. *Pak. J. Pl. Sci.*, 11: 93-101.
- Zhang, Y., L. Y. Xie, B. Q. Xiong and MZeng (2003). Effect of polyamine on growth and development of arbuscular mycorrhizal fungi in vitro culture condition. *Mycosyst.* 22: 417-423.

(Accepted for publication March 2016)