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BIODIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI ASSOCIATED WITH *ACACIA GERRARDII* BENTH IN DIFFERENT HABITATS OF SAUDI ARABIA

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Abstract

Arbuscular mycorrhizal fungi (AMF) are the most influential and ubiquitous rhizosphere microbiome. AMF improve the soil characteristics and assist the symbiotic plants by improving plant absorption of soil nutrients particularly phosphorus. The biodiversity of native AMF highly influenced by soil nature and plant composition. The present investigation studied the enumeration and biodiversity of AMF associated with rhizosphere soil and roots of *Acacia gerrardii* (Talh trees) grown natively in different habitats of Saudi Arabia (SA). Soil analyses were varied with locations nonetheless, there are no distinct correlations has been estimated among the root colonization with AMF, spores number of AMF and soil properties. Fifteen mycorrhizal fungal species belong to seven genus (Funneliformis; Glomus; Rhizophagus; Septoglomus; Acaulospora; Claroideoglomus; Archaeospora) and four families (Glomeraceae; Acaulosporaceae; Claroideoglomeraceae; Archaeosporaceae) were identified from forty soil samples collected from four different locations belong to Riyadh region (Rawdhat Khuraim, Houta Bani Tamim) and Holy Madina region (Ola city, Werqaan Mountain) in SA. The present investigation extends our knowledge on the biodiversity of AMF associated with rhizosphere soil of Talh trees (*A. gerrardii*) grown natively in different Saudi locations.

Key words: Soil microbiome, Biodiversity of Arbuscular mycorrhizal fungi, *Acacia gerrardii*.

Introduction

Land degradation due to salinity and drought negatively affect plant vegetation and causes disturbances in plant microbe interactions, which are important factors in helping plant to withstand stress factors (Requena *et al*., 2001). Acacia species, considered as salt and drought tolerant trees are thus good candidates for reforestation of degraded lands (Chaudhary, 1983). They are leguminous plants and form root nodules in symbiosis with rhizobia. It has been reported that stress factors such as salinity and drought inhibited nodulation process and also reduces N_2 fixation of the legumes (Hungria & Vargas, 2000; Ghorbanpour *et al*., 2013). The salt tolerant microbes associated with halophytic plants adapt to high osmotic stress and have the capacity to survive in hostile environmental conditions. They are able to stimulate plant growth and resistance to stress factors through their production of biological active compounds, such as phytohormones, and osmoprotectants. However, abiotic factors were also found to affect microbial composition and activities within plant environment (Thrall *et al*., 2008). It has been demonstrated that the content of plant exudates effect soil rhizosphere microbiome and their beneficial properties (Faure *et al*., 2009; Doornbos *et al*., 2012). The soil rhizosphere is colonized more intensively by microorganisms than the other regions of the soil and may form symbiotic relationship with the plant (rhizobia, AMF) and those that are free-living in the soil and the rhizosphere, phyllosphere of plants (Lugtenberg & Kamilova, 2009; Hameed *et al*., 2014). It is well documented that the use of stress-tolerant microbial strain including AMF stimulate plant growth, protect plants from soil borne disease, improve stress tolerance of plants and stimulate soil microbial activity, which can lead to improved fertility of salt affected soils (Alqarawi *et al*., 2014; Hashem *et al*., 2014, 2015a). The possible mechanisms of plant growth stimulation, tolerance of plant to various abiotic stresses, and biological control of plant disease by PGPR are i) production of phytohormones (Ghorbanpour *et al*., 2013) ii), solubilisation of minerals such as phosphorus, potassium, oxidation of sulphur (Lugtenberg and Kamilova 2009), iii) extra cellular production of antibiotics, lytic enzymes (Berg *et al*., 2014), iv) induction of systemic resistance (ISR) in host plants to a broad spectrum of pathogens (Fürnkranz *et al*., 2012), v) production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase to reduce the level of ethylene in the roots of developing plants (Glick *et al*., 2007), vi) competition for nutrients and niches (Kamilova *et al*., 2005), vii) and production of exopolysaccharides (Upadhyay *et al*., 2011).

The structure and function of the plant microbiome is driven by plant species and prevailing environmental conditions (Berg *et al*., 2014). The rhizosphere microbiome of *Salicornia* plants grown in hypersaline ecosystems in Tunisia support high bacterial diversity and these bacteria were characterized by their resistance to temperature, osmotic and saline stresses, and plant growth promotion (PGP) features (Mapelli *et al*., 2013). The host plants and soil properties have a strong effect on root associated microorganisms and their activities within plant rhizosphere (Macia Vicente *et al*., 2012). Although, several

studies analyzing plant-associated bacterial communities already exist (Fürnkranz *et al*., 2012; Berg *et al*., 2014), little is known about the micro-biome of desert halophytic plants and their activities within hostile condition. In particular, it is poorly explored whether desert plants may promote the selection of microbes capable of enhancing a plant resistance to salinity and water stress.

Little information is currently available on the taxonomic and functional diversity of AMF communities associated with the endosphere and root system of *A. gerrardii*. An improved understanding of the composition of mycorrhizal community associated with *A.gerrardii* (Talh trees), however, may open new opportunities to broaden plant growth in fragile environments.

In the current study, our goal was to investigate on morphological basis the diversity of AMF associated with root system of *A. gerrardii* grown at different locations of Saudi Arabia (SA).

Materials and Methods

Plant materials and sampling: Ten replicates of each root and rhizosphere soil sample were collected from *Acacia gerrardii* growing wildly in different locations of Saudi Arabia. The locations were Rawdhat Khuraim; Houta Bani Tamim (Riyadh region), Ola city, Werqaan Mountain (Holy Madina region). The samples were collected at 30-40 cm depth. All the samples were collected under sterile conditions using sterile tools. Chemical analysis (soluble cations, soluble anions, TDA, EC, pH) of some part of the rhizosphere soil was carried out according to Allison and Moodie (1965). Remained samples were stored at −20°C in the laboratory for microbiological analysis or at 4°C for isolation and further processes.

Isolation and identification of AMF: The wet sieving and decanting method (Gerdemann & Nicolson, 1963) was used for extraction of the spores using sequentially sieving mechanism through different sieves. The sieved residues were filtered through Whatman filter paper No. 1. After water filtration, the filter paper was examined under a stereo-binocular microscope at 25 × magnification. Morphologically similar spores were selected for identification. AMF species were identified based on the description of subcellular structures (spore color, shape, surface ornamentation, spore contents, and wall structures) of asexual spores provided by the International Culture Collection of Vesicular and Arbuscular Mycorrhizal Fungi (Anon., 2014) and other descriptive protocols (Schüßler & Walker, 2010; Redecker *et al*., 2013).

Propagation of AMF in trap cultures: The trap culture protocol described by Stutz & Morton (1996) was used in the current study to propagate the mycorrhizal isolates using surface-sterilized seeds [0.5% (v/v) NaOCl used] of *Sorghum sudanense* as described in details in our previous study (Hashem *et al*., 2016a).

Determination of arbuscular mycorrhizal colonization: Root samples (fine) of *Acacia gerrardii* from different locations of Saudi Arabia as described above were collected and fixed in FAA solution (formalin/acetic acid/alcohol, 10, 0.5, 0.5; v/v/v) for further processes. Roots were stained with trypan-blue in lactophenol (Phillips & Hayman, 1970) and assessed for mycorrhizal infection. Pigmented roots after clearing, were bleached in alkaline hydrogen peroxide (0.5% NH_4OH and 0.5% H_2O_2 v/v in water) to remove any phenolic compounds (Kormanik & McGraw, 1982) before acidification (0.05 M HCl). To assess the mycorrhizal colonization, stained root segments (one cm in length) were mounted on glass slides with lactophenol and were observed under a digital computerized microscope (model DP-72, Olympus) at 20 × magnification. A minimum of 50 segments for each replicate sample were observed to assess structural colonization of AMF associated with roots. Twenty or more segments were mounted on each slide and examined under the microscope. The presence of mycelia, vesicles and arbuscules was recorded and analyzed to assess structural colonization.

Results and Discussion

Sample collections and soil chemical properties: Samples (rhizosphere soil, roots) of native Talh trees (*A. gerrardii*) were grown in two main locations namely Riyadh region (Rawdhat Khuraim, Houta Bani Tamim) and Holy Madina region (Ola city and Werqaan Mountain) (Table 1 and Fig. 1). The locations are suggested based on previous local studies (Al Shahrani & Shetta, 2011; Waly & Emad, 2012; Al-Watban *et al*., 2013; Al-Barakah & Mridha, 2014). The soil of Ola city location was more saline with higher concentration of sodium, potassium, magnesium, and calcium. However, the soil of Rawdhat Khuraim, Houta Bani Tamim, and Werqaan Mountain were lesser, but all are stressed soil as shown in Table 1. Our results were in agreement with other previous soil analysis of these locations in Saudi Arabia (Al-Kadeeb, 2007; Adetunji *et al*., 2008; Omar, 2013; Suliman *et al*., 2017).

Table 1. Chemical analysis of rhizosphere soil associated with Acacia trees in Saudi Arabia.

Area and location of rhizosphere soil		*Soil chemical properties									
		pH	EC (dS/m)	TDS (ppm)	Soluble anions (meq/ l)			Soluble cations (meq/ l)			
					HCO_3^+ CO3	Cl	SO_4	Ca	Mg	Na	K
Riyadh	Rawdhat Khuraim	8.23	0.18	120.66	1.056	0.44	0.58	0.81	0.166	0.803	0.076
	Houta Bani Tamim	7.93	0.12	78.33	0.133	0.547	0.59	0.28	0.126	0.253	0.12
Holy Madina	Ola city	8.43	1.61	10.35	0.412	0.38	14.12	1.57	14.186	0.681	0.253
	Werqaan Mountain	8.86	0.117	82.33	0.1067	0.457	0.66	0.35	0.103	0.616	0.146
LSD at: 0.05		0.721	0.034	3.97	0.019	0.008	0.014	0.092	0.037	0.042	0.183

dS/m: (deciSiemens/m); meq/ l: (milliequivalents/liter)

*The soil samples were collected at 30-40 cm depth and will be taken to laboratory in polyethylene bags

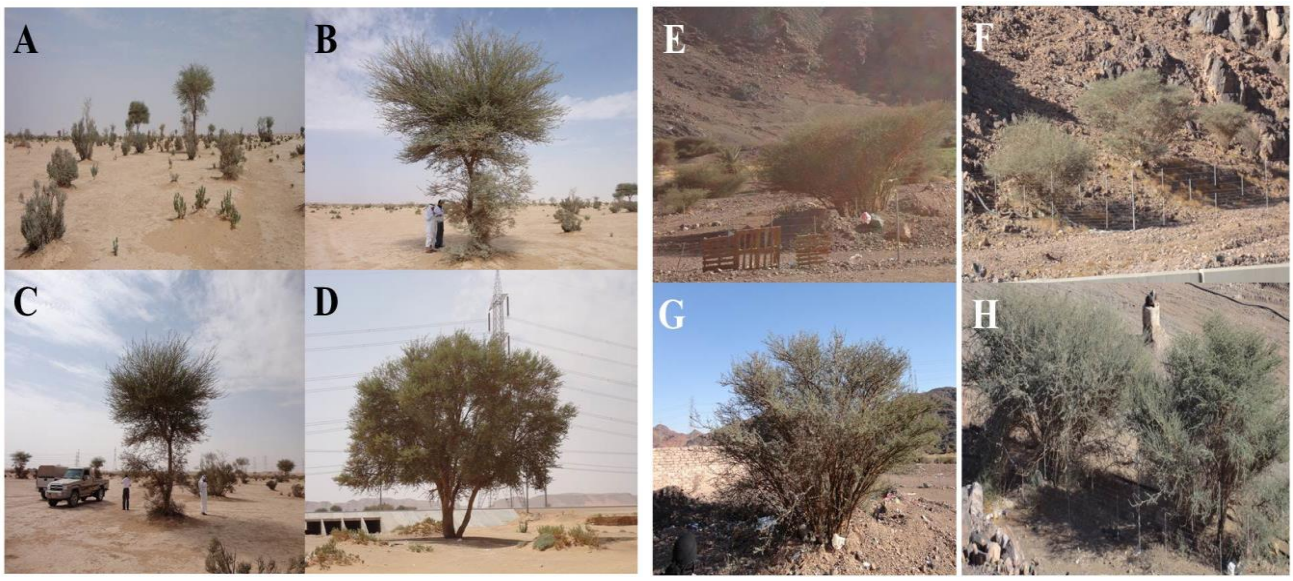

Fig. 1. (A-H). Native Performance of Talh trees (*A. gerrardii*) in different habitats of Saudi Arabia.

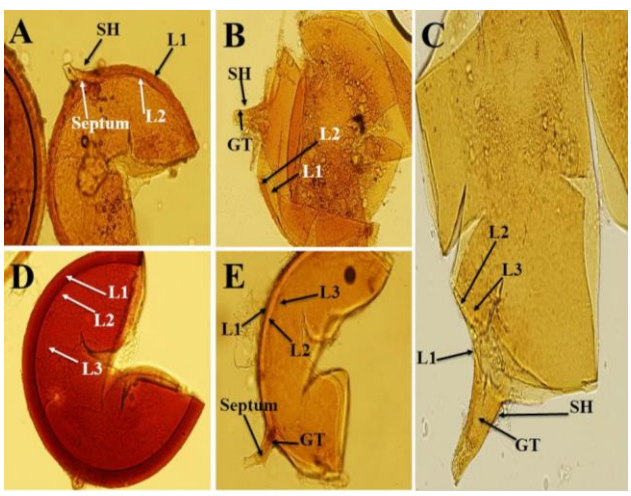

Fig. 2. (A-E): Illustration of different arbuscular mycorrhizal fungal spores shows various structural morphology of their crushed spores. A, *Claroideoglomus etunicatum* with subtending Hyphae (SH), septum, L_1 and L_2 of spore wall. B, *Funneliformis coronatum* with subtending hypha (SH), germ tube (GT), L_1 and L_2 of spore wall. C, *Funneliformis mosseae* with subtending hypha (SH), germ tube (GT), L_1, L_2, L_3 of spore wall. D, Rhizophagus fasciculatus with L_1, L_2, L_3 of spore wall. E, *Rhizophagus intraradices* with subtending hypha (SH), germ tube (GT), septum and L_1, L_2, L_3 of spore wall.

Mycorrhizal diversity and species composition: The community of AM fungi colonizing the roots of different Talh trees (*A. gerrardii*) at different locations was characterized (Table 2). A total of 7 genera and 14 species belonging to 4 different families of AM fungi were investigated in the present study (Table 2 and Fig. 2). Fifteen mycorrhizal fungal species (*Funneliformis verruculosum*; *Funneliformis badium*; *Funneliformis mosseae*; *Funneliformis geosporum*; *Glomus segmentatum*; *Glomus arenarium*; *Glomus pansihalos*; *Glomus sinuosum*; *Rhizophagus fasciculatus*; *Rhizophagus aggregatus*; *Funneliformis constrictum*; *Acaulospora denticulata*; *Acaulospora mellea*; *Claroideoglomus etunicatum*; *Archaeospora trappei*) belong to seven genus (Funneliformis; Glomus; Rhizophagus; Septoglomus; Acaulospora; Claroideoglomus; Archaeospora) and four families (Glomeraceae; Acaulosporaceae; Claroideoglomeraceae; Archaeosporaceae) were identified from 40 soil samples collected from four different locations belong to Riyadh region (Rawdhat Khuraim, Houta Bani Tamim) and Holy Madina region (Ola city, Werqaan Mountain) in SA.

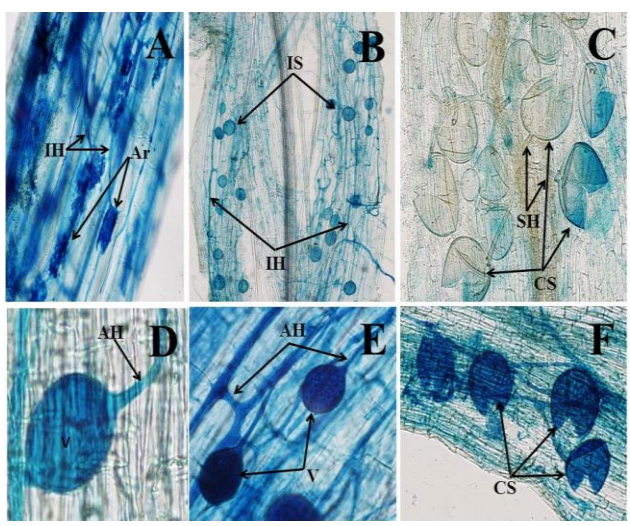

Fig. 3. (A-F). Photomicrographs of structural colonization of AMF in the roots of *A. gerrardii*. A, intraradical hypha (IH); B, Intact spore (IS); C, Subtending hypha (SH); D & E, Attached hypha (AH) and C, Crushed spore (CS).

The AMF root colonization of Acacia trees collected from four different locations viz., Rawdhat Khuraim, Houta Bani Tamim, Ola city, and Werqaan Mountain,of Saudi Arabia is shown in (Table 3). All the plants from all the sites showed AMF colonization (Fig. 3, A-F). The highest mycelial colonization was found in the roots collected from Rawdhat Khuraim (196.33%) followed by Houta Bani Tamim (87.67%) and Ola city (79.00%). The lowest mycelial infection was recorded in the roots from Werqaan Mountain (61.33). The vesicle formation showed a

significant difference. The maximum vesicle formation was found in Houta Bani Tamim (59.00%) which was followed by Rawdhat Khuraim (55.67%). Werqaan Mountain (6.67%) showed the lowest vesicle formation. In case of total infection with arbuscules the highest percentage was recoreded in Rawdhat Khuraim (75.67%) and the second highest was shown by Houta Bani Tamim (71.00%) which was followed by Werqaan Mountains (53.33%). The lowest Abuscular formation was found in Ola city (51.00%). The intensity of infection in each location with mycelium, vesicles and arbuscules was estimated as Poor (P), Moderate (M) and Abundant (A) as shown in Table 4. The intensity of infection varied significantly in each location. in case of intensity of infection with mycelium, the highest infection as poor type was shown by Werqaan Mountain (85.67%) while as moderate (42.00%) and abundant (29.67%) were recorded in Rawdhat. Similarly, the vesicle formation as poor type was highest in Werqaan Mountain (100%) while as moderate (17%) and abundant (2.67%) in Rawdhat Khuraim. In case of arbuscules, the highest percent of poor type of infection was found in Werqaan Mountain (86.67%) while as highest moderate and abundant type were shown by Houta Bani Tamim (34.33%) and Rawdhat Khuraim (15.00%) respectively as shown in Table 5. Phylogenetic tree analysis of different arbuscular mycorrhizal fungi associated with root of Talh trees (*A. gerrardii*) in different habitats of Saudi Arabia (Fig. 4). Jaccard's similarity dendrograms (Fig. 4) of AM fungi generated by RAPD data of fungal flora associated with root of Talh trees (*A. gerrardii*) in different habitats of Saudi Arabia. The current study confirmed the colonization of AM fungi in most roots of Acacia trees (*A. gerrardii*) in different locations of SA. Mycorrhizal occurrence in Saudi soil was previously reported in previous studies (Al-Whaibi, 2009; Alqarawi & Alshahrani, 2010; Al-Khaliel, 2010; Dhar *et al.*, 2015). The Mycorrhizal colonization reported in roots of Talh trees supports the previous studies in many countries viz., Ethiopia (Belay *et al*., 2013), Bangladesh (Dhar & Mridha, 2012), France (Remigi *et al*., 2008), Senegal (Sene *et al*., 2012) and SA (Al-Whaibi, 2009; Hashem *et al*., 2016a, b; Suliman *et al*., 2017). The root colonization level was strongly influenced by edaphic factors and plant phenological events (Bellgard & Williams, 2011), whereas, the spore population was dependent on water availability (Remigi *et al*., 2008; Bouamri *et al*., 2014; Hashem *et al*., 2016a; Mosbah *et al*., 2017; Suliman *et al*., 2017).

Table 2. Arbuscular mycorrhizal fungi composition associated with rhizosphere of Acacia trees collected from different locations of Saudi Arabia.

Family	Genus	Arbuscular mycorrhizal fungi
Glomeraceae	Funneliformis	*Funneliformis verruculosum* (syn. *Glomus verruculosum*)
		Funneliformis badium (syn. *Glomus badium*)
		Funneliformis mosseae (syn. *Glomus mosseae*)
		Funneliformis geosporum (syn. *Glomus macrocarpum* var. *geosporus*)
	Glomus	*Glomus segmentatum*
		Glomus arenarium (syn. *Diversispora arenaria*)
		Glomus pansihalos
		Glomus sinuosum
	Rhizophagus	*Rhizophagus fasciculatus*
		Rhizophagus aggregatus
	Septoglomus	*Funneliformis constrictum (syn. Glomus constrictum)*
Acaulosporaceae	Acaulospora	*Acaulospora denticulata*
		Acaulospora mellea
Claroideoglomeraceae	Claroideoglomus	*Claroideoglomus etunicatum*
Archaeosporaceae	Archaeospora	*Archaeospora trappei*

Table 3. Total Spore population (spore/ 100 g soil) and total structural colonization (%) of arbuscular mycorrhizal fungi associated with rhizosphere and roots of Acacia trees in Saudi Arabia.

Area and location of soil		Spore population (%)	Total structural colonization (%)		
Area	Location		M	V	A
Riyadh	Rawdhat Khuraim	196.33	94.33	55.67	75.67
	Houta Bani Tamim	145.34	87.67	59.00	71.00
Holy Madina	Ola city	93.31	79.00	25.33	51.00
	Werqaan Mountain	82.30	61.33	6.67	53.33
LSD at: 0.05		8.263	4.117	3.01	3.72

Total colonization (%); M: Mycelium; V: Vesicules; A: Arbuscules
Spore population: (spore/ 100 g soil)

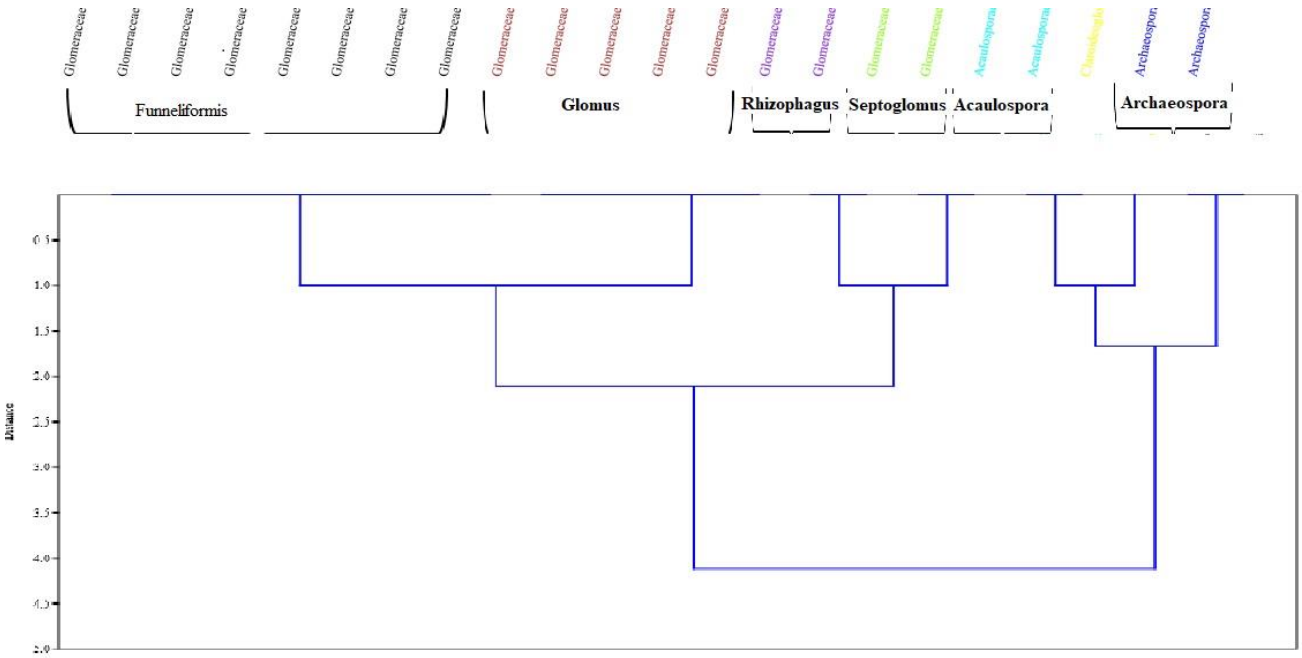

Fig. 4. Phylogenetic tree analysis of different arbuscular mycorrhizal fungi associated with root of Talh trees (*A. gerrardii*) in different habitats of Saudi Arabia.

Table 4. Intensity of structural colonization (%) of arbuscular mycorrhizal fungi (AMF) as mycelia (M), vesicles (V), and arbuscules (A) associated with different roots of Acacia trees in Saudi Arabia.

Area and location of soil		Intensity of structural colonization (%)								
		Mycelium			Vesicules			Arbuscules		
Area	Location	P	M	A	P	M	A	P	M	A
Riyadh	Rawdhat Khuraim	28.31	42.00	29.67	80.33	17.00	2.67	69.67	15.33	15.00
	Houta Bani Tamim	36.00	40.67	23.33	84.31	15.67	0.00	52.31	34.33	13.33
Holy Madina	Ola city	51.00	29.33	19.67	91.67	8.32	0.00	82.67	14.30	3.00
	Werqaan Mountain	85.67	7.30	7.00	100.00	0.00	0.00	86.67	9.67	3.67
LSD at: 0.05		4.21	1.07	3.82	3.76	1.34	0.53	2.78	0.36	0.24

P: Poor, M: Medium, A: Abundance

Table 5. Relative frequency (per 100g soil) of arbuscular mycorrhizal fungi (AMF) associated with different rhizosphere soil of Acacia trees in Saudi Arabia.

Area and location of soil		Relative frequency of AMF (per 100g soil)											
		Glomeraceae								Clae	Acaulosporaceae		
Area	Location	Funneliformis		Rhizoglomus			Gsp1	Gsp2	Gsp3	Clus	Acaulospora		
		Fm	Fb	Ra	Rf	Ri				Ce	Ak	Am	Acaul. Sp1
Riyadh	Rawdhat Khuraim	25.33	7.67	19.31	18.67	10.67	2.00	5.00	1.67	1.34	3.00	3.00	2.33
	Houta Bani Tamim	14.35	3.68	10.00	15.00	21.33	10.33	11.38	6.00	3.00	3.00	1.00	1.00
Holy Madina	Ola city	11.00	1.31	6.332	24.00	16.00	12.00	1.67	10.00	8.32	1.30	3. 67	4.33
	Werqaan Mountain	16.00	3.62	5.30	10.00	25.33	9.67	8.00	2.67	14.31	2.68	1.34	1.00

LSD at: 0.05

Fm: *Funneliformis mosseae* (syn. *Glomus mosseae*); *Rhizophagus irregularis* (syn. *Glomus intraradices*); *Funneliformis badium* (syn. *Glomus badium*); *Claroideoglomus etunicatum* (syn. *Glomus etunicatum*); Fb: *Funneliformis badium* (syn. *Glomus badium*); Ra: *Rhizoglomus aggregatum* (syn. *Glomus aggregatum*); Rf: *Rhizophagus fasciculatus* (syn. *Glomus fasciculatum*); Ri: *Rhizophagus irregularis* (syn. *Glomus intraradices*); Ce: *Claroideoglomus etunicatum* (syn. *Glomus etunicatum*); Sc: *Scutellospora calospora* (syn. *Gigaspora calospora*); Sh: *Scutellospora heterogama* (syn. *Gigaspora heterogama*); Ak: *Acaulospora kentinensis* (syn. *Entrophospora kentinensis*); Am: *Acaulospora morrowiae* (syn. *Acaulospora morrowae*)

Conclusions

Our observations in this study indicate that the endophytic arbuscular mycorrhizal fungi living in the rhizosphere and within the plant tissues of *A. gerrardii* are coordinately involved in plant adaptation against salt stress. There is wide diversity in AMF associated with rhizosphere and roots within the same genus and species based on morphological basis. The knowledge about the community of AMF associated with roots of *A. gerrardii* in different habitats of Saudi Arabia are important in lieu of its every possibility to use such association as an alternative biological mechanism to alleviate the adverse impact of abiotic stress. This way the rehabilitation of Saudi deserts with *A. gerrardii*c can be improved.

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