Status of arbuscular mycorrhizal fungi in different plants of date palm plantation of Al-jamil farm at Qassim, Saudi Arabia

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ABSTRACT

Arbuscular mycorrhizal fungal association were assessed in different plant species namely Allium sativum, Cenchrus ciliaris, Cynodon dactylon, Helianthemun sp, Malva parviflora, Medicago sativa, Trigonella foenumgraceum, and Zea mays collected from date palm plantation of Al-jamil Farm at Qassim, Saudi Arabia. Roots and rhizosphere soils were processed by following the standard methods. Total colonization, spore population and diversity significantly varied in different plant species. Total mycelial colonization varied from 28-72% with the highest in A. sativum (72%) and the lowest in Helianthemum sp. (28%). Maximum vesicular colonization was in Zea mays (60%) and minimum was in Cynodon dactylon(31%). The highest arbuscular colonization was found in Trigonella foenumgraceum (59%) and the lowest was in Helianthemun sp. (26%). Arbuscular mycorrhizal fungal spore population varied 95-130/100g dry soil. The highest number was recorded in Trigonella foenumgraceum (130) and the lowest was in Medicago sativa (95). Funneliformis mosseae (8-42%), Glomus etunicatum (5-26%), Glomus intraradices (14-50%), Glomus sp (3-21%). Gigaspora sp. (8-62%) and Sclerocystis sp. (4-42%) were identified from the rhizosphere soils. A few spores were unidentified.

Keywords: Arbuscule, Colonization, Diversity, Mycorrhiza, Mycelium, Vesicles, Spore population.

INTRODUCTION

Date palm, *Phoenix dactylifera* L., is one of the firstborn perennial fruit trees and it stands tall with their branches outstretched and their roots anchored deep into the earth in the sanctuaries of Saudi Arabia. Date palms have been a treasured part of the Saudi landscape for their beauty as well as their utility. Since ancient times, the date palm has been a source of food for Arabian people, and its branches provide with shade from the sturdy desert sunlight. The Kingdom of Saudi Arabia is the world's second largest producer of dates, supplying 17.6 percent of the world market. The estimated annual production of dates in Saudi Arabia is 1,008,105 tons occupying an area of 156,023 hectares with 23,742,593 date palm trees (MOA, 2012).

In the arid ecosystems of Saudi Arabia, drought is an important abiotic factor and liable for limiting plant growth and yield (Kramer and Boyer, 1997). Plants growing in these stressed conditions form mutualistic mycorrhizal interaction to survive the drought stress (Auge, 2001; Ruiz-Lozano *et al.,* 2001) and to adapt themselves by their morphological, anatomical and physiological responses (Bray, 1997) in addition. Arbuscular Mycorrhiza Fungi (AMF), belonging to Phylum-Glomeromycota (Redecker *et al.,* 2013), are important constituents of the soil microbial community in terrestrial ecosystems forming mutualistic symbiotic association with most of the terrestrial plants (Trappe, 1987). AMF have been shown to promote plant growth by uptake of slow releasing nutrients (Newsham *et al.,* 1995), drought

tolerance (Auge, 2001), salinity tolerance (Evelin *et al.,* 2009), establishment and growth in harsh environments (Koske and Polson, 1984), protection to roots against soil borne pathogens (Azcon-Aguilar and Barea, 1996), improve host physiological processes, promote plant diversity (van der Heijden *et al.,* 1998) etc. AM fungi are important to the persistence of vegetation in harsh environment conditions. However, little is known about the biodiversity of AM colonization and spore population in Saudi vegetation (Khaleil, 1989; Malibari *et al.,* 1990; Al-Garni, 2001; Al-Whaibi, 2009; see also Al-Qarawi *et al.,* 2012). The mycorrhizal association of different crops and weeds in various agro-ecosystems are well known (see Muthukumar and Prakash, 2009), no specific reports on mycorrhizal association in different plants growing in the farming systems of Saudi agro-ecosystems. The present study was undertaken to test our hypothesis that plant species growing in various farms of Saudi agro-ecosystems may demonstrate the arbuscular mycorrhizal association. We examined different plants growing in the date palm plantation at Qassim, Saudi Arabia for their arbuscular mycorrhizal association and diversity of arbuscular mycorrhizal fungi in the soil.

MATERIALS AND METHODS

Root and soil sample collection and preservation

Different agricultural and non-target plants were growing in the date palm plantations of Qassim. Roots and rhizosphere soil samples of *Allium sativum, Cenchrus ciliaris*, *Cynodon dactylon*, *Helianthemun* sp, *Malva parviflora*, *Medicago sativa*, *Trigonella foenumgraceum* growing in the date palm plantation were collected. Removing the gravels from top soils, roots and rhizosphere soils were collected from 5−30 cm soil layer. Roots were preserved in 50% alcohol after cleaning and washing. Preserved roots were cleaned, chopped into 1cm pieces and stained with 0.05% aniline blue (Philips and Hayman, 1970; Koske and Gemma, 1989) with modifications. Assessment of AM colonization was followed under digital computerized microscope. Data were recorded on total colonization, intensity (poor, moderate and abundant) (Dhar and Mridha, 2006) of AM structural (mycelium, vesicle and arbuscule) colonization. Mycelial colonization was regarded as total colonization. Percent colonization and intensity of AM structural colonization were calculated (Dhar and Mridha, 2006).

Processing of soil samples and spore isolation

From each sample, 100g soil was processed by wet sieving and decanting method (Gerdemann and Nicolson, 1963) with some modifications. The series of ASTM-60, ASTM-100, ASTM-270 and ASTM-400 sieves were used to extract the spores. Part of residues on the sieves was used for isolation of the spores through centrifugation with 60% sugar solution and other part was used for filtration method to have intact spores with morphological structures (Gerdemann and Nicolson, 1963). Spore suspension was filtered through gridded Whatman filter paper No-1 facilitating the easy counting of the spores. After filtration the paper was examined under the stereo-binocular microscope at 2.5´10 magnification and spore number was recorded. The total number of spore population in each individual sample was calculated per 100g dry soil basis.

Identification of AMF spores

Morphologically similar spores were separated and observed under computerized compound microscope mounting on PVLG and Melzer's reagent to identify by following the established literatures (INVAM, 2013; Schenck and Perez, 1990; Schüßler and Walker, 2010; Redecker *et al.,* 2013). Total spore population, species richness and Shannon's diversity index (Hs) of AM fungal species were calculated (see Dhar and Mridha, 2006).

Statistical analysis

Data were analyzed by One Way Anova and means were compared using the SPSS 21 at 0.05% level.

RESULTS

Data on the arbuscular mycorrhizal (AM) colonization have been presented in the Table-1. The range of AM colonization varied from 28-72%. The highest was recorded with *Allium sativum* (72%) followed by *Zea mays* (70%) and *Trigonella foenumgraceum* (69%). Vesicular colonization was recorded 31-60%. Maximum vesicular colonization was recorded in *Zea mays* (60%) which was followed by *Trigonella foenumgraceum* (58%). Minimum was recorded in *Cynodon dactylon* (31%). Arbuscular colonization was observed 26-59%. The highest arbuscular colonization was found in *Trigonella foenumgraceum* (59%) and the lowest was in *Helianthemun* sp (26%). Intensity of mycelial, vesicular and arbuscular colonization varied independently (Table-1).

Data on the total AM fungal spore population and AM fungal species richness in the rhizosphere soil of different plant species under study have been presented in the Table-2. The highest AM fungal spore population was counted in the rhizosphere soil of *Trigonella foenumgraceum* (130) which was followed by *Zea mays* (125)*, Allium sativum* (121) and *Helianthemun* sp (116). The lowest spore population was counted in the rhizosphere soil of *Medicago sativa* (95). *Funneliformis mosseae, Glomus etunicatum, Glomus intraradices, Glomus fasciculatum, Glomus* sp*, Gigaspora* sp*, Sclerocystis* sp were identified. *Funneliformis mosseae* was recorded 8-42% in six samples: maximum in *Cenchrus ciliaris* (42%) and minimum in *Medicago sativa* (8%).

Glomus etunicatum was observed 5-26%. The highest was recorded in the sample of *Malva parviflora* (26%) and the lowest was in the sample of *Allium sativum* (5%). Glomus sp-1 was counted 3-21%. *Gigaspora* sp was observed in five soil samples (5-62%). The highest population was recorded in the soil sample of *Medicago sativa* (62%) and the lowest was recorded in the soil sample of *Cynodon dactylon* (5%). *Sclerocystis* sp was recorded 4-42% from five soil samples. Maximum was in the soil of *Trigonella foenumgraceum* (42%) and minimum was in the soil of *Malva parviflora* (4%). A few spore remained unidentified.

Figure-1 represents the data on the Shannon's diversity index (Hs) of AM fungi in the rhizosphere soils of different plants growing in the date palm plantation of Al-Jamil Farm at Qassim of Saudi Arabia. The highest Hs was calculated in the soil of *Helianthemun* sp and it was followed by *Cenchrus ciliaris, Cynodon dactylon* and *Malva parviflora.* The lowest was in the soil sample of *Medicago sativa*.

DISCUSSION

All the plant species under study were observed to be associated with arbuscular mycorrhizal fungi. Occurrence of different arbuscular mycorrhizal fungal species has been confirmed in the date palm plantation of Al-Jamil Farm at Qassim, Saudi Arabia. Different AM fungal structures viz: coenocytic mycelium, vesicles and intracellular arbuscules were observed and their presence confirmed the occurrence and association of arbuscular mycorrhizal fungi with the growing plant species in the date palm plantation. *Allium sativum, Trigonella foenumgraceum* and *Zea mays* were observed to be highly colonized. They have no significant difference in case of total colonization. These agricultural crop plants were previously reported to be highly mycorrhizal (Christopher and Vyan, 2008; Chu et al, 2013; Gill *et al.,* 2013; Tuncturk, 2011). Variation in total AM colonization was significant among other plants species. Similar variation of AM colonization in different desert plants were reported in the earlier studies (see Al-Qarawi *et al.,* 2012). Arbuscular colonization in the plant species indicates the active role of mycorrhizal symbionts in the date palm plantation. Intensity of mycelial, vesicular and arbuscular colonization were variable in different plant species.

Total spore population was variable in all soil samples which was statistically significant ($p<0.05$). Most of the AMF species were under *Glomus*. Arbuscular mycorrhizal fungal species richness and distribution varied independently in different rhizosphere soil samples. It may remarkably be mentioned that agricultural crops growing in the date palm plantation showed lower species richness and diversity index. It may be due to the different cultural practices in the agro-ecosystem. It is reported that cultural practices may be responsible for lower species richness, spore density and diversity of AM fungi (Abbott and Robson, 1991). Whereas naturally growing non-target plants showed comparatively higher species richness and diversity index. Such phenomenon may be the result of the plant's survival strategy in the adverse condition.

Funneliformis mosseae was also recorded. Different recorded species of *Glomus* were more or less similar in all the samples. Frequency distribution (species richness) of different AMF species varied significantly. It is notable that *Gigaspora* spp were higher *Glomus* spp were almost lower in the agricultural plants and whereas in the non-target plants *Gigaspora* sp was lower and *Glomus* was frequently occurring. The reason of this inverse interaction is unknown. More and extensive studied are emphasized hereby to understand any positive or negative interrelationship between *Gigaspora* and *Glomus* in the agroecosystem at Qassim of Saudi Arabia. Species composition of AMF community may be influenced by the host species (see Kumar *et al.,* 2012). Growth and development habits of the host and AM fungi may also influence the variation of AMF structural colonization and sporulation of the fungal symbionts (see Kumar et al,.2012). Diversity of AM fungal species is important in the soil systems for the best nutrient uptaking.

Arbuscular mycorrhizal fungi spread extraradical mycelia network in the pedosphere binding the microparticles by filamentous hyphae constituting persistent aggregates of the soil particles. Stability of soil aggregates is related to the soil density, root length and extra-radical mycorrhizal mycelium in the rhizosphere soils (see Graf and Frei, 2013). Mycorrhizal fungal mycelial mass in the soils of date palm farm increase soil stabilization and thus reduce the soil erosion in Saudi agro-ecosystems. Arbuscular mycorrhizal inocula are dispersed by the windflow in the desert fields and they are deposited near the gravels on the soil surface. New borne seedling of different non-target plants facilitate the colonization and spreading of these deposited mycorrhizal propagules (Koske and Polson, 1984) which help to nutrient enrichment, soil improvement, and microbial development in the date palm rhizosphere thus favouring the date palm trees.

In the abiotically stressed habitats like arid ecosystems of Saudi Arabia, AM dependent plants acquire mycorrhizal symbiosis for their survival and nutritional requirement. Estimation of AM fungal inoculum potential in the non-target plants and their influence in the nutrient cycling, nutrient management, and maintenance of the balanced soil health of the date palm plantation in the harsh conditions of Al-Qassim might be important. As such these non-target plants, supporting the date palm trees by maintaining the diversity of AMF species, providing with sufficient nutrients and managing the soil health system, may be regarded as helpful friends to the date palm trees in the abiotically

stressed agroecosystems of Saudi Arabia with the importance of nutritional, ecological and evoltuionary standpoint.

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Tables

Table 1: Total colonization and intensity of different AM structural colonization in the roots of various plants species growing in the date palm plantation of Al-Jamil farm at Qassim, Saudi Arabia.

Plant species	Total Colonization (%)			Intensity of AM structural colonization (%)								
				Mycelium			Vesicles			Arbuscules		
	Mycelium	Vesicles	Arbuscules	P**	M	A	P	M	A	P	M	A
A. sativum	72 a*	42 c	27 e	42	41	17	53	43	4	32	34	34
C. ciliaris	45 d	40 c	36 c	27	53	20	38	47	15	35	52	13
C. dactylon	52 c	31 d	31 d	25	75	--	--	100	--	100	--	--
Helianthemun sp	28 e	35 d	26 e	32	47	21	46	54	--	47	51	2
M. parviflora	66 b	53 b	48 b	25	46	29	32	62	6	50	24	26
M. sativa	46 d	42 c	33d	24	53	23	54	23	23	25	53	22
T. foenumgraceum	69 ab	58 a	59 a	21	57	22	54	40	6	15	53	32
Z. mays	70 ab	60 a	57 a	36	43	21	20	46	34	--	100	--

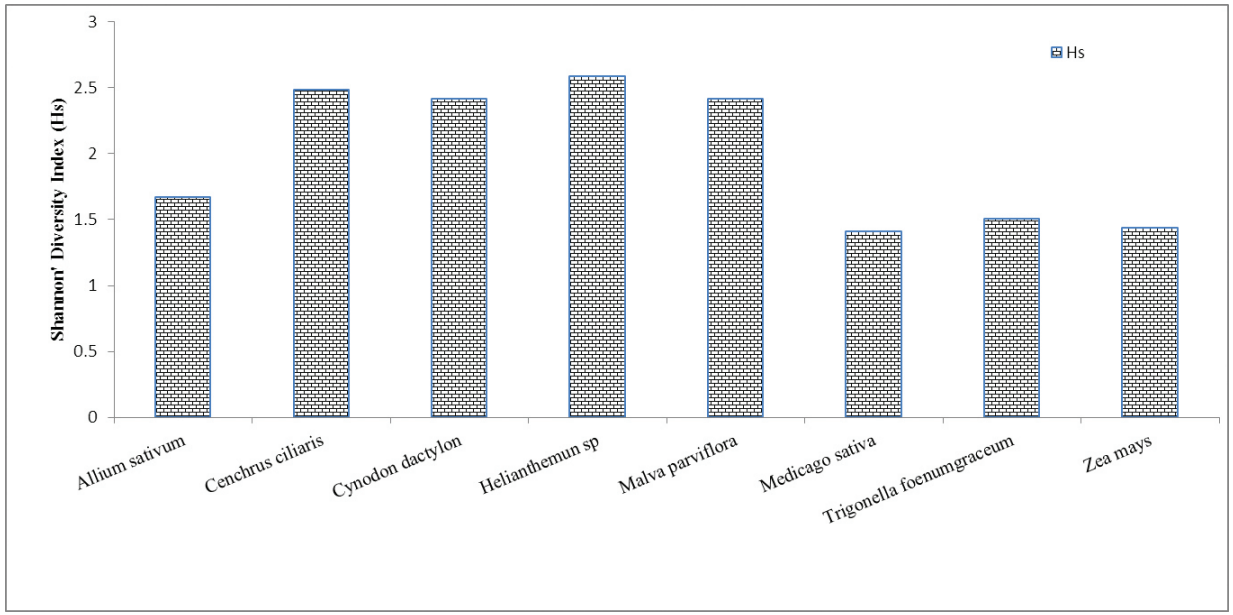
*Different letters indicate the significant variation as shown by DMRT ($p<0.05$).
**P-Poor, M-Moderate, A-Abundant.

Table-2: Total spore population and percent population of different AM fungal species in the rhizosphere soil of various plant species growing in the date palm plantation of Al-Jamil farm at Qassim, Saudi Arabia.

Plant species	Total population	% population of different AM fungal species						
		F. mos**	G. etu	G. intra	Glom. sp.-1	*Gig.*	*Scl.*	Unidfd
A. sativum	121 c*	35	5	50	3	--	--	7
C. ciliaris	105 e	42	14	20	11	8	--	5
C. dactylon	97 g	38	21	14	13	5	--	9
Helianthemun sp	116 d	21	21	22	12	--	5	19
M. parviflora	102 f	25	26	19	21	--	4	5
M. sativa	95 g	8	--	--	--	62	26	4
T. foenumgraceum	130 a	--	--	--	19	39	42	--
Z. mays	125 b	--	--	--	16	52	32	--

*Different letters indicate the significant variation as shown by DMRT ($p<0.05$).
**F. mos =*Funneliformis mosseae*, G. etu =*Glomus etunicatum*, G. intra =*Glomus intraradices*, Gig.=*Gigaspora* sp, Scl.=*Sclerocystis* sp., Unidfd=Unidentified.

Figure:



Shannon's Diversity Index (Hs) of AM fungi in the soil samples of different plants growing in the Date Palm Plantation