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Diversity of Structural Colonization and Spore Population of Arbuscular Mycorrhizal Fungi in Some Plants from Riyadh, Saudi Arabia

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The percentage infection in the roots of different species with the arbuscular mycorrhizal fungi varied widely and independently, irrespective of plant species. The overall highest infection was recorded in *Petunia hybrida* and *Gaillardia pulchella* (97%), which was followed by *Calendula officinalis (90%), Cynodon dactylon* and *Ocimum sanctum* (87%), *Convolvulus arvensis* (70%), *Phoenix dactylifera* (53%), *Tagetes patula (*43%) and the lowest infection was found in *Sesuvium portulacastrum* (33%). The maximum vesicles were found in *P. hybrida* (97%), which was followed by *G. pulchella* (80%), *C. officinalis (67%), O. sanctum* (60%) and the minimum was in *P. dactylifera* (7%). In case of total infection with arbuscules, again the highest percentage of infection was recorded with *P. hybrida* (73%) and arbuscules were not found with *C. arvensis, S. portulacastrum*. The second and third highest percentage infection of arbuscule was recorded with *C. officinalis (63%) and O. sanctum* (57%). The intensity of infection also varied widely and independently in each individual plant species and it was not always comparable to the percentage infection with different structure of the AMF. Spore population also varied irrespective of plant species. Highest number was recorded from the rhizosphere soils of *P. hybrida* and the lowest number was found with *S. portulacastrum.* So far, this is the first report of extensive study on structural colonization and spore population study of AM fungi from a large number of plant species from Riyadh, Saudi Arabia.

Key words: Arbuscular mycorrhizal fungi, structural colonization, spore population, Saudi Arabia.

The Kingdom of Saudi Arabia comprises of 225,000sq. kilometer area and occupies four fifths of the Arabian Peninsula. The climate of Saudi Arabia is hot but not humid for the greater part of the year, relative humidity is low except along the coastal zones where it reaches over 90%. The average annual temperature is 33.4°C in summer (a maximum of 50°C) and 14°C in winter (below zero at night). Rainfall in the greater part of Saudi Arabia is scanty, unpredictable and irregular[1]. Saudi Arabia has diverse vegetation because of the diverse physiographic features coupled with diverse climatic differences[2,3]. Desert (semi- and arid lands) covers about 40% of land surface[4]. Desert land is characterized by little precipitation, hence there is limited moisture, and high temperature at day time at least part of the year, and moreover high incidence of light are prevailing. In many areas of the desert, the soil is sandy and poor in organic nutrients.

Arbuscular Mycorrhiza Fungi (AMF) are widespread throughout the world and found in

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the majority of terrestrial ecosystems. They are an important component of the soil microbial community. These fungi, belonging to phylum Glomeromycota, form mutualistic symbiotic association with most of the land plants[5]. They are obligate symbiont and survive in exchange for carbon from plant hosts. Arbuscular mycorrhizal fungi play an important role in the uptake of water and increase drought tolerance[6]; help in increasing uptake of slow release nutrients[7], especially in phosphorus deficient soils and help plant establishment and growth in harsh environments[8]; help to control pests and fungal pathogens by enhancing the resistance to disease[9] and affect the fitness of plants in polluted environments as well as promote plant diversity[10] etc. The association is essential for plant ecosystem function because the great majority of plant species depend on it for mineral nutrient uptake. This task is efficiently performed by the extensive extraradical mycelium of the fungal symbionts. Within root cells, arbuscular mycorrhizal fungi form typical tree-like structures, the arbuscules and hyphal coils. Some also produce storage organs, termed vesicles. Mycorrhizal plants are effective colonizers of disturbed habitats and the lack of mycorrhizal fungi influences plant species composition. Although AM fungi are important to the persistence of vegetation in harsh environment conditions, little is known about the diversity of this beneficial symbiosis in Saudi vegetation[11-15]. The understanding of mycorrhizal associations in plants growing in harsh soil conditions as found in Saudi Arabia and their distribution in the soil is necessary for the sustainable management of these habitats.

So far very limited works[11-15] have been done in Saudi Arabia in different aspect of AMF. It is urgently needed to perform some studies in relation to the practical application of mycorrhizal fungi in Saudi Agriculture, Range land, Forestry, and other vegetations. Starting with the activities of mycorrhizal study, the present research programme was initiated to assess the structural colonization of AM fungi and spore population of AM fungi associated with some plant species in Dirab, King Saud University Farms, Riyadh, Saudi Arabia, to understand the status of AMF with Saudi plants.

MATERIALS AND METHODS

Structural colonization of AMF in roots

Collection and processing of root samples

Roots along with rhizosphere soils of *Calendula officinalis* L., *Catharanthus roseus* (L.) G. Don, *Convolvulus arvensis* L., *Cynodon dactylon* (L.) Pers., *Petunia hybrida* (L.) Mill. *Gaillardia pulchella* Foug., *Ocimum sanctum* L., *Phoenix dactylifera* L., *Sesuvium portulacastrum* (L.) L., *Tagetes patula* L., were collected from the King Saud University Farm, at Dirab, Riyadh, Saudi Arabia. Fine and feeder roots were collected from 0-30 cm soil layer digging the soil with a soil corer. In the laboratory, roots of each plant species were separated from the soil, washed to free the roots from soil and debris and preserved in 50% alcohol. Preserved roots were washed with care to remove the alcohol. Root segments of a length of 1 cm (approx.) were chopped for AM fungal structural analysis.

Clearing roots and staining of mycorrhiza

The washed and cleaned root segments were placed in 10% KOH and heated to 90°C for 30-60 minutes depending on the colour and thickness of the roots, they were then washed in distilled water and immersed in 3% H_2O_2 for 5-10 minutes. After that they were washed in distilled water and acidified with 5N HCl for 2-3 minutes. The root segments were stained with 0.05% aniline blue in acidic glycerol for 30 minutes at 90°C in a water bath and after staining, the excess stain was removed with clear acidic glycerol[16, 17].

Assessment of structural colonization in roots

The stained root segments were mounted in lacto glycerol solution on glass slides. Ten segments were mounted on each slide. After mounting the segments, the cover slip was gently pressed to facilitate the observation of different type of structures present in the whole root segment under compound microscope. A minimum of 50 segments from each samples were observed for the assessment of structural colonization of AM fungi associated with roots. Presence of mycelium and coiled hyphae, vesicles, as well as arbuscules was recorded and analyzed to determine the structural colonization. To estimate the percentage of mycorrhizal colonization , intensity of infection (mycelium, vesicles and

arbuscular development) in the infected region of the roots was estimated in root samples stained for total infection by the method of Trouvelot *et al.,*[18] and Giovannetti and Mosse,[19]. A root segment was considered to be infected if it showed mycelium, coiled hyphae, vesicles, arbuscules, or any other combination of these structural characteristics of AM fungi. The intensity of colonization was measured as poor, moderate and abundant[20] type of colonization with each of the individual structure. When any of these were found in sample, the intensity of infection of AM fungi was estimated as: poor- (if only mycelia were present); moderate-(if mycelium and vesicles or arbuscules were present) and abundant- (if mycelium, vesicles and arbuscules were present). Mycelial colonization was regarded as total AM colonization. Percent colonization was calculated by following Dhar and Mridha[21].

Isolation and identification of AMF from soils

Collection and processing of soil samples

For enumeration of arbuscular mycorrhizal fungi available in rhizosphere soil, the collected samples were used. Before collecting the samples, to avoid the undesirable particles and plant debris, top soil (approximately 1cm) was removed first and then sufficient soils were collected from 0-30 cm depth and was kept in zipped polybags individually and brought to the laboratory for isolation of AMF propagules. Before spore isolation, the unwanted particles were removed by sieving the soils with 2mm sieve. Spores were isolated immediately after collection and/or preserved at low temperature in the laboratory for future use.

Spore isolation

Spores were separated by wet sieving and decanting method[22] with some modifications as followed by Dhar and Mridha[21]. From each sample, 100g soil was taken in a bucket of 10-litre capacity and 5-litre of water was added to the soil. The soil was mixed well with water to make a soil-water suspension. According to the soil physical structure (clay or sandy), the suspension was left for 3(sandy soil) -5(clay soil) minutes for settling down of insoluble and heavy soil particles. The suspension was passed through the ASTM-60, ASTM-100, ASTM-240 and ASTM-400 sieves gradually to extract the spores. The residues of the individual sieves were washed with water jet and collected individually in a small beaker by backwashing. Fifty percent of the washed were used for isolation of the spores through centrifugation at 2000 rpm by following Gerdemann and Nicolson[22] for 2 minutes. Other 50% of the solution was used for filtration method to have intact spores with morphological structures. The individual collection of spore suspension was filtered through gridded Whatman filter paper No.1 (to facilitate the easy counting of the spore) placed in a funnel fitted with conical flask (vacuum). The supernatants of the sieves were examined under stereo-binocular microscope to observe the presence of sporocarps and larger spores. After water filtration, the paper was examined under the stereo-binocular microscope at 2.5x10 magnification and the number was counted. The total number of spore population in each individual sample was calculated per 100g dry soil basis. Some spores were tightly grouped in sporocarps and it was difficult to count the number of spores per sporocarp. So, to simplify this procedure, we referred to a sporocarp as one spore.

Identification

Spores were separated on the basis of morphological characters and each type of spore was observed under compound microscope mounting in water, lacto glycerol, PVLG and PVLG +Melzer's reagent[23, 24] for identification. The identification was based on spore colour, size, surface ornamentation and wall structure with reference to the descriptions and pictures provided by INVAM[25]; ZUT (Zachodniopomorski UniwersytetTechnologiczny)[26] and Schenck and Perez[27] as well as originally published species descriptions, wherever possible.

The spores were placed in PVLG and PVLG+Melzer's reagent on the two ends of a slide separately. Cover slip was placed gently to observe the whole spore. After recording the characteristics, the cover slip was pressed gently to observe the internal structures and chemical reaction with mountant, if any. Some spore specimens could not be identified to species as only a few spores were isolated, or the spores lacked distinguishable, fine taxonomic characters.

Statistical analysis

Variation of structural colonization was calculated by DMRT at $P<0.05$ using the SPSS-11.5 software.

RESULTS AND DISCUSSION

Structural colonization of AMF in roots

The percentage infection in the roots of different species with the mycorrhizal fungi varied significantly (Table 1). A wide and independent variation was recorded irrespective of plant species and types. The mycelium infection was regarded as the total infection of each of the plant species. The range of variation of total infection was 33% - 97%. The overall highest infection was recorded with *P. hybrida* and *G. pulchella* (97%), which was followed by *C. officinalis (90%), C. dactylon* and *O. sanctum (87%)*, *C. arvensis (70%), P. dactylifera (53%)* , *T. patula (43%)* and the lowest infection was found with *S. portulacastrum* (33%). The total percentage infection of mycelium was not reflected with the infection of vesicles and arbuscules in individual sample. The range of infection with vesicles was 7 % with *P. dactylifera* and 97% with *P. hybrida*. The maximum vesicles were found with *P. hybrida* (97 %), which was followed by *G. pulchella* (80%), *C. officinalis (67%), O. sanctum* (60%) and the minimum was with *P. dactylifera*(7%). In case of total infection with arbuscules the highest percentage of infection was recorded with *P. hybrida* (73%) and the second and third highest infection with arbuscules was recorded with *C. officinalis (63%) and O. sanctum* (57%). No infection with arbuscules was found in *C. arvensis* and *S. portulacastrum*.

The intensity of infection in individual plant species with mycelium along with coiled hyphae, vesicles, and arbuscules was estimated as poor, moderate and abundant in each case. The intensity of infection also varied significantly in each individual plant species and was not always comparable to the percentage infection with different structure of the AMF (Table 1). The samples which had high total infection percent may not show the high intensity of infection with vesicles and arbuscules. It varied from plant species to species. In case of intensity of infection with mycelium, the maximum infection as poor, moderate and abundant types was recorded with *C. arvensis (*60%), *P. hybrida* (37%), *C. officinalis* (70%) respectively and the minimum was recorded with *T. patula* (0%) for mycelium; both *C. roseus* (7%), *C. dactylon* (7%) for vesicles. And no arbuscular

Table 1. Structural colonization of Arbuscular Mycorrhizal Fungi in different plant species in Dirab, King Saud University Farm, Riyadh

Plant species	Total colonization (%)			Intensity of Colonization (%)								
	Mycelium	Vesicles	Arbuscules	Mycelium			Vesicles			Arbuscules		
				P	M	A	P	M	A	P	M	A
Calendula officinalis	90a	67c	63b	7	13	70	7	3	57	13	0	50
Catharanthus roseus	37d	30d	3f	30	7	0	20	10	0	3	0	0
Convolvulus arvensis	70b	23d	0f	60	10	0	23	3	0	0	0	0
Cynodon dactylon	87a	33d	10e	53	7	27	20	0	13	3	7	0
Petunia hybrida	97a	97a	73a	27	37	33	27	27	43	33	13	27
Gaillardia pulchella	97a	80b	33c	30	10	57	20	3	57	10	20	3
Ocimum sanctum	87a	60c	57b	13	23	51	17	7	36	17	7	33
Phoenix dactylifera	53c	7e	10e	37	13	3	3	4	0	10	0	0
Sesuvium portulacastrum	33d	30d	0f	23	10	0	13	10	7	0	0	0
Tagetes patula	43cd	30d	20d	0	16	27	13	3	14	7	0	13

Means with the same letter are not significantly different
P= Poor; M= Moderate; A= Abundant

colonization was found in *C. arvensis, C. roseus*, *S. portulacastrum*. In the same way, the intensity of infection with vesicles , the highest percentage of poor, moderate and abundant types was found with *P. hybrida* (27%), *P. hybrida* (27%) and *C. officinalis* (57%) respectively and the lowest percent of poor type of infection was recorded with *P. dactylifera* (3%), and most of species produced the moderate type of infection with very less amount of infection, but the maximum type of abundant infection with vesicles was recorded with both *C. officinalis and G. pulchella* (57%), and the minimum was recorded with three different species (*C. roseus, C. arvensis* and *P. dactylifera)* with no infection with the vesicles. The intensity of infection with arbuscules was less in comparison to mycelium and vesicles. Most of the samples were found to have no arbuscular presence in their root systems. Poor type of percent infection was recorded in 8 plant species with maximum (33%) in *P. hybrida*, moderate type was found in four plant species only with highest (20%) in *G. pulchella* and abundant type of infection was recorded with five plant species with highest (50%) in *C. officinalis.*

The mycorrhizal colonization for the selected plant species growing at Riyadh was not studied before for their structural colonization with AMF. Khaleil[11] from Riyadh, Saudi Arabia studied the soil and root infection of some plants such as *Anisosciadium lanatum, Korwoodia dicksoniae, tripleurospermum auriculatum, Anthemis deserti, Rhazya stricta* and *Panicum turgidum* and found the presence of AMF belonging to two species: *Glomus fasciculatum* and *G. mosseae*. He also mentioned that the domination of *G. mosseae* is due to the alkalinity of the soil[11]. Again Khaleil and Abu-Heilah[12] from Saudi Arabia reported the presence of mycorrhizae with date palm growing in Qassim. The occurrence of AMF at different soils of Saudi Arabia was reported by few other researchers[13, 14, 15, 28, 29] but no one has mentioned the structural colonization of AMF with Saudi Plants.

AMF spore population

Spore population varied from 59-216/100g dry soils, irrespective of plant species. The highest spore population was recorded with *P. hybrida* (216) which was followed by *C. officinalis* (204), *G. pulchella* (184), *O. sanctum* (174), *C. arvensis* (150), *T. patula* (147), *P. dactylifera* (120), *C. dactylon* (106) and *C. roseus* (87) and the lowest number was found with *S. portulacastrum* (59). Out of the different species of AMF recorded during the present study, *G. mosseae, G. etunicatum, G. aggregatum, G. intraradices, , G. fasciculatum, G. macrocarpum* were found and a few spore were unidentified. *G. fasciculatum* and *G. etunicatum* were very common and was recorded from most of the soil samples studied in the present investigation.

Al Garni[15] reported a wide variation among the samples for spore populations with the field soils and infectivity of AMF with roots from a study with Taif soils and standing crops. But there was no mention of structural variation with AMF in any individual plant species. Al-Whaibi[30] in his mini –review on Desert plants and mycorrhizae, mentioned the occurrence and diversity of mycorrhizae in desert plants (e.g. [31-41] and also see Zhang *et al*., [42]) throughout the desert world but very few references are available with structural colonization studies and spore population in different countries with desert plants especially from Saudi Arabia.

From the present assessment of colonization and spore population study, it is important to note that most of the selected plants species growing in the farm under control conditions were highly mycorrhizal. Generally the plant species which have high infection in the roots have also produced higher number of spore from the rhizosphere soils with little exceptions. All the different mycorrhizal fungal structures like mycelium, coiled hyphae, different types of vesicles, different types of arbuscules, spores inside the root segments etc were found in most of the plant species studied in the present investigation. So far this is the first report of extensive study on structural colonization and spore population study of AMF in large number of different types plant species from Riyadh, Saudi Arabia.

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