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MANAGEMENT OF ARBUSCULAR MYCORRHIZAL FUNGI BY GROWING *PETUNIA HYBRIDA* (L.) MILL. AS AN ORNAMENTAL PLANT IN SAUDI ARABIA - A CASE STUDY

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Abstract

Arbuscular mycorrhizal fungi (AMF) regarded as ubiquitous soil fungi which help in improving plant growth under harsh conditions. *Petunia hybrida* is one of the most favorite ornamental plants growing all over the Riyadh city of Saudi Arabia. In the present study, we would like to highlight the *Petunia* as a mycotrophic plant for the management of mycorrhizal fungi under field conditions. Roots along with rhizosphere soils of *P. hybrida* were collected from various sites in Riyadh, Saudi Arabia to study AM colonization and biodiversity of AMF. The data obtained in this study indicated that *P. hybrida* is a very highly mycotrophic plants, and all the samples produced very high colonization with mycelium, vesicles, coiled hyphae and arbuscules. The significant variation was found with the occurrence of mycelium and vesicles among the locations but in case of arbuscules more or less same range of occurrence was found. Only different species of *Glomus* were observed in all the locations. *Glomus* showed diversity in all the locations as indicated by the Shanon Diversity Index. As the *P. hybrida* is a highly mycotrophic plant, so this plant may be grown under harsh condition of Saudi Arabia to manage the plant growth under different stresses viz., water stress, saline soils and heavy metal toxicity conditions.

Introduction

Riyadh covers over 700km^2 and lies in the center of Arab Peninsula on latitude 34°-38^o north and longitude 46^o-43^o east and approximately 1,950 feet (600 meters) above sea , in the centre of the Arabian Peninsula. The Riyadh region features very harsh and dry weather, and has extreme temperatures during summer and winter (Anon., 2002).

Arbuscular Mycorrhizal Fungi (AMF) is widespread throughout the world and found in the majority of terrestrial ecosystems (Smith & Read, 2008). They are obligate symbionts can improve plant growth by up taking P and to help to absorb N, K, Ca, S, Cu, and Zn (Tinker & Gildon, 1983; Requena *et al*., 2001; Jiang, *et al*., 2013); produce glomalin (Wright & Upadhyaya, 1998; Guo *et al*., 2012) that reduces soil erosion by bringing together microaggregates of soil particles to form macroaggregates (Miller & Jastro, 1994); improving Azcon-Aguilar & Barea, 1996; water absorption (see Dhar & Mridha, 2003); decrease disease incidence (Linderman, 1994); enhance the salt tolerance (Evlin *et al*., 2009); heavy metal sequestration (Tonin *et al*., 2001); and showed diversity of structural colonization (Chaudhry, *et al.*,2012) etc.

Petunia (*Petunia hybrida* (L.) Mill.) is one of the most popular and beautiful ornamental plant species growing in road side, avenues, gardens, road divider etc in all over Riyadh because of its ability to thrive under deficit irrigation and at high summer temperatures. There is little information on the ability of petunia to form symbiotic associations with AMF (Daft & Okusanya, 1973; Gaur *et al*., 2004) and only Shamshiri *et al*., (2011) reported the response to Phosphorous and Drought Stress. Reddy *et al*., (2009) studied the development and function of the arbuscular mycorrhizal symbiosis in Petunia and focused as a model species of AM research regarding nutrient acquisition, transportation and interaction between different micro and macronutrients in the soil due to its high mutant varieties and genetical characteristics. But no study was found on the infectivity of different varieties of Petunia grown in different habitats with AMF under field conditions and also there is no report of association of AMF species in petunia grown soils.

Here, we would like to highlight the *Petunia* as a mycotrophic plant for the management of mycorrhizal fungi in the soil systems of Saudi Arabia. Therefore the present study was undertaken to observe the status of structural colonization of different varieties of Petunia and mycorrhizal spore available in the rhizosphere soils and a case study of *P. hybrida* has been mentioned in the present study.

Materials and Methods

Collection of root samples: Roots along with rhizosphere soils *P. hybrida* were collected from various places (Al Qasab, Derab, Umm Al Hammam, Solaimania, Riyadh centre**)** in Riyadh city, Saudi Arabia to study AM colonization in the roots and AMF spore population in the rhizosphere soil. Soil samples were collected from 0−30cm soil layer. In the laboratory, roots were separated from the soil samples, washed to free of soil and debris and preserved in 5% formalin.

Staining and assessment of roots: Preserved root segments were stained by following the methods of Phillips & Hayman, (1970) and Koske & Gemma, (1989). The stained root segments were mounted in lacto glycerol solution on glass slides for observation of different AM structures (mycelium, vesicles, arbuscules, hyphal coils). Percentage of mycorrhizal colonization, intensity of AM structural colonization (mycelium, vesicles and arbuscular colonization) were estimated in the stained roots for total infection. The intensity of colonization was measured as poor, moderate and abundant (Dhar & Mridha, 2003) types of colonization with each of the individual structure. The intensity of infection of AM fungi was estimated as: poor-(if only mycelia were present); moderate-(if mycelia 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 the following formula:

$$\% \text{ Colonization} = \frac{\text{Total number of AM positive segments}}{\text{Total number of segments studied}} \times 100$$

Assessment of rhizosphere soil for spore population and AMF diversity: Soils were assessed following wet sieving and decanting (Gerdemann & Nicolson, 1963) with some modifications (Dhar & Mridha, 2003) to enumerate the total spore population and biodiversity of AMF spore in the soils. Morphologically similar spores were picked up and they were identified by following the established literature (INVAM; ZUT; Schenck & Perez, 1990; Schüßler & Walker, 2010). Percent population of individual species was calculated by the following formula:

$$\% \text{ Species} = \frac{\text{Number of individual species}}{\text{Total number of spores}} \times 100$$

Species diversity of AM fungi was assessed by the Shannon index as follows:

$$\text{Shannon's Index (Hs)} = \frac{C}{N} \{(N \log 10N) - \Sigma n_i \log 10\ n_i\}$$

where 'C' =3.321928 (constant used in converting log10 to log2), "n_i" is the number of species in the '*i*th' species and "N" is the total number of individual (Simpson, 1949; Lloyd *et al*., 1968).

Statistical analysis: The data were statistically analyzed using analysis of variance (ANOVA) for a completely randomized design using the program SAS (SAS, v.9.1) and the differences in means were determined by the least significant differences (LSD) (α=0.05) test.

Results and Discussion

The root samples of *P. hybrida* collected from many different locations of Riyadh indicated that all the samples are infective with AM Fungi but the degree of colonization varied in different samples.

The data obtained in this study indicated that *P. hybrida* is a very highly mycotrophic plants, as almost all the samples showed a very high infection with mycelium, vesicles, coiled hyphae, arbuscules etc. and in some of the samples intraradical spores were also found . The significant variation was found with the occurrence of mycelium and vesicles among the locations but in case of arbuscules more or less same range of occurrence was found (Fig. 1). In case of total Mycelial colonization, the highest infection was found with root samples from Solaimania (75.67%) followed by Derab (75.33%) and the lowest was recorded from Al Qasab (68.33%). Total vesicular colonization was observed highest in Solaimania (66.00%) followed by Derab (62.67%) and the lowest was in Riyadh centre (55.00%) and total arbuscular colonization was recorded highest from Umm al Hammam (74.33%) followed by Solaimania (71.00%) and the lowest was in Al Qasab (65.67%).

Data on the root colonization for different verities of *Petunia* collected from different locations are summarized in the Fig. 2. The mixed colored variety collected from Al Qasab produced highest total colonization (78.33%) which was followed by white and violet colored varieties. Vesicular colonization was recorded highest (75.33%) in the mixed colored variety and the lowest (67.67%) was in the white variety. Arbuscular colonization was highest in the violet and mixed colored varieties (79.00%) and the lowest was in the white variety (74.67%). Again, mixed colored variety showed the highest total colonization (95.57%) and violet colored variety showed the lowest (78.67%). Vesicular colonization was observed highest in the mixed variety (96.67%) and the lowest was in the violet variety (74.00%). Arbuscular colonization was observed highest in the mixed variety (85.67%) and the lowest was in the violet variety (75.33%).

Not much research was available on the infectivity of Petunia with AMF under field conditions and also with different varieties of petunia. There is no report of association of AMF species in petunia grown soils. Daft & Okusanya, (1973) and Gaur *et al*., (2004) reported the symbiotic associations with AMF in petunia. Our present results are in agreement with them. Shamshiri *et al.,* (2011) reported the response to phosphorous and drought stress and Reddy *et al*., (2009) studied the Development and Function of the Arbuscular Mycorrhizal symbiosis in Petunia and different mutant varietal efficiency of AM colonization relates to the genetically attributes of Petunia varieties (Reddy *et al*., 2007). This is for the first time we reported the AMF structural colonization in *P. hybrida* grown under field conditions in Saudi Arabia.

Total spore population from different locations is presented in the Fig. 3. The range of spore population was 136-180/100g dry soil. The highest was observed in the soil from Derab and the lowest was from Solaimania. Data on percent population of different AM fungal species are presented in the Table 1. All the AM fungal species were of genus *Glomus*. *G. etunicatum, Funneliformis mosseae* (=*G. mosseae,* Schüßler and Walker, 2010), *G. fasciculatum, G. mulituculis,* and *G. aggregatum* were observed. A few *Glomus spp*. were unidentified. From Al Qasab, *G. etunicatum, F. mosseae* and G. *multiculis* were identified. Soil samples from Derab produced *G. etunicatum, G. mosseae, G. fasciculatum* and *G. aggregatum. F. mosseae, G. fasciculatum, G. aggregatum,* Glomus sp.-1 and *Glomus* sp-2 were from Umm Al Hammam. Soil samples from Solaimania showed *G. etunicatum*, *G. fasciculatum, G. mulituculis* and *Glomus* sp-2. Samples of Riyadh centre produced *F. mosseae, G. fasciculatum, G. mulituculis* and *Glomus* sp.2. Diversity Index of different AM fungal species in the Petunia growing soils of Riyadh is summarized in the Fig. 4. The highest Shannon Diversity Index (Ds) of AM fungal species was in Umm al Hamm (2.12) followed by Solaimania (1.82) and the lowest Ds were in Al Qasab (1.46). The occurrence of different species of AMF from *P. hybrida* has also been reported for the first time from petunia grown soils and although AMF spore were reported with other crops in Saudi Arabia (Al-Qarawi *et al*., 2012 & 2013).

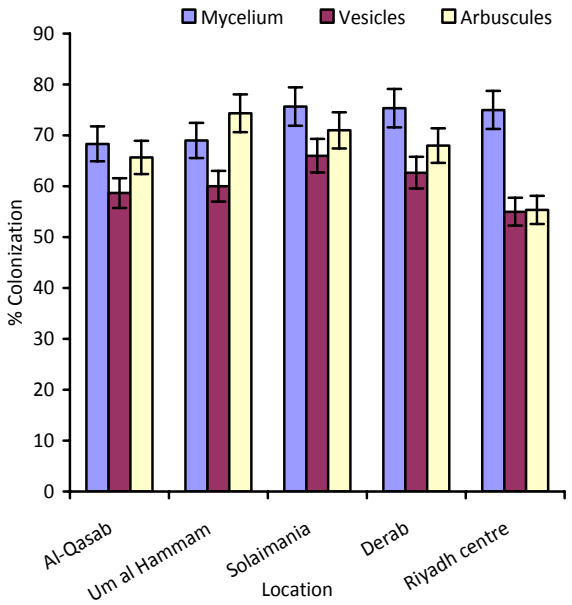

Fig. 1. Arbuscular mycorrhizal structural colonization in the roots of *P. hybrida* collected from different locations of Riyadh, Saudi Arabia.

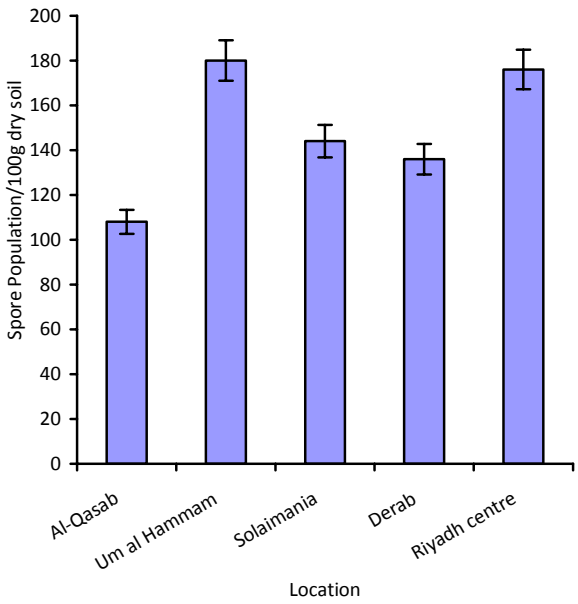

Fig. 3. Total spore population of arbuscular mycorrhizal fungi in the rhizosphere soil of *P. hybrida* collected from different locations of Riyadh, Saudi Arabia

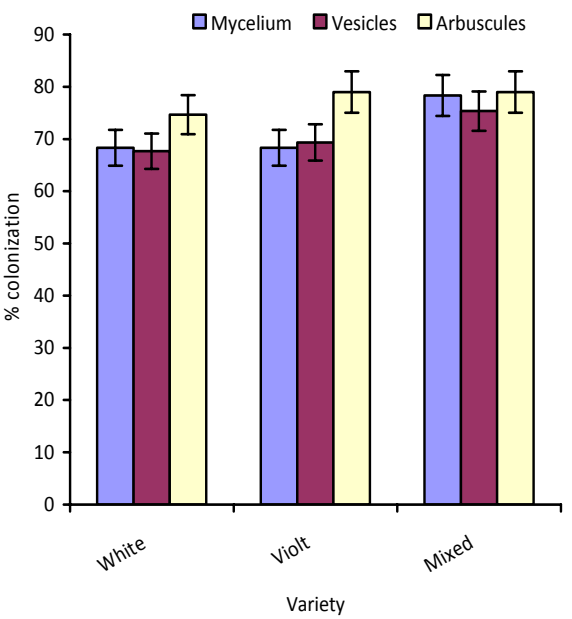

Fig. 2. Arbuscular mycorrhizal structural colonization in the roots of different varieties of *P. hybrida* collected from different locations of Riyadh, Saudi Arabia.

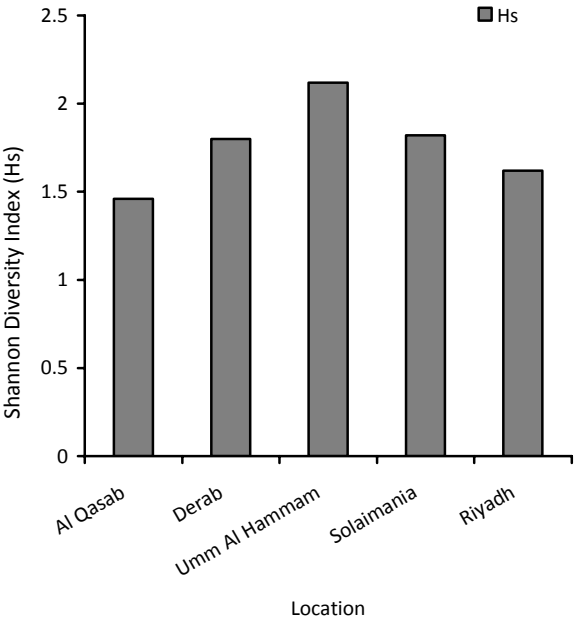

Fig. 4. Shannon Diversity Index of arbuscular mycorrhizal fungi obtained from the rhizosphere soil of *P. hybrida* collected from different locations of Riyadh, Saudi Arabia.

Table 1. Percent population of different species of arbuscular mycorrhizal fungi in the rhizosphere soil of *P. hybrida* grown in different locations of Riyadh, Saudi Arabia.

Locations	% Population of AM fungal species						
	G. etu	F. moss	G. fas	G. multi	G. agg	G. sp-1	G. sp-2
Al Qasab	33ab	50a	00	17a	00b	00b	00b
Derab	40a	15b	35ab	00b	10b	00b	00b
Umm Al Hammam	00c	18b	36ab	00b	27a	10a	9a
Solaimania	28b	00c	42a	22a	00b	00b	8a
Riyadh centre	00c	47a	32ab	18a	00b	00b	3b

G.etu=*G. etunicatum;* F. moss= *F. mosseae;* G. fasci=*G. fasciculatum;* G. multi=*G. multiculis;* G. agg=*G. aggregatum; G.* sp-1= *Glomus sp. 1;* and *G.sp-2= Glomus sp.2* (Means with the same letter are not significantly different $p<0.05$)

Conclusion (management of AMF): *P. hybrida* survive comfortably because of it's highly mycotrophic nature as reported during the present study. *P. hybrida* has the potentiality to improve the water relation; protect from the soil borne plant pathogens; reduces the erosion; work as an agent of phyto-remediation of heavy metals; to alleviate saline conditions in Saudi Arabia. As the mycotrophic plant has the ability to improve soil physical condition by binding soil particles and under harsh condition of Saudi Arabia, soil biological activity is less prominent because of shortage of water and availability of different types of microorganisms, under this condition *P. hybrida* as a mycotrophic plant are helpful in improving the plant community in arid and semiarid conditions in Saudi Arabia and in improving the biological activity of soil which directly or indirectly will be favorable for growth of the other plants.

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