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ORIGINAL PAPER

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OCCURRENCE OF ARBUSCULAR MYCORRHIZAL FUNGI AND NODULES IN THE ROOTS OF TWELVE LEGUME SPECIES IN SOUTH-WESTERN SAUDI ARABIA

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ABSTRACT

The tripartite associations of arbuscular mycorrhizal fungi (AMF), rhizobia and legumes play a vital role in preserving and even restoring fertility of poor and eroded soils. The present study attempted to quantify relationship between legumes and symbiotic microorganisms (rhizobia and mycorrhizal fungi) by describing the mycorrhizal status and the occurrence of nodualtion of legumes growing in different areas of Jazan. The effect of legume species on soil microbial biomass was also investigated. Mycorrhizal and nodulation intensity varied greatly between legume species. The higher number of nodules (14 nodules per plant) and mycorrhization intensity (54%) were registered in root of *Argyrolobium arabicum*. Rhizosphere soils of all legume species harbored higher AMF fungal spores than bulk soils. Our results suggest also a significant effect of legumes species on soil microbial biomass. Thus, legume species investigated in this study are potentially useful for replanting and soil protection of most degraded regions of Saudi Arabia.

Key words: arbuscular mycorrhizal fungi, rhizobia, legumes, soil microbial biomass, soil

INTRODUCTION

Improving food productivity is one of the major focus areas of agricultural sciences in order to feed the ever increasing world population which is expected to reach 10 billion within the next 50 years. Abiotic stresses caused by complex environmental conditions are the major constraints to crop productivity worldwide [Araus et al. 2002], they also play a significant role in establishing the geographic distribution of plant species in different types of ecosystems. Abiotic stress effects on plants are receiving increasing attentiveness from the scientific community because of the important influence of climate change on rainfall patterns, salinization of farm land by irrigation, and the global necessity to retain or increase agricultural productivity on marginal lands [Patanè et al. 2013]. Under both natural and agricultural conditions plants are exposed to various environmental stresses, either concomitantly or serially throughout their growing season. Drought and salinity are considered among the most brutal factors affecting crop productivity by inhibiting photosynthesis and decreasing plants growth [Verslues et al. 2006]. The problem is becoming more acute as the area affected by salinity and drought is increasing day after day.

In nature, plants may establish symbioses with soil microorganisms, such as arbuscular mycorrhizal fungi (AMF) and various N2-fixing bacteria. Both symbioses are recognized to enhance plant growth under environmental conditions [Azcón and El-Atrash 1997].

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Legumes play an important economic and ecological role, as natural fertilizer sources and a food source for humans. They provide high quality forage and contribute to soil stabilization and erosion prevention [Mahdhi et al. 2008]. Legumes are also a major source of timber, phytochemicals, phytomedicines and nitrogen fertility in agrosystems because of their capacity to establish a nitrogen fixing symbiosis with soil bacteria known as rhizobia. In extreme ecosystemic conditions, rhizobia and AM fungi have been shown to enhance the growth of roots and to help plants to use more efficiently soil nutrients and to grow under hard conditions such as drought, salinity and nutrient deficiency [Honrubia 2009]. It is assumed that AM fungi are able to increase nodulation and N fixation by supplying high levels of P to the nodules [George et al. 1995].

Most regions of Saudi Arabia have been subjected to accelerated desertification due to increasing grazing intensity, wood gethering, urban development and other human activities. Because of their capacity to establish dual symbioses with rhizobia and AM fungi, legume plants can be used as an alternative to slow desertification and to preserve fragile ecosystems in southwestern Saudi Arabia. In this respect, rhizobia and AM fungi could play a key role in increasing trees tolerance to drought or at least in improving the survival of seedling up on transplantation from nurseries to the field.

The purpose of this study is to identify legume species that could be used as an alternative to slow desertification and to preserve fragile ecosystems in southwestern Saudi Arabia. Therfore, the objective of this investigation was to study the occurrence of nodulation, spore density and AMF colonization of twelve legumes species growing in Jazan and to evaluate their effect on soil microbiological biomass.

MATERIALS AND METHODS

Study area. The study area is located in South-Western Saudi Arabia (Jazan). Jazan province is situated in the south-western part of Saudi Arabia. Its area is 13,500 km^2. It is located between 16°20'N to 17°40'N and 41°55'E to 43°20'E. The region can be roughly divided into three regions: Tihama, the escarpments, and the Islands. The region has about 260 km long coastal area on the western side. Twelve legume species were investigated in this study: *Rhynchosia minima*, *Indigofera hochstteri*, *Crotalaria retusa*, *Vernifrux abyssinica*, *Vigna membranacea*, *Argyrolobium arabicum*, *Microcharis tritoides*, *Acacia ehrenbergiana*, *Acacia tortilis*, *Acacia orfeta*, *Senna italic* and *Prosopis juliflora*.

Occurrence of nodulation and sampling procedure. Spontaneous nodulation of legume species was prospected. The intensity of nodulation (number of nodules per plant) and the morphology of nodules (shape and color) were investigated visually. Five plants of each species were considered. Soil samples were obtained from the top 10–25 cm from underneath plant canopies and open areas. Soil samples were passed through a 2-mm sieve and stored in cool room until examination. Fine root samples were collected from at least three individual plants of each legume species and stored at 4°C until examination.

Soil microbiological analysis. Soil microbial biomass (Cmic) of soil sub-samples collected from under each legume species and open areas was determined by the fumigation extraction method [Amato and Ladd 1988] using ninhydrin-N reactive compounds extracted from the soils with KCl after a 10-day fumigation period.

AMF spore isolation. Spores of AMF were extracted from 100 g soil collected under from each legume species and open areas. AMF spores were isolated by wet-sieving and sucrose centrifugation [Gerdemann and Nicolson 1963]. Quantification was carried out in petri dishes under a stereoscopic microscope. The spore density was expressed as the total number of spores per 100 g of soil.

Determination of the mycorrhizal status. The roots samples were carefully washed with sterile water, cleared by heating in 10% KOH at 90°C for 1h, bleached by immersion in 10% H_2O_2 for 5 min, acidified in dilute HCl and stained with 0.05% trypan blue in lactophenol [Phillips and Hayman 1970]. Stained roots were checked for AMF colonization by examination under a compound microscope and the mycorrhizal colonization percentage was obtained by the root slide technique [Giovannetti and Mosse 1980]. A minimum of 90 root segments per plant were counted. The intensity (M%) and frequency (F%) of mycorrhization was assessed.

RESULTS

Occurrence of nodulation. The twelve legumes species were nodulated and the number of nodules varied significantly between species ($P \leq 0.05$). The highest number of nodules (14 ±0.4) was observed for *A. arabicum* (fig. 1) and the lowest (5 nodules per plant) was for *A. tortilis.*

The external color of the nodules was white or brown. The internal color of all nodules was pink-red. The size of the nodules with globular shapes varied from 0.1 to 0.4 cm, and could exceed the diameter of the root of their host plant.

Assessment of root colonization by AM fungi. The colonization rate of roots of the twelve legume species by AMF is shown in Figure 2. The roots were colonized with different AMF morphological structures such as intraradical hypae, arbuscules, vesicles and extra-radical spores. Extra-radical spores have also been observed in some root segments. Result showed that the mycorrhizal frequency and intensity of legume roots differed among species ($P \leq 0.05$). Mycorrhizal intensity and frequency ranged from 20 to 54% and from 46 to 65%, respectively. The highest colonization was recorded in *A. arabicum* and the lowest was found in *R. minima.*

Number of spores. Spores of AMF were present in all soil samples and the density of spore differed among legume species (fig. 3). For all legume species investigated in this study the number of spores in the rhizosphere of each legume was higher in the bulk soil. The AM fungal spores numbers ranged from 88 per 100 g of soil (soil collected under *R. minima*) to 188 spores per 100 g of soil (soil collected under *A. arabicum*). The majority of spores we collected were small with diameter less than 70 µm, comparable in shape and size to those of *Glomus.*

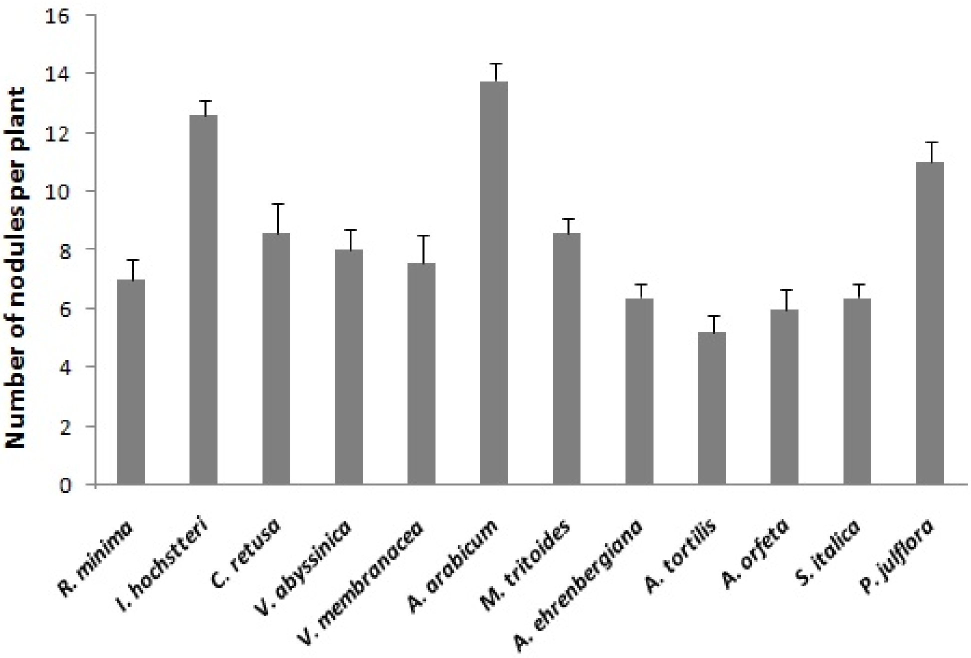

Fig. 1. Number of nodules per plant of legume species in natural conditions. Error lines correspond to standard deviation (n = 5)

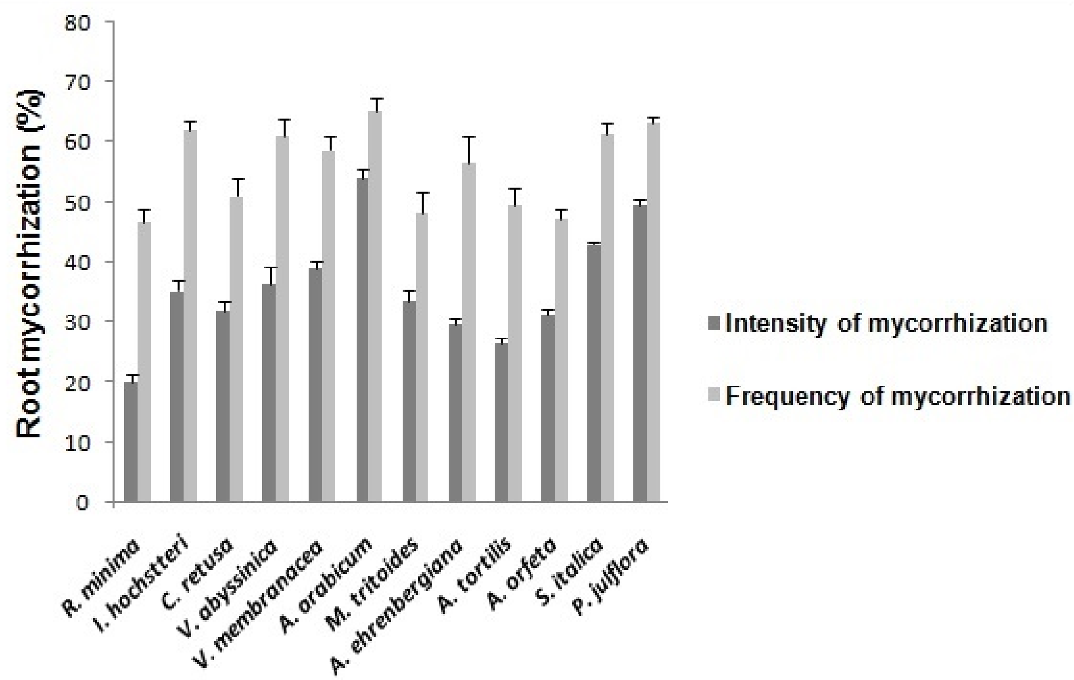

Fig. 2. AMF root colonization (mycorhizal intensity (M) and mycorhizal frenquency (F)) of legume species. Error lines correspond to standard deviation of the means (n = 3)

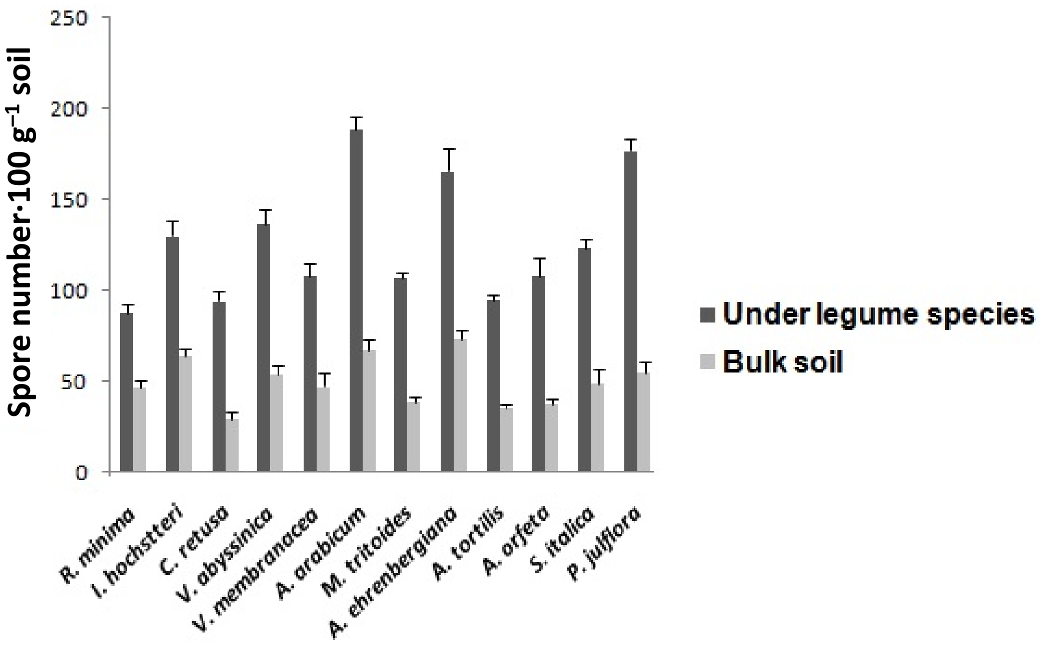

Fig. 3. Spore number of AMF in Jazan soils. Error lines correspond to standard deviation of the means (n = 3)

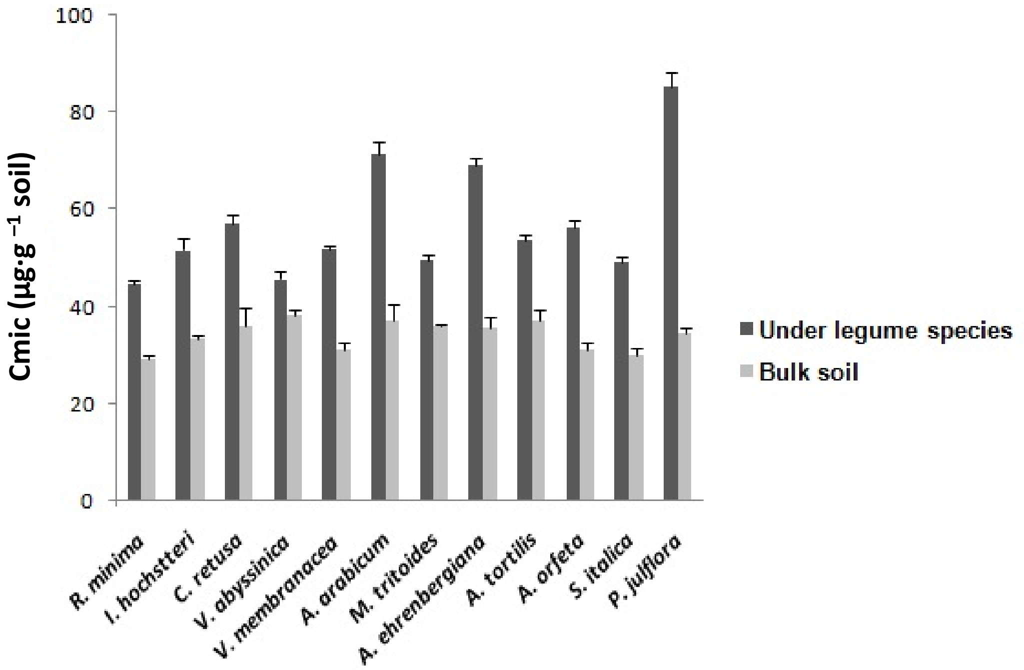

Fig. 4. Soil microbial biomass carbon (Cmic) in the studied soils. Error lines correspond to standard deviation (n = 3)

Soil microbiological biomass. Soil microbial biomass (Cmic) differed significantly between legume species (fig. 4). For all legume species, Cmic was greater in the rhizosphere of each legume than in bulk soil. The highest value of Cmic was recorded in the rhizosphere of *P. juliflora* from (85.3 ±3.1). The lowest Cmic was recorded in the rhizosphere of *A. minima* (44.6 ±0.8).

DISCUSSION

The ecosystem surveyed in this study is characterized by high temperature and stress for most of the year. These conditions limit plant establishment and growth and as a consequence they accelerated soil degradation and microbial communities [Viana et al. 2011]. Beneficial soil microorganisms such as rhizobia and AMF are recognized to enhance plant growth [Abdalla et al. 2014]. In this study we investigated the occurrence of nodulation, AMF colonization in roots of 12 legume species growing in Jazan region and evaluated the effects of legume species on soil microbiological properties.

Result showed that all legume species investigated in this study have the possibility to associate with both rhizobia and AM fungi. This makes these legumes as an important plant functional group since they can form a tripartite symbiosis with nitrogen-fixing rhizobium bacteria and phosphorus-acquiring AMF. Nodulation was found in roots of all species and the number nodules varied greatly between species as was found by Mahdhi et al. [2012] for legume species growing in arid regions of Tunisia. The number of nodules is very low (<14 nodules per plant). The poor nodulation observed can be explained by the effect of several environmental conditions. Previous studies [Bécquer et al. 2014] showed that nodule formation is very sensitive to salt and osmotic stress. In arid regions, drought and soil salinity limit rhizobial population proliferation which affects the infectivity of plant roots. Furthermore, even when nodules were formed, the symbiosis is less effective. The lowest number of nodules was recorded in roots of *A. tortilis* as reported by Mahdhi et al. [2017]. The shape, color and size of nodules varied between legume species. These nodules morphology depends more on soil quality and color, and on the presence of leg- haemoglobin in the nodules.

The survey of the mycorrhizal status indicated that AMF colonization was observed in the roots of all legume species investigated in this study. This suggests that AMF play an important role for the sustainability of legume species in this arid environment. These results agree with others reports [Sun et al. 2016] which found that legumes plants are more mycotrophic than Poaceae and other families. Kannenberg et al. [1998] reported that the genetic pathway of AMF symbiosis is shared in part by N2-fixing bacteria. Previous reports [Abd-Alla et al. 2014] show that dual inoculation with rhizobia and AMF had a synergistic effect on nodulation, plant growth, dry matter production and nitrogen fixation.

Our results show that AMF colonization varied greatly between legume species. This variability in colonization of AMF may depend on host species and their phenology [Smith and Read 2008]. It was reported that AMF colonization was influenced by plant communities, water availability, ambient temperature and soil nutrient levels [Treseder 2004]. Garrido et al. [2010] suggested that root colonization by AMF was positively related to the availability of infective AMF units (e.g. spores) in the soil. A higher rate of mycorrhizal colonization was recorded in roots of *A. arabicum.* This gives a particular interest of this leguminous herbaceous to the fertility of the arid soils. *P. juliflora* showed also a higher AMF root colonization. This could be explained by the invasive character of this legume. Several studies [de Souza et al. 2016] reported that invasive plants increased mycorrhizal fungal abundance and diversity.

Results showed that the density of AMF spores was relatively low, which is common for arid lands [Shi et al. 2007]. Most of the AMF spores obtained in this study by wet-sieving were small (diameter less than 100 μm). The dominance of small spores may be a selective adaptation to water stress. The number of spores in the rhizosphere of legume species is higher than in bulk soils. This result agrees with previous studies which showed that plant species have the ability to promote the development and the diversity of fungal propagules in their rhizosphere [Wang et al. 2016; Turrinia et al. 2017].

Biological indicators such as microbial biomass have been suggested as good indicators of soil quality and productivity [Garcia et al. 2000]. Parameters inferred by the study of microbiological soil states are important to establish reforestation programs and decide about the type of suitable plants for restoration of degraded soils [Traoré et al. 2007]. In our study, result show that soil microbiological biomass was affected by legume species canopies. Compared with bulk soils, canopied soils were characterized by higher soil microbial biomass. This result may indicate that these legume species can be considered as candidate legumes for soil fertility improvement. Similar results were reported by Fterich et al. [2011] about the positive effect of legumes canopy on soil microbial properties. Results showed that the higher soil Cmic was recorded in the rhizosphere of *P. juliflora* as suggested by Solís-Domínguez et al. [2011]. Liao and Boutton [2008] reported that the modification of the quantity or quality of litter exerted by *P. juliflora* affect positively the microbial biomass. In our study, *P. juliflora* microbial alterations are mainly driven by leaf litter produced extensively by this legume.

CONCLUSION

In the present investigation, the occurrence of nodulation and mycorhizal status of most these legume species in Saudi Arabia were documented for the first time. Results showed that roots of all legume species were colonized by both rhizobia and AMF. The highest mycorrhizal and nodulation intensity were observed for *A. arabicum.* Furthermore, the present study reported that soil microbial properties were improved with the presence of leguminous species in comparison with bare areas. These results may have wider implication for improving restoration success of soil fertility in degraded ecosystems.

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