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Occurrence of Arbuscular Mycorrhizal Fungi and Nodules in the Roots of Three Acacia Species in South-western Saudi Arabia

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ABSTRACT

Acacia trees play an important role in the restoration and maintenance of soil fertility. The aims of this study were to evaluate natural nodulation and arbuscular mycorrhizal fungi **(***AMF) colonization of the roots of three Acacia species (Acacia tortilis, A. ehrenbergiana, A. negrii) growing in South-western Saudi Arabia. Mycorrhizal status was assessed by microscopic observation of root fragments after trypan blue staining. Spore density was quantified and was expressed as the total number of spores per 100 g of soil. The occurrence of natural nodulation was also investigated. Mycorrhizal and nodulation intensity varied greatly between species. The roots of A. negrii had the highest AMF root colonization and nodulation (47±2.2 % and 27 nodules, respectively). Rhizosphere soils harbored AMF fungal spores ranging from 161.3 spore per 100 g of soil in A. tortilis to* 423.3 *spore per 100 g of soil in A. negrii. Thus, Acacia species investigated in this study are potentially useful for replanting degraded unfertile soils prevalent in most regions of Saudi Arabia.*

Key words: *Arbuscular Mycorrhizal Fungi, Acacia, nodulation, Saudi Arabia*

INTRODUCTION

Desertification has become a major issue of concern around the world. The causes favoring desert formation include drought, climate change and human activities such as over-cultivation of land, over-grazing, clearing of forests and the use of poor quality water for irrigation[1,2]. Solutions to these problems require integrated activities aiming at the sustainable management of affected areas. The priority in combating desertification should be to implement preventive measures for lands that are not yet or only slightly degraded as recommanded by the United Nations' ICCD. In ligne with these recomandations, efforts to raise production and productivity of rangeland and to improve rangeland management have been carried out in many countries. Reseeding affected areas, planting of fodder shrubs and trees, introduction of appropriate grazing management systems are among the practices used in several countries[3].

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The use of native species for re-vegetation is advocated especially in countries with dry climatic conditions. Specifically, native legume species are recommended for inclusion in re-vegetation programs. Indeed many projects used legumes to combat desertification across the world in recent years. For instance, it is well documented that Acacia increases soil fertility by boosting soil nitrogen content[4]. It is also a source of high quality fodder for livestock and food for local communities, and a mean to prevent erosion of the soil in degraded ecosystems. It also provides gum Arabic, shade timber and fuel wood[5].

Plant cover in Saudi Arabia is limited and sparse, made essentially of annual species except in the southwestern and western regions where trees and shrubs represent about two thirds of the flora[6,7]. Acacias are generally the most dominant tree species in Saudi Arabia and elsewhere in the Arabian Peninsula. Among tree genera, Acacia contains the highest number of species (13 species: *A. abyssinica, A. asak, A. etbaica, A. gerrardii, A. hamulosa, A. latea, A. mellifera, A. negrii, A. Oerfota, A. johnwoodii, A. ehrenbergiana A. raddiana* and *A. seyal*)[7]. Some of these species are endemic to Saudi Arabia and are well adapted to arid climates hence their high ecological value. These multipurpose trees are useful for the restoration of degraded ecosystems generally low in organic matter, nitrogen and phosphorus because of their ability to form symbiotic associations with both rhizobial bacteria and mycorrhizal fungi[8,9,10].

Symbiotic relationships between plants and mycorrhizal fungi are ubiquitous and play a very important ecological role in many ecosystems. Differences in species responses to mycorrhizal colonization can have a significant influence on plant community structure. AM fungi interact with all rhizospheric microflora including beneficial and plant pathogenic microbes. Mycorrhizal colonization alters positively nodules formation and N_2 fixation[11]. Several studies showed that AMF enhance nutrient, particularly (P), and water uptake by Acacia species plants[12,13]. It was reported that AMF help Acacia species grow well on poor and degraded under dry and saline conditions[14,15]. Rhizobia strains showing high tolerance to extreme temperature, pH and salinity were isolated from Acacia species[16] (Shetta and Alshahrani, 2016). Consequently, Acacia species have been recommended for inclusion in re-vegetation programs and many projects used Acacia to combat desertification across the world in recent years.

The purpose of this study is to explain the capacity of three Acacia species to survive in arid regions and specially the role of their symbiotic associations with nitrogen-fixing bacteria (rhizobia) and mycorrhizal fungi in this adaptation. Therfore, the objective of this investigation was to study the spore density, AMF colonization and diversity of three Acacia species (*A. tortilis, A. ehrenbergiana, A. negrii*) that grow in different land in south western Saudi Arabia.

MATERIALS AND METHODS

Study area

The study area is located in South-western Saudi Arabia. We collected the plant material from two sites in Tihama plains of Jazan province and one in higher areas (2200m) located in Abha province (Table 1). Rainfall in the entire Tihama region is too scanty to support any appreciable vegetation. The annual rainfall do not exceed 140 mm. Vegetation is dominated by *Leptadenia pyrotechnica* and *A. tortilis*. In Tihama plains, two sites were selected. Site 1 (JAZADCO) is a shallow depression where flood waters pool. The soil is deep and sandy. *A. ehrenbergiana* is dominant in this site. Site 2 (Sabia) is a slightly elevated flat section of the plain where the soil is gravely and shallow. It is dominated by *A. tortilis*. Site 3 is located on the eastern escarpments of Souda Mountains (Assir region) at an elevation of 2596m. The average annual rainfall at this site can reach more than 270 mm. The vegetation is an open forest of *Juniperus prosera* and *A. negrii*. The prospection was carried out in December 2015.

Occurrence of nodulation and sampling procedure

The intensity of root nodulation (number of nodules per plant) and the morphology of nodules were investigated visually. Soil samples were obtained from the top 10-25 cm at each site from underneath at least five large trees of the three Acacia species (*A. tortilis*, *A. ehrenbergiana*, *A. negrii*). Soil samples were passed through a 2-mm sieve and stored in cool room until examination. Fine root samples were collected from at least five individual plants of each species at a depth of approx. 25 cm and stored at 4 °C until examination.

Assessment of root colonization by AM fungi

The roots samples were carefully washed with sterile water and cut into 1 cm-long segments, cleared by heating in 10% (w/v) KOH solution at 90°C in water bath for 1h, then acidified with 1% HCl for 5 min. Finally, the roots were stained with 0.05% trypan blue for 30 min[17]. Stained roots were checked for AMF infection by examination under a compound microscope and the mycorrhizal colonization percentage were obtained by the root slide technique[18]. Ninety root pieces per plant were counted. The frequency (F) and intensity (M) of mycorrhization were assessed following the method of Trouvelot et al.[19].

Isolation, enumeration of AM fungal spores

AMF spores occurring in the soil samples were extracted by the wet sieving and and sucrose centrifugation technique[20]. Supernatant was decanted into a 32 mm sieve, washed with running tap water and transferred to Petri dishes. Spore density was expressed as the total number of spores per 100 g of soil[21].

Statistical analyses

Statistical analyses were performed with a SAS statistical package. The data were subjected to ANOVA test. Comparisons among means were made using the Least Significant test at the 5% level of significance (P< 0.05).

RESULT

Occurrence of nodulation

The three Acacia species were nodulated and the number of nodules varied significantly between species (P≤0.05). The highest number of nodules (7.5±0.4) was observed for *A. negrii* (Fig.1) and the lowest (<11 nodules per plant) was recorded for *A. tortilis.* Nodules were multishaped and undeterminated. The size of the nodules varied from 5 to 14 mm. The external colour of the nodules is white-brown. The majority of nodules from *A. negrii* were pink indicative of the presence of leghaemoglobin

AMF spore density

Spores density in the rhizosphere soil varied significantly between Acacia species (Fig. 2). The higher number of spores was counted in the rhizosphere soil of *A. negrii* (423.3 spores/ 100 g soil) and the lowest was recorded under *A. tortilis* (161.3 spores/ 100 g soil). Microscopic observation showed a high variation in shape, size and color of spores with a clear dominance of yellow-colored and small spores in all samples (Table 2).

AMF colonization

The colonization rate of the three Acacia roots by AMF is shown in Fig. 3. The roots were colonized with different AMF morphological structures such as intraradical hypae, arbuscules, vesicules and extra-radical spores. Statistical analysis showed that the mycorrhizal frequency and intensity of Acacia roots differed among species (P≤0.05). Mycorrhizal intensity and frequency ranged from 29.5 to 47% and from 65 to 83.3, respectively. The highest colonization was recorded in *A. negrii* and the lowest was found in *A. tortilis.* There was no significant difference in F and M between roots of *A. tortilis* and *A. ehrenbergiana.*

Table 1: Description of the three sites where the tree roots and soil samples were collected

Site	Elevation (m)	Soil characteristics	Annual rainfall (mm)	Average Maximum temperature	Average minimum temperature
Site 1: JAZDCO (17°11’54’’N, 42°37’3’’E)	37	Deep, sandy	140	37°C	31°C
Site 2: Sabia (17°13’42’’N, 42°51’16’’E)	64	Shallow, sandy	133	46°C	34°C
Site 3: Souda (18°13'29.6"N 42°26'11.8"E)	2596	Shallow, sandy loam	278	31.3°C	8°C

Table 2: Morphological characters of AMF spores in studied plants

	Shape	Color	Sporecarp	Size (µm)
A. tortilis	Globase to avoid, subglobose, ellipsoidal, irregular	Pale yellow, light yellow, pastel yellow, orange yellow, Brown, red brown, dark	Presence, none	50 to 100 (40 %) 100 to 200 (30 %) 200 to 300 (25 %) 300 to 350 (5 %)
A. ehrenbergiana	Globase to avoid, subglobose, ellipsoidal, irregular	Pale yellow, light yellow, orange yellow, brown, red brown dark	Presence, none	50 to 100 (35 %) 100 to 200 (30 %) 200 to 300 (25 %) 300 to 350 (10 %)
A. negrii	Globase to avoid, subglobose, ellipsoidal, irregular	Pale yellow, light yellow, Brown, red brown, dark	Presence, none	50 to 100 (35 %) 100 to 200 (25 %) 200 to 300 (25 %) 300 to 350 (15 %)

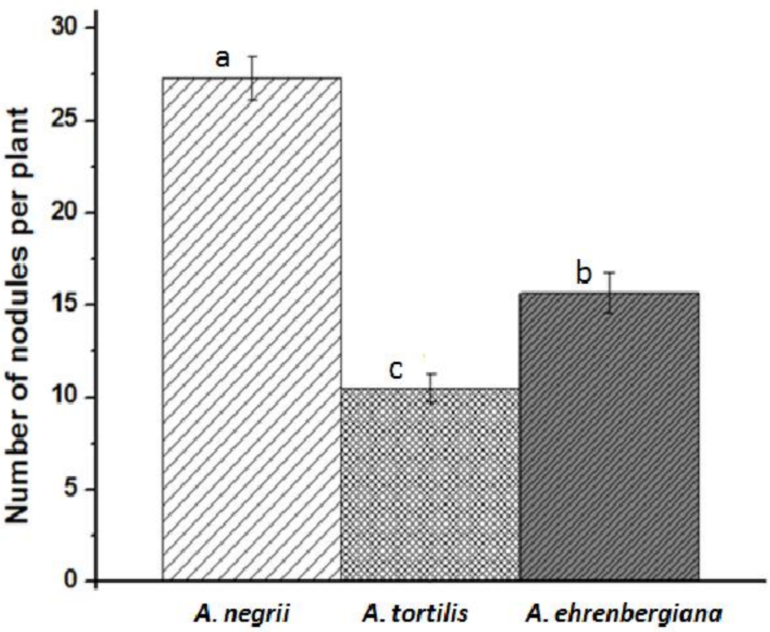

Fig. 1: Number of nodules per plant of three Acacia species in natural conditions. Different letters on top of bars indicate significant differences (*P*<0.05, mean and standard error, n=6)

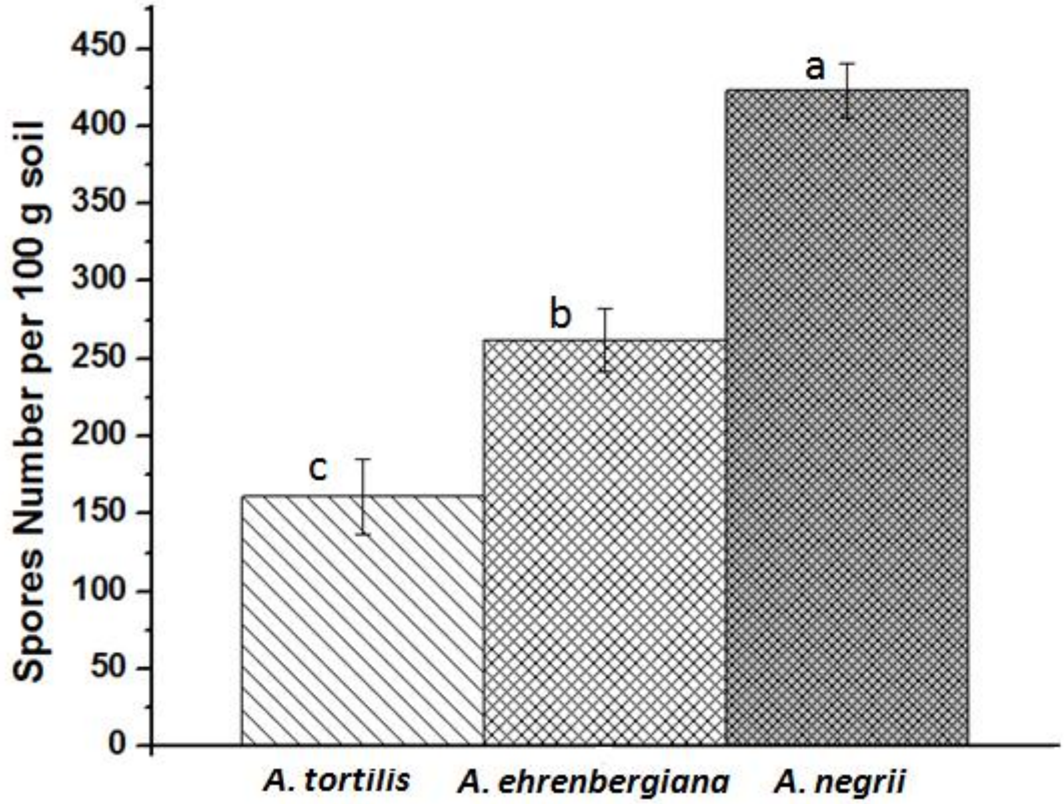

Fig. 2: AMF spore abundance in rhizosphere soil of three Acacia species. Different letters on top of bars indicate significant differences (*P*<0.05, mean and standard error, n=3)

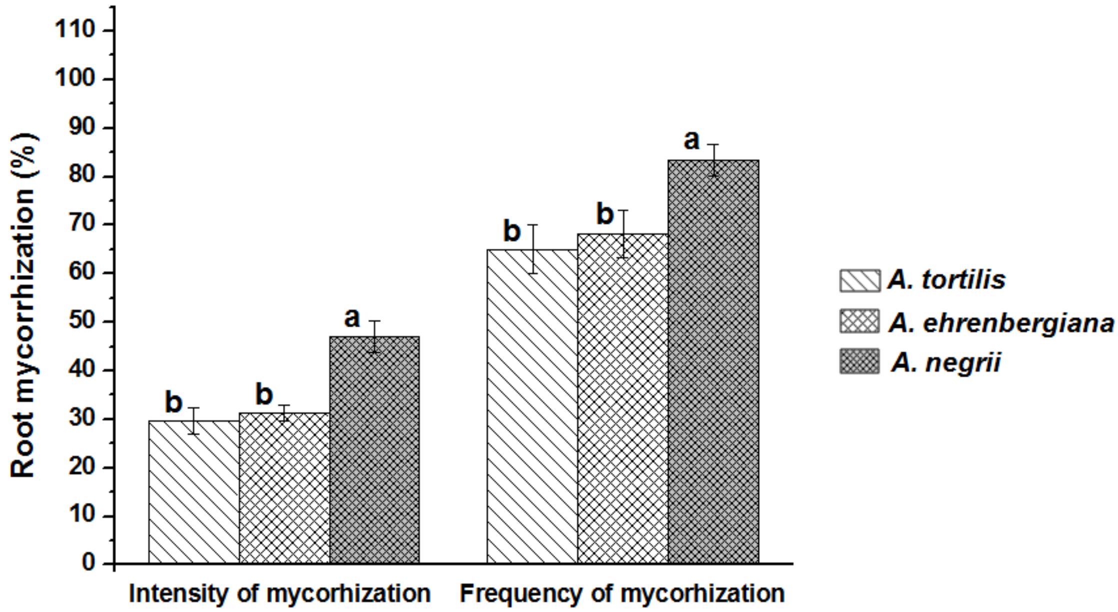

Fig. 3: AMF root colonization (mycorhization intensity (M) and mycorhization frenquency (F)) of three Acacia species. Different letters on top of bars indicate significant differences (*P*<0.05, mean and standard error, n=3)

DISCUSSION

Acacia species have the possibility to associate with both rhizobia and AMF. Thus, these legumes form an important plant functional group since they can form a tripartite symbiosis with nitrogen-fixing bacteria and phosphorus-acquiring AMF. In extreme ecosystemic conditions such as those prevailing in most of the Arabian Peninsula, rhizobia and AMF have been shown to enhance the growth of roots and to help plants use more efficiently soil nutrients and to grow under harsh environmental conditions such as salinity, drought and nutrient deficiency[22,23]. Our results indicate that Acacia species have a good mycotrophic status, and arbuscules and vesicles are found in their roots. This suggests that AMF play an important role for the sustainability of Acacia species in this arid environment. These findings are consistent with earlier reports on AMF associated with the roots of Acacia species trees in Saudi Arabia[24,25]. These results agree with others reports[26,27] which found that legumes plants are more mycotrophic than Poaceae and other families. A higher spore density and mycorrhizal colonization was registered in the rhizosphere and roots of *A. negrii* growing in the elevated areas of Assir (Site 3). This variability in spore density and colonization of AMF may depend on host species and their phenology, the differences in the physico-chemical properties of substrates, temperature and rainfall[15,28]. Facelli et al.[29] showed that AMF colonization levels can decrease with increasing vegetation biomass because of increased competition for infective AMF units. In the present study, the trees at all three sites were stating to bloom when we collected root and soil samples in December. However, the soil at Sites 1 and 3 were wetter than that at Site 2. As compared to *A. negri*, a low spore density and low AMF root colonization were observed for *A. tortilis* and *A. ehrenbergiana*, investigated in Tihama plains which are characterized by a low rainfall and sandy soils. However, Diop et al.[8] suggested that AMF spore density was greater in dry regions than in wet region. In wet soils, water availability would increase fungal mycelium growth, leading to a decrease in spore germination. 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[30].

There were differences in nodulation between the three Acacia species. The lowest number of nodules (<11 nodules per plant) was recorded for *A. tortilis.* This can be explained by the effect of several environmental conditions. Others reports[31,32] showed that legume rhizobia association and especially nodule formation are very sensitive to salt and osmotic stress. In arid regions, several environmental conditions affects both the free-living and symbiotic life of rhizobia themselves. In this study, *A. tortilis* and *A. ehrenbergiana* roots were colledted from the arid plain of Tihama where total annual rainfall does not exceed 170 mm and soil temperature often exceeds 35 °C in summer.

In conclusion, results reported the presence of tripartite symbiosis (acacia–rhizobia-AMF) in all studied sites. The highest mycorrhizal and nodulation intensity were observed for *A. negrii* endemic for elevate area. Our study showed relatively higer mycorrhizal colonization and AMF diversity in the roots of different Acacia species growing in Saudi Arabia. These results may have wider implication for improving restoration success of soil fertility degraded ecosystems.

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