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Full Length Research Paper

Arbuscular mycorrhizal fungi species associated with rhizosphere of *Phoenix dactylifera* L. in Morocco

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A survey of arbuscular mycorrhizal fungi (AMF) diversity and date palm (*Phoenix dactylifera* L.) tree root colonization in arid areas was undertaken in ten palm groves located along the Ziz valley (Tafilalet, south-west Morocco). The frequency and the mean intensity of root colonization reached 72 and 43% respectively and the spore population varied from 238 to 1840 spores/10g of soil. The AMF colonization levels of field date palm roots were found to be negatively correlated with soil phosphorus content (r2 = -0,64). A total of ten AMF species were trapped from the 10 studied sites with a population of 4 to 7 species per sites. The isolated species included: *Glomus mosseae*, *G. fasciculatum*, *G. constrictum*, G. aggregatum, *G. macrocarpum*, 3 undescribed species of Acaulospora and two of *Scutellospora genera*. The latter two species were trapped only at the second trapping cycle.

Keywords: AMF, diversity, Morocco, palm grove, *Phoenix dactylifer*,.

INTRODUCTION

In the arid Moroccan region area, date palm trees (*Phoenix* L.,), are considered crucial to the ecosystem as they protect the surrounding vegetation against desert influences and provide adequate microclimate to the under storey crops. However, drought, soil salinity and fungal disease such as vascular wilt (*Fusarium oxysporum* sp. albedenis) considerably decrease the palm grove yields (Haddouch, 1997).

The arbuscular mycorrhizal fungi (AMF) found worldwide establish a symbiotic association with the majority of land plants including those of the arid areas (Brundrett, 1991; Stutz et al., 2000); once established, AMF enhance root mineral nutrition, especially phosphorus, and favor plant growth. Moreover, AMF may protect plants against environmental stress such as soil salinity (Klironomos et al., 2001; Giri et al., 2003), drought (Al Karaki et al., 2004; Guissou et al., 2001) and pathogens such as *Fusarium* wilt (Hwang et al., 1992; St-Arnaud et al., 1995; St-Arnaud et al., 1997; Jaizme-Vega et al., 1997 ; Habte et al., 1999). In an extensive survey on the mycorrhizal status of xerophytic and halophytic plants Khan (1974) did not detected any mycorrhizal colonization in naturally grown *P. dactylifera* root systems although their rhizosphere contained AMF spores. Since that time, the natural association of date palm trees with AMF has never been confirmed as the few investigations on palm trees symbiotic status were done under greenhouse using artificially inoculated plants (Al-Whaibi and Khaliel 1994; Jaizme Vega and Dias Perez 1999; Morte and Honrubia 2002). The date palm AMF association was found to promote palm growth especially on nutrient poor soil and was recognised as positively significant for their establishment and survival (Khaliel and Abou-Heilah., 1985; Al-Whaibi and Khaliel, 1994). The objectives of the present study deal with the evaluation of the mycorrhizal status of date palm roots and the survey of AMF species in palm grove soils.

MATERIAL AND METHODS

Sampling sites

The survey was done in an arid zone of the pre-Saharan region located along the Ziz Valley in the palm grove of Tafilatet, South-West of Morocco. The area is characterized by annual rainfall of 60 to 265 mm from north to south with a mean of 25 rainy days/year, mean temperature of -1.5°C (January) to 50°C (July), and an annual mean of potential evaporation of 2500 mm (ORMVAT, 2002). Ten soil sampling sites were retained on the basis of one palm tree rhizosphere sample per site (Figure 1, Table 1). For each tree, 4 soil and root samples were harvested around

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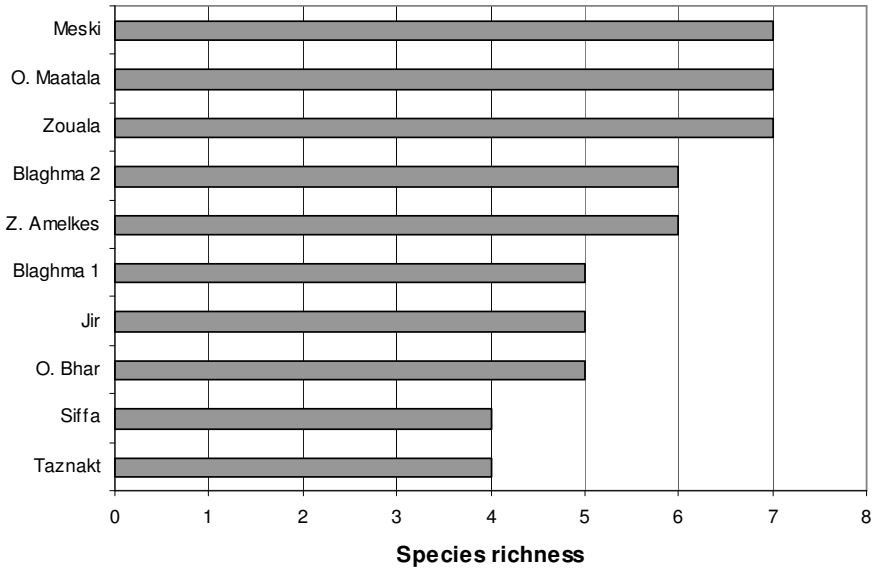

Figure 1. AMF species richness for the 10 AM fungi detected in successive trap cultures from 10 sampling sites

Table 1. Physical and chemical properties of soil samples.

Localities	pH_{eau}	Organic matter (%)	N- NH_4 mg/100 g	N- NO_3 mg/100g	P available µg/g	Soil texture		
						Sand (%)	Limon (%)	Argile (%)
Taznakt	8,30	1,91	0,03	3	164,2	33,2	51,6	15,2
Amelkes	8,40	1,07	0,02	1,87	16,6	51,3	38,4	10,4
Blaghma1	8,70	1,64	0,06	1,04	10,.4	39,5	41,2	19,3
Blaghma2	8,60	2,38	0,03	0,94	40,5	38,3	40,9	20,8
O. Bhar	8,40	1,58	0,04	0,16	24,.3	33,6	51,9	14,4
Meski	8,50	1,08	0,03	1,49	23,9	74,4	18,9	6,7
Zouala	8,20	1,41	0,02	4,57	31,4	86,1	9,6	4,3
Maatala	8,40	1,30	0,14	2,57	71	57,6	31,5	10,9
Jir	8,60	0,37	0.21	1.22	17,7	10,9	55,2	33,8
Siffa	8,40	1,60	0.05	0.54	26,5	15	55,7	29,4

the tree from 10 to 40 cm depth, and mixed together. Sampling was done in February 2003 and the samples preserved at 4°C until use.

Physical and chemical analysis of soil

Field soil sub-samples from each location were analysed. Soil properties measured were the pH in 1 :1 water, available phosphorus (Olsen et al., 1954), nitrogen N- NH4 N- NO3 (Bremner and Keeny, 1966), soil texture and organic matter. This later soil property was indirectly measured by comparing the dry weight after 6 hrs at 105°C with the dry weight after 4 hrs at 550°C, and soil texture (Klute, 1986; Page et al., 1982).

Root staining for the evaluation of AMF root colonization

Roots staining procedures were adapted from Koske and Gemma (1989) methodology. Roots were rinsed to remove adhering soil and debris, and cut into 0.5 to 1 cm long segments bleached once in a KOH (10%) solution for 45 minutes at 90□C, rinsed in water, immersed in an HCl (1%) solution for 4 min to acidify the tissues, and then stained in Trypan Blue (0.05%) 15 min at 90°C. Root colonization levels were estimated according the Trouvelot et al., (1986) method on 3 lots of 30 root segments randomly chosen and observed under the light microscope. Two parameters retained were the colonization intensity i.e. the proportion of cortical cells colonized by the fungi, and the colonization frequency corresponding to the ratio of colonized versus non colonized root fragments.

Spore extraction

Spores were isolated from their substrate using a mix of wet-sieving and sucrose gradient techniques (Brundrett et al., 1996). 100 g of soil was rinsed in through 850, 500, 250, 100 and 50 µm sieves; soil material was recovered from each sieve, suspended in water, and centrifuge at 3000 RPM for 3 min. The supernatant removed and the soil material re-suspended in a sucrose solution

Table 2. Root colonization levels and spore density.

Localities	Indigenous Soil		
	Spore Density /100 g of soil	Root colonisation frequency (%)	Root colonization rate(%)
Taznakt	328,25 ^{d}	82,6^{c}	5,26 ^{e}
Meski	454,16 ^{d}	84,61^{c}	17,07 ^{bc}
Z. Amelkes	966,66 ^{b}	95,45 ^{a}	31,47 ^{a}
Zouala	235,41 ^{d}	81,81^{b}	22,45^{b}
Blaghma 1	931,25 ^{b}	90,47^{b}	35 ^{a}
Blaghma 2	1839,5 ^{a}	100 ^{a}	29,52 ^{ab}
O. Bhar	914,58 ^{b}	82,66 ^{b}	23,66 ^{b}
Maatala	958,33 ^{b}	72,5 ^{d}	21,55 ^{b}
Siffa	768,75 ^{c}	83,71 ^{b}	43^{a}
Jir	720,31^{c}	77,56 ^{cd}	10,95 ^{d}

Table 3. Root colonization levels of sorghum during the two successive trapping cycles

Sites	Root colonization fréquency (%)		Root colonization rate (%)	
	1^{rst} cycle	2nd cycle	1 ^{rst} cycle	2 nd cycle
Taznakt	90,32	90,45	55,87	60,22
Meski	100	100	81,96	85,85
Z. Amelkes	100	100	75,23	81,45
Zouala	96,15	100	71,92	70,92
Blaghma 1	90,62	89,22	52,37	60,75
Blaghma 2	92,85	88,44	67,32	70
O. Bhar	100	94	78,14	80,45
Maatala	46,15	87,14	2	20,44
Siffa	100	100	64	71,85
Jir	92,59	96,50	56,14	70,52

(60%) and centrifuge at 1000 RPM for 2 min. The supernatant containing spores is filtered under vacuum on filter paper (Whatman # 2). The spores are recovered one by one under the dissecting microscope, separated according their morphotype and evaluated for their respective abundance.

AMF trapping from soil samples

Sorghum (*Sorghum vulgare* L.) was used as the trap plant. Seeds were surface sterilized in calcium hypochlorite (1%) for 10 min. and rinsed three times in sterile distilled water. Pot-cultures were established in growth chambers using original soil samples mixed with autoclaved sand 1: 1 (v/v) as the substrate for growth of sorghum to propagate inhabiting AMF. Pot-cultures were grown during two successive cycles of 4 months each under controlled growing conditions of 20-25°C temperature, 50% humidity, and day/light period of 16/8 period. Plants were watered 2-3 times a week and bi-monthly fertilized with Long Ashton's no-P fertilizer. Phosphorus was added only if signs of deficiency symptoms appeared in host leaves (Brundrett et al., 1996). The most abundant AMF spore morphotypes were recovered and used to start monospecific culture on 12 day old sorghum seedlings grown in a sterile mix of sand-perlite (2/3 V : 1/3 V). Watering and fertilization were done similarly as for the AMF trapping.

AMF characterization and identification

Spores with similar morphotypes were isolated from pot culture, and mounted between slide and coverslips in PVLG (Polyvinyl Alcohol - Lactic acid- Glycerin), and in PVLG-Melzer's reagent (1 : 1/ v :v) for the detection of dextrinoid reaction (Koske and Tessier, 1983). Species identification was based on original descriptions, on type specimens when available, and on descriptions provided by International culture collection of (Vesicular) arbuscular mycorrhizal (INVAM) and Dr J. Blaszkowski, websites. The spore morphological characters were also compared with reference material from the National Mycology Herbarium (DAOM). The evaluation of diversity was concerned by the species frequency and the specific richness (total number of species per site).

Statistical analyses

ANOVA 1 analyses were done with the SPSS statistics software. When significant differences were noticed, a mean comparison between studied parameters was done using Student-Newman-Keuls test at 5%. Pearson's correlation coefficients were employed to determine the relationships between mycorrhizal colonization and available P.

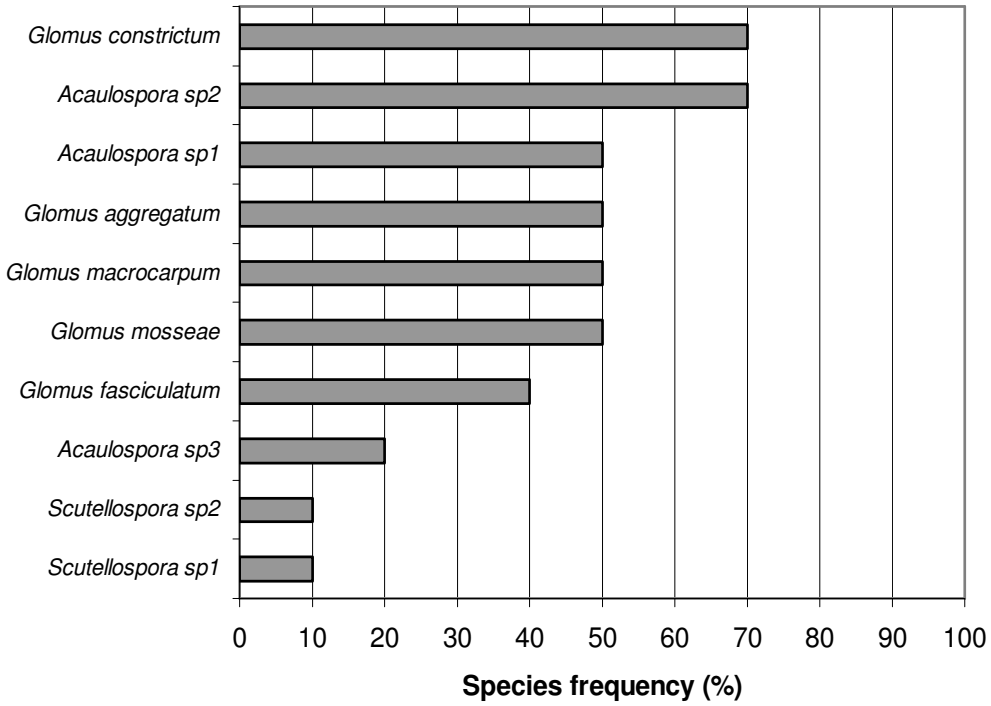

Figure 2. AMF species frequency for the 10 AM fungi detected in successive trap cultures from 10 samplin

RESULTS

Physical and chemical properties of soil

The soil analyses revealed strong alkaline soil (Table 1) low nitrogen concentration below 0.21 mg/kg for N-NH4 and 4.57 mg/kg for N-NO3, extremely low available P concentrations (10-40 µg/g) except for the Taznakt site which harbored a medium concentration of 164.5 µg/g, and a low level of organic matter between 0.37 and 2.38%. As a whole the palm grove rhizosphere is considered poor for a cultivated soil.

Mycorrhizal status and AMF soil diversity

Intraradical hypha, vesicles, and arbuscules were detected in all date palm root samples surveyed. The root colonization pattern clearly belongs to the Arum type. The statistical analyses revealed a sampling site effect for both frequency and intensity of root colonization. Frequency data varied from 72 to 100% and root colonization reached between 5 and 43%. The highest mycorrhizal frequency was observed in the Blaghma 2 site in a relatively low P soil; the maximum root colonization registered was found in the low P, N, and organic matter soil of Siffa. At the opposite extreme the lowest level of root colonization were observed in the two P richest soils of Maatala and Taznakt. As a result, a strong negative correlation was found between the root colonization levels and the available P concentration in soil (r2 = - 0.64). Similar to root colonization, significant differences were observed between sites for spore density parameter (Table 2). At the Blaghma 2 site, spore density reached 238 spores/100 g of soil whereas spore density reached 1840 spores/100 g of soil at the Zouala site (low N and P soil).

Root colonisation levels of sorghum plant

The sorghum root colonization levels varied according to the origin of soil sample. The colonization rates remained high for the two successive cycles of 4 months except with Maatala samples (Table 3). The mean frequency of colonization and root colonization levels remained quite stable during both cycles (90.8 and 95% respectively for frequency; 64.4 and 67.24% respectively for root colonization). With Maatala soil, root colonization was almost absent for the first cycle but reached 20% in the second one. Diversity in AMF associated with palm grove soils.

Diversity of AMF

Ten distinct AMF species were isolated from the ten palm grove sites. The genus Glomus was represented by 5 species: *Glomus mosseae* (Nicol and Gerd.) Gerd.and Trappe, *G. fasciculatum* Gerd. And Trappe emend.

Walker and Koske, *G. constrictum* Trappe, *G. aggregatum* Schenck and Smith emend. Koske, *G. macrocarpum* Tulasne and Tulasne. Three Acaulospora species and two *Scutellospora* species remained unidentified and do not correspond to any described species Glomus constrictum and Acaulospora sp 2 were the most abundant and frequently observed AMF when the two *Scutellospora* species showed up respectively at only one collecting site. Acaulospora sp1, G. macrocarpum, *G. aggregatum* and *G. mosseae* were found in 50% of the sites (Figure 2). Up to seven species shared the same rhizosphere at Zouala, Meski, and Maatala and only four species were found simultaneously at Taznakt and Siffa sites (Figure 1).

DISCUSSION

Sustained detection of AMF structures inside date palm roots harvested from palm groves confirms the arbuscular mycorrhizal status of date palm trees. The uniform distribution of mycorrhizal roots throughout the ten collecting sites together with a relatively constant frequency and root colonization intensity revealed that the arbuscular mycorrhizal association is naturally established and represents the standard in the studied palm groves (Khaliel and Abou-Heilah, 1985; Al-Whaibi and Khaliel, 1994). The negative correlation registered between root colonization levels and the available P concentration (Table 2) confirmed the AMF adaptation to low P soil (Kaushal, 2000 ; Mohammad et al., 2003 ; Smith and Read, 1997) as the mycorrhizal receptivity to the symbiosis (Khaliel and Abou-Heilah, 1985). However, contrary to the positive correlation observed by Mohammad et al. (2003) between the levels of organic matter and AMF spore density, no such correlation was detected in the Siffa low organic matter soil compared to the other collecting sites.

In semi-arid zones, AMF populations usually reached their maximum during the rainy season (Kaushal, 2000) which corresponds in Tafilalet to the period between October and May. The relatively important root colonization levels registered in date palm roots harvested in February were associated to high spore density and reasonable species diversity. The mean spore densities measured in palm grove rhizospheres reached 812 spores /100 g of soil and was found comparable to spore densities registered under similar habitats associated with other hosts, e.g. argan trees (Argania spinosa (L.) Skeels) from south-west Morocco (900 to 2080 spores/100g of soil) (Nouaim, 1994) and Acacia albida Del. in Senegal (775 to 1240 spores/100g of soil) (Diop et al., 1994). The spore density variability between samples were considered minor and may be due to microclimate (Koske, 1987), to physico-chemical and microbiological properties (Anderson et al., 1984 ; Johnson et al., 1991) and also to the sampling season (Gemma et al., 1989).

Of the ten AMF species trapped and isolated from pot-cultures, five belonged to Glomus, three to Acaulospora and two to *Scutellospora*. Glomus species were predominant ranging in frequency from 40 to 70%, a spore population characteristic frequently observed under semi-arid and arid habitats (Dalpé et al., 2000; Kennedy et al., 2002; Muthukumar and Udaiyan, 2002 ; Friberg, 2001 ; Stutz et al., 2000). Because of their dominance under those ecosystems, species of Glomus have been considered as the best adapted genus for habitats subjected to drought and soil salinity stresses (Haas and Menge, 1990; Blaszkowski et al., 2002). The recorded AMF species diversity was found comparable to the one observed with several other arid and semi-arid adapted plant species (Stutz et al., 2000; Duponnois et al., 2001; Muthukumar and Udaiyan, 2002; Mohammad et al., 2003; Tao and Zhiwei, 2005). However, the observed taxonomical diversity in the palm grove soil remains relatively low when compared to the diversity found in other dry habitats such as sand dunes and long-term meadows (Hamel et al.,1994; Dalpé, 1989; Stürmer and Bellei, 1994).

As far as fungal species distribution is concerned, *G. mosseae*, *G. aggregatum*, *G. constrictum* and *G. macrocarpum* have been repetitively detected under semi-arid zones of Africa, America, and India (Stutz et al., 2000 ; Mohammad et al., 2003 ; Muthukumar and Udaiyan, 2002) simultaneously with a variety of Acaulospora and *Scutellospora* species (Duponnois et al., 2001 ; McGee, 1989). Moreover, all Glomus species isolated from the Tafilalet palm grove exhibited a worldwide distribution and has been found associated with a diversity of plant communities.

The two successive trapping of AMF species on sorghum plants resulted in a total of ten fungal species. This probably gives a good representative picture of the major AMF species inhabiting palm grove soils. Considered for long as non-specific with their host plant, it has been gradually demonstrated that AMF possess a certain specificity toward their plant partners which has an influence of the composition and the distribution of plant communities (Harnett and Wilson, 1999). Using different host plants for trapping may have eventually reveal the presence of additional AMF that are not trapped with Sorghum. However, previous trapping investigations with 4 different plant hosts did not changed the AMF diversity of tested soils substantially (Vestberg, 1995). All the AMF species isolated from palm grove soil, with the exception of the two *Scutellospora* species were detected at the first trapping cycle. The *Scutellospora* species only sporulated during the second trapping cycle due probably to spore dormancy, and late root colonization followed by a delay in sporulation. Such sporulation behavior with *Scutellospora* species has been previously observed under pot-culture and under root-organ culture conditions (Dalpé et al., 2005).

Such results highlight the usefulness of the trapping technique for the detection of low or late sporulating species or for slow growing and low viability strains (Stutz and Morton, 1996).

Among the AMF species listed, only the mycorrhizal potential of *G. mosseae* has been tested using date palm seedlings (Khaliel and Abou-Heilah, 1985) and moreover a powerful effect on plant growth was demonstrated. A systematic evaluation of the mycorrhizal potential of all the other AMF strains isolated from palm grove is undertaken. Such data on the AMF strain efficiency will allow for the formulation of a mycorrhizal inoculum capable of improving the mycorrhizal potential of the natural mycorrhizal flora, support plant growth, and counteract, in a sustainable way, the decrease in yield caused by drought, soil salinity, and *Fusarium* wilt.

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