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Monitoring the evolution of the arbuscular mycorrhizal fungi associated with date palm

B. ZOUGARI-ELWEDI [1*], W. ISSAMI [2], A. MSETRA [1], M. SANAA [3], D. YOLANDE [4], A. LOUNES-HAJ SAHRAOUI [5]

[1] Regional Research Center on Oases Agriculture of Degache- Tozeur. Road of Tozeur Km 1, Degache 2260, Tunisia.
[2] Faculty of Mathematical, Physical and Natural Sciences. University of Tunisia El Manar, 2092. EL MANAR 2 Tunisia.
[3] National Institute of Agronomy of Tunisia. Soil Sciences and Environment Laboratory, Tunis Mahrajene, Tunisia, 43, Av. Charles Nicole, 1082.
[4] Eastern Cereal and Oilseed Research Center (ECORC) Ottawa. 960 Carling Avenue, ON K1A 0C6 Canada.
[5] Unité de Chimie Environnementale et Interaction sur le Vivant (UCEIV). Université du littoral Côte d'Opale (ULCO). 50 rue Ferdinand Buisson.62228 CALAIS cedex.

*Corresponding author: zougarib@yahoo.fr

Abstract - In the Djerid palm groves, soils are often poor in mineral nutrients. Therefore, mycorrhizal symbiosis has a beneficial effect on date palm growth.But studies made on this symbiosis remains again very limited and even absent in Tunisia.

In fact, 27date palms divided in to nine palm groves, have been studied for their rhizospheres and we can notice the presence in the roots of different date palm cultivars, four genera mycorrhizal fungi (*Glomus, Acaulospora, Sclerocystis* and *Scutellospora*). The predominant genus identified was *Glomus*.Eight species ofarbuscular mycorrhizal fungihave been recorded in all study sites: *Glomus mosseae*, *Glomus constrictum*, *Glomu stortuosum*, *Glomus irregulare*, *Glomus sp.*, *Acaulospora cavernata*, *Scutellospora calospora* and *Sclerocysti srubiformis.* Their frequency and their rates vary depending on the sporulation sites and seasons.

Seasonal variation of colonization by arbuscular mycorrhizal fungal (AMF) hyphae in all 8species has been characterized in vertical profiles and at different lateral distances from the date palm trunk. We can notice also that, the AMF colonization levels of field date palm roots were found to be according to the seasons. The peaks of colonization observed during autumn could be due to the sporadic rains that can activate fungus colonization and the tendency to associate depression with winter. The most important populations reached their maximum at the aplomb of the date palm crown and mainly 50 cm from the date palm trunk.The different AMF distributions were recorded at 20–40 cm soil depth; they refer to the most common roots of date palm.

Keywords: Mycorrhiza, colonization, symbiosis, season, date palm.

1. Introduction

Arbuscular mycorrhizal fungi (AMF) are common in almost all eco systems and can form associations with over than 80% of higher plants (Leake et al. 2004and Marcel et al.2015).The AMF confers numerous benefits to host plants including improved plant growth and mineral nutrition (Ghazi 2013);it is conceivable to use this symbiosis in favor of the date palm. It thus becomes possible to promote healthier farming systems, reduce the use of chemical inputs (pesticides, fertilizers), and while ensuring the profitability of crops and the quality of the environment in preserving sustainable long-term soil fertility and ecosystem stability (Al-Whaibi and Khaliel 1994 and Carrenho et al. 2007). Mycorrhizae play an important role in the mineral nutrition of many plant species including those that grow in marginal conditions of soil fertility (El Beker 2002).

The date palm is widely cultivated on poor skeletal soils with extreme pH conditions, which in turn affect their nutrient status. Overall, the date palm may not show any visual symptoms of nutrient deficiency.

However, despite the repeated experimental demonstration of the benefits of using mycorrhizae in agriculture, this biotechnology remains badly known and under-utilized, except by the scientific community and a few leading-edge industries.
This work shall consist of highlight the presence and the importance of AMF associated with date palm

2. Materials and Methods

The material used in this workis the AM fungal biomass of the host plant: *Phoenix dactilifera L.*(Date palm).Within the framework of this present study and to verify the presence of mycorrhizal fungi, several experimental sites (Figure 1) were established in the Djerid region of Tunisia (Tozeur, Deguèhe, Nefta, Hazoua and Tamaghza).

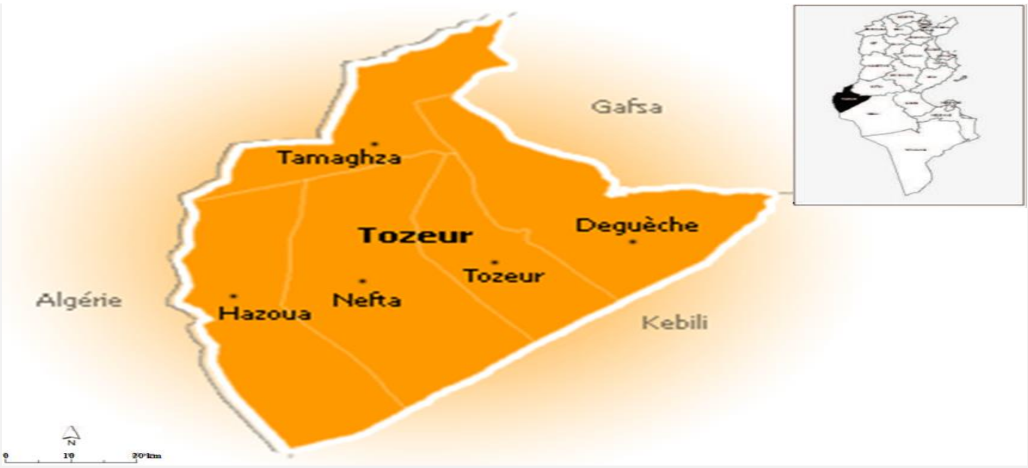

Figure 1 Sampling stations

Soil samplesfor each date palm were collected on site using a hand auger from 3differentdepths;0-20cm, 20-40cm and 40-60cmat different lateral distances from the date palm trunk, to develop and evaluate an effective sampling strategy. At each site, varieties of dates (Deglet Nour, Aligue, Kinta, Hamraya and Halwaya) were taken. For spatial characterization and over time, only 3 sites were considered (Tozeur, Degache and Nefta) over a two-year period. The palm trees selected are grouped to remove as much variability as possible within the same site (irrigation, types of soil, influence of neighboring cultures if they exist).Date palm of uniform age (20-year old), size (4-5 m), palm number (150/date palm) and bunch number (10-12/ date palm); were selected to control the maximum variability factors. Samplings of soil were collected simultaneously.
Samples are taken at four easily identifiable phenological stages:

1) The test starts in different experimental sites at harvest and at the beginning of the rainy season (October).
2) The second sampling date during the winter rest (January).
3) The third sampling date during pollination (April)
4) The fourth sampling will take place at the beginning of the critical period (stage “Bsir”) (July)

Samples of soil and roots are taken on the same dates, on all selected date palms.
The root samples were first cleared and stained before microscopic observation. A staining technique based on methods developed by Kormanik et al. (1980) is used with a slight modification suited to date palm roots which are thick and woody.
A method described by McGonigle et al. (1990) is used to examine roots and quantify AM fungi, at defined intersections.

Statistical analysis

The statistical treatment of results is achieved through the STATISTICA Version 5 software, Beaux et al. (1991). The measures have been an analysis of variance of two factors by Fisher's F test to verify the equality of the means of hypothesis risk threshold of 5%. It is supplemented by multiple comparisons of means by the Newman Keuls test when the equality of averages hypothesis is rejected, according to Robert et al. (1980) and Dagnelie (1986).

3. Results

3.1. Physico-chemical properties of oasis soil

Study on mycorhizal symbiosis with date palm must be accompanied by the determination of the main characteristics of the corresponding soil samples (Table 1). The soils studied at all depths were recovered for analysis (Pauwels et al., 1992) after sieving to 2 mm.

Table 1. Soils properties of date palm groves studied

Soils	Depth (cm)	pH	EC(mmhos/cm)	CEC (meq/100g soil)	$CaCO_3$% TotalActive	Texture Class
palm groves of Tozeur	0 – 20	8.8±0.02	7.4±0.12	5.3	12.7± 1.017.3±0.09	SCL
	20 – 40	8.7±0.05	5.3±0.01	4.5	12.5±1.178.1±0.38	SL
	40 – 60	7.4±0.01	5.2±0.16	4.5	10.1±1.208.0±1.07	SL
palm groves of Degache	0 – 20	8.5±0.03	1.6±0.08	4.9	12.1±0.887.7±0.29	SL
	20 – 40	8.4±0.05	4.8±0.01	4.4	13.1±1.27 8.1±0.48	SL
	40 – 60	7.9±0.08	7.6±0.22	4	12.2±1.30 8.0±0.75	SL
palm groves of Nefta	0 – 20	8.7±0.02	2.2±2.03	5	13.5±0.49 8.5±0.39	SL
	20 – 40	8.4±0.04	5.1±0.46	4.8	11.1±1.228.8±0.66	SL
	40 – 60	8.0±0.00	12.0±1.40	4.5	10.5±1.077.3±0.60	SL

SCL: Sandy Cay Loam,SL: Sandy Loam, EC: electrical conductivity, $CaCO_3$: calcium carbonate

The results show that the soils studied at all depths considered are generally characterized by coarse textures, slightly alkaline to alkaline pH values(7.4 – 8.8),very low cation exchange capacity (CEC) (4.0 – 5.3),the percentage of active limestone is moderately high (7.3 et 8.8%).

Other soil fertility parameters are determined (Table 2)

Table 2. Soil fertility parameters

Soils	Depth (cm)	N (mg kg^{-1})	P (mg kg^{-1})	K meq/100g soil	Ca meq/100g soil	Mg meq/100g soil	Cu (mg kg^{-1})	Zn (mg kg^{-1})	OM (%)
palm groves of Tozeur	0 – 20	0.12±0.01	13.70 ±1.03	0.82 ±0.02	4.83 ±0.09	6.8 ±0.13	0.46±0.01	3.5 ±0.06	0.35 ±0.02
	20 – 40	0.07±0.01	14.74 ±0.20	0.79±0.06	5.13 ±0.17	7.5 ±0.15	0.38 ±0.01	3.9 ±0.03	0.76 ±0.05
	40 – 60	0.03±0.00	16.29± 1.07	0.75± 0.04	5.71 ±0.24	8.3 ±0.21	0.35±0.03	3.1 ±0.07	0.94 ±0.08
palm groves of Degache	0 – 20	0.09±0.01	9.76 ±0.92	0.77 ±0.08	3.21 ±0.18	4.6± 0.17	0.40 ±0.03	2.8 ±0.08	0.60 ±0.13
	20 – 40	0.09± 0.00	11.83 ±0.51	0.82 ±0.10	4.74 ±0.24	3.9 ±0.12	0.42 ±0.01	3.1 ±0.02	0.69 ±0.04
	40 – 60	0.08±0.00	17.22± 0.11	0.86 ±0.04	6.05 ±0.12	6.1 ±0.08	0.36 ±0.00	3.5 ±0.10	0.83 ±0.04
palm groves of Nefta	0 – 20	0.09±0.00	10.89± 1.50	0.83 ±0.03	3.78 ±0.05	6.4 ±0.15	0.48 ±0.02	3.8 ±0.08	0.52 ±0.03
	20 – 40	0.05±0.00	12.28±0.88	0.91 ±0.05	5.23 ±0.27	5.2 ±0.09	0.41 ±0.03	4.0 ±0.12	0.65 ±0.08
	40 – 60	0.08±0.00	14.91±0.09	0.80 ±0.07	5.82 ±0.24	7.2 ±0.06	0.31± 0.04	3.4 ±0.06	0.81 ±0.04

The analysis results shown that, the palm groves are generallycharacterized by notably low concentrations of certain minerals. Therefore the rhizosphere of date palm is considered poor for a cultivated soil

3.2. Demonstration of the presence of arbuscular mycorrhizal fungi in studied oasis soils

Inspired of root dissection and microscopic observationsof all the treated cultivars (Deglet Nour, Alig, Kinta and Hamraya)as well as those of the corresponding soils of the underground part of the date

palm,the presence of endomycorrhizal fungi that live in association with the roots was revealed.
The presence of these endomycorrhizal fungi is illustrated by the root colonization by fungal hyphae (transport fungal structures), arbuscules (branched hyphal structures) and vesicles (lipid storage organs). These mycorrhizal structures were observed under an optical microscope (Figure2).

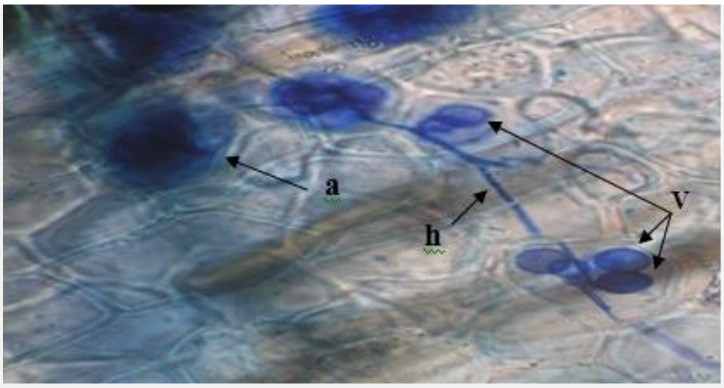

Figure 2. Different mycorrhizal structures in fragments of date palm roots (h: hypha; a: arbuscule and v: vesicle).

Statistical analysis shows that traditional palm groves have mycorrhizal rates (hyphal. arbuscular and vesicular) higher than recent palm groves (Figure3).

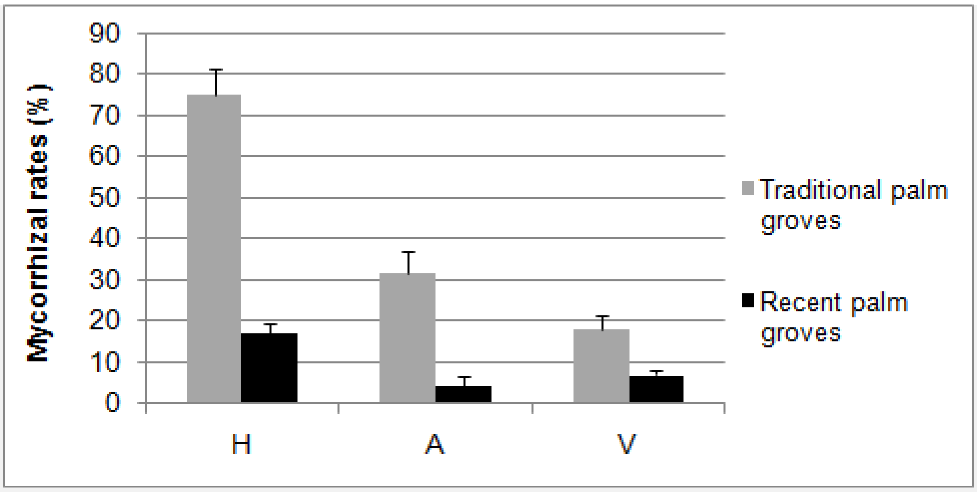

Figure 3 Effect of types of palm groves on mycorrhizal rates (hyphal (H). arbuscular (A) and vesicular (V)) of date palm.

3.3. The horizontal distribution of mycorrhizae

Mycorrhization was observed directly below the crown of the palm tree and the populations of the most important mycorrhizal fungi are located 50 cm from the trunk (Figure4).Beyond 1m from the trunk, population of arbuscular mycorrhizal fungi in soils decrease related to lower feeder roots of date palm (Figure 4).

Figure 4 The root system of date palm cultivated in the oasis of Tozeur (Djerid region)

3.4. The vertical distribution of mycorrhizae

Mycorrhizal fungi are aerobic. Their adaptation to extreme temperatures; to pH and osmotic pressure of the medium is remarkable.For this distribution deep into the palm grove is found in the soil levels where the rootlets are the most abundant.This depth is between 20 and 40 cm (Figure 4).

The second level of sampling (20-40 cm) is the most recommended for the root removalbecause it is considered the most favorable area for the development of arbuscular mycorrhizal fungi.

3.5. Seasonal evolution of arbuscular mycorrhizal colonization in date palm

This study show that colonization of date palm root by arbuscular mycorrhizal fungi is affected by the changing of seasons with a corresponding peak in the fall and depression during the winter (Figure 5)

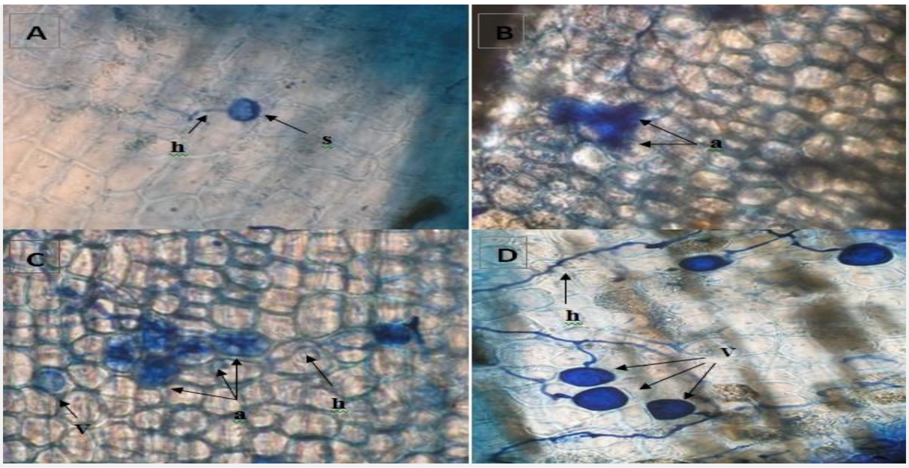

Figure 5. Seasonal evolution of arbuscular mycorrhizal colonization in date palm roots.(A: winter; B: Spring; C: Summer and D: Autumn; h: hyphea; s: spore; a: arbuscule and v: vesicle)

Microscopic examination of date palm roots stained with trypan blue showed that in winter, the mycelial activity is low or absent.All that remains true place of mycorrhizal fungi survival (Figure5A).
When the spring soil temperature increases, arbuscular mycorrhizal hyphae colonize roots and penetrate the inner cortical cells, where they form arbuscules (Figure5B).
In summer, the mycelial activity is important. The mycorrhizal fungus grows in soil as filaments; it develops its potential of mycelium. Furthermore, we note the presence of arbuscular, hyphae and a few vesicles in date palm roots (Figure5C).
At the beginning of autumn, the number of vesicles increase and the mycelial concentration process is initiated; this is related to the arrival of the first rains that caused a heat shock (Figure5D).
A few days after the heat shock,the mycelium is concentrated in the ground and having almost disappearedonly remain mycorrhizae and the cycle repeats.

3.6. Evaluation of arbuscular mycorrhizal fungal colonization

The chosen date palms roots have mycorrhizal rates (hyphal. arbuscular and vesicular)low or none in winter. A significant increase of mycorrhizal hyphal rate approximately 1.2 times was observed going from spring to summer and summer to autumn (Figure 6).

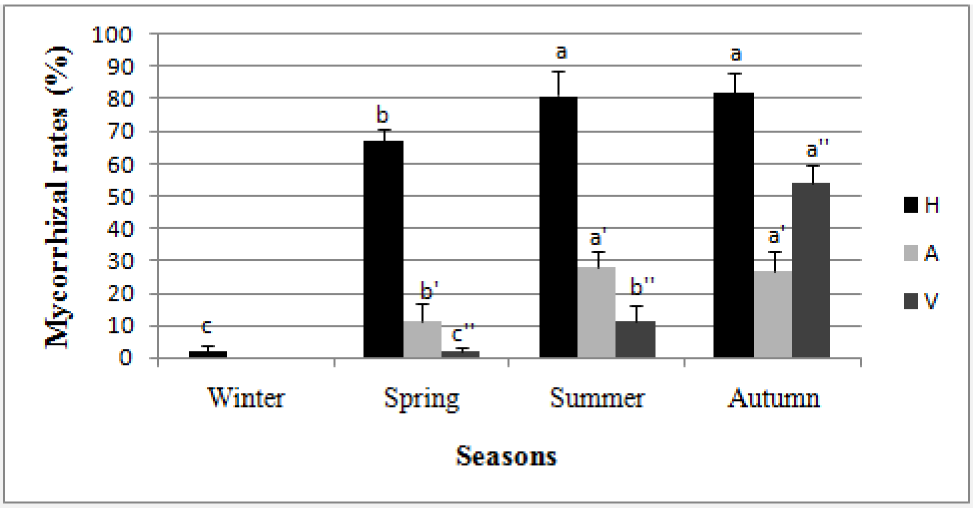

Figure 6. Effects of seasons on mycorrhization rate hyphal (■) arbuscular (■) and vesicular (■)in date palm roots.Bars with different letters represent statistical significant differences mycorrhization rate hyphal (a. b. c); mycorrhization rate arbuscular (a'. b'. c') and mycorrhization rate vesicular (a". b". c") according to Newman Keuls test($P \leq 0.05$).

The same result was observed for the mycorrhizal vesicles rate. Law increases of 5 and 4.9 times were recorded from spring to summer and summer to autumn.
The highest percentage of arbuscules was observed in summer (Figure6).
Root colonization of date palm was also evaluated using the following parameters; the average frequencies (F) and intensities of colonization (I) (Figure 7).

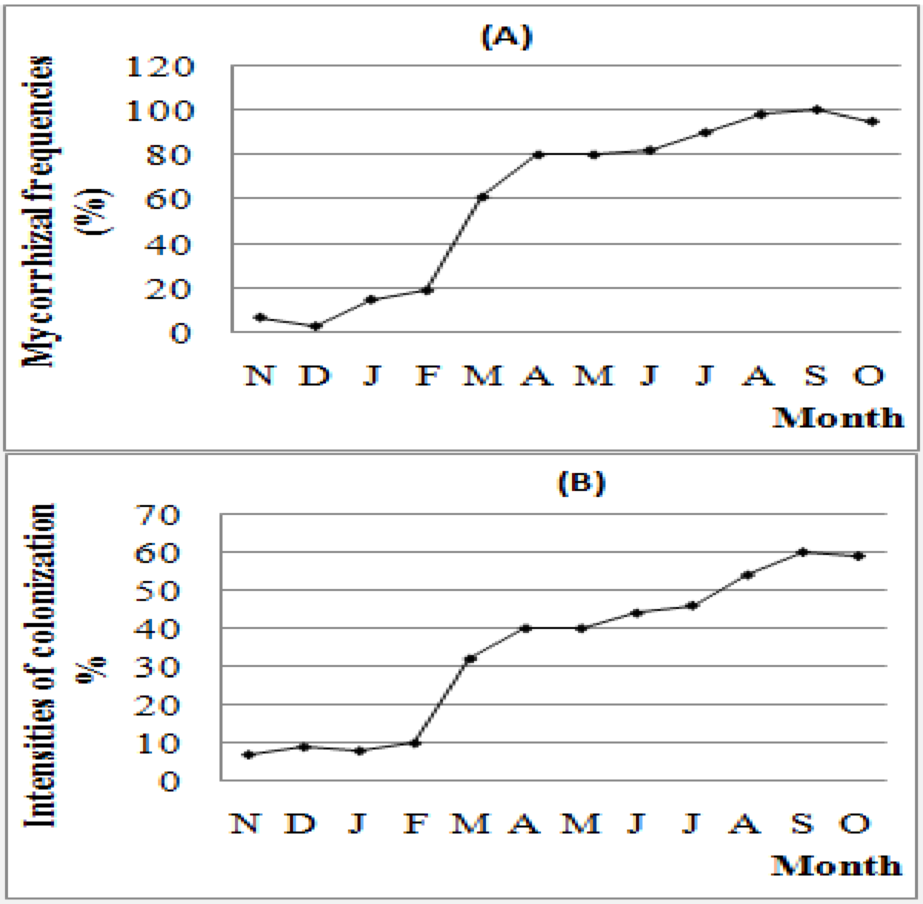

Figure 7 Monitoring of average mycorrhizal frequencies (A) and intensities (B) of date palm roots over time

The results shown in details in figure 7 also verified the effect of the sampling time on the frequency and intensity of root colonization. They believe in a remarkable way from winter to autumn.

3.7. The frequency of spores

The frequency of spores is given by the average of the nine samples taken from each palm grove.The values variy with the seasons (Figure 8).

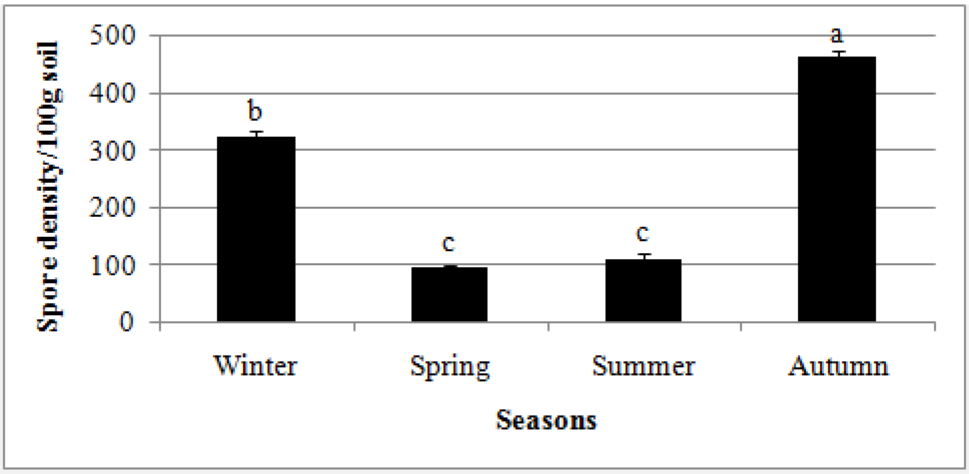

Figure 8 Impact of seasons on spore density of AM fungi in the rhizosphere of date palm in the sites studied. Bars with different letters represent statistical significant differences (a, b and c) according to Newman Keuls test ($P \leq 0.05$).

Analysis of arbuscular mycorrhizal fungal (AMF) spore communities found in the rhizosphere of date palm showed their frequency of occurrence vary depending on the season.
In Djerid region, mycorrhizal spore counts steadily increased from spring reaching a maximum value (460 spores/100g of soil) in autumn (rainy season). After November, spore abundance decreased in

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winter (320 spores/100g of soil). Spore counts were higher in autumn and winter than in spring and summer season. Thus, maximum abundance of AM fungi spores was recorded during the rainy season. Extraction of spores from soil shows that there is a wide variety of mycorrhizal fungi species.
All morphological characteristics of spores described by Giovannetti and Gianinazzi-Pearson (1994) and Dalpe(1995)play an important role in the identification, description and classification of new arbuscular mycorrhizal fungal species.
The collected spores are identified and classified into 4 mycorrhizal fungal genera (*Glomus, Acaulospora, sclerocystis* and *Scutellospora*).Among them, Glomus was the dominant genera.8 species belonging to 4 genera were found at all sites studied:*Glomus mosseae, Glomus constrictum, Glomus tortuosum, Glomus irregular, Glomus sp., Acaulospora cavernat, Scutellospora calospora and Sclerocystis rubiformis* (Figure 9).

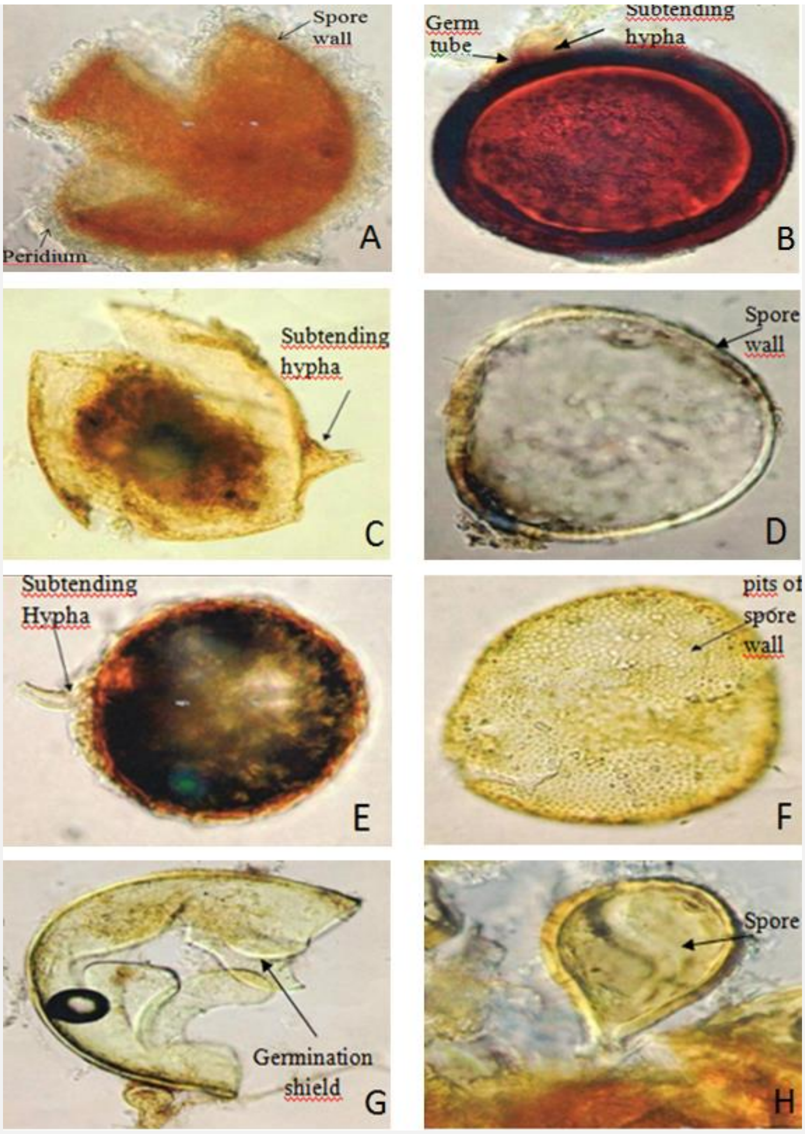

Figure 9 Diversity of arbuscular mycorrhizal fungal (AMF) spore in the rhizosphere of date palm in the sites studied: *Glomus tortuosum* **(A),** *Glomus constrictum* **(B),** *Glomus mosseae***(C),** *Glomus irregular* **(D),***Glomus sp.* **(E),***Acaulospora cavernata* **(F),***Scutellospora calospora* **(G)** and *Sclerocystis rubiformis* **(H).**

4. Discussion

A study on the presence of different mycorrhizal structures in the rhizosphere soil collected from the studied sites confirmed the mycorrhizal status of date palm, considered as a mycothrophic species (Bouamri et al. 2006). The roots of date palm were receptive to arbuscular mycorrhizal fungi (Khaliel and Abu Heilah 1985 and Oihabi 1991). The mycorrhizal frequency of date palm roots showing more than 60% and capable of growing in arid climates with poor sandy soil.

This study has highlighted the AMF at the rhizosphere of date palm oasis in the region of Djerid. The traditional palm groves have mycorrhizal rates (hyphal, arbuscular and vesicular) higher than recent palm groves. This result may be related to recent operating modes based on monoculture palm plantations that reduce the microbial communities in a soil (Lin et al. 2015).

Indeed, some of these microorganisms secrete compounds capable of increasing the permeability of the cell membrane (Perroto et al. 1994 and Wyss et al. 1992),these compounds exceed the cell membrane of roots and increase exudates production,t hus stimulating growth and penetration hyphae enter the root cortex (Bécard et al. 1992).

The medium mycorrhizal intensity and frequency obtained seem similar to those recorded by Bouamri et al. (2006).

Our results indicate that mycorrhization of date palm is affected by operating modes of palm plantation and it is also affected by abiotic factors such as temperature and humidity. This report is also found by Bansal et al.(2012) and Ellouz (2011)on several fruit trees.

A study on mycorrhizal structures show that colonization of date palm root by arbuscular mycorrhizal fungi is affected by the changing of seasons with a corresponding peak in the fall and depression during the winter. Controvert, the result appears to contradict those of Lugo et al. (2003) and Sharma et al. (2005) because they have studied mycorrhizal fungi in conditions where summer is the rainy season, while autumn and winter are dry with occasional rain in spring. According to the results of their direct comparisonone will talk about rainy season and dry season instead of talking about winter and summer Several hypotheses have been put forward to explain the reduction in mycorrhizal population in the winter months. This might be due to reduced translocation of carbohydrates towards roots (Sharma et al. 2005). Sanon (2009) thinks that this dropping in population is related to the reduction of the availability of feeder roots required for normal penetration and development of hyphae. However, Satyanarayana and Johri (2005) think that the reduction in mycorrhizal population caused by unavoidable invasion of hyphae into non-host plant roots due to intercropping with host plants. It may take even months with mycorrhizal host to rebuild the reduced mycorrhizal population to the original level. Further, some plants do not become colonized by AM fungi and therefore will depress populations of these fungi.

Experimental observation of the root systems of several date palm cultivars harvested in the Djerid region showed higher mycorrhizal ability (100% of traditional palm groves).The intensity of root colonization by mycorrhizal fungi was higher in autumn than in winter.

The collected spores are identified and classified into four mycorrhizal fungal genera (*Glomus*, *Acaulospora, sclerocystis* and *Scutellospora*).Among them, Glomus was the dominant genera.

Note also that the second sampling level (20-40cm) is the most recommended for the root removal because it is considered the most favorable area for the development of arbuscular mycorrhizal fungi associated with date palm. The populations of the most important mycorrhizal fungi are located 50 cm from the trunk. Indeed the date palm root colonization rate is based on the spatial variability in density of arbuscular mycorrhizal fungi. The frequency of occurrence of these fungi and their sporulation rates vary depending on the sites and seasons.

Further long-term studies are necessary to elucidate optimal conditions of this mycorrhizal symbiosis for the benefit of date palms.

5. References

Al-Whaibi MH, Khaliel AS (1994) The effect of Mg and Ca, K and P content of date palm seedlings under mycorrhizal and nonmycorrhizal conditions. Mycoscience; 35: 213-217. Doi: 10.1007/BF02268440

Bansal M, Kukreja K, Dudeja SS (2012) Diversity of arbuscular mycorhizal fungi, prevalent in rhizosphere of different crops grown in the university farm. African journal of microbiology Research vol. 6(21): 4557-4566. DOI: 10.5897/AJMR12.222

Beaux MF, Gouet H, Gouet JP, Morghem P, Philippeau G, Tranchefort J, Verneau M (1991). Manuel d'utilisation du Logiciel STAT-ITCF.1st ed. Paris. Imprimerie Institut Technique des Céréales et des Fourrages

Becard G, Douds DD, Pfeffer PE (1992) Extensive in vitro hyphal growth of vesicular-arbuscular mycorrhizal fungi in presence of CO_2 and flavonols. *Appl Environ Microbiol* 58*:*821–825. Doi: 0099-2240/92/030821-05$02.00/0

Bouamri R, Dalpé Y, Serrhini MN, Bennani A (2006) Arbuscular mycorrhizal fungi species associated with rhizosphere of *Phoenix dactylifera* L. in Morocco. African Journal of Biotechnology. Vol. 5 (6):510-516. doi: 10.5897/AJMR2015.7472

Carrenho R, Trufem SFB, Bononi VLR, Silva ES (2007) The effect of different soil properties on arbuscular mycorrhizal colonization of peanuts, sorghum and maize. Acta Bot Bras21:723–730. doi : 10.1590/S0102-33062007000300018

Dagnelie P (1986) Théorie et méthodes statistiques. 2nd ed. Gembloux, Belgique :Presses agronomiques

Dalpé Y (1995). Systématique des endomycorhizes à arbuscules. De la mycopaléontologie à la biochimie. Ontario, Ottawa : Orbis Press.

El Beker A (2002) La fertilisation. In : El Beker A (ed) Le palmier dattier: Son passé, son présent et les nouveautés dans la culture. 2 nd ed. El achour, Alger, pp 178-190.

Ellouz O (2011) Diversité des champignons endophytes mycorhiziens et de classe II chez le pois chiche et influence du génotype de la plante. PhD, Université de Montréal, Canada.

Ghazi N Al-Karaki (2013) Application of mycorrhizae in sustainable date palm cultivation. Emir. J. Food Agric. 25 (11): 854-862. Doi:10.9755/ejfa.v25i11.16499

Giovannetti M, Gianinazzi-Pearson V (1994) Biodiversity in arbuscular mycorrhizal fungi. Mycol. Res. 98: 705-715. doi: 10.1016/S0953-7562(09)81041-1

Khaliel AS, Abou Heilah AN (1985) Formation of vesicular mycorrhizae in phoenix dactylifera L. cultivated in Qassim region; Saudi Arabia. Pakistan J. Bot. 17:267-270.

Kormanik PP, Bryan WC and Schultz RC (1980) Procedures and equipment for staining large of plant roots for endomycorrhizal assay. Can. J. Microbiol.26: 536-538

Leake JR, Johnson D, Donnelly DP, Muckle GE, Boddy L, Read DJ (2004) Networks of power and influence: the role of mycorrhizal mycelium in controlling plant communities and agroecosystem functioning. Can J Bot. 82: 1016–1045. doi. org/10.1139/b04-060

Lin S, Huangpu JJ, Chen T, Wu LK, Zhang ZY, Lin WX (2015) Analysis of soil microbial community structure and enzyme activities associated with negative effects of *pseudostellaria heterophylla* consecutive monoculture on yield. Pak. J. Bot., 47(2): 761-769

Lugo MA, Maza EGM, Cabello MN (2003) Arbuscular mycorrhizal fungi in a mountain grassland II: Seasonal variation of colonization studied, along with its relation to grazing and metabolic host type *Mycologia.*vol. 95 (3): 407-415. doi: 10.1111/j.1574-6941.2012.01373.x.

Marcel GA, Francis MM, Marc-André S, Ian RS(2015). Mycorrhizal ecology and evolution: the past, the present, and the future. New phytologist.205:1406-1423. doi: 10.1111/nph.13288

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol. 115:495-501. doi : 10.1111/j.1469-8137.1990.

Oihabi A (1991) Étude de l'influence des endomycorhizes à vésicules et arbuscules sur le Bayoud et la nutrition du palmier dattier. PhD, University Cadi Ayyed, Marrakech, Maroc.

Pauwels JM, Van Ranst E, Verloo M, Mvondo ZA (1992) Méthodes d'analyses d'éléments majeurs dans la plante. Manuel de laboratoire de pédologie : Méthodes d'analyses des sols et des plantes. Equipement, gestion des stocks de verrerie et produits chimiques. 1st ed. AGCD, Dschang-Bruxelles. Publications agricoles

Perotto S, Brewin NJ, Bonfante P (1994) Colonization of pea roots by the mycorrhizal fungus Glomus versiforme and Rhizobium bacteria: Immunological comparison using monoclonal antibodies as probes for plant cell surface components. Mol Plant-Microbe Interact 7:91–98. doi: 10.1094/MPMI-7-0091

Robert G, Steel D, Hiram J (1980) Principles and procedures of statistics a biometrical approach.3rd ed New York, NY, USA : Cambridge University Press.

Sanon A (2009) Le concept de niche écologique associé à la co-existence des espèces végétales: mise en évidence du rôle de la symbiose mycorhizienne et de sa microflore associée dans la structuration de la strate herbacée en milieu tropical. PhD, Henri Poincaré University, Nancy, Paris.

Satyanarayana T, Johri BN (2005) Microbial diversity: Current perspectives and potential applications. 1 st ed. New Delhi, India: International Publishing House.

Sharma S, Aggarwal A, Parkash V, Mehrotra RS (2005) Seasonal population dynamics of vesicular arbuscular mycorrhizal fungi associated with Tectona grandis L. and Dendrocalamus strictus (Roxb.) Nees. Indian Phytopath. 58 (2): 163-166.

Wyss M, Smeitink J, Wevers RA, Wallimann T (1992) Mitochondrial creatine kinase: a key enzyme of aerobic energy metabolism. Biochim. Biophys. Acta1102: 119-166.doi: 10.1016/0167-4838(92)90506-9.