Plant Archives
Journal home page: www.plantarchives.org
DOI Url: https://doi.org/10.51470/PLANTARCHIVES.2021.v21.no1.201

ISOLATION AND CHARACTERIZATION OF ARBUSCULAR MYCORRHIZAL FUNGI SPORES FROM DATE PALM RHIZOSPHERE IN AN ARID REGION

Oumaima Harkousse[1,2], Aitboulahsen Mohamed[3], MazriMouaad Amine[4], Lahcen Ouahmane[5*], Tayeb Koussa[2] and Mohamed Najib Al Feddy[1]

[1]National Institute of Agronomic Research, Plant Protection Unit, Laboratory of Phyto-Bacteriology, Marrakech, Morocco
[2]Laboratory of plant biotechnology, ecology and ecosystem exploitation, Department of biology, faculty of sciences, El Jadida, Morocco
[3]Department of Biology, Polydisciplinary Faculty of Larache, Abdelmalek Essaâdi University, B.P 745, Larache, Morocco
[4]National Institute of Agronomic Research, UR Agro-Biotechnology, Laboratory of Plant Biotechnology, Marrakech, Morocco
[5]Cadi Ayyad University, Laboratory of Microbial Biotechnologies, Agro-sciences and Environment, Marrakesh, Morocco

(Date of Receiving-03-02-2021; Date of Acceptance17-04-2021)

ABSTRACT

The arid lands are the most disturbed and degraded, mainly because of the wind, water shortage, and salinization. With the loss of vegetation and loss of productive surface soil as primary factors. In these areas, the protection and regeneration of degraded lands are critical for sustainable agriculture, and dryland ecosystem's enhancement. Mycorrhizal fungi take up a substantial portion of soil rhizosphere microflora and play an essential role in plant development. In this context, a study of mycorrhizal spores isolated from the date palm rhizosphere located in the Zagora palm groves (Moroccan desert) was conducted. The collected soil samples were analyzed for chemical and physical properties and spores were separated from the soil and examined. Root colonization, spore density, abundance, and morphological studies, were the considered parameters. The mean frequencies and intensities of colonization were 98% and 73% respectively. The maximum spore density was at 34,165 spores / 20 g of soil, and the lowest was at 22,015 spores / 20g according to the site. Twenty-eight arbuscular mycorrhizal fungi species belonging to seven genera were identified in all the sites studied. These endomycorrhizal were significantly similar in the four different soil samples. The "Zagora" inoculum contains a mixture of the mycorrhizal spores previously mentioned.

Keywords: arid, mycorrhizal fungi, spores, rhizosphere, *Phoenix dactylifera*

INTRODUCTION

Arid regions represent nearly 40% of the earth's land area and endorse two billion people, 90% of whom live in developing countries. Dryland populations are generally among the world's poorest countries, with many surviving with much less than one US dollar per day (UN 2020; White *et al.,* 2002). These areas receive extremely low total precipitations in the form of rainfall or snow. The African Sahel, Australia Outback, South American Patagonia, and North American Great Plains constitute the widely known drylands (White and Nackoney, 2003).

Regardless of their severe conditions, these lands are considered remarkable ecosystems with a wide range of plant diversity. Dryland species must adapt to harsh climate conditions, including extreme temperatures and water shortage. Some areas were identified as particularly valuable for the survival of these distinctively adapted plants. The conservation status of this biodiversity is being collected in some locations but is still not available for many sites (White *et al.,* 2002). Among the drylands of the world, those in the western Mediterranean are highly susceptible to abiotic and biotic stress because of the climate variability, the constant drought, and demographic disequilibrium (Kouba *et al.,* 2018).

Located in southeastern Morocco, Zagora Province is characterized by its desert environment as well as its culture, people, and rich biodiversity (Lahmidi *et al.,* 2020). In the drylands of the Maghreb, over one-quarter of the population derives their livelihood directly from the land-based activities of mixed farming and cattle breeding. Among the cultures best adapted to a hot and severe climate, Date palm (*Phoenix dactylifera L.*) has long been the primary fruit crop in the semi-arid regions of southern Morocco (Carr, 2013). These trees can resist heat and severe environmental stresses, but it is in constant need of water source. It is said in Arabic that palm trees must have their feet in the water and its head in the fire of the sky (Carr, 2013). The root system of this tree is very dense, fasciculate, and fibrous; wish allows the development of a diverse rhizosphere. If there is a near water source, it can reach with its roots. Thus, surviving and forming a well diverse ecosystem near it granting the development of other plants in such extreme conditions.

The soil surrounding the plant's roots is an underground mystery filled with life. For years now, and since Hiltner used the term rhizosphere for the first time in 1904, scientists tried first to identify the components of this sphere, and then tried to make good use of it. And then the focus of using the beneficial proprieties of the soil microorganisms started to make an echo in sustainable

agriculture since the addition of these microorganisms to the plants is purely organic and without chemical interference (Brink, 2016).

Mycorrhizal is a term introduced for the first time in 1885 by the botanist Albert Bernhard Frank (Trappe, 2005), which represents a beneficial fungal-plant relationship influencing the physiological characteristics of many crops, including food production and quality. Most plant roots form a symbiotic relationship with mycorrhizal fungi and their coexistence benefit generally both the fungi and the host plant. Mycorrhizae fungi can colonize the roots from three main sources of inoculum in the soil: spores, infected root fragments, and hyphae. The large spores with strong resistant walls and multiple nuclei are structures of long-term survival with some potential for wind and water dispersal (Koske, 1982; Friese and Allen, 1991). Outside the roots, the fungiform extra-radical hyphae that penetrate the soil and form a wide-reaching network referred to it by the AM hyphal network (Selosse *et al.,* 2006). Hyphae can be rather thin and thick, forming a branched absorbing structure. Water and minerals are more proficiently take in by this hyphal network than by the plant roots. It can stretch from the roots permitting the host plant to explore a large amount of soil and thus reducing the side effects imposed by the slow dispersion of inorganic phosphate (Pi) in the soil (Schachtman *et al.,* 1998)Reid, and Ayling 1998. As for the Arbuscular mycorrhizal fungi (AMF) spores, they are formed both outside and inside the roots (Drew *et al.,* 2006). It is known that AMF inoculation can substantially increase the concentration of different macro-nutrients and micro-nutrients, resulting in higher production of photosynthesis and thus increased accumulation of biomass (Chen *et al.,* 2017; Mitra *et al.,* 2019). AMF's can boost inorganic nutrient absorption in almost all plants, particularly phosphate (Smith and Read, 2009). In addition to macronutrients, AMF has been documented to increase the phyto-availability of micronutrients such as zinc and copper (Smith and Read, 2009).

It has been reported in serval studies, that AMF's help agricultural plants to increase their tolerance and adaptation to abiotic stresses (Shahane *et al.,* 2020; Belimov *et al.,* 2020; Li *et al., 2020; Nanjundappa et al., 2019)*New Delhi in the winter seasons of 2013–14 and 2014–15. The objective of the study was to evaluate the significance of three crop establishment methods viz., conventional drill sown wheat, the system of wheat intensifica tion, and zero tillage wheat and rates of nitrogen, phosphorus (P. The complex and dynamic interactions between these microorganisms and plant roots under harsh environments disturb not only the plants but also the structural, physical, and chemical properties of soil (Ech-Cheddadi *et al.,* 2019). The application of microorganisms in agriculture opens a new chapter for abiotic stress control in plants. Several AMF species may play a significant role in explaining the stress tolerance of plants, adaptation to stress, and mechanisms that develop under stress conditions in plants. For a better understanding of these mechanisms, the AMF population in the rhizosphere must be identified.

Despite the importance and wide occurrence of AMF's, the description and identification of indigenous AM fungi species that are present in the Zagora rhizosphere are very limited. Based on the current literature published so far and to our knowledge, only a few findings about the AMF diversity associated with date palm in the desert soil of Zagora. Hence, this study aims to isolate and characterize the native mycorrhizal fungi spore population from date palm rhizosphere planted in the Zagora experimental domain, Morocco (Regional Center for Agronomic Research of Errachidia).

MATERIALS AND METHODS

Study site

Four date palm groves were subjected in this study; all situated in the same location where the climate and soil conditions were nearly the same across all palm groves. The groves are located in the experimental domains of l'INRA (Regional Centre for Agronomic Research of Errachidia, Experimental Domain of Zagora), in the Drâa-Tafilalet region of southeastern Morocco (30°19′50″N 5°50′17″W). The climate in this region is known as a hot desert climate by Köppen climate classification (Köppen, 2010).

Root and soil sampling

Soil samples containing root fragments were taken near the rhizosphere of date palm trees in Zagora, at a depth of approximately 10cm to 40cm, with five samples for each type of rhizospheric soil (INVAM, 2020).

Trap culture

The trap cultures are prepared as follows, the plant debris and shoots are removed from the soil samples collected, and the roots fragments are cut into small fragments and mixed with the associated soil. After that, the blend is mixed 1:1 (v/v) with autoclaved soil and then transferred into plastic pots with barley seeds (80-100 seeds/pot). Barley is used as a host plant for the trapping cultures, the pots are grown in a greenhouse for at least four months before being used(INVAM, 2020).

Spores extraction

The procedure detailed here focuses on the extraction of spores from soil samples and greenhouse-grown pot cultures. Following two steps the wet sieving method described by Gerdemann and Nicolson (1963) and sucrose gradient techniques (Brundrett *et al.,* 1996). The supernatant containing spores is filtered under vacuum

on filter paper (Whatman # 2). The spores are recovered one by one under a stereoscope, to be identified and to calculate their abundance and density.

Spores abundance and density

The ecological diversity indexes for the description of mycorrhizal community structure and concentration are spore abundance and density. The spore's abundance can be estimated by direct counting of spore's numbers present in the soil (Gerdemann and Nicolson, 1963; Brundrett *et al.,* 1996).

After extraction and direct counting of the spores from soil and from trapping culture, results of the spores abundance and density in soil samples were calculated, according to equation 1 and 2:

$$\text{Abondance} = {}^{(Mi + \text{Mî})}/_{2}$$

(Spores) (eq.1)

Were Mi is the number of spores from soil samples and Mî is the number of spores from trapping culture

$$\text{Density} = \frac{Abondance}{soilquantity}$$

(Spores/g of soil) (eq.2)

Morphological identification of mycorrhizal spores

The spore's morphological characters (color, shape, hypha of attachment, and consistency) were determined under a stereomicroscope (Olympus SZ H10 research Stereomicroscope, China). Spore wall structures and other attributes were observed on permanent slides prepared according to the methods on the West Virginia University International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi(WVU-INVAM, 2020) site, explaining the procedure to mount spores on slides based on the application of one drop of PVLG and PVLG + Melzer reagent. All the morphological features are compared to original descriptions of the species database provided on the Website of the INVAM (http://fungi.invam.wvu.edu/the-fungi/species-descriptions.html).

Physical and chemical analysis of soil samples

Soil texture analysis

Soil texture is an important parameter to be identified as it affects physical, chemical, and biological processes in the soil environment (Ritchey and McGrath 2015). It was studied by applying the 'Feel Method' as detailed by Ritchey and McGrath (2015).

Chemical analysis

All the soil's chemical analyses were made following the book of physical and chemical methods in soil analyses by Sarkar and Haldar (2005). All calculations were made based on oven-dry soil weight. A saturated soil paste was prepared to more closely mimic the water content of the soil under field conditions. The saturation percentage was determined by drying the paste in an oven at 105 °C to constant weight. The soil pH was measured as described by Sarkar and Haldar (2005). The electrical conductivity (EC in dS m^{-1}) of the saturation extract was measured using a conductivity meter (Benchtop meters for conductivity measurements – the inoLab® series) after calibrating the instrument. The total nitrogen was determined by modified Kjeldah's method(Sarkar and Haldar, 2005). The total content of the elemental phosphorus in soils can be extracted and determined by per-chloric acid digestion followed by spectrophotometric determination (Sarkar and Haldar, 2005)

Mycorrhizal inoculum multiplication

The AMF inoculation was stored in the form of barley root segments (*Hordeum vulgare L.)* infected by the above AM fungi complex from the morphological identification. The barley plants were placed in plastic pots dimension: 13×09 cm with various fungi spores to be tested, regularly irrigated with distilled water. The parameters of barley root infection were calculated after 4 months of culture using the technique stated by Trouvelot *et al.,*(1986). The barley roots were then disinfected for 10 min, rinsed with sterile distilled water three times for 10 min, and cut into 1–2 mm long fragments to be used as an inoculum based on the Strullu *et al.,* (1986) method.

Determination of root mycorrhizal colonization

A part of the roots from the lateral root system is properly rinsed from soil remains and then cleaned using 10% KOH at 90 °C for 30 min, and acidified afterward with 1% HCl for 10 min and finely stained with Trypan blue at 90 °C for 20 min as described by Phillips and Hayman (1970). The evaluation of the mycorrhization parameters was conducted for 30 root fragments of 1.0 cm in length using a *Zeiss Axioskop* 40 microscope at 40–100× magnification, according to Trouvelot *et al.,* (1986). Two mycorrhizal parameters the AMF infection frequency (Fa %) and the AMF infection intensity (Ma %) was calculated, according to equation 3 and 4:

$$\text{AMF infection frequency (Fa \%)} = \frac{\text{Infected root segments}}{\text{Total root segments}} \times 100$$

(eq.3)

$$\text{AMF infection intensity (Ma\%)} = \frac{(95n5 + 70n4 + 30n3 + 5n2 + n1)}{\text{Total root segments}}$$

(eq.4)

With

n5= the number of roots with infection level of 5 (infection

rate 90–100%).

n4= the number of roots at level 4 (infection rate 50–90%).

n3= the number of roots at level 3 (infection rate at 10–50%).

n2= the number of roots at infection level 2 (infection rate 1–10%).

n1= the number of roots at level 1 (infection rate 0–1%).

Statistical analysis

All measurements were done in five repetitions (n=5). Data analysis was performed on a one-way variance analysis (ANOVA) backed by Tukey's post hoc tests to evaluate significant differences among the samples, at a 95% confidence interval using the statistical software IBM SPSS, version 20. 0. The significance level was $p \leq 0.05$.

The correlation relationship between the soil pH, total P, organic matter, abundance, and density was studied to analyze the effect of soil characteristics on the AM fungi. The data collected were subjected to Pearson's correlation analysis using the same software.

RESULTS

Physical and chemical analysis of soil samples

In this study, the rhizosphere soil was analyzed for the physicochemical properties, as it will exert significant impacts on the dispersion and abundance of AM fungi (Reddy *et al.,* 2007). The soil texture results based on the "Feel Method" of four sample studies were regrouped in Table 1. The "Feel Method" was described by Thien (1979). From the results shown in Table 1, there are four different soil textures obtained.

The measured characteristics (physical and chemical of soil), were slightly variable. The soil pH differed significantly from one soil sample to another, as well as the organic matter percentage, the first two first soil samples showed higher organic matter percentage than the two last ones (Table2). As for the electric conductivity (EC), the total Azote (N) and total phosphorus (P) values did not show a significant high variation between the four soil samples.

The correlation between the soil pH, total P, organic matter, abundance, and densitywas identified and represented in Table 3. The r-value +0.997 indicated a positive and strong correlation between soil pH and the spore abundance and density in the soil rhizosphere ($p<0.01$). The same correlation was between soil Organic matter and the spore abundance and density with r-value +0.968. Meanwhile, the r-value -0.419 of soil total P showed a negative correlation with the spore abundance and density but not as strongly significant as the previous ones.

Table 1: Soil texture analysis based on the "feel method"

Soil sample	Forming of a ball	Forming of ribbon	Soil feel			Type of soil
			Gritty	Equally	Smooth	
1	yes	Yes	yes	No	No	Sandy loam
2	yes	Yes	yes	No	No	Sandy clay loam
3	yes	No	yes	No	No	Loamy sand
4	no	No	yes	No	No	Sand

Table 2: Chemical analysis of soil samples

Soil Samples	Soil pH	EC (mmhos/cm)	Total N (%)	Total P (mg kg−1)	Organic Matter (%)
1	8,401±0.001a	0,9848±0.004a	14,416±0.008a	286,6±1.00a	2,686±0.005a
2	8,358±0.008b	0,9734±0.001b	14,044±0.011b	288,2±0.577bc	2,558±0.013b
3	8,314±0.011c	0,91±0.01c	14,022±0.019a	290,4±0,547ab	1,642±0.008c
4	8,29±0.007d	0,3624±0.005c	14,0156±0.015a	291,6±1,14c	1,58±0.007d

Different small letters within a column indicate significant differences among soil samples within the same parameter ($p < 0.05$). Values are represented as Mean±SD (n=5).

Table 3: Pearson correlation among soil pH, Total P, Organic matter, abundance, and density of AM fungi in soil samples.

		Soil pH	Total P (mg kg−1)	Organic matter (%)	Abundance (spores)	Density (Spores/g)
Soil pH	Pearson correlation	1	-0,455	,948*	,997**	,997**
	Sig. (one-tailed)		0,273	0,026	0,001	0,001
Total P (mg kg−1)	Pearson correlation	-0,455	1	-0,395	-0,419	-0,419
	Sig. (one-tailed)	0,273		0,302	0,291	0,291
Organic matter (%)	Pearson correlation	,948*	-0,395	1	,968*	,968*
	Sig. (one-tailed)	0,026	0,302		0,016	0,016
Abundance (spores)	Pearson correlation	,997**	-0,419	,968*	1	1,000**
	Sig. (one-tailed)	0,001	0,291	0,016		0,000
Density (Spores/g)	Pearson correlation	,997**	-0,419	,968*	1,000**	1
	Sig. (one-tailed)	0,001	0,291	0,016	0,000	

*. The correlation is significant at the level 0.05 (one-tailed).
**. The correlation is significant at the level 0.01 (one-tailed).

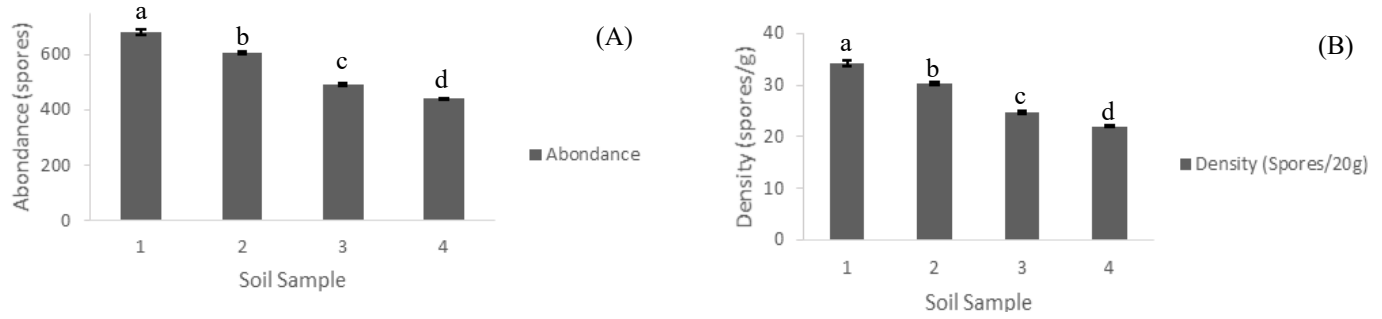

Figure 1: Spores abundance (A) and density (B) of the four soil samples. Different small letters within a column indicate significant differences among soil samples within the same parameter ($p < 0.05$). Values are represented as Mean±SD (n=3).

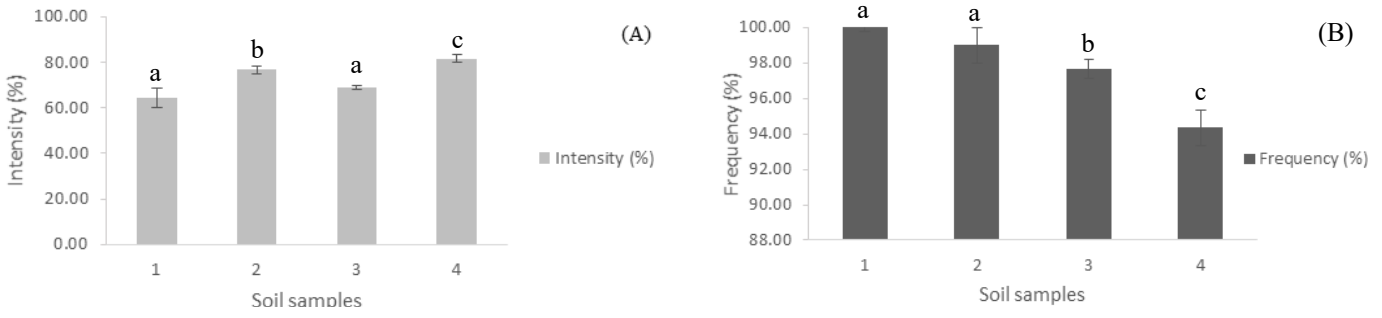

Figure 2: Intensity (A) and frequency (B) of barley root mycorrhization. Different small letters within a column indicate significant differences among soil samples within the same parameter ($p < 0.05$). Values are represented as Mean±SD (n=3).

Spores abundance and density

The average abundance and density vary from one site to another (Figure. 1-A-B). The soil sample 1 and 2 showed more AMF spores abundance and density compared to soil sample.3 and 4. The highest abundance values were reached by the soil sample.1 (683.3 ±10.053 spores) and the lowest by soil sample.4 (440.3±5.529 spores). In addition, the same pattern was observed for the density of the spores (soil sample.1=34.165±0.503, sample.4=22.015±0.276). A strong correlation between the abundance and density of the spores can be observed from these results. A high spore's abundance is accompanied by a high spore's density (Figure. 1-A-B), and as previously mentioned,

Table 4: Endomycorrhizal fungi isolated from the date palm groves in Zagora.

Spore	Form	Color	Diameter	Surface	Wall thick-ness	Wallnumber	Hypha size	Species
1	Globular	Yellow-brown	220	Granular	5.11	3	_	*Acaulosporaelegans*
2	Globular	light brown	164	Granular	6	3	_	*Acaulosporascorbiculata*
3	Subglobular	reddish-yellow	106	Granular	9.49	3	_	*Acaulosporalacunosa*
4	Globose	pale orange-brown	125.45	Irregular	4.76	3	_	*Acaulosporalaevis*
5	Globular	Brown	131.5	Granular		3	_	*Acaulosporabireticulata*
6	Ellipsoid or oval	Yellow-brown	136	Granular	1.81	2.3	_	*Acaulosporarehmii*
7	Oval	Marron claire	44.4	Smooth	1.37	2		*Acaulosporalacunosa*
8	Subglobular	Marron foncé	341.60	Granular	2.91	1.2		*Diversisporatortuosa*
9	pyriform	Light yellow	133	Granular		_	_	*Entrophosporasp*
10	Globular	Yellow-brown	_	Smooth	9.75	3	81	*Funneliformismossae*
11	Subglobular	Dark yellow	117.5	Smooth	2.51	2	_	*Glomus sp*
12	Globular	Light brown	96	Granular	3.52	3	_	*Glomus globiferum*
13	Globular	Orange	64	Smooth	2.5	2	24	*Glomus sp*
14	Globular	Yellow	122	Granular	3.21	3	_	*Glomus globiferum*
15	Subglobular	Light yellow beige	77	Irregular	2.19	2	_	*Glomus aurantium*
16	Oval	Orange-brown	108	Granular	5.40	3.4!	_	*Glomus trufemii*
17	Globular	Orange-brown	133	Granular	5.61	3	47.2	*Glomus macrocarpum*
18	Globular	Brown	106.7	Smooth	4.45	3	_	*Glomus sp*
19	Ellipsoid	Light brown	110	Smooth	1.09	2	_	*Glomus sp*
20	Globular	Light yellow	65.7	Granular	0.99	2	_	*Glomus versiformis*
21	Subglobular	Orang- brown	98	Granular	2.94	3	_	*Glomus macrocarpum*
22	Globular	yellow	65.75	Granular	1.3	2	69	*Glomus*
23	Oval	Marron Claire	116	Granular	2.16	2.3	5.08	*Glomus sp*
24	Oval	Brown	95	Smooth	2.92	3	54.9	*Rhizophagusintraradices*
25	Globular	Marron	116.6	Granular	15.81	3.4	_	*Rhizophagusreticulatum*
26	Subglobular	Yellow	65	Irregular	2.69	3	38	*Scutellosporasp*
27	Subglobular	Orang- brown	118.2	Irregular	4.45	3	_	*Scutellosporasp*
28	Globular	Orang-yellow	75	smooth	1.95	3	23.37	*Septoglomussp*

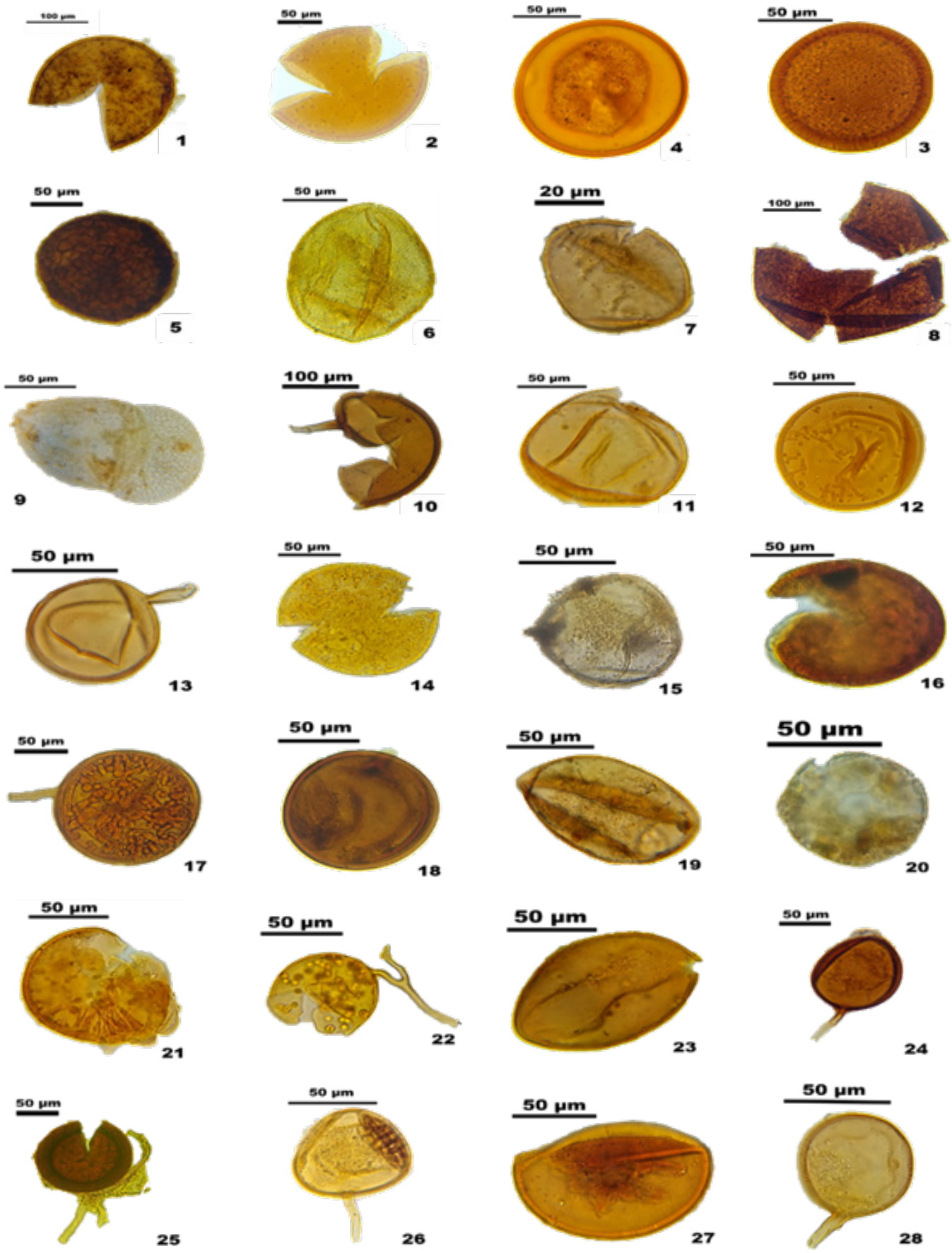

Figure 3: Mycorrhizal spores isolated from Zagora palm grove rhizosphere. *(1) Acaulosporaelegans. (2) Acaulosporascorbiculata. (3) Acaulospora lacunose. (4) Acaulosporalaevis. (5) Acaulosporabireticulata. (6) Acaulosporarehmii. (7) Acaulosporalacunose. (8) Diversisporatortuosa. (9) Entrophosporasp. (10) Funneliformismossae. (11) Glomus sp. (12) Glomus globiferum. (13) Glomus sp. (14) Glomus globiferum. (15) Glomus aurantium. (16) Glomus trufemii. (17) Glomus macrocarpum. (18) Glomus sp. (19) Glomus sp. (20) Glomus versiformis. (21) Glomus macrocarpum. (22) Glomus sp. (23) Glomus sp. (24) Rhizophagusintraradices. (25) Rhizophagusreticulatum. (26) Scutellosporasp. (27) Scutellosporasp. (28) Septoglomussp*

the results in Table.3 showed a strong correlation between spore abundance and density and the soil physicochemical characteristics.

Frequency and Intensity of barley root mycorrhization

Microscopic examination of barley root samples confirmed the presence of substantial hyphae, vesicular and arbuscular aspects of AM fungal colonization and the appearance of root spores. Statistical analysis of the mycorrhization frequency indicated no significant effect between the soil samples that were taken from the first and second sites ($P<0.05$) (Figure.2-A-B). In addition, there was no significant variation of the mycorrhizal intensity between the first and the third site. The roots from sample 1 showed the lowest values of mycorrhizal intensity (64%) compared to the other samples. As for the highest value, it was recorded by sample 4 (84%).

Morphological identification of mycorrhizal spores

The isolated spores were examined for some properties determined by the INVAM web site and some other references. The morphological identification of isolated spores revealed the presence of 28 species belonging to seven genera: *Acaulospora* (6 species), *Diversispora* (1 specie), *Entrophospora* (1 specie), *Funneliformis* (1 specie), *Glomus* (7 species), *Rhizophagus* (2 species), *Scutellospora* (1 specie), and *Septoglomus* (1 specie) (Table 4, Figure 3). *Acaulospora* and *Glomus* genera were the most common ones in all of the soil samples. The appearance of these endomycorrhizal was significantly similar in the four different soil samples. The "Zagora" inoculum contains a mixture of the mycorrhizal spore previously mentioned.

DISCUSSION

The present study highlights the morphological identification of Mycorrhizal fungi associated with the date palm rhizosphere in the arid and semi-arid regions of the Moroccan desert. The rhizosphere of the plant is an extraordinary ecological environment with different flora and fauna. To harness all the beneficial properties that the symbiotic relationship of mycorrhizal fungi has to offer to the plant, the mycorrhizal statue must be studied. When faced with abiotic stress, plants develop several strategies to avoid this stress or increase tolerance; many detailed studies have reported some strategies for alleviating the stress-induced adverse effects on plant growth (Evelin *et al.,* 2009; Glick *et al.,* 2007; Saharan and Nehra, 2011). Oyediran *et al.,*(2015) have reported that the peculiar capacity of plants in dry lands to produce high amounts of sugars and amino acids to cope with abiotic stress paired with the low phosphor content of arid soils, favors the plant/ fungi symbiotic relationships, and may increase the mycorrhizal fungi spore diversity in these areas. These interpretations were demonstrated in our spores abundance and density results (Figure.1). Spore densities found in this study can be considered relatively large since all of the soil samples are from an arid region. The soil samples were also tested for physiochemical properties (Table 1 and 2). Rhizosphere soil texture directly influences soil water holding capacity, aeration, root movement, and nutrient uptake (Motsara and Roy, 2008). Soil aggregates that are better in quality and stability are considered a better choice for AM fungi colonization (Motsara and Roy, 2008). The Pearson correlation represented in Table 3 suggested that the soil pH had significant effects on both spore abundance and density in the soil samples, when the soil pH increased the spore's abundance and density also increased. The same correlation was observed with the soil Organic matter and the spore's abundance and density. Toh *et al.,* (2018) and Bhat *et al.,* (2011) reported similar observations and findings. Furthermore, the large sand composition for all soil samples (Table 1) may have allowed greater drainage for the decomposition of the soil organic matter and hyphae penetration leading to an enhanced fungal spread and thus more spore population and AMF diversity (Oyediran *et al.,* 2015). Belay and Vestberg, (2013) uncovered a positive correlation between sand fraction and spore density among acacia species in the central highlands of Ethiopia. Sand fractions are considered to have many macro-pores, these macro-pore spaces are essential for increased AMF density (Belay and Vestberg, 2013). The colonization parameter (intensity and frequency) in this study were significantly high in all of the soil samples (Figure.2). This may be largely attributed to a combination of factors predominant in the semi-arid regions, higher organic matter, ideal levels of nutrients (available N and P), favorable E.C, and soil pH (Table 2).Similar to our results, Toh *et al.,* (2018) suggested that the pH of the rhizosphere soil had significant effects on both spore numbers in soil and the colonization rate in roots. Also, Bhat *et al.,*(2014) revealed that there may be significant relationship among available soil potassium/ phosphorous and AMF root colonization.

Several genera of AM fungi spores were isolated and characterized from the crop plants and soil samples studied as shown in Table 4 and Figure 3. The characterization of the spores was mainly focusedon the color and shape of the spores, the number of wall layers, and any other form of structures associated with the AM fungi. The identification also was based on several registered sites such as the West Virginia University International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi, The Mycorrhiza Biology Laboratory page, and the Mycorrhizal Associations: Web Resource (INVAM, 2020). From Table.4, AM fungi isolated from the trap plants were *Glomus spp., Acaulospora spp., Funneliformis spp., Rhizophagus spp.,*and*Diversispora spp.Scutellospora spp. Septoglomus spp. Entrophospora spp*.These species are typical ofthe arid and semi-arid environments. The presence of four different strains *Funneliformis spp. Rhizoglomus spp. Dominikia spp.*

Albahypha spp.) within the date palm rhizosphere of the Tunisian desert was reported by Chebaane, (2020). Symanczik *et al.,* (2014) described the traits of the three AMF genera collected from the date palm rhizosphere, notably*Claroideoglomus spp., Diversispora spp., and Funneliformis spp*. In the desert oasis of Saudi Arabia, 25 species have been identified: 18 species member of the genus *Glomus,* two species of the genus *Scutellospora, Racocetra*, one species of the genus *Acaulospora, Paraglomus*, and *Ambiospora* (Al-Yahya'ei *et al.,* 2011). Spores of eight species belonging to the Glomus spp., and *Acaulospora spp*. were found in the desert lands in Jordan (Mohammad *et al.,* 2003). Among various AM fungi species isolated, genera *Glomus spp.*was found to be the predominant species followed by *Acaulospora spp.* in this research study. The*Glomus* species are found to be the most abundantgenera inthe AMF assemblage. This could be attributed to their distinctive ability to survive in both acidic and alkaline soils, to co-adapt with plants, to resist the environmental challenges, and to produce excellent inoculum under constraining environmental conditions.

To sum up, the results from this study confirm several previous ones (Chebaane *et al.,* 2020; Belay *et al.,* 2013; Symanczik *et al.,* 2014; Al-Yahya'ei *et al.,* 2011), but the AM fungi population found, along with the soil characteristics, gives a new understanding of the mycorrhizal status of the Zagora desert and may lead to further investigations.

CONCLUSION

This experiment has highlighted the presence and diversity of AMF inside the date palm rhizosphere in the region of Zagora. The richness of the desert regions soil with endomycorrhizalgrantgoodconditions for the growth and development of date palm trees by promoting their access to minerals and water and increasing the plant's tolerance to abiotic stress (drought, the salinity of water or soil). Various indigenous AM fungi species such as *Acaulospora* (6 species), *Diversispora* (1 specie), *Entrophospora* (1 specie),*Funneliformis* (1 specie), Glomus (7 species), *Rhizophagus*(2 species), *Scutellospora* (1 specie), and *Septoglomus* (1 specie), were isolated from the crop plants and *Glomus* genera was identified as the predominant genera followed by *Acaulospora* in these selected plants. These findings suggested that the date palm plant might be a suitable host plant for the AM fungi. Apart from that, soil characteristics may also have a positive influence on AM fungi by affecting the root colonization rate in crop plants and spore number. These findings would also provide researchers with fundamental tools and a descriptive profile of AMF fungi in the Zagora desert in order to put to use the beneficial aspect of the AMF fungi. The knowledge provided on the diversity and drivers of AMF in the ecologically stressed ecosystem of arid and semi-arid regions may be a new sustainable approach in dealing with environmental challenges. Date palm rhizosphere is potentially a source of mycorrhizal fungi that can be isolated and used to preserve the palm groves and the oasis ecosystem, in addition to increasing the yield of the date palm and potentially other plantations.

REFERENCES

Belimov, A.A. Shaposhnikov, A.I. Azarova, T.S. Makarova, N.M. Safronova, V.I. Litvinskiy, V.A. Nosikov, V.V. Zavalin, A.A. Tikhonovich, I.A. 2020. Microbial Consortium of PGPR, Rhizobia and Arbuscular Mycorrhizal Fungus Makes Pea Mutant SGECdt Comparable with Indian Mustard in Cadmium Tolerance and Accumulation. *Plants* 9 (8): 975.

Brink, S.C. 2016. Unlocking the Secrets of the Rhizosphere. *Trends in Plant Science* 21 (3): 169–70.

Brundrett, M. Bougher, N. Dell, B. Grove, T. Malajczuk, N. 1996. Working with Mycorrhizas in Forestry and Agriculture. International Mycorrhizal Workshop in Kaiping, China in *1994*. ACIAR Monograph Series. Canberra: Australian Centre for International Agricultural Research.

Carr, M.K.V. 2013. The water relations and irrigation requirements of the date palm (*Phoenix Dactylifera* L.): A REVIEW. *Experimental Agriculture* 49 (1): 91–113.

Chen, S. Zhao, H. Zou, C. Li, Y. Chen, Y. Wang, Z. Jiang, Y. 2017. Combined Inoculation with Multiple Arbuscular Mycorrhizal Fungi Improves Growth, Nutrient Uptake and Photosynthesis in Cucumber Seedlings.*Frontiers in Microbiology* 8.

Climate-data.org. Climat Zagora: Pluviométrie et Température Moyenne Zagora, Diagramme Ombrothermique Pour Zagora - Climate-Data.Org'. Accessed 10 September 2020.

Drew, E.A. Murray, R.S. Smith, S.E. 2006. Functional Diversity of External Hyphae of AM Fungi: Ability to Colonise New Hosts Is Influenced by Fungal Species, Distance and Soil Conditions. *Applied Soil Ecology* 32 (3): 350–65.

Ech-cheddadi, S. Ouallal, O. Fatine, M. Moussa, O. El yacoubi, H. El goumi, Y. Atmane, R. 2019. Soil Physicochemical Properties and Diversity of Arbuscular Mycorrhizal Fungi in the Rhizosphere of Sugarcane in the Gharb Region of Morocco. *Plant cell biotechnology and molecular biology*. 828–845–828–845.

Friese, C.F. Michael, F.A. 1991. The Spread of VA Mycorrhizal Fungal Hyphae in the Soil: Inoculum Types and External Hyphal Architecture. *Mycologia* 83 (4): 409.

Gerdemann, J.W. Nicolson, T.H. 1963. Spores of Mycorrhizal Endogone Species Extracted from Soil by Wet Sieving and Decanting. *Transactions of the British Mycological Society* 46 (2): 235–44.

Invam. 2020. Trap Culture | INVAM International Culture Collection of (Vesicular) Arbuscular Mycorrhizal

Fungi | West Virginia University. 2020.

Köppen. 2010. World Maps of the Köppen-Geiger Climate Classification Updated. 6 September 2010.

Kouba, Y. Gartzia, M. El aich, A. Alados, C.L. 2018. Deserts Do Not Advance, They Are Created: Land Degradation and Desertification in Semiarid Environments in the Middle Atlas, Morocco. *Journal of Arid Environments* 158 (November): 1–8.

Koske, R.E. 1982. Evidence for a Volatile Attractant from Plant Roots Affecting Germ Tubes of a VA Mycorrhizal Fungus. *Transactions British Mycological Society.* 79 (2), 305-310.

Lahmidi, S. Lagnaoui, L. Bahaj, T. El adnani, A. FADLI, D. 2020. First Inventory and Assessment of the Geoheritage of Zagora Province from the Project Bani Geopark (South-Eastern Morocco). *Proceedings of the Geologists' Association*.

Li, Z. Wu, N. Meng, S. Wu, F. Liu, T. 2020. Arbuscular Mycorrhizal Fungi (AMF) Enhance the Tolerance of Euonymus *Maackii Rupr* at a Moderate Level of Salinity.*plos one* 15 (4): e0231497.

Mitra, D. Uniyal, N. Ppanneerselvam, P. Senapati, A. 2019. Role of Mycorrhiza and Its Associated Bacteria on Plant Growth Promotion and Nutrient Management in Sustainable Agriculture, 10.

Nanjundappa, A. Davis, J.B. Anil, K.S. Murugan, K. Hillol, C. 2019. Interaction between Arbuscular Mycorrhizal Fungi and Bacillus Spp. in Soil Enhancing Growth of Crop Plants. *Fungal Biology and Biotechnology* 6(23) (1): 1–20.

Phillips, J.M. Hayman, D.S. 1970. Improved Procedures for Clearing Roots, Staining Parasitic, and Vesicular-Arbuscular Mycorrhizal Fungi for Rapid Assessment of Infection. *Transactions British Mycological Society.*55 (1)

Ritchey, E.L. Mcgrath, J.M. Gehring, D. 2015. Determining Soil Texture by Feel. Agriculture and Natural Resources Publications. 139.

Sarkar, D. Haldar, A. 2005. Physical and Chemical Methods in Soil Analysis: Fundamental Concepts of Analytical Chemistry and Instrumental Techniques. New Delhi: *New Age International*. 193 pp.

Schachtman, D.P. Reid, R.J. Ayling, S.M. 1998. Phosphorus Uptake by Plants: From Soil to Cell. *Plant Physiology* 116 (2): 447–53.

Selosse, M.A. Richard, F. Xinhua, H. Simard, S.W. 2006. Mycorrhizal Networks: Des Liaisons Dangereuses? *Trends in Ecology & Evolution* 21 (11): 621–28.

Shahane, A.A. Shivay, Y.S. Prasanna, R. 2020. Enhancing Phosphorus and Iron Nutrition of Wheat through Crop Establishment Techniques and Microbial Inoculations in Conjunction with Fertilization. *Soil Science and Plant Nutrition*, August, 1–9.

Smith, S.E. Read, D.J. 2009. Mycorrhizal Symbiosis. 3. ed., Repr. Amsterdam: Elsevier/Acad. Press.

Strullu, D.G. Grellier, B. Marciniak, D. Letouzé, R. 1986. Micropropagation of Chestnut and Conditions of Mycorrhizal Syntheses *in Vitro*. *New Phytologist* 102 (1): 95–101.

Trouvelot, A. Kough, J.L. Gianinazzi-pearson, V. 1986. Mesure du taux de mycorhization VA d'un systeme radiculaire. Recherche de methodes d'estimation ayant une signification fonctionnelle. Physiological and genetical aspects of mycorrhizae = Aspects physiologiques et genetiques des mycorhizes : proceedings of the *1st European Symposium on Mycorrhizae, Dijon*, 1-5 July 1985.

White, R.P. Nackoney, J. 2003. Drylands, People, and Ecosystem Goods and Services.

White, R.P. Tunstall, D. Henninger, N. 2002. Building Support for New Development Policies. *World Resources Institute.* 1(1) 14.

WVU, and INVAM. 2020. Home | INVAM International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi | West Virginia University. 2020.