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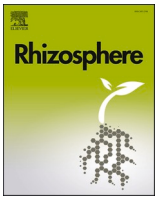

Cultivation, identification, and application of arbuscular mycorrhizal fungi associated with date palm plants in Drâa-Tafilalet oasis

Rania El Hilali [a,b], Sarah Symanczik [c], Said El kinany [a,d], Fritz Oehl [e], Lahcen Ouahmane [b], Rachid Bouamri [a,*]

[a] Department of Environment and Plant Protection, Ecole Nationale D'Agriculture de Meknès, Meknès, Morocco
[b] Laboratory of Microbial Biotechnologies, Agro-sciences and Environment, Faculty of Sciences, Cadi Ayyad University, Marrakesh, Morocco
[c] Soil Science Department, Research Institute of Organic Agriculture (FiBL), Ackerstrasse 113, 5070, Frick, Switzerland
[d] Laboratory of Microbial Biotechnology, Faculty of Sciences and Techniques, Sidi Mohamed Ben Abdellah University, Fez, Morocco
[e] Agroscope, Competence Division for Plants and Plant Products, Ecotoxicology, Müller-Thurgau-Strasse 29, CH-8820, Wädenswil, Switzerland

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) have been frequently reported as effective tools for alleviating environmental stresses and promoting plant growth and yield. In this study, we aimed to isolate, culture, and identify molecular and morphological AMF species associated with date palms and spontaneous plants present at eight sites in the arid agroecosystem of the Drâa-Tafilalet oasis of Morocco. We tested the capacity of AMF to colonize micropropagated date palm seedlings at their first acclimatization stage. Soil and root samples were collected to propagate indigenous AMF strains using trap culture techniques under greenhouse conditions and, at the same time, their root colonization potential was evaluated. We used freshly propagated spores to establish a collection of single spore-derived cultures. Morphological, microscopic, and molecular approaches were adopted to quantify AMF communities in the roots and rhizosphere and identify the recovered AMF species present at the eight sites. In an inoculation experiment, a micropropagated date palm was inoculated with a consortium of four cultured AMF strains, alone or in combination with synthetic fertilizer or compost. Our results showed that after two cycles of trap culturing, the frequency and intensity of AMF colonizing host plant roots significantly increased, exceeding 91% and 50%, respectively. Using three trap plant species and favorable growing conditions helped increase root colonization rates and AMF proliferation. AMF propagation resulted in the cultivation of 13 AMF strains. Molecular and morphological analyses revealed six different AMF species within our cultures: *Pervetustus simplex*, *Claroideoglomus etunicatum*, *Albahypha drummondii, Septoglomus xanthium, Funneliformis mosseae,* and *Rhizoglomus irregulare*. Results of the inoculation experiment revealed that root colonization was higher in treatments augmented with synthetic fertilizers than those supplemented with compost with 84.4% as against 46.7% and 26.1% as against 12.3%, respectively, for colonization frequency and intensity. In contrast, shoot length and stem diameter of date palms were significantly higher in treatments augmented with compost and AMF than that with synthetic fertilizers. Synthetic fertilizers might have been partially immobilized directly after application, limiting availability and resulting in lower growth performance of date palms. These findings indicated that date palm groves are a niche for efficient indigenous AMF strains that can colonize and enhance date palm growth at the early stages.

1. Introduction

The date palm (*Phoenix dactylifera* L.) is one of the most important species of the palm family (Palmaceae), which includes 200 genera and more than 2500 species (El Hadrami and Al-Khayri, 2012; Hadrami and Hadrami, 2009). Date palm is the main crop and source of economy for the oasis population (Al Antary et al., 2015). It is also of ecological importance as it provides a microclimate conducive to cultivating subjacent crops such as olives, wheat, lucerne, and barley under the Saharan climate (El Bouhssini and Faleiro, 2018). However, oases are progressively subjected to various stresses that are further accelerated by climate change, reduced groundwater levels, and increased salt stress

* Corresponding author.
E-mail address: rbouamri@enameknes.ac.ma (R. Bouamri).

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(Mainguet et al., 2011). Moreover, Moroccan oases are threatened by desertification, low soil organic matter content, and the spread of vascular fusariosis, the most destructive disease of date palm (El Bouhssini and Faleiro, 2018; Mainguet et al., 2011; Ou-Zine et al., 2021).

Several studies have reported the association between beneficial root symbionts, arbuscular mycorrhizal fungi (AMF), and date palms. The occurrence of AMF in date palm groves was discovered in the Crescent desert near Baghdad and in the oases of Qassim (Saudi Arabia), where their contribution to plant mineral nutrition and water supply was recognized (Khudairi, 1969). In 2006, a study investigating the diversity of AMF communities associated with date palms using morphological and molecular approaches was conducted for the first time in Morocco (Bouamri et al., 2006). Subsequently, researchers discovered eight AMF species from desert ecosystems in southern Arabia: *Claroideoglomus drummondii, Diversispora aurantia, Diversispora spurca*, *Funneliformis africanum,* and the new AMF species *Diversispora omaniana, Septoglomus nakheelum, Rhizophagus arabicus,* and *Pervetustus simplex* (Błaszkowski et al., 2017; Symanczik et al., 2014a, 2014b). A few studies investigating AMF associated with date palms in southeastern Morocco found ten AMF species according to morphological identification (Bouamri et al., 2014; Sghir et al., 2014). These ten species were identified as belonging to *Glomus* (five), *Acaulospora* (three), and *Scutellospora* (two). Spontaneous plants, particularly those growing in arid habitats, have recently served as biotopes for undescribed and locally adapted AMF species, as observed in Oman (Al-Yahya'ei et al., 2011a; 2011b). Our study is the first to investigate AMF species associated with spontaneous plants in Morocco.

Previous studies have indicated that AMF can improve the survival and growth of date palms (Baslam et al., 2014; Benhiba et al., 2015; El Kinany et al., 2019). AMF has been found to increase date palm nutrient uptake and alleviate drought and salinity stress, thereby improving plant productivity (Ait-El-Mokhtar et al., 2019; Anli et al., 2020; Toubali et al., 2020). Albers (2011) found that commercial AMF as an inoculum for tissue-cultured seedlings of micropropagated date palms resulted in larger shoots and roots than in non-inoculated seedlings. Another recent study showed that the inoculation of micropropagated date palm plants with *Glomus iranicum* improved their growth and nutrition uptake (El Kinany et al., 2019). However, indigenous AMF species, which rapidly colonize the root system of inoculated olive plants, improve plant growth more efficiently than commercial AMF (Chenchouni et al., 2020). Hence, we aimed to isolate, culture, and identify AMF species associated with date palms and spontaneous plants at eight sites in the arid Moroccan agroecosystem of the Drâa-Tafilalet oases. In addition, a consortium of four AMF strains was used to assess their potential for application in date palm cultivation to improve the growth and performance of date palms during acclimatization under nursery conditions.

2. Materials and methods

2.1. AMF cultivation

The study area, the Drâa-Tafilalet region of Morocco, is the largest oasis ecosystem in the Saharan Desert (Lamqadem et al., 2019). This region is one of the most arid regions, with low aridity index (0.05–0.20), water scarcity, and high salinity levels (FAO, 2019). Rhizosphere soil and root sampling were conducted during the winter season using completely randomized sampling. Samples (soil and root) were collected from eight different sites in the rhizosphere of date palm (*Phoenix dactylifera* L.) and two spontaneous plants (*Ziziphus lotus* and *Retama blanca*; Table 1). From each site, 1 kg of rhizosphere soil and fresh roots of date palm (three date palms per site) were collected from a depth of 10–40 cm using a soil corer. Samples were placed immediately in a zip-lock freezer bag and stored at a 4 °C cooler. Roots were removed by sieving and stored in 50% ethanol until further analysis. Soil samples were homogenized, sieved at 2 mm, and stored at 4 °C until use. A subsample of 500 g/site was sent to a private laboratory (LaboMag, Casablanca, Morocco) for physical and chemical analyses (soil texture, soil organic matter (SOM) content (C x1.724 NF ISO 14235: 1998), pH_w (ISO 10390: 2005), and P content (P_2O_5 Olsen mg/kg, NF ISO11263:1995).

2.2. Soil characteristics

All the soils were alkaline, with pH values ranging from 8 to 8.4. Soils from traditional palm groves were characterized by a sandy loam texture, whereas soils from modern palm groves and non-agricultural sites were characterized by a clay texture (Table 2). Accordingly, soil organic matter varied significantly between sites and ranged from 0.7% recorded in non-agricultural sites (7 and 8) to 4.9% in agricultural sites (site 4) of modern palm groves (Table 2). For most soils, P contents significantly differed among sites and ranged between 12.41 μg P/g and 37.41 μg P/g of dry weight soil except for site 3, the traditional grove with 70.8 μg P/g dry weight soil.

2.3. Trap and single spore-derived cultures

Trap cultures were established using rhizosphere soil samples from the date palm and spontaneous plants collected from eight sites (Table 1). A total of 300 g of rhizosphere soil (roots and soil) per disinfected pot (0.5 L) was used to initiate trap culture for each site. Disinfected sorghum, maize, and leek seeds were planted on the surface and covered with 100 g of sterilized sandy soil. Using multiple host plants increases the probability of AMF diversity and quantity (Bouamri et al., 2006).

Two cycles of multiplication of AMF in trap cultures were conducted under greenhouse conditions (temperature: 27 ± 2 °C, natural photoperiod, and humidity). Three pots of trap culture were selected for each

Table 1
Coordinates and description of sampling sites in Drâa-Tafilalet oasis and list of arbuscular mycorrhizal fungal (AMF) strains recovered from each site.

Site	Coordinates	Host plants	Plantation type	Strain ID	AMF species
1	31°51′33.2″N4°16′13.5″W	*Phoenix. Dactylifera*	Traditional grove (Date palm with subjacent culture)	Mena 0166	*Pervetustus simplex*
				Mena 1626	*Claroideoglomus etunicatum*
				Mena 1627	*Albahypha drummondii*
2	31°47′21.9″N4°14′39.0″W	*Phoenix dactylifera*	Traditional grove (Date palm with subjacent culture)	Mena 1629	*Claroideoglomus etunicatum*
				Mena 0164	*Septoglomus xanthium*
3	31°41′42.9″N4°09′20.7″W	*Phoenix dactylifera*	Traditional grove (Date palm with subjacent culture)	Mena 0165	*Albahypha drummondii*
				Mena 0167	*Funneliformis mosseae*
4	31°41′12.4″N4°54′36.3″W	*Phoenix dactylifera*	Modern grove (Monoculture without subjacent culture)	Mena 1622	*Albahypha drummondii*
5	31°41′15.0″N4°54′32.4″W	*Phoenix dactylifera*	Modern grove (Monoculture without subjacent culture)	Mena 0163	*Albahypha drummondii*
6	31°41′16.0″N4°54′40.5″W	*Phoenix dactylifera*	Modern grove (Monoculture without subjacent culture)	Mena 1628	*Albahypha drummondii*
7	31°42′28.2″N4°08′19.8″W	*Zizyphus lotus*	Non-agriculturally managed site (Desert habitat)	Mena 1623	*Funneliformis mosseae*
8	31°42′28.2″N4°08′19.8″W	*Retama blanca*	Non-agriculturally managed site (Desert habitat)	Mena 0161	*Rhizoglomus irregulare*
				Mena 1630	*Rhizoglomus irregulare*

Table 2
Characteristics of soil samples.

Site N°	Sand (%)	Silt (%)	Clay (%)	pH	SOM (%)	P (µg/g)
1	70.51 ± 1.39a	23.61 ± 1.13 d	6.42 ± 0.61e	8.33 ± 0.30a	1.06 ± 0.07 b	32.4 ± 0.84c
2	61.19 ± 1.057 b	29.46 ± 1.00c	9.16 ± 0.34 d	8.23 ± 0.25a	1.27 ± 0.03 b	24.39 ± 1.09 d
3	57.36 ± 1.28c	32.14 ± 0.76 b	10.11 ± 1.02 d	8.46 ± 0.83a	1.29 ± 0.04 b	70.78 ± 1.94a
4	30.40 ± 0.84e	11.50 ± 0.63e	57.7 ± 0.66 b	8.26 ± 0.15a	4.89 ± 0.18a	37.41 ± 0.71 b
5	28.49 ± 0.50e	10.54 ± 1.44e	61.37 ± 1.14a	8.20 ± 0.10a	4.81 ± 0.24a	37.38 ± 0.71 b
6	29.88 ± 2.30e	10.33 ± 1.05e	60.13 ± 1,.80a	8.20 ± 0.20a	4.83 ± 0.30a	37.32 ± 0.78 b
7	44.51 ± 1.96 d	40.22 ± 1.61a	15.92 ± 1.12c	8.37 ± 0.21a	0.69 ± 0.17c	12.46 ± 1.24e
8	45.15 ± 1.70 d	39.85 ± 2.12a	15.36 ± 0.78c	8.26 ± 0.25a	0.68 ± 0.13c	12.40 ± 1.21e
F	343.97	287.14	1820.99	0.19	417.07	803.09
p-value	0.000	0.000	0.000	0.981	0.000	0.000

SOM: soil organic matter, P: phosphorus. The values present means ± standard deviation of 3 replicates. Different letters indicate significant differences between sites according to Duncan's test ($p < 0.05$).

site. The aerial parts of the host plants were cut and removed after the first trap cycle (four months). From each pot, the entire soil containing AMF propagules was mixed with 2 kg of sterilized sandy soil and transferred into a new 3 L pot to further amplify indigenous AMF. Disinfected sorghum, maize, and leek seeds were planted on the surface and covered with 100 g of sterilized sandy soil. The pots were maintained under the same growth conditions as described above. Plants were irrigated as required and fertilized with the nutrient solution every 15 days (Hewitt and Smith, 2002).

AMF spores were extracted by wet sieving 100 g of soil (Gerdemann and Nicolson, 1963), followed by sucrose density gradient centrifugation (Walker et al., 1982) for examination. Quantification was performed under a stereoscopic microscope in 9 cm diameter Petri dishes containing sterile water. As previously described, a pipette-tip technique was used to produce single-spore cultures using sorghum as the host plant (Tchabi et al., 2010). After spore extraction, only the vital and intact spores were selected to initiate AMF single-species cultures by placing one AMF spore below one sorghum seed per individual pipette tip under a microscope. In total, 90 tips were prepared, placed in a pipette tip box, and cultured in a growth chamber (light: 16 h; temperature: 21 ± 2 °C; relative humidity: 70%). After the germination of the seeds, each tip was transplanted into a 0.5 L pot containing the same sterilized soil used in the trap culture and grown under greenhouse conditions (temperature: 27 ± 2 °C). Similarly, two four-month cycles were conducted to multiply AMF strains using a mixture of sorghum, maize, and leek as host plants and fertilized with a Long Ashton nutrient solution once every 15 days (Hewitt and Smith, 2002).

2.4. AMF species colonization

Colonization frequency, intensity, and spore densities were assessed for field samples, trap cultures, and two single-spore derived cultures. Mycorrhizal colonization was estimated using a staining technique (Koske and Gemma, 1989). The stained roots were observed under a compound light microscope (Olympus, Tokyo, Japan). Colonization frequency and intensity were calculated using the formula described by Trouvelot et al. (1986). Spore densities were counted after wet sieving and sucrose density gradient centrifugation as described above.

2.5. Morphological identification

Spores of single-species cultures were separated from the culture substrate, as described by Sieverding (1991). The defined morphological spore characteristics and their subcellular structures are based on observations of specimens mounted in polyvinyl alcohol-lactic acid--glycerol (PVLG) (Koske and Tessier, 1983) and a mixture of PVLG and Melzer's reagent (Brundrett, 1996). The terminology of spore structure followed (Błaszkowski et al., 2012) for species with glomoid spore formation. Photographs were taken with a digital camera (Leika DFC 295) on a compound microscope (Leitz Laborlux S) using Leica Application Suite Version V 4.1 software. Reference specimens for each isolate were deposited at Z + ZT (ETH Zurich, Switzerland).

2.6. Molecular analyses and phylogeny

Molecular and phylogenetic analyses were performed on spores extracted from single-spore derived cultures. In brief, a single spore was transferred into a 0.2 mL polymerase chain reaction (PCR) vial and crushed with a pipette tip. Then 2 µL of 0.25 M NaOH were added to the crushed spores and incubated in a T3 thermocycler (Biometra GmbH, Goettinger, Germany) at 95 °C for 2 min. One µL of 0.5 M Tris-HCl (pH 8) and 2 µL of 0.25 M HCl were added to the extract and incubated at 95 °C for 2 min. Extracts were then used as templates for nested PCR amplification to obtain a fragment covering the partial small subunit (SSU), internal transcribed spacer (ITS), and partial large subunit (LSU) of ribosomal DNA (rDNA). The primer pairs, SSUmAf-LSUmAr and SSUmCf-LSUmBr, were used for the first and second nested PCR, respectively (Krüger et al., 2009). The final concentration of the master mix contained 1 U Phusion HF DNA polymerase (Thermofisher, Switzerland), 1.5 mM $MgCl_2$, 500 nM of each primer, 200 µM of each deoxynucleotide, and 3 µg BSA (Bioconcept, Allschwil, Switzerland). Nested PCR was done in a T3 thermocycler with the following PCR cycling conditions: for the first PCR, 5 min initial denaturation at 99 °C, 40 cycles of 10 s denaturation at 99 °C, 30s annealing at 60 °C, 1 min elongation at 72 °C, and 10 min at 72 °C for final elongation. The same conditions were used for the nested PCR, except that we used 30 cycles, and the annealing temperature was 63 °C. PCR products were visualized on 1.5% agarose gels with 1X TAE buffer and 1X Midori green for staining (NIPPON Genetics EUROPE GmbH, Dueren, Germany). PCR products with expected size bands of 1500 bp were purified with a PCR purification kit (Roche Diagnostics GmbH, Mannheim, Germany) following the manufacturer's protocols. PCR products were cloned into a pJET1.2/blunt cloning vector (Promega, Madison, Wisconsin, USA) and transformed into Dh5α competent cells (Promega) following the manufacturer's protocols. Four positive clones from each plate were confirmed by colony PCR using PCR nested as described by Krüger et al. (2009). Colonies yielding PCR products of expected size bands were used to set up overnight cultures, followed by plasmid extraction using the Plasmid Miniprep Kit 1 (peqGOLD, VWR) following the manufacturer's instructions. Plasmids were sequenced using the primers ITS1/ITS3/ITS4 (White et al., 2009) and Microsynth AG (Balgach, Switzerland). The electropherograms were processed and analyzed with ChromasPro Version 1.5 (www.technelysium.com.au). Sequences were initially sequenced and analyzed by BLAST (https://blast.ncbi.nlm.nih.gov) to extract sequences of closely related AMF strains, which were then aligned with their sequences in MegaX using ClustalW. The phylogenetic tree was inferred using the maximum likelihood criteria, as implemented in MegaX. Sequences generated in this study were registered in GenBank under the accession numbers ON033152-ON033175.

2.7. Inoculation experiment

2.7.1. Plant material

Micropropagated date palms (cv. Mejhoul) at the first acclimatization stage were provided by a private tissue culture laboratory (Oasis Biotechnology, Erfoud, Morocco, USA).

2.7.2. Mycorrhizal inoculants

A consortium of four indigenous AMF strains, namely *Septoglomus xanthium*, *Albahypha drummondii,* and *Pervetustus simplex* species (MENA 0164, MENA 0165, MENA 0166, MENA 01628) was inoculated directly adjacent to the root system of each plant as previously described (Haghighi et al., 2015). The strains were chosen based on their spore density in the second cycle of the single-spore culture (Supplementary Table S1). The inoculum was a mixture of roots and soil (50 g/strain/pot).

2.7.3. Experimental setup

Micropropagated date palms were individually transplanted into 6 L pots containing sterilized sandy soil, as described above. The experimental treatments were synthetic fertilizers (M), compost (C), synthetic fertilizers plus AMF consortium (MA), and compost plus AMF consortium (CA). Each treatment was replicated six times. The micropropagated date palm was grown for 18 months under greenhouse conditions set at 28 ± 3 °C and humidity of 52%. Compost was used as a source of organic matter. It was produced at a local private composting unit using olive mill waste and sheep manure, analyzed in a private laboratory (Table 3), and mixed manually into sandy soil (1:6 (v/v): 100 g/pot) during the transfer of date palms. Date palm plants were fertilized with a nutrient solution every 20 days, providing 1.2, 0.3, and 0.8 g per pot of N, P, and K, respectively. The plants were irrigated twice a week.

2.7.4. Evaluation of date palm growth

Plant growth was estimated every six months by measuring (i) shoot length using a decameter, (ii) collar diameter using sliding calipers, and (iii) leaf number. Mycorrhizal root colonization was assessed six and 18 months after the experimental setup. Roots were sampled from two opposite sides in the pot using a soil corer, gently washed to remove adhering soil, cleaned with deionized water, and stained with trypan blue (0.05%) prepared in a Lacto-glycerol solution (Koske and Gemma, 1989). The frequency (% F) and intensity (% I) of root colonization were further evaluated following the method described by Trouvelot et al. (1986).

2.7.5. Statistical analysis

Data were analyzed using analysis of variance (ANOVA), followed by Duncan's test at a significance level of $\alpha = 0.05$. The normality of the residuals was tested using the Shapiro-Wilk test. The AMF colonization frequency and intensity data were arcsin (× /100) transformed. All analyses were conducted using SPSS V21 software, and graphics were generated using GraphPad Prism 8 software.

Table 3
Chemical characteristics of compost.

Parameter	Compost
pH $_{H2O}$	8.26
Humidity (%)	32.56
Organic matter (%)	32.30
Organic carbon (g/kg)	187.9
Nitrogen (g/kg)	9.30
Carbon/Nitrogen	20.86
Phosphorus (%)	2.17
Potassium (%)	1.60
Copper (mg/kg)	70.56
Manganese (mg/kg)	300.45
Iron (mg/kg)	7164.76
Zinc (mg/kg)	200.42
Boron (mg/kg)	29.45

3. Results

3.1. Root colonization in field and trap cultures

Root colonization frequency, intensity, and spore density differed significantly between sites ($F = 38.940$, $P < 0.001$; $F = 878.667$, $P < 0.001$; $F = 680.51$, $P < 0.001$) and significantly increased after two trap-culture cycles ($F = 11.838$, $P < 0.001$; $F = 34.784$, $P < 0.001$; $F = 136.829$, $P < 0.001$), as illustrated in Fig. 1.

For instance, the frequency under field conditions at sites 1, 2, and 3 was 72.3%, 89.0%, and 75.3%, respectively, whereas it reached 78.4%, 100.0%, and 93.0% under trap culture conditions, respectively (Fig. 1a). Similarly, mycorrhization intensity increased and reached 40.2%, 63.9%, and 32.0% at the same sites, respectively (Fig. 1b). Furthermore, spore densities significantly increased in trap cultures obtained from all sites, except site 2, reaching 500–1000 spores/100 g soil, while they

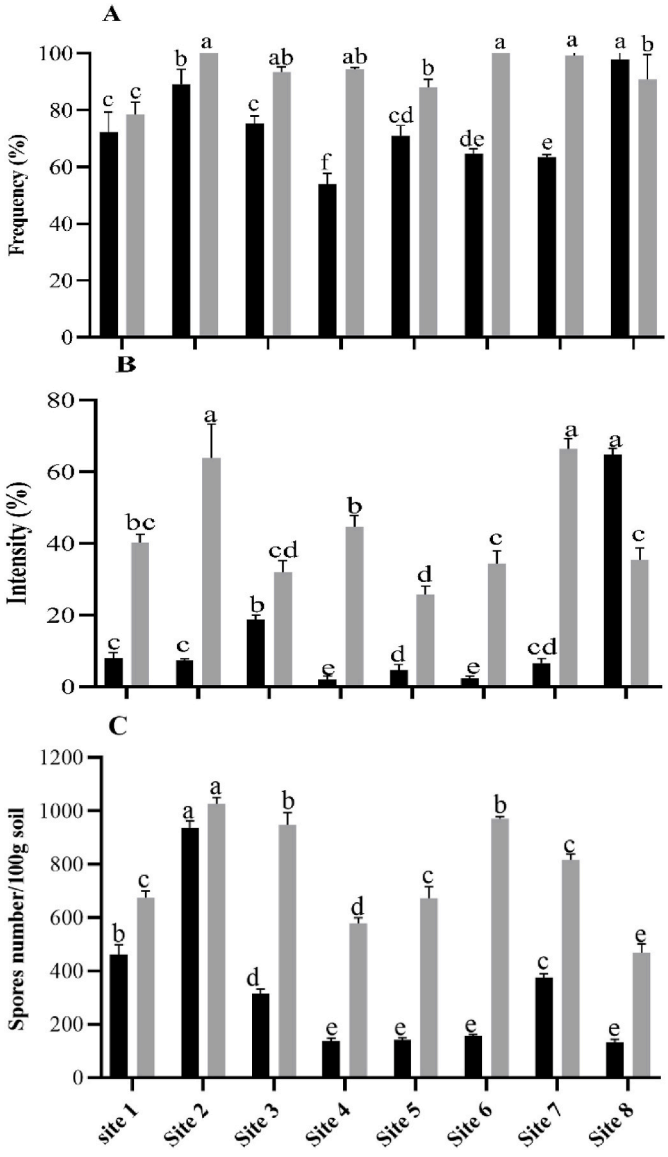

Fig. 1. Colonization frequencies (A), intensities (B) and spores densities (C) of roots sampled in the field (black bars) and after two trap culture cycles (grey bars). The values present means ± standard deviation of 3 replicates. Different letters indicate significant differences between sites individually under field and trap culture conditions according to Duncan test ($p < 0.05$).

only reached 140–450 spores/100 g soil under field conditions (Fig. 1c).

3.2. Single spore-derived cultures

Thirteen AMF strains were successfully isolated from 90 single-spore cultures. Three AMF cultures were recovered from site 1; two from sites 2, 3, and 8; and a single culture from sites 4, 5, 6, and 7 (Table 1). More spores germinated from traditional groves than from other sites. Some single spores did not germinate; therefore, no trace of AMF was observed in the host plant roots.

During multiplication, different root colonization frequencies, intensities, and spore densities were observed between the AMF strains (Supplementary Table S1). In the first cycle, mycorrhization frequency, intensity, and spore density ranged between 30.3 and 77.0%, 14.6–55.3%, and 4.0–18.6 spores/10 g soil, respectively. In the second cycle, mycorrhization frequency, intensity, and spore density ranged between 47.3 and 93.3%, 12.3–52.6%, and 3.6–43.6 spores/10 g soil, respectively.

3.3. Morphological and molecular identification

Morphological and molecular phylogenetic analyses based on LSU rDNA sequences obtained from isolated AMF spores revealed that the 13 AMF strains belonged to six genera of *Glomeromycota* (Schüßler and Walker, 2010) (Supplement Figure 1 and 2). Seven and three AMF strains were recovered from the date palm rhizosphere of traditional and modern groves, respectively, while three strains were recovered from the rhizosphere of the two spontaneous plants *R. blanca* and *Z. lotus,* growing on adjacent undisturbed sites (Table 1).

Phylogenetic analyses of LSU sequences of single spores recovered from Mena 0166 and Mena 0164 strains were identified as *Pervetustus simplex* and *Septoglomus xanthium*, respectively, and clustered the closest to *P. simplex* (99% identity) and with *S. xanthium* (98% identity), respectively. Sequences obtained from the single spores of Mena 01626 and Mena 01629 strains were identified as *Claroideoglomus etunicatum* and clustered the closest to *Glomus etunicatum* (99% identity). Sequences obtained from Mena 0167 and Mena 01623 were identified as *Funneliformis mosseae* and clustered the closest to *F. mosseae* (99% identity). Two of our single spore cultures, Mena 0161 and Mena 01630, belonged to *Rhizoglomus irregulare* and clustered the closest to *Rhizophagus irregularis* (99% identity), and five cultures, Mena 01627, 0165, 01622, 0163, and 01628, were identified as *Albahypha drummondii* and clustered the closest to *Claroideoglomus drummondii* (99% identity) (Supplement Figure 2). The spores of six recovered AMF species are shown in Supplement Figure 1.

A. drummondii was the only AMF species recovered from modern groves characterized by monoculture without subjacent culture. In contrast, five different AMF species were recovered from traditional groves: *P. simplex, C. etunicatum, A. drummondii, S. xanthium,* and *F. mosseae* characterized by a monoculture with subjacent culture, and two species were recovered from the desert habitat, namely, *R. irregulare* and *F. mosseae* (Table 1).

3.4. Inoculation experiment

3.4.1. Root colonization with indigenous mycorrhizal inoculant

Six months after the inoculation of micropropagated date palms with the indigenous AMF consortium, the frequency and intensity of mycorrhizal root colonization were high in MA and CA treatments, reaching values of 40.0% ± 13.3% and 6.24% ± 4.31%, and 24.4% ± 3.84% and 2.3% ± 1.83%, respectively. Significant differences were not detected between treatments. In contrast, after 18 months, significant differences between treatments were observed in mycorrhizal frequency ($F = 28.84$, $P = 0.006$) and intensity ($F = 18.45$, $P = 0.01$). The MA treatment showed higher frequency and intensity of 84.4% ± 3.84% and 26.1% ± 2.39%, respectively, than 46.6% ± 13.33% and 12.3% ± 5.07%, respectively, observed in the CA treatment. No traces of AMF colonization were detected in treatments M or C.

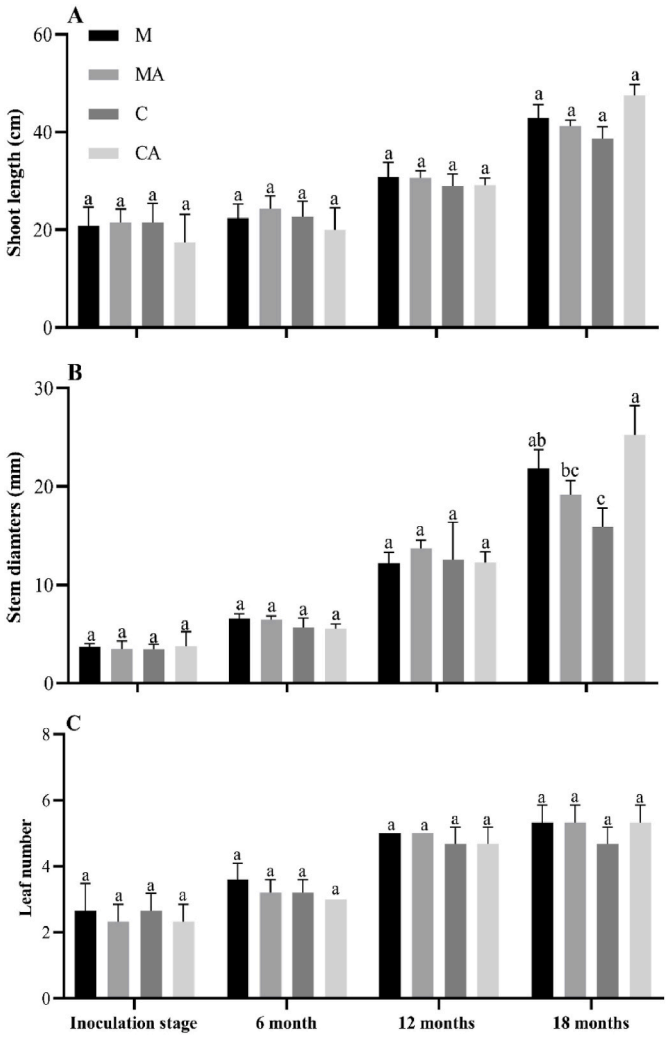

Fig. 2. Shoot length (A), stem diameter (B), and number of leaves (C) of micro propagated date palm cv. Mejhoul at inoculation stage and after 6, 12, and 18 months after inoculation with arbuscular mycorrhizal fungi (AMF). Date palms were amended with either mineral fertilizers (M), mineral fertilizers and indigenous AMF consortium (MA), compost (C) or compost and indigenous AMF consortium (CA). Data represent means of six-replicates ± standard deviation Different letters indicate significant differences between treatments of the same measurement time point according to Duncan test ($p < 0.05$).

3.5. Growth of micro-propagated date palms during acclimatization

After 6 and 12 months of growth, no significant differences between treatments were observed in shoot length, stem diameter, and leaf number (Fig. 2). However, at the end of the experiment, after 18 months, significant differences between treatments were observed in shoot length ($F = 6.51$, $P = 0.015$) and stem diameter ($F = 8.41$, $P = 0.007$). The highest shoot length was observed in the CA treatment (47.5 ± 2.57 cm) followed by the M, MA, and C treatments (Fig. 2a). Similarly, the highest stem diameter was recorded in the CA treatment with 25.3 ± 3.29 mm, followed by the M treatment with 21.8 ± 2.15 mm (Fig. 2b).

4. Discussion

4.1. Characterization, cultivation, and identification of native AMF

We investigated AMF associated with date palms and spontaneous plants at eight sites in the arid Moroccan agroecosystem of the Drâa-Tafilalet oasis. The colonization frequency in roots collected from sites 1 and 2 was consistent with that reported by Bouamri et al. (2006) and Sghir et al. (2014). They also observed root colonization frequency ranging between 65% and 85% at sites 1 and 2. By contrast, the colonization intensity was lower, ranging between 7% and 22.5% for sites 1 and 2. Bouamri et al. (2006) also reported high spore densities of 454/100 g at site 1. The reason for the high root colonization levels and spore densities observed at sites 1, 2, and 3 might be the advanced age of the plantation, higher plant diversity due to subjacent crops, and cultivation of different date palm cultivars managed by agro-ecological farming practices, as reported by Sangabriel-Conde et al. (2015) and Trejo et al. (2016). The low spore numbers observed in modern monoculture systems are consistent with the results obtained by Sangabriel-Conde et al. (2015) and Trejo et al. (2016), who also observed a decrease in AMF species abundance in monoculture systems. Mixed-culture systems have been shown to harbor abundant and diverse AMF communities when compared to monoculture systems (Guzman et al., 2021). According to Trejo et al. (2016) and Gosling et al. (2006), soil disturbance and farm management types are important factors affecting the functionality and diversity of AMF species. In addition, fertilization, the use of readily soluble fertilizers and high dosages of organic supplements, has been shown to reduce root colonization and spore abundance, as previously observed by Johnson et al. (1993), Bhadalung et al. (2005), and Oehl et al. (2004).

Root colonization and spore density significantly increased in trap cultures for almost all sites. These results might be explained by the involvement of three host plants, *Zea mays, Sorghum bicolor,* and *Allium porrum,* as mixed cultures favor abundant and diverse AMF communities, as reported earlier (Bouamri et al., 2014; Guzman et al., 2021).

Thirteen AMF strains were successfully isolated from a single-spore derived culture. Despite similar growth conditions in the greenhouse during the two cycles of single-spore derived culture multiplication, AMF strains behaved differently according to their natural site. Previous studies have shown that many factors significantly affect the multiplication of AMF strains, such as AMF strain-plant species compatibility, response to the target environment, competition with other soil microbes, soil type, and inoculation timing (Berruti et al., 2018; Selvakumar et al., 2018). In addition, the two multiplication cycles increased the inoculum volume of each AMF strain for morphological and molecular identification and had a suitable starter inoculum (Symanczik et al., 2014a; 2014b).

Following to the morphological and molecular analyses, five AMF species were recovered from traditional palm groves, two from non-agriculturally managed sites, and only one AMF species from modern palm groves. The low plant diversity of non-agriculturally managed sites and modern plantations might be the strongest driver of the low recovery rate of AMF species. Guzman et al. (2021), who assessed AMF communities across 31 field sites, observed that mixed culture systems favor richer and more diverse AMF communities than monoculture systems. Similarly, Dietrich et al. (2020) found that various plant mixtures maintained greater viability of AMF spores than monocultures. AMF spores from mixed culture systems showed higher colonization potential than AMF spores from monoculture systems. According to Johnson (1993), fertilization reduced colonization, propagule numbers, and the abundance of selected AMF species. Previous studies have indicated that the use of readily soluble fertilizers, particularly N, or the overuse of organic supplements, especially those high in P, such as chicken manure, can negatively impact AMF diversity and colonization (Gosling et al., 2006; Jordan et al., 2000; Oehl et al., 2004). Furthermore, modern sites are characterized by high percentages of organic matter compared to traditional groves and non-agricultural sites. Ou-Zine et al. (2021), who studied the same modern sites, explained the high rate of organic matter as due to the application of manure. Adding organic supplements to soils in the form of compost or manure has been shown to increase organic matter content (Grandy et al., 2002). As previously reported by Jiang et al. (2020), excessive fertilization with manure can negatively affect AMF species richness via the integrated effect of soil organic carbon on soil fertility. This finding agrees with Liu et al. (2017), who observed that soil organic carbon negatively impacted AMF richness.

Based on previous studies, all species described here have a worldwide distribution in Central Europe, tropical forests, New Caledonia, and Oman (Baltruschat et al., 2019; Błaszkowski et al., 2017; Marinho et al., 2018). In comparison with the works of Bouamri et al. (2006) and Sghir et al. (2014), which were conducted in the same region, the species discovered in this study were identified for the first time in Drâa-Tafilalet oases.

4.2. Inoculation experiment

In the inoculation experiment, the indigenous AMF consortium (*Septoglomus xanthium*, *Albahypha drummondii,* and *Pervetustus simplex* species) was able to colonize date palm roots in the early stage, namely the first acclimatization stage, irrespective of the fertilization practice. El Kinany et al. (2019) observed similar colonization frequencies of 35.3% when inoculating micropropagated date palms with a commercial AMF strain (*Glomus iranicum*) grown for 12 months in a substrate augmented with compost under greenhouse conditions. In addition, we observed lower root colonization rates in date palms that received compost than in those that received synthetic fertilizers. This is in accordance with the results of Douds et al. (2007), who found that compost applied at a ratio of 1:9 (v/v) resulted in lower AMF colonization rates than synthetic fertilization. The amount of P added via compost (2.17 g P per pot) compared to P added via synthetic fertilizers (0.3 g P per pot) might have exceeded a critical threshold limiting AMF root colonization, as already observed by Anli et al. (2020). Their results showed that colonization of date palm roots by AMF was inhibited when plants were supplemented with a compost dosage of 20% compared to that in plants supplemented with or without a compost dosage of 5%. Furthermore, synthetic fertilizer P might have been immobilized directly after mixing with sandy soil, as observed previously by Devau et al. (2011), which might have reduced plant-available P, counteracting the inhibitory effect of mineral P on AMF root colonization. Several studies have shown that a high soil available P content can decrease the root colonization potential of AMF (Del Mar Alguacil et al., 2010; Allison and Goldberg, 2002; Lekberg and Koide, 2005).

The differences in shoot length and stem diameter were evident 18 months after inoculation. The treatment that combined indigenous AMF and compost yielded the best results. Similarly, El Kinany et al. (2019) observed that compost and commercial AMF improved the growth and nutrition of micropropagated date palm plants grown under greenhouse conditions. Several studies have demonstrated the importance of the use of compost and AMF (indigenous or commercial) for plant growth in several plant species, such as *Solanum lycopersicum*, *Argania spinosa*, *Triticum aestivum*, and *Medicago polymorpha* (Akhter et al., 2015; Akhzari et al., 2015; Jan, 2014; Mrabet al., 2014). Our results highlight the synergetic effects of AMF and compost on the growth performance of micropropagated date palms during the first acclimatization stage.

5. Conclusion

AMF is considered an alternative to synthetic fertilizers for developing eco-friendly approaches to agriculture. Therefore, we aimed to isolate and multiply AMF present at different sites in the Drâa-Tafilalet region (Morocco) using trap culture and single-spore culture. In this study, morphological and molecular tools were used for the first time to

identify 13 AMF species derived from a single-spore culture in the Drâa-Tafilalet region (Morocco). The collected soil colonization potential and sporulation significantly increased in trap culture by using three trap plant species and favorable growing conditions and differed between sampling sites. In addition, more AMF species were recovered from traditional date palm groves than from modern date palm groves, most likely owing to the higher plant diversity in traditional date palm groves. The inoculation experiment highlighted the potential of cultivated AMF species to colonize date palm roots in the first acclimatization stage while simultaneously enhancing their growth in the long term. Thus, our results suggested that the combined application of compost and indigenous AMF strains during acclimation could effectively accelerate the growth of micropropagated date palms to reduce the cultivation time under nursery conditions and thus reduce cultivation costs. The identified strains constitute an AMF culture collection that will be multiplied on a large scale for further use in *vitro* and *in vivo* experiments.

Authors contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by all authors. The first draft of the manuscript was written by **Rania EL HILALI** and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rhisph.2022.100521.

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