Pistacia atlantica, a spontaneous hypermycotrophic phanerophyte: could be a natural tool to enhance the potential of mycorrhizal infectivity (PMI) of soils in arid regions?

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Pistacia atlantica, a spontaneous hypermycotrophic phanerophyte: could be a natural tool to enhance the potential of mycorrhizal infectivity (PMI) of soils in arid regions?

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Abstract. In our drylands, spontaneous perennials species should be used to play the role of facilitator species in the success of plurispecific agro-ecosystems. It's the case of Atlas pistachio (the main spontaneous phanerophyte in Algerian pastoral steppe). The mycorrhizal status of this species could potentially increase the potential of mycorrhizal infectivity (PMI) of soils in drylands and benefit these poor soils by its rhizospheric effect. In Algeria, we have chosen for this study, two populations of *Pistacia atlantica*. The first one is located in semi-arid region (province of Médéa) and the other in hyper arid region (province of Béchar). We found that all the roots of the studied samples were infected by Arbuscular Mycorrhizal Fungi (AMF). Identification of their spores showed that Atlas pistachio is a hypermycotrophic species. At least we found 5 different species of Glomeromycetes in the first population (Médéa) and 3 different species in the second one (Béchar). It may be a reservoir of AMF propagules which will potentially infect cultivated species and thus will enhance their yields.

Key words. Arid soils – PMI – *Pistacia atlantica* – Rhizospheric effect – AMF – Glomeromycetes – Propagules – Algeria.

Pistacia atlantica, phanérophyte spontanée hypermycotrophe : peut-être un outil naturel pour améliorer le potentiel infectieux mycorhizogène des sols des régions arides ?

Résumé. *Dans nos zones arides, les espèces pérennes spontanées doivent être utilisées pour jouer le rôle d'espèces facilitatrices dans le succès des agroécosystèmes plurispécifiques. C'est le cas du pistachier de l'Atlas, espèce par excellence de la steppe pastorale algérienne. Le statut mycorhizien de cette essence est potentiellement prometteur pour augmenter le potentiel infectieux mycorhizogène (PIM) des sols arides et bénéficier de l'effet rhizosphérique sur ces sols indigents. En Algérie, nous avons choisi pour étude, deux populations situées, l'une en milieu semi-aride (Médéa) et l'autre en milieu hyperaride (Béchar). Nous avons constaté que toutes les racines des échantillons étudiées sont endomycorhizées par des Glomeromycètes. L'identification de leurs spores a montré que le pistachier de l'Atlas est une espèce hypermycotrophe (au moins : 5 espèces différentes chez la population en semi-aride et 3 espèces différentes chez celle en milieu hyperaride). Il pourra être un réservoir à propagules d'AMF qui s'associera potentiellement aux espèces cultivées et améliorerai ainsi leurs rendements.*

Mots-clés. *Sols arides – PIM –* Pistacia atlantica *– Effet rhizosphérique – AMF – Glomeromycetes – Propagules – Algérie.*

I – Introduction

Among the microbial components involved in soil biofunctioning, mycorrhizal fungi are considered as major elements in the soil / plant interface (Duponnois *et al,*. 2012). In fact, their key roles are the mobilization of soil nutrients that have low mobility, especially phosphorus (Duponnois *et al*., 2005a; Lambers *et al*., 2008); improving plant hydration (Augé, 2001); and the reduction or even total inhibition of the negative effects of some pathogenic agents (Smith and Read, 2008).The colonization of the soil by extramatrical mycelium and the production of a glycoprotein (glomalin) by mycorrhizal hyphae generate better soil structure by forming more stable aggregates (Lovelock *et al*., 2004; Rillig and Mummey, 2006).These fungi promote coexistence between different plant species, improving productivity and plant biodiversity in the ecosystems where they are present (van der Heijden *et al*., 1998 a,b; Sanon *et al*., 2006; Kisa *et al*., 2007).The presence of mycorrhizal plants can act as a reservoir of mycorrhizal propagules, and thus should be a very effective means of ensuring the establishment of young regeneration by facilitating the infection of seedlings, and thus their survival in these often-hostile environments (Newman, 1988; Simard and Durall, 2004).

In the arid regions of Algeria, there exists one of the rare spontaneous phanerophytes which could allow a natural approach to increasing the potential of mycorrhizal infectivity (PMI) of those soils: it is the Atlas pistachio (*Pistacia atlantica* subsp. *atlantica*). This phanerophyte colonizes disparate habitats, constituting an important metapopulation which ranges from the Mediterranean coast to the heart of the Hoggar (in the extreme south of the Algerian Sahara), where some old individuals are regarded almost as relics (Monjauze, 1967).

The aim of this work is to try to establish for the first time (to our knowledge), the mycorrhizal status (root colonization and spores) of Atlas pistachios belonging to two spontaneous populations: one located in a semi-arid environment, and another located in a hyper-arid environment.

II – Materials and methods

We sampled two spontaneous populations of Atlas pistachio, one located in Sidi Naamane (SN) in the province of Medea and the other in Beni Ounif (BO) in the province of Béchar. These two populations are situated on a gradient of increasing climatic and edaphic aridity (Table 1).

We took samples of roots with diameter less than 1 cm from the 0-20 cm soil level, along with their ramifications and their rhizospheric soil. We sampled six individuals from the SN population and six individuals from the BO population. In the laboratory, we gently released the roots from their rhizospheric soil. Using a digital caliper, we selected fine roots of less than 1 mm of diameter. The rhizospheric soil collected was admixed to form two composite samples: one for SN and one for BO.

Table 1. Climatic data of the two stations

Stations	P mm/year	T (°C) (average annual)	PET mm/year	AI	Ecoclimatic zonation (UNEP, 1992)	LDS (Bagnouls and Gaussen, 1953)
Sidi Naamane (Médéa)	628	15	1597	0.39	Semiarid	4.5
Béni Ounif (Béchar)	76	22	2366	0.03	Hyper arid	12

P: Precipitations; T: Temperatures; PET: Potential Evapotranspirational; AI: Aridity Indices; LDS: Longer of Dry Saison.

The roots of the Atlas pistachio are very dark because of their high tannin content. Therefore, we have adapted the protocol of Brundrett *et al*. (1996) by increasing the bleaching time to as much as 8 days for the darkest samples. After rinsing roots with tap water to remove the fixative solution (formalin), we separately placed root samples of each station in heat-resistant containers. The selected roots were completely immersed in a solution of KOH (10% w / v) and baked at 90°C for one hour. After that, we left the roots immersed in the KOH overnight. The next day, we replaced the now-brown KOH with fresh solution, and began a new cycle of bleaching. This process was repeated until the roots became decoloured. The bleached roots were immersed in a 2% HCl solution for 30 minutes to neutralize the KOH. After rinsing with tap water, we put the roots in a trypan blue solution (0.05% w / v in lactoglycerol) and baked at 90°C for 4 hours. The roots were then preserved in a solution of 50% glycerol. The prepared slides were observed using an optical microscope.

The spores were separated from the rhizospheric soil by a wet-sieving and decanting technique (Gerdemann and Nicolson, 1963). Under a stereomicroscope, the spores were manually extracted using fine forceps and micropipettes, and sorted by morphotypes. They were then transferred to microscope slides and covered with PVLG. The same steps were performed for the mounting of spores in the PVLG-Melzer (1: 1). Under the microscope, we measured their diameters, examined their shapes and when possible, their walls, suspending hyphae, and ornamentation, using the identification keys from Blaszkowski (2012).

III – Results and discussion

No ectomycorrhizae were detected in either sample population. However, all of the roots presented abundant endomycorrhizae (Fig. 1).

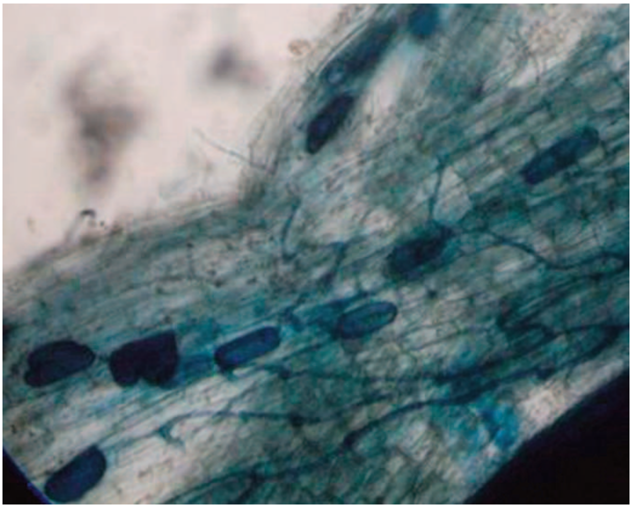

Fig. 1. *Pistacia atlantica* root colonisation by AM fungi (x 100).

The examination of spores allowed the identification of 4 species belonging to *Glomus* genus and one belonging to Scutellospora genus in the rhizospheric soil of the SN sample. In the rhizospheric soil of the BO sample, we identified 3 species from *Glomus* genus and 2 unidentified morphotypes (Figs. 2, 3, 4 and 5).

Despite the disparity between the sampled habitats (semi-arid for SN and hyper-arid for BO), these results show that the Atlas pistachio is a hypermycotrophic species (a plant associated with abundant and diverse mycorrhizal fungi). Therefore, it has the ability to promote the growth of fungal symbionts and may constitute an AMF propagules reservoir likely to be associated with cultivated plants, as well as improved crop yields (Duponnois and *al*., 2012).

The Atlas pistachio is naturally adapted to these arid environments due in part to its highly flexible and efficient root system (Limane *et al*., 2014). Its roots can reach 6 meters deep (Monjauze, 1968), and can expand horizontally to more than 12 meters (personal data). With such a large volume of soil influenced, it is able to increase the PMI of these soils. It should thus be considered as a native facilitative tool for integration in agro-ecosystems, especially in arid environments.

Fig. 2. **Spore of *Glomus* sp. 1 mounted in PVLG.**

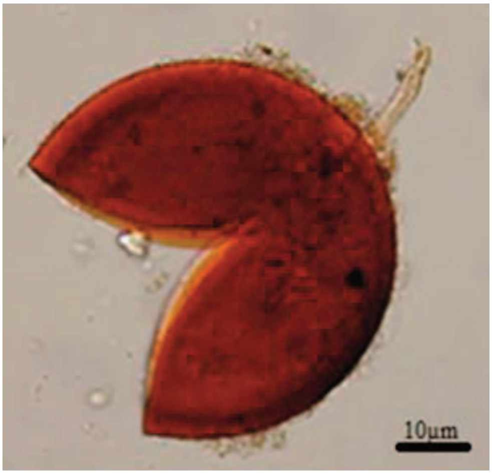

Fig. 3. **Spore of *Glomus* sp. 2 mounted in PVLG+Melzer.**

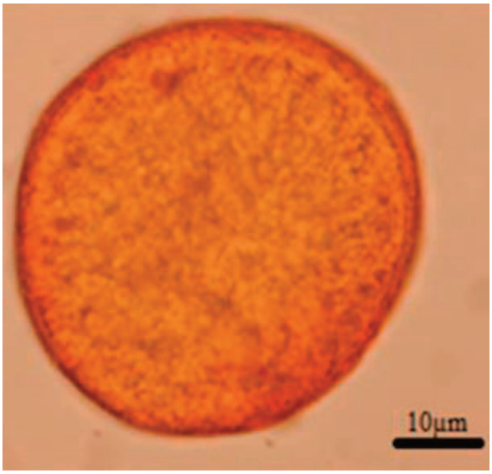

Fig. 4. **Spore of *Scutellospora* sp. mounted in PVLG.**

Fig. 5. **Spore of *Glomus* sp. 3 mounted in PVLG.**

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