

Seedlings responses to arbuscular mycorrhizal inoculation of the Atlas Cypress, *Cupressus atlantica* Gaussen, from various Moroccan Western High Atlas origins

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Photo 1.
Monumental Atlas Cypress Tree in Mzouzit station.
Photo L. Ouahmane.

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RÉSUMÉ

Réponses des plantules du cyprès de l'Atlas, *Cupressus atlantica* Gaussen, à l'inoculation de mycorrhizienne en provenance de diverses origines de l'ouest du Haut Atlas au Maroc

Le cyprès de l'Atlas, *Cupressus atlantica* Gaussen, est endémique stricte de l'ouest du Haut Atlas marocain. Cette espèce joue un rôle écologique et socio-économique très important dans son aire de distribution : pour produire du bois de feu, aider à protéger les sols contre l'érosion, et comme plante médicinale chez les populations locales. Malheureusement, subissant diverses pressions, principalement le surpâturage et les activités humaines excessives, l'espèce souffre d'une détérioration alarmante et de perte de territoire. La variabilité des champignons mycorrhiziens arbusculaires et sa réponse à l'inoculation mycorrhizienne des plantules sous serre ont été étudiées pour différentes provenances. Douze populations naturelles représentant l'aire de distribution du cyprès de l'Atlas ont été sondées, à Adebdi, Aghbar, Allous, Idni, Ighil, Ighzer, Mzouzit, Rikt, Tiouna, Taghzout, Talat N'os and Taws. L'analyse des variations des champignons mycorrhiziens associés aux racines de cyprès de l'Atlas ont révélé la présence d'au moins sept morphotypes appartenant aux genres *Rhizophagus*, *Glomus*, *Gigaspora* et *Acaulospora*. Le taux de colonisation mycorrhizienne dans les racines de cyprès de l'Atlas a été examiné dans son milieu naturel, montrant une variation de 25 % à 45 %. Dans les conditions sous serre, la croissance des plantules, avec ou sans inoculation avec un complexe mycorrhizien, a été surveillée pour les plantules provenant des douze lieux échantillonnés. Les résultats montrent qu'en absence des champignons bénéfiques dans le substrat de culture, la croissance et la nutrition des plantules ont été hautement dépendantes de l'origine des graines. En revanche, en présence du champignon dans le substrat de culture, les paramètres de croissance et de nutrition des plantules des douze provenances ne différaient pas de manière significative. Deux groupes principaux ont été identifiés par l'analyse en composantes principales et la classification ascendante hiérarchique basées sur les caractères analysés. Le premier groupe comprend les populations de la vallée de N'Fis, à savoir Allous, Idni, Ighil, Ighzer, Mzouzit, Rikt, Talat N'os et Taws, alors que le second groupe est composé des populations du côté sud, Aghbar, Adebdi, Taghzout et Tiouna.

Mots-clés : *Cupressus atlantica*, *Rhizophagus*, *Glomus*, *Gigaspora*, *Acaulospora*, cyprès de l'Atlas, spores de champignons mycorrhiziens, colonisation des racines, croissance des plants, Maroc.

ABSTRACT

Seedling responses to arbuscular mycorrhizal inoculation of the Atlas cypress, *Cupressus atlantica* Gaussen, from various Moroccan Western High Atlas origins

The Atlas cypress, *Cupressus atlantica* Gaussen, is a strict endemic of the Moroccan Western High Atlas. The species plays a very important ecological and socio-economic role in its area of distribution: it is used in the production of firewood, helps to protect soils against erosion and is also used as a medicinal plant by the local population. Unfortunately, due to various pressures, mainly overgrazing and overuse by humans, the species is experiencing alarming deterioration and loss of territory. The variability of arbuscular mycorrhizal fungi in Atlas cypress of different origins and the response of its seedlings to mycorrhizal inoculation in greenhouse conditions were studied. Twelve natural populations representing the distribution area of the Atlas cypress were surveyed, at Adebdi, Aghbar, Allous, Idni, Ighil, Ighzer, Mzouzit, Rikt, Tiouna, Taghzout, Talat N'os and Taws. The analysis of variations in the mycorrhizal fungi associated with the roots of the Atlas cypress has shown the presence of at least seven morphotypes belonging to the *Rhizophagus*, *Glomus*, *Gigaspora* and *Acaulospora* genera. The rates of mycorrhizal colonization in the roots of the Atlas cypress examined in its ecosystem varied from 25% to 45%. In greenhouse conditions, the growth of seedlings from seeds originating in the twelve locations was monitored with and without inoculation of a mycorrhizal fungal complex. The results show that in the absence of these beneficial fungi in the culture substrate, the growth and nutrition of the seedlings was highly dependent on the origins of the seeds. However, in the presence of mycorrhizal fungi in the culture substrate, the growth and nutrition parameters for the seedlings from the twelve populations did not show significant differences. Two main groups were identified by principal component analysis and hierarchical classification based on all the characters analyzed. The first comprises the populations of the N'Fis valley, namely Allous, Idni, Ighil, Ighzer, Mzouzit, Rikt, Talat N'os and Taws, while the second group is made up of the populations from the southern side, namely Aghbar, Adebdi, Taghzout and Tiouna.

Keywords: *Cupressus atlantica*, *Rhizophagus*, *Glomus*, *Gigaspora*, *Acaulospora*, Atlas cypress, mycorrhizal fungi spores, root colonization, seedling growth, Morocco.

RESUMEN

Respuesta a la inoculación de micorriza arbuscular en el plantío de ciprés del Atlas, *Cupressus atlantica* Gaussen, proveniente de varios lugares del alto Atlas occidental marroquí

El ciprés del Atlas, *Cupressus atlantica* Gaussen, es estrictamente endémico del alto Atlas occidental marroquí. La especie juega un rol ecológico y socioeconómico muy importante en esta área de distribución: se utiliza en la producción de leña, contribuye a la protección de suelos frente a la erosión y la población local también la utiliza como planta medicinal. Por varios motivos, principalmente el sobrepastoreo y la actividad humana excesiva, la especie sufre un deterioro alarmante y una pérdida de territorio. Se estudió la variabilidad del hongo micorriza arbuscular para diferentes orígenes del ciprés del Atlas, así como la respuesta de las plantaciones a la inoculación de micorriza en condiciones de invernadero. Se realizó un seguimiento de doce poblaciones naturales que representan el área de distribución del ciprés del Atlas: Adebdi, Aghbar, Allous, Idni, Ighil, Ighzer, Mzouzit, Rikt, Tiouna, Taghzout, Talat N'os y Taws. El análisis de la variación del hongo micorriza asociado a las raíces del ciprés del Atlas ha mostrado la presencia de al menos siete morfotipos pertenecientes a los géneros *Rhizophagus*, *Glomus*, *Gigaspora* y *Acaulospora*. Las tasas de colonización de micorriza en las raíces examinadas del ciprés del Atlas en su ecosistema varían entre el 25 % y el 45 %. En condiciones de invernadero, el crecimiento de los plantíos de semillas originales de las doce ubicaciones estudiadas se monitorizó con o sin inoculación de complejo fúngico micorrízico. Los resultados muestran que, en ausencia de estos hongos benéficos en el sustrato de cultivo, el crecimiento y nutrición del plantío tenía una fuerte dependencia con el origen de las semillas. Sin embargo, con la presencia de hongos micorrízicos en el sustrato de cultivo, los parámetros de crecimiento y nutrición entre plantíos originarios de las doce poblaciones no mostró diferencias significativas. El análisis del componente principal y la clasificación basada en todos los caracteres analizados permitieron identificar dos grupos principales. El primer grupo incluye las poblaciones del valle N'Fis, Allous, Idni, Ighil, Ighzer, Mzouzit, Rikt, Talat N'os y Taws; mientras que el segundo grupo incluye las poblaciones del lado sur, concretamente Aghbar, Adebdi, Taghzout y Tiouna.

Palabras clave: *Cupressus atlantica*, *Rhizophagus*, *Glomus*, *Gigaspora*, *Acaulospora*, ciprés del Atlas, esporas de hongos micorrízicos, colonización de raíces, crecimiento de plantío, Marruecos.

Introduction

Mycorrhizal symbioses are key elements in natural systems (Brundrett *et al.*, 1991). They play a major role in water absorption as well as in the uptake of important nutrients (Kormanik *et al.*, 1982; Fontana, 1985; Strullu, 1990). They also contribute to the plants' protection against microbial pathogens (Whipps, 2004; Pozo *et al.*, 1999) while improving multi-trophic interactions within the soil. The symbiotic relationship between trees and their mycorrhizal fungi thus contributes to the establishment of the forest ecosystem and to its longevity. The climate in the Mediterranean regions is characterized by long periods of drought and hot summers; precipitations are rare, irregular and often times torrential (López-Bermúdez and Albaladejo, 1990; Vallejo *et al.*, 1999). These conditions render Mediterranean ecosystems particularly precarious and highly sensitive to degradation; this in turn causes the loss or reduction of mycorrhizal diversity in the soil and the decrease of the mycorrhizal potential in the affected areas (Mc Lellan *et al.*, 1995). As a result, the restoration of native plants in these regions is compromised and subject to serious limitations (Requena *et al.*, 2001). The importance of mycorrhizal fungi in the preservation of biodiversity in forest ecosystems is indeed undeniable (Van der heijden *et al.*, 1998). Their ability to control the productivity and diversity of the seedlings has a direct effect on the diversity of plant communities (Burrows and Pflieger, 2002; Kernaghan *et al.*, 2003). This mutual influence between the plant and fungal communities can play a fundamental role in the determination of the existing diversity of both plants and fungi. Mycorrhizal symbioses affect the maintenance of plant density in natural habitats (Requena *et al.*, 2001; Peterson *et al.*, 1985) and modify the structure and function of plant communities (Douds and Millner, 1999); they are essential indicators of the evolution of ecosystems.

Mycorrhizal fungi play the role of "ecosystem engineers" and can be used for the improvement of the productivity of natural and anthropized ecosystems. However, the production of high quality plants with high nutritional status and resistance to biotic and abiotic stressors through the introduction of selected mycorrhizal fungi can be challenging. To circumvent these limitations, mycorrhizal fungi can be used in combination with culture substrate in forest nurseries which would allow the production of vigorous forest seedlings capable of resisting in reforestation perimeters and withstanding transplantation choc (Caravaca *et al.*, 2005; Rincón *et al.*, 2006; Ouahmane *et al.*, 2006). Indeed, in the Atlas Cypress, the rates of reintroduction of young plants are mediocre, which leads to increased costs of reforestation. These reforestation costs are further exacerbated by soil constraints: the soils are highly calcareous, with low mineral concentration and very little water content. These difficult environmental conditions have a direct impact on the Atlas Cypress seedling mortality. To alleviate these constraints, multiple strategies can be employed to improve success rates, one of which would be the mycorrhization of the Atlas Cypress seedlings in forest nurseries. However, to ensure the success of the use of beneficial fungi in an artificial inoculation program, the

characterization and proper selection of the mycorrhizal fungi is of utmost importance (Francis and Thornes, 1990; Vallejo *et al.*, 1999). Therefore, as a first step in the controlled mycorrhization of the Atlas Cypress, the population of mycorrhizal fungi associated with the Atlas Cypress in its natural habitat is analyzed in order to select the fungi capable of improving the quality of Cypress seedlings. The study of the mycorrhizal fungi associated with the Atlas Cypress will focus on the evaluation of the mycorrhizal infectivity potential of the soils and the identification of the different species encountered. The characterization of these populations is essential to the implementation of a plant improvement program in nurseries (Borchers and Perry, 1990; Miller *et al.*, 1992; Roth and Berch, 1992; Ouahmane, 2007).

This study had the following objectives: (i) to estimate the diversity of mycorrhizal fungi associated with the roots of *Cupressus atlantica* in twelve different soils where the plant is naturally found; (ii) to estimate the variability in forest nurseries of the plants' response to mycorrhizal inoculation of young seedlings from seeds collected from the twelve different origins.



Photo 2.
Spores of Arbuscular Mycorrhizal fungi in soil under the Atlas Cypress tree.
Photo L. Ouahmane.

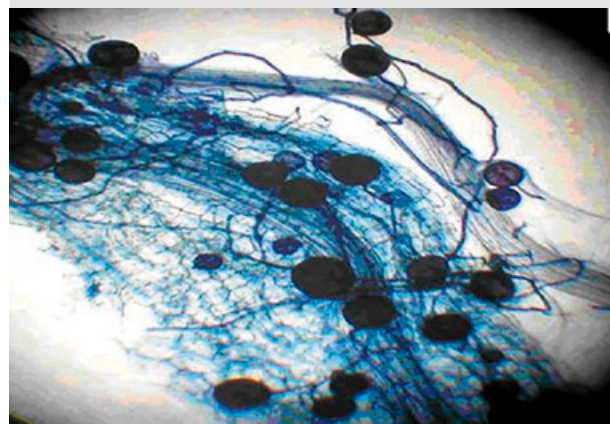


Photo 3.
Atlas Cypress roots colonized by Arbuscular Mycorrhizal Fungi.
Photo L. Ouahmane.

Materials and methods

Seed collection

Atlas Cypress cone collection was conducted for 12 natural populations, representative of the natural distribution, in September 2010. Composite samples of cones from terminal branches of 10 different trees were randomly selected in each of the 12 origins: Adebdi, Aghbar, Allous, Idni, Ighil, Ighzer, Mzouzit, Rikt, Tiouna, Taghzout, Talat N'os and Taws (table I).

Soil sampling

For each origin, 10 kg of rhizospheric soil were randomly collected at depths varying between 10 cm and 40 cm. In total, 10 samples were collected for each origin of the Atlas Cypress. The diversity and abundance of mycorrhizal fungi associated with the Atlas Cypress were evaluated. In the same samples, roots were examined in order to estimate the extent of root infection by the mycorrhizal fungi.

Extraction and identification of the populations of mycorrhizal fungi in the soil

The extraction of spores from the collected soils was conducted in two steps; the first involves the isolation of spores from the soil through a water sieving and decantation technique (Gerdemann and Nicholson, 1963) whereas the second involves the use of centrifugation in a 65% saccharose solution to isolate the spores (Brundrett *et al.*, 1985).

Spores isolation

After thorough homogenization of each soil specimen, 100 g were screened through a series of sieves (800 µm and 50 µm) under water jets. The resulting sample was transferred to a 200 mL beaker, the beaker was filled with water and the solution was allowed a 15 min. sedimentation time after which the supernatant and floating particles were eliminated. The soil-water solution was centrifuged at 3000 rpm for 5 min., the supernatant was removed and replaced by a 65% saccharose solution (w:v) and the resulting mixture of sediments and saccharose was once again centrifuged at 1000 rpm for 3 min. The supernatant was filtered under vacuum filtration using Whatman filters. The spores were then isolated using a thin brush under a binocular magnifier and carefully washed then preserved in physiological liquid. The obtained spores were examined and counted under a binocular magnifier at 40x and the different morphotypes were classified and identified at the species level and for some, at the genus level. The isolated spores were conserved in polyvinyl-lacto-glycerin (PVLG) which allows the preservation of the spores' initial features, their long term conservation and better visualization of the spore walls (Estaun *et al.*, 1997).

Identification of arbuscular mycorrhizal fungi

The spores were separated under binocular magnification according to the phenotypic characteristics: color, shape, consistency, attachment to hyphae. The diameters of the spores were measured under a microscope at 40x magnification along with the thickness of the wall and the attached hyphae. The measurements were conducted with a microscope connected to digital image processing software and the essential characteristics of the spores and associated hyphae were evaluated: spore wall thickness, lamellar structure, shape of the attachment zone between the spore and hypha, thickness of the hyphae and its wall structure. Direct count of the number of spores in 100 g of soil was also conducted in order to estimate their abundance in the soil as well as the relative abundance of each spore type in relation to the total number of spores.

In order to identify the fungi, about 20 spores of each morphotype recovered from the collected soils were placed on a microscope slide with a cover slip. Half was placed in a 1:1 volume mixture of PVLG and Melzer reagent, while the other half was placed in PVLG. The original descriptions of the species as well as the descriptions available on the INVAM website (1997) served as a reference for species identification. The morphological characteristics of the spores were compared to the type specimens and reference strains.

Determination of the infection rate of the Atlas Cypress roots by mycorrhizal fungi

In order to clearly determine the presence of endomycorrhizal structures, the cellular contents of the roots were eliminated and stained with a specific coloring agent such as acid Fuchsin or Trypan blue. The finest roots from each origin were mixed and distributed into four batches that would each be used to prepare one slide. To test for the presence of an endomycorrhizal infection, the roots of the collected

Table I.
Geographical characteristics of the different Atlas Cypress provenances.

Collection site	Latitude/ Longitude	Altitude (m)
Adebdi	N 30°50' / W 08°29'	1300
Aghbar	N 30°55' / W 08°22'	1750
Alouss	N 30°59' / W 08°19'	1650
Idni	N 30°54' / W 08°17'	1600
Ighil	N 30°57' / W 08°15'	1315
Ighzer	N 30°55' / W 08°17'	1551
Mzouzit	N 30°57' / W 08°14'	1280
Rikt	N 30°59' / W 08°11'	1173
Tiouna	N 30°52' / W 08°53'	1581
Taghzout	N 30°54' / W 08°51'	1839
Talat N'os	N 31°50' / W 08°08'	1150
Taws	N 30°56' / W 08°16'	1391

plants were colored according to the Phillips and Hayman method (1970). For each origin of the Atlas Cypress, fine roots were collected from 10 randomly selected trees at 2 m from the trunk basis, the minimal distance between selected trees was fixed beforehand to at least 100 m. The Atlas Cypress roots were easily recognized thanks to their very high abundance and their horizontal distribution under the tree canopy. After thorough washing, the roots were placed in 10% Potassium hydroxide solution (KOH) for 60 min. at 90°C; this allows the plant cells to be rid of their cytoplasm and pigments. The roots were then washed, placed in 5% lactic acid solution in order to neutralize the remaining potassium hydroxide solution then rewashed and rinsed in room temperature water. The roots were then washed with distilled water and placed in 0.05% Trypan blue solution diluted in lactoglycerol (1/3 water, 1/3 glycerol, 1/3 lactic acid) for 15 min. at 90°C. After cooling, the roots were rinsed then conserved in lactoglycerol solution. Fungal structures such as arbuscules, vesicles and mycelia, were then blue colored. Roots were cut into about 1 cm in length fragments and two squash slides were prepared from 40 randomly selected fragments. The slides were then observed under a microscope at 40x. By counting the number of fragments displaying arbuscules and/or vesicles in relationship to the total number of fragments, infection rate was estimated. In order to estimate the endomycorrhizal colonization of the roots, the method described by Trouvelot *et al.* (1986) was employed. Overall, we have estimated infection parameters such as mycorrhizal infection frequency, defined as the percentage of mycorrhized root fragments relative to the total number of observed fragments, and the intensity of the infection developed by the entire root system.

Production of the inoculum

The arbuscular mycorrhizal inoculum production was conducted in a glasshouse. Maize (*Zea mays* L.), a highly mycotrophic plant, was used as an endophyte for the production of endomycorrhizal inoculum for the indigenous mycorrhizal complex naturally associated with the Atlas Cypress. Maize seeds were sterilized in 30% oxygenated water for 30 min. then rinsed multiple times with distilled water. They were then set for germination in plastic pots containing 1.5 kg of rhizospheric soil collected around the roots of Atlas Cypress in the 12 origins, the pots were watered regularly. The mycorrhization status of the trapped plants was then verified under a binocular magnifier through nondestructive observation at 40x.

Seed pregermination and seedlings inoculation

The Atlas Cypress cones (*Cupressus atlantica* Gaussen) were collected from the 12 origins during the month of September. The seeds were then extracted and separated according to their origin, disinfected in 90% bleach solution diluted to 50% for 3 to 4 min., thoroughly rinsed, then placed in sterile water for 30 min. They were then subjected to scarification pretreatment. The pretreated seeds were then placed in Petri dishes (30 seeds per dish) containing

filter paper washed with 5 mL of sterile water. The petri dishes were then incubated for 10 to 15 days in the dark at 20°C, which is considered to be the species' optimal temperature for germination (Bechir, 2004). After germination had occurred and rootlets were visible, the pregerminated seeds were planted in pots. After 3 months of growth, the Maize roots were harvested and disinfected in a mixture of chloramine T (2%) and streptomycin 0,2 g/L (Mosse, 1973) then rinsed with sterile distilled water. They were then cut into 1 cm fragments and used as inoculum for young seedlings of the Atlas Cypress. The Maize root fragments used for inoculation had a 75% mycorrhization rate according the method developed by Trouvelot *et al.* (1986) and contained approximately 250 vesicles/cm. Once the rootlets had reached length of 1 to 2 cm, the seeding was conducted in plastic pots (1 L volume) containing a mixture of sand and peat with a 2:1 volume ratio. The sand was run through a screen with a mesh size of 5 mm then covered with water to which 1 L of commercial bleach solution was added and left for 3 days. The sand was then thoroughly rinsed then sterilized for 3 hours in an autoclave at 120°C; the peat was also autoclaved for 3 hours at the same temperature. The inoculation of pregerminated seeds was done with the addition of 1 g of Maize root fragments in the dip (1 cm x 5 cm) where the pregerminated seeds were sown. The control plants that did not receive the inoculum and the pregerminated seeds were placed directly on the substrate with the addition of fungi. The seedlings were grown in a greenhouse at a temperature of 27 ± 2°C, a 65% relative humidity and a photoperiod of 16/8 (day/night). They were watered regularly without fertilizers addition. The trials were conducted using a randomized block design with 40 repetitions per treatment.

Dendrometric parameters

During seedlings growth under greenhouse conditions, dendrometric parameters were measured: the height of the different resulting seedlings from the bottom of the crown to the apex, and the diameter of the stem. After six months of culture, the shoot dry biomass (SDB) and the root dry biomass (RDB) were reported. The dry weights were measured after the drying process was conducted in a heat chamber at 65°C for 72 hours.

Mineral nutrition of the Atlas Cypress seedlings: N, P, K

Three mineral elements were used in the analysis of the nutritional effect of the mycorrhizal fungi on the growth of the Atlas Cypress. Considering its importance as limiting factor in plant production, the total phosphorus in the seedlings was measured by applying a colorimetric method after calcination of the Atlas Cypress plants using Barton reagent containing ammonium molybdate and ammonium metavanadate (NH_4VO_3). The reading was done using a spectrophotometer at 820 nm with the use of a standard range using Potassium dihydrogen phosphate (KH_2PO_4). The total nitrogen was measured using the Kjeldahl method and the potassium was measured in the mineral solution obtained from the calcination using a colorimetric technique and flame emission spectrometry.

Statistical analysis

In order to compare the heterogeneity of the different parameters measured in the population, a variance analysis (ANOVA) was conducted for each factor using Windows SPSS software (version 12.0). The averages were compared using the Newman Keuls test with 0.05 probability threshold for each variance analysis series. For each morphological characteristic, the correlations between the averages of the populations were analyzed using a correlation table. In order to understand the overall relationships within the population, a principal component analysis (PCA) was conducted associated with an Ascending Hierarchical Classification (AHC). A Pearson 'chi-squared test' was used to check the dependence between Mycorrhizal frequency and Mycorrhizal colonization of roots from different origins.

Results

Diversity of mycorrhizal fungi

In the analysis of the diversity of mycorrhizal fungi associated with the Atlas Cypress in the twelve surveyed origins, seven taxa were identified. *Rhizophagus aggregatus* was the dominant species in this study followed by *Rhizophagus fasciculatus*, *Rhizophagus intraradices*, and *Rhizophagus manihotis* (*Rhizophagus clarus*), three other unidentified species belonging to the *Glomus*, *Gigaspora* and *Acaulospora* genera were also encountered. The number of spores per 100 g of soil varied between 100 spores at the Talat N'os station and 608 spores at the Mzouzit station (table II). Additionally, it was determined that each of the

six sites Aghbar, Allous, Idni, Ighil, Ighzer and Mzouzit, were home to seven species, the Rikt site to six species whereas, the Taghzout and Tiouna sites were home to five species. Four mycorrhizal species were found at the Adebdi and Talat N'os sites and only three species were found in the Taws site which had the lowest number of mycorrhizal species encountered in this study (table III). Dominance analysis had shown that the species relative importance was similar in soils of the major of provenances except for the Mzouzit station which presents in addition the higher total number of spores/100 g soil (table III). Furthermore, this same result was noted when analyzing the equitability that concerned distribution of individuals per species (table III).

Root mycorrhizal colonization

Examination of the roots of Atlas Cypress trees from the different locations has shown that 35% of the examined roots from the Tiouna site display mycorrhizal infections, at the Allous site, 70% of the roots were infected. On average, 55% of the examined roots from all origins showed signs of mycorrhizal infection. In order to estimate the total volume occupied by the fungi in the roots, mycorrhizal infection intensity was estimated through microscopic observation of the specifically stained root fragments. The results indicate that roots from the Talat N'os sites show 20% colonization intensity, whereas roots from the Rikt and Ighil sites had a colonization intensity of 45%, the average value for all 12 sites was 32.2%. Nevertheless the Pearson 'chi-squared test' had shown no dependence between Mycorrhizal frequency and Mycorrhizal colonization of roots from different origins (table IV).

Table II.

Abundance and diversity of arbuscular mycorrhizal fungi in the rhizospheric soils of the Atlas Cypress trees from twelve provenances. In the same column, numbers followed by the same letter are not considered to be significantly different according to the Newman and Keuls method with a 0.05 threshold.

Sites	<i>Rhizophagus aggregatus</i>	<i>Rhizophagus fasciculatus</i>	<i>Rhizophagus intraradices</i>	<i>Rhizophagus clarus</i>	<i>Glomus</i> sp.	<i>Gigaspora</i> sp.	<i>Acaulospora</i> sp.	Total number of spores/100g soil
Adebdi	64 ^d	92 ^a	0 ^f	0 ^d	160 ^a	0 ^d	4 ^b	320
Aghbar	24 ^e	104 ^a	36 ^d	8 ^c	48 ^b	48 ^a	8 ^b	276
Allous	96 ^c	48 ^c	24 ^e	20 ^b	32 ^b	52 ^a	8 ^b	280
Idni	124 ^c	92 ^a	52 ^c	24 ^b	24 ^b	36 ^a	12 ^a	364
Ighil	112 ^c	80 ^b	72 ^b	24 ^b	8 ^c	12 ^c	8 ^b	316
Ighzer	44 ^d	52 ^c	136 ^a	32 ^a	28 ^b	32 ^a	16 ^a	340
Mzouzit	424 ^a	32 ^c	60 ^b	44 ^a	32 ^b	4 ^c	12 ^a	608
Rikt	172 ^b	76 ^b	44 ^c	12 ^c	8 ^c	8 ^c	0 ^c	320
Taghzout	52 ^d	40 ^c	32 ^d	52 ^a	0 ^d	8 ^c	0 ^c	184
Talat N'os	16 ^e	32 ^c	36 ^d	16 ^c	0 ^d	0 ^d	0 ^c	100
Taws	52 ^d	28 ^c	0 ^f	0 ^d	0 ^d	24 ^b	0 ^c	104
Tiouna	104 ^c	88 ^a	16 ^e	44 ^a	8 ^c	0 ^d	0 ^c	260
Total Number of fungal spores /100 g soil	1284	764	508	276	348	224	68	3472

Table III.
 Diversity analysis of arbuscular mycorrhizal fungi from twelve different origins of the Atlas Cypress.

	Sites											
	Adebdi	Aghbar	Allous	Idni	Ighil	Ighzer	Mzouzit	Rikt	Taghzout	Talat N'os	Taws	Tiouna
Total number of morphotypes per origin	4	7	7	7	7	7	7	6	5	4	3	5
Total number of spores/100 g soil	320	276	280	364	316	340	608	320	184	100	104	260
Dominance (species relative importance)	0.3728	0.2287	0.2078	0.2199	0.2501	0.2269	0.5073	0.3669	0.2391	0.2832	0.3757	0.3079
Shannon indice	1.082	1.66	1.731	1.692	1.559	1.713	1.09	1.256	1.487	1.319	1.038	1.313
Simpson indice	0.6272	0.7713	0.7922	0.7801	0.7499	0.7731	0.4927	0.6331	0.7609	0.7168	0.6243	0.6921
Equitability (Distribution of individual/Species)	0.7802	0.8528	0.8893	0.8697	0.8009	0.8801	0.5604	0.7007	0.9236	0.9513	0.945	0.8155

Table IV.
 Mycorrhizal frequency and roots colonization intensity from the twelve provenances of the Atlas Cypress. In the same column, numbers followed by the same letter are not considered to be significantly different according to the Newman and Keuls method with a 0.05 threshold.

Sites	Frequency %	Root Colonization %
Adebdi	65 ^c	35 ^b
Aghbar	50 ^d	35 ^b
Allous	70 ^b	30 ^c
Idni	60 ^d	35 ^b
Ighil	55 ^d	45 ^a
Ighzer	85 ^a	30 ^c
Mzouzit	65 ^c	35 ^b
Rikt	45 ^e	45 ^a
Taghzout	45 ^e	35 ^b
Talat N'os	45 ^e	25 ^d
Taws	40 ^f	20 ^e
Tiouna	35 ^f	35 ^b
Average	55	32,25

Growth parameters

In the analysis of the effect of the seed origin on growth parameters in the absence of any other treatment, it was observed that the height and shoot dry biomass (SDB) were the two parameters influenced by the origin. The height of the plants varied between 8.12 cm at the Ighzer site and 14.37 cm at the Adebdi site, whereas the shoot dry biomass

varied between 0.72 mg per plant for the seedlings from the Tiouna and Taghzout sites and 1.57 mg per plant for the seedlings from the Aghbar site. The analysis of nutritional parameters (total nitrogen and phosphorus in the plants) has shown significant differences between plants resulting from seeds of different origins. The total nitrogen content of the seedlings varied between 0.65 mg per plant in the Taws site and 1 mg per plant for most other sites. On the other hand, the total phosphorus content of the seedlings varied between 0.3 mg per plant for the Adebdi and Aghbar sites and 0.75 mg for the Ighil, Ighzer and Taghzout sites.

Growth parameters of the six-month-old greenhouse plants resulting from seeds that were inoculated by the fungal complex isolated from the rhizospheric soil of the Atlas Cypress in the twelve sites had shown that the effect of the origin was not significant for all the analyzed parameters (table V). In fact, in the presence of mycorrhizal fungi following inoculation in the greenhouse cultures, the observed differences between seedlings of different origins were no longer present (table V). The parameters that showed the most significant effect were the shoot wet biomass and the shoot dry biomass whereas, the height and stem diameter were not influenced by the origin of the seeds in the presence of mycorrhizal fungi (table V). Additionally, it was shown that phosphorus content varied from one origin to another whereas the nitrogen content was less influenced.

Mycorrhization parameters

After six months growth under greenhouse conditions, seedlings from the 12 different origins, displayed that mycorrhization frequency, root colonization and mycorrhizal dependence varied greatly from one origin to another. The observation of the root system of the seedlings has shown that over 60% of the examined root fragments presented signs of mycorrhizal infection. In the seedlings grown from seeds collected in Allous, Ighzer and Mzouzit, all the analyzed fragments have displayed at least one sign of the presence of mycorrhizal structures. The average root

Table V.

Effects of the Mycorrhizal inoculation on the growth and nutrition status of seedlings from the 12 provenances of *Cupressus atlantica* after 6 months of growth under greenhouse conditions. My = Mycorrhizal inoculation, NMy = No Mycorrhizal inoculation, H: Height (cm), D = Stem diameter (cm), WAGB = Wet above ground Biomass, WRB = Wet root Biomass, DAGB = Dry above ground Biomass, DRB = Dry root Biomass, N = Total Nitrogen (mg/plant), P = Total Phosphorus (mg/plant). Numbers followed by the same letter are not considered to be significantly different according to the Newman and Keuls method with a 0.05 threshold.

Sites	Treatment	H	D	WAGB	WRB	DAGB	DRB	N	P
Adebdi	My	16.75 ^a	3.06 ^a	2.8 ^a	3.87 ^a	1.92 ^a	1.8 ^a	1.25 ^a	0.52 ^a
	Nmy	14.37 ^b	2.8 ^b	2.52 ^b	3.32 ^b	1.55 ^b	1.45 ^b	0.7 ^b	0.3 ^b
Aghbar	My	13.12 ^a	2.9 ^a	2.8 ^a	3.15 ^a	1.85 ^a	1.37 ^a	0.85 ^a	0.75 ^a
	Nmy	12.37 ^b	2.4 ^b	2.75 ^a	2.25 ^b	1.57 ^b	1.17 ^b	0.67 ^b	0.3 ^b
Allous	My	14.5 ^a	3.05 ^a	2.47 ^a	3.25 ^a	1.95 ^a	2.47 ^a	1.75 ^a	0.7 ^a
	Nmy	11.25 ^b	2.86 ^a	2.22 ^b	3.17 ^b	0.95 ^b	1.82 ^b	1 ^b	0.37 ^b
Idni	My	16 ^a	2.67 ^a	2.72 ^a	2.6 ^a	2.37 ^a	1.72 ^a	2 ^a	1 ^a
	Nmy	8.12 ^b	2.58 ^b	1.82 ^b	2.52 ^a	1.35 ^b	1.17 ^b	0.62 ^b	0.6 ^b
Ighil	My	15.6 ^a	2.9 ^a	3.05 ^a	3 ^a	2.8 ^a	1.85 ^a	1.55 ^a	0.87 ^a
	Nmy	12.8 ^b	2.7 ^a	2.32 ^b	2.45 ^b	1.8 ^b	1.25 ^b	0.85 ^b	0.75 ^b
Ighzer	My	16 ^a	2.67 ^a	2.72 ^a	2.6 ^a	2.37 ^a	1.72 ^a	1.75 ^a	0.8 ^a
	Nmy	8.12 ^b	2.58 ^a	1.82 ^b	2.52 ^b	1.37 ^b	1.17 ^b	1 ^b	0.75 ^a
Mzouzit	My	14.6 ^a	2.8 ^a	2.2 ^a	2.95 ^a	1.95 ^a	1.42 ^a	1.35 ^a	0.75 ^a
	Nmy	8.5 ^b	2.17 ^b	1.07 ^b	1.37 ^b	0.95 ^b	1 ^b	0.82 ^b	0.37 ^b
Rikt	My	15.25 ^a	3.5 ^a	2.9 ^a	2.15 ^a	1.67 ^a	0.82 ^a	1.55 ^a	0.75 ^a
	Nmy	12 ^b	3.29 ^b	2.3 ^a	2 ^b	0.75 ^b	0.67 ^b	1 ^b	0.35 ^b
Taghzout	My	13.25 ^a	2.87 ^a	2.2 ^a	2.35 ^a	0.84 ^a	0.55 ^a	1.82 ^a	1 ^a
	Nmy	12.25 ^q	2.57 ^b	1.8 ^a	2.15 ^b	0.72 ^b	0.45 ^a	0.87 ^b	0.75 ^b
Talat N'os	My	14.25 ^a	2.57 ^a	2.85 ^a	2.8 ^a	1.2 ^a	0.95 ^a	1.82 ^a	0.77 ^a
	Nmy	12 ^b	2.51 ^a	2.18 ^b	2.57 ^b	0.8 ^b	0.67 ^b	1 ^b	0.35 ^b
Taws	My	13.25 ^a	2.58 ^a	2.15 ^a	2.8 ^a	1.2 ^a	0.97 ^a	2 ^a	1.25 ^a
	Nmy	12 ^b	2.51 ^a	1.8 ^b	2.57 ^b	0.8 ^b	0.67 ^b	0.65 ^b	0.55 ^b
Tiouna	My	14 ^a	2.9 ^a	2.7 ^a	2.35 ^a	0.86 ^a	0.55 ^a	1.35 ^a	0.85 ^a
	Nmy	12.25 ^b	2.6 ^b	2.2 ^b	2.15 ^b	0.72 ^a	0.35 ^b	1 ^b	0.55 ^b

mycorrhizal colonization intensity for all examined seedlings has a value of 46.6%, it varied between 28.5% for Taws originated seedlings and 59.1% for the Allous originated seedlings. Mycorrhizal dependence expressed by the seedlings from the difference origins had an average value of 37.2%; it varied between 16% for the Tiouna origin and 71% for the Mzouzit origin (table VI).

Global analysis of the response to natural or artificial mycorrhizal inoculation of the Atlas Cypress

Principal component analysis (PCA) of the effect of seeds origin and mycorrhization on growth parameters, nutritional status, root colonization by mycorrhizal fungi and mycorrhizal dependence of the seedlings from different origins (figure 1) allowed the classification of the provenances into two main groups. The first group was made of the following locations: Taws, Taghzout, Tiouna, Talat N'os, Adebdi

and Aghbar, whereas the Rikt, Ighzer, Ighil, Idni, Allous and Mzouzit provenance made up the second group. In fact, mycorrhizal dependence, which indicates the overall mycorrhizal effect on growth and biomass production, showed a clear distinction between the two groups: mycorrhizal dependency varied between 15 and 33% in the first group and 43 and 71% in the second group (table VI).

In order to classify the different provenances based on their response to growth in a forest greenhouse, a hierarchical data analysis based on the similarities between the different regions was conducted. The results allowed the establishment of two distinct groups. The first group was made of the Tiouna, Aghbar, Taghzout and Adebdi provenances which correspond, from a geographic perspective, mainly to the high N'Fis valley. The second group correspond to the low and middle valley of N'Fis and is made of the Mzouzit, Allous, Idni, Talat N'os, Taws, Ighzer, Ighil and Rikt locations (figure 2).

Table VI.

Mycorrhization frequency, root colonization and mycorrhizal dependence in 12 provenances of *C. atlantica* after six months growth under greenhouse conditions. Numbers followed by the same letter are not considered to be significantly different according to the Newman and Keuls method with a 0.05 threshold.

Sites	Mycorrhizal frequency (%)	Root colonization (%)	Mycorrhizal dependence (%)
Adebdi	85 ^d	45 ^c	19.2 ^f
Aghbar	80 ^e	47.5 ^c	15.1 ^g
Allous	100 ^a	59.12 ^a	51 ^c
Idni	90 ^c	45.5 ^c	43 ^d
Ighil	85 ^d	52 ^b	53 ^b
Ighzer	100 ^a	46.75 ^c	43 ^d
Mzouzit	100 ^a	38 ^d	71 ^a
Rikt	95 ^b	61 ^a	55 ^b
Taghzout	70 ^g	52 ^b	14 ^g
Talat N'os	75 ^f	29 ^e	33 ^e
Taws	60 ^h	28.5 ^e	33 ^e
Tiouna	60 ^h	55 ^b	16 ^g
Average	83.34	46.61	37.19

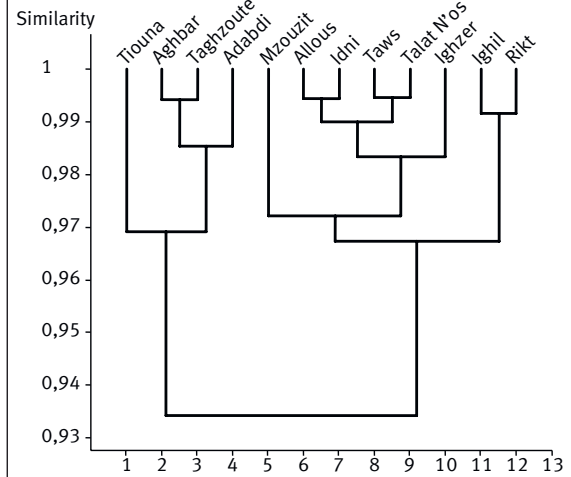


Figure 2. Hierarchical Analysis of growth parameters, nutritional status and mycorrhizal root colonization of the seedlings from 12 origins of *Cupressus atlantica* after six months of growth under greenhouse conditions.

Discussion

The analysis of the diversity of mycorrhizal fungi associated with the Atlas Cypress in the twelve locations representative of the geographic distribution of the species has shown considerable variation in their abundance from one population to another. The seven described species coexist in six of the twelve different populations that were studied with a higher dominance observed at the Mzouzit site due to the high concentration of spores seen at this location. *Rhizophagus aggregatus* was the dominant species in this study followed by *Rhizophagus fasciculatus*. This result does not corroborate previous study at the Idni location (Ouahmane *et al.*, 2007) where *Rhizophagus fasciculatus* was the dominant species. Nonetheless, the number of collected spores per 100 g of rhizospheric soil of the Atlas Cypress at the different locations was comparable to the results obtained by Ouahmane *et al.* (2006). In this study, the Talat N'os and Taws stations were the poorest in terms of mycorrhizal fungi spores which is seemingly due to the steep gradient of the soils and lead to the formation of a deeper root system and taproots unfit for mycorrhizal development. On the other hand, the Mzouzit site was the richest in terms of spore numbers which might be due to its flat terrain, well-formed soil and denser root system. The observation of the roots from the different Atlas Cypress sites has shown that the Talat N'os and Taws sites have the lowest colonization intensity relatively to the other populations. This difference to the previously addressed conditions at those sites, the two populations grow on rocky substrates with a very high gradient. The highest levels of colonization were observed for the more

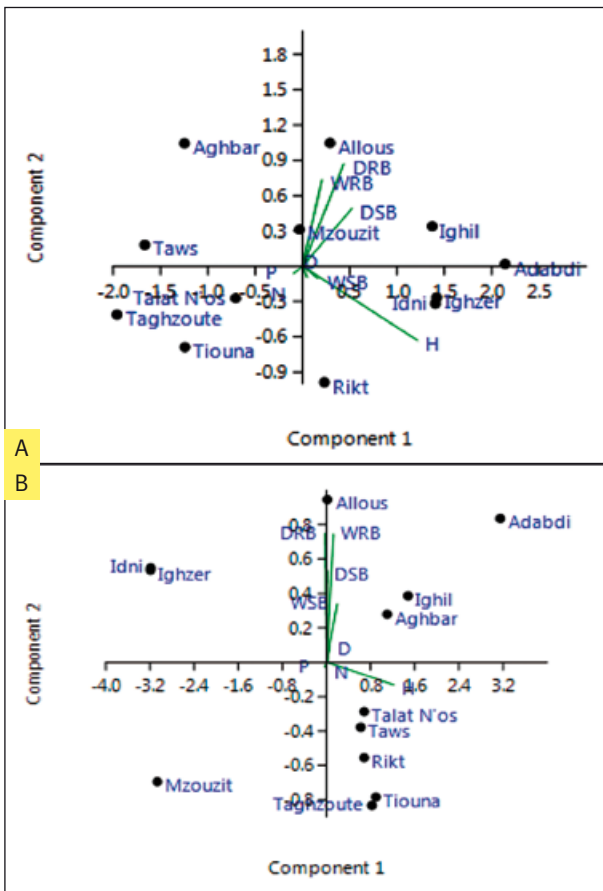


Figure 1. Principal component analysis (PCA) of growth parameters, nutritional status of the seedlings from the 12 origins after 6 months of growth in forest greenhouse. A: Inoculated seedling with Mycorrhizal fungi; B: Non inoculated seedling.



Photo 4.
Atlas Cypress seedlings under greenhouse conditions.
Photo L. Ouahmane.



Photo 5.
Atlas Cypress trees in Idni Station.
Photo L. Ouahmane.

stable populations where alluvium accumulation is the highest thus allowing a finer, more fasciculate and more superficial root system that is in constant contact with surface fungal microflora. The different populations have consequently shown significant statistical differences for the studied parameters. These results related to composition of fungal soil communities with plant abundance and geographic origin are well documented (Reinin-ger *et al.*, 2015; Thioye *et al.*, 2017).

In order to further characterize the diversity of the Atlas Cypress populations, the physiological and nutritional behavior of the seedlings from the different locations was studied through forest greenhouse cultures. The first analysis of the differences between the greenhouse-grown seedlings focused on the effect of the origin of the seeds in the absence of any treatment. The results show a significant effect on all growth and nutritional parameters: plant height, stem diameter, above ground and root wet and dry biomass and nitrogen and phosphorus content of the plant tissues. An important height variation was observed between the Ighzer and Adebdi locations where the height of the plant increased by a factor of 1.8. The shoot dry biomass also increased by a factor of 2 when

going from the Tiouna and Taghzout locations to the Aghbar location. Based on these dendrometric measurements, the plants from the Talat N'Os, Taws, Tiouna, Taghzout and Rikt provenances were the poorest in above ground and root biomass. The second series of seedlings in this study came from seeds from different origins of *C. atlantica* that were inoculated at an early stage by a mixture of arbuscular mycorrhizal fungi. The seedlings were first compared to inoculated seedlings from the same origin after six months of growth under greenhouse conditions, the results showed a huge improvement in the growth and nutrition of the inoculated plants for all origins. This further confirms results obtained by Ouahmane *et al.* (2007) where a strong dependence of the Atlas Cypress on mycorrhizal symbiosis was noticed. Similarly, when the inoculated seedlings from the different origins were compared to each other after 6 months of greenhouse culture, the origin of the seeds was shown to be less significant for all analyzed parameters. The above ground biomass was the only parameter that varied from one location to another. All the mycorrhized plants in this experience had a large size and had similar morphologies and the statistical analysis did not show any significant differences between the mycorrhized plants for all the examined parameters. In fact, the more spectacular effects of the mycorrhizal inoculation on the growth and nutrition of the plants have already been shown for many species in Morocco (Ouahmane, 2007; El Mrabet *et al.*, 2012; Abbas *et al.*, 2013) and also in other Mediterranean countries (Plenchette and Strullu, 1996; Azcón and Barea, 1997; Azcon-Aguilar *et al.*, 2003; Bonfonte and Genre, 2010). In this study, the results have clearly shown that the growth of the Atlas Cypress and its nitrogen and phosphate nutrition were greatly improved by mycorrhizal fungi. However, this high level of mycorrhizal dependency displayed by *C. atlantica* has shown great variation from one origin to another. The seedlings from seeds collected from the Taghzout, Tiouna and Aghbar provenances were found to be least dependent on mycorrhizal symbiosis.

In order to synthesize all the differences observed for the different parameters in the field and in greenhouse cultures, a principal component analysis of the effect of the origin and mycorrhizal inoculation on the plant's growth and nutrition parameters, root colonization by mycorrhizal fungi and mycorrhizal dependency was conducted. The analysis has shown that plants obtained from seeds originating from the different provenances showed important variation and allowed the establishment of two main groups. The first group is made of the Taws, Taghzout, Tiouna, Talat N'os, Adebdi and Aghbar provenances and the second is made of the Rikt, Ighzer, Ighil, Idni, Allous and Mzouzit provenances. Mycorrhizal dependency differed greatly between the two groups. The hierarchical analysis of the data has also allowed the establishment of two distinct groups. The first group is made of Tiouna, Aghbar Taghzout and Adebdi whereas the second one is made of the Mzouzit, Allous, Idni, Talat N'os, Taws, Ighzer, Ighil and Rikt sites. Geographically, the first group corresponds to the high N'Fis valley and the Souss plains whereas the second group corresponds to the low and middle N'Fis valleys. The differences in the geographic and bioclimatic conditions between the different populations seem to have a direct effect on the physiological behavior of the seeds and seedlings of the Atlas Cypress.

Conclusion

The soils hosting the Atlas Cypress, *Cupressus atlantica*, were poor in terms of mycorrhizal fungi spores probably due to the harsh environmental conditions occurring in these areas like drought, strong slope, rocky and superficial soils, and strong water erosion. Nevertheless, mycorrhizal fungi are associated with the roots even at an advanced age, the strength of this association could contribute to the protection of the Atlas Cypress trees from the environmental constraints occurring in their habitat such as drought and fungal pathogens. The Atlas Cypress is thus heavily dependent on its fungal partnerships for its development in all studied provenances. Under nursery conditions without mycorrhizal fungi inoculation, the high variability among different origins was directly tied to genetic variation between and among the different populations. When mycorrhized plants were compared to each other, the growth and nutrition response of the Atlas Cypress is hence dependent on its origin and on the interaction between the different provenances and the fungal symbiont. The combination of strong, resilient genotypes and powerful mycorrhizal partners could guarantee the wider distribution and longevity of the Atlas Cypress. The mycorrhization of the Atlas Cypress seedlings in nurseries before their transfer to reforestation perimeters could contribute to a greater success of reforestation programs based on *Cupressus atlantica*.

References

- Abbas Y., Bakkali Y. S., Prin Y., Arahou M., Abourouh M., Duponnois R., 2013. Growth and nutrition of *Tetraclinis articulata* (Vahl) Mast. cultivated in different rhizosphere soils collected from *Tetraclinis* stand. *Biotechnologie, Agronomie, Société et Environnement*, 17 (1): 3-11. <http://agritrop.cirad.fr/569483/>
- Azcon-Aguilar C., Palenzuela J., Roldan A., Bautist S., Vallejo R., Barea J. M., 2003. Analysis of the mycorrhizal potential in the rhizosphere of representative plant species from desertification-threatened Mediterranean shrublands. *Applied Soil Ecology*, 22: 29-37. [https://doi.org/10.1016/S0929-1393\(02\)00107-5](https://doi.org/10.1016/S0929-1393(02)00107-5)
- Azcón R., Barea J. M., 1997. Mycorrhizal dependency of a representative plant species in mediterranean shrublands (*Lavandula spica* L.) as a key factor to its use for revegetation strategies in desertification-threatened areas. *Applied Soil Ecology*, 7: 83-92. [https://doi.org/10.1016/S0929-1393\(97\)00013-9](https://doi.org/10.1016/S0929-1393(97)00013-9)
- Bechir A., 2004. Évaluation de la variabilité génétique à l'aide de marqueurs adaptatifs et moléculaires du cyprès de l'Atlas (*Cupressus atlantica* Gaussen) ; impact sur la gestion des ressources génétiques endémiques et introduites (*C. sempervirens* L. & *C. arizonica* Green). Thèse Doctorat ès-sciences, Biologie Appliquée, Université Ibn Zohr, Faculté des Sciences, Agadir, Maroc, 135 p.
- Bonfante P., Genre A., 2010. Mechanisms underlying beneficial plant - fungus interactions in mycorrhizal symbiosis. *Nature Communications*, 1 (48). <https://doi.org/10.1038/ncomms1046>
- Borchers S. L., Perry D. A., 1990. Growth and ectomycorrhiza formation of Douglas-fir seedlings grown in soils collected at different distances from pioneering hardwoods in southwest Oregon clear-cuts. *Revue Canadienne de Recherche Forestière*, 20: 712-721. <https://doi.org/10.1139/x90-094>
- Brundrett M. C., 1991. Mycorrhizas in natural ecosystems. *In: Macfayden A., Begon, M., Fitter A. H. (Eds.), Advances in Ecological Research*, vol. 21, Academic Press Ltd., United Kingdom, 171-313.
- Brundrett M. C., Piche Y. R. L., Peterson, 1985. A developmental study of the early stages in vesicular arbuscular mycorrhiza formation. *Revue Canadienne de Botanique*, 63 (2): 184-194. <https://doi.org/10.1139/b85-021>
- Burrows R., Pflieger F., 2002. Arbuscular mycorrhizal fungi respond to increasing plant diversity. *Revue Canadienne de Botanique*, 80 (2): 120-130. <https://doi.org/10.1139/b01-138>
- Caravaca F., Alguacil M. M., Barea J. M., Roldan A., 2005. Survival of inocula and native AM fungi species associated with shrubs in a degraded Mediterranean ecosystem. *Soil Biology and Biochemistry*, 37: 227-233. <https://doi.org/10.1016/j.soilbio.2004.06.019>
- Douds D. D., Millner P. D., 1999. Biodiversity of arbuscular mycorrhizal fungi in agroecosystems. *Agriculture Ecosystems and Environment*, 74: 77-93. [https://doi.org/10.1016/S0167-8809\(99\)00031-6](https://doi.org/10.1016/S0167-8809(99)00031-6)
- El Mrabet S., Ouahmane L., El Mousadik A., Msanda F., Abbas Y., 2012. The Effectiveness of Arbuscular Mycorrhizal Inoculation and Bio-Compost Addition for Enhancing Reforestation with *Argania spinosa* in Morocco. *Open Journal of Forestry*, 4 (1): 14-23.
- Estaun V., Save R., Biel C., 1997. AM inoculation as a biological tool to improve plant re-vegetation of a disturbed soil with *Rosmarinus officinalis* under semi-arid conditions. *Applied Soil Ecology*, 6: 223-229. [https://doi.org/10.1016/S0929-1393\(97\)00014-0](https://doi.org/10.1016/S0929-1393(97)00014-0)
- Fontana A., 1985. Vesicular arbuscular mycorrhizas of *Ginkgo biloba* L. in natural and controlled conditions. *New Phytologist*, 99: 441-447. <https://doi.org/10.1111/j.1469-8137.1985.tb03671.x>
- Francis D. F., Thornes J. B., 1990. Matorral: erosion and reclamation. *In: Albaladejo J., Stocking M. A., Diaz E. (Eds.), Soil Degradation and Rehabilitation in Mediterranean Environmental Conditions*. Consejo Superior de Investigaciones Científicas, Murcia, Spain, 87-115.
- 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: 235-244. [https://doi.org/10.1016/S0007-1536\(63\)80079-0](https://doi.org/10.1016/S0007-1536(63)80079-0)
- INVAM website, 1997. Species Descriptions from Reference Cultures. The morphological characteristics of the spores were compared to the type specimens and reference strains. <http://fungi.invam.wvu.edu/the-fungi/species-descriptions.html>

- Kernaghan G., Widden P., Bergeron Y., Légaré S., Paré D., 2003. Biotic and abiotic factors affecting ectomycorrhizal diversity in boreal mixed-woods. *Oikos*, 102 (3): 497-505. https://www.jstor.org/stable/3547932?seq=1#page_scan_tab_contents
- Kormanik P. P., Schultz R. C., Bryan W. C., 1982. The influence of vesicular arbuscular mycorrhizae on the growth and development of eight hardwood tree species. *Forest Science*, 28: 531-539. https://lib.dr.iastate.edu/for_pubs/15
- López-Bermúdez F., Albaladejo J., 1990. Factores ambientales de la degradación del suelo en el área mediterránea. In: Albadejo J., Stocking M. A., Diaz E. (Eds.), *Soil Degradation and Rehabilitation in Mediterranean Environmental Conditions*. Consejo Superior de Investigaciones Científicas, Murcia, Spain 15-45.
- Mc Lellan A. J., Fitter A. H., Law R., 1995. On decaying roots, mycorrhizal colonization and the design of removal experiments. *Journal of Ecology*, 83 (2): 225-230. <http://www.jstor.org/stable/2261561>
- Miller S. L., Koo C. D., Molina R., 1992. Early colonization of red alder and Douglas fir by ectomycorrhizal fungi and Frankia in soils from the Oregon coast range. *Mycorrhiza*, 2: 53-61. <https://doi.org/10.1007/BF00203250>
- Mosse B., 1973. Advances in the study of vesicular-arbuscular mycorrhiza. *Annual Review of Phytopathology*, 11: 171-196. <https://doi.org/10.1146/annurev.py.11.090173.001131>
- Ouahmane L., 2007. Rôles de la mycorrhization et des plantes associées (lavande et thym) dans la croissance du cyprès de l'Atlas (*Cupressus atlantica* G.) : conséquences sur la biodiversité rhizosphérique et la réhabilitation des milieux dégradés. Faculté des Sciences SEMLALIA, Université Cadi Ayyad, Marrakech, Maroc.
- Ouahmane L., Hafidi M., Kisa M., Boumezzough A., Thioulouse J., Duponnois R., 2006. *Lavandula* species as accompanying plants in *Cupressus* replanting strategies: Effect on plant growth, mycorrhizal soil infectivity and soil microbial catabolic diversity. *Applied Soil Ecology*, 34: 190-199. <https://doi.org/10.1016/j.apsoil.2006.02.002>
- Ouahmane L., Hafidi M., Thioulouse J., Ducouso M., Kisa M., Prin Y., et al., 2007. Improvement of *Cupressus atlantica* Gaussen growth by inoculation with native Arbuscular mycorrhizal fungi. *Journal of Applied Microbiology*, 103: 683-690. DOI: <https://doi.org/10.1111/j.1365-2672.2007.03296.x>
- Peterson R. I., Ashford A. E., Allaway W. G., 1985. Vesicular arbuscular mycorrhizal association of vascular plants on Heron Island, a Great Barrier Reef Coral cay. *Australian Journal of Botany*, 33: 669-676. <https://doi.org/10.1071/BT9850669>
- Phillips J. M., Hayman D. S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society*, 55: 158-161. [https://doi.org/10.1016/S0007-1536\(70\)80110-3](https://doi.org/10.1016/S0007-1536(70)80110-3)
- Plenchette C., Strullu D. G., 1996. Les mycorrhizes, situation et perspectives pour le pépiniériste et l'horticulteur. *PHM. Revue Horticole*, 365 : 72-76.
- Pozo M. J., Azcón-Aguillar C., Dumas-Gaudot E., Barea J. M., 1999. β -1,3-Glucanase activities in Toato roots inoculated with arbuscular mycorrhizal fungi and/or *Phytophthora parasitica* and their possible involvement in bioprotection. *Plant Science*, 141: 149-157. [https://doi.org/10.1016/S0168-9452\(98\)00243-X](https://doi.org/10.1016/S0168-9452(98)00243-X)
- Reininger V., Martinez-Garcia L. B., Sanderson L., Antunes P. M., 2015. Composition of fungal soil communities varies with plant abundance and geographic origin. *AOB PLANTS*, 7: plv110. <https://dx.doi.org/10.1093%2Faobpla%2Fplv110>
- Requena N., Perez-Solis E., Azcón-Aguilar C., Jeffries P., Barea J. M., 2001. Management of indigenous plant-microbe symbioses aids restoration of desertified ecosystems. *Applied and Environmental Microbiology*, 67: 495-498. <http://aem.asm.org/content/67/2/495.full>
- Rincón A., Ruiz-Diez B., Fernandez-Pascual M., Probanza Pozuelo J. M., de Felipe M. R., 2006. Afforestation of degraded soils with *Pinus halepensis* Mill.: effects of inoculation with selected microorganisms and soil amendment on plant growth, rhizospheric microbial activity and ectomycorrhizal formation. *Applied Soil Ecology*, 34: 42-51. <https://doi.org/10.1016/j.apsoil.2005.12.004>
- Roth A. L., Berch S. M., 1992. Ectomycorrhizae Douglas fir and western hemlock seedlings out planted on eastern Vancouver Island. *Revue Canadienne de Recherche Forestière*, 22: 1646-1655. <https://doi.org/10.1139/x92-218>
- Strullu D. G., 1990. Les mycorrhizes des arbres et plantes cultivées. Collection TEC et DOC, Lavoisier, Paris, 250 p.
- Thioye B., Mania de Faria S., Kane A., Ndiaye C., Ould Soule A., Fall D., et al., 2017. Growth response of different species of *Ziziphus* to inoculation with arbuscular mycorrhizal fungi. *Fruits*, 72 (3): 174-181.
- Trouvelot A., Kough J. L., Gianinazzi-Pearson V., 1986. Mesure du taux de mycorrhization ayant une signification fonctionnelle. In: *Aspects physiologiques et génétiques des mycorrhizes*, Dijon, 1985. INRA, 217-221.
- Vallejo V. R., Bautista S., Cortina J., 1999. Restoration for soil protection after disturbances. In: Trabaud L. (ed.), *Life and Environment in Mediterranean Ecosystems, Advances in Ecological Sciences*. Wit Press, Southampton, United Kingdom, 301-343.
- Van der Heijden M., Klironomos J., Ursic M., Moutoglis P., Streitwolf-Engel R., Boller T., et al., 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, 396: 69-72. <https://www.nature.com/articles/23932>
- Whipps J. M., 2004. Prospects and limitations for mycorrhizas in biocontrol of root pathogens. *Revue Canadienne de Botanique*, 82 (8): 1198-1227. <https://doi.org/10.1139/b04-082>

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