IMPACT OF INOCULATION BY NATIVE ENDOMYCORRHIZAL FUNGI ASSOCIATED WITH *TETRACLINIS ARTICULATA* ON PLANT GROWTH AND MYCORRHIZAL DIVERSITY IN THE FOREST NURSERY

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Link to this article: https://doi.org/10.11118/actaun.2022.019
Received: 18. 1. 2022, Accepted: 21. 6. 2022

Abstract

In Morocco, *Tetraclinis articulata* is one of the species of great socio-economic value. Improving the resistance and survival of thuya to sometimes extreme environmental conditions is a concern of managers. For this, the use of arbuscular mycorrhizal fungi can promote the good growth of this species. We aim to study the effect of a native endomycorrhizal inoculum on the growth of *T. articulata* plants under nursery conditions. Our results showed that after eighteen months of inoculation, there was a significant effect on the growth of the mycorrhizal plants as compared to the non-mycorrhizal plants. Concerning the root fresh weight (8.58 g), the root system length (34 cm), the collar diameter (5.44 mm), and the number of branches (27) of the mycorrhizal plants are higher than those observed in the non-mycorrhizal plants, 7.67 g, 30 cm, 4.13 mm, and 24, respectively. However, the results of the height and the fresh weight of the shoot part of the non-mycorrhizal plant are superior to those observed in the mycorrhizal plants. The number of spores formed in the rhizosphere of plants inoculated was 135/100 g of soil. And they were represented by 29 endomycorrhizal species belonging to ten different genera: *Glomus* (6 species), *Acaulospora* (8 species), *Rhizophagus* (3 species), *Diversispora* (1 species), *Funneliformis* (3 species), *Septoglomus* (2 species), *Scutellospora* (2 species), *Claroideoglomus* (1 species), *Entrophospora* (1 species) and *Gigaspora* (2 species). *Diversispora versiformis* is the most abundant species; its frequency of occurrence reached 30%. The results of this inoculation study highlight the importance of native endomycorrhizal fungi isolated from the rhizosphere of Aderj in increasing the root system and improving the growth of *T. articulata* plants.

Keywords: diversity, endomycorrhizal fungi, nursery, mycorrhizae, plant growth, *Tetraclinis articulata*

INTRODUCTION

In Morocco, *Tetraclinis articulata* is considered a solution for protecting soil against erosion and plays a very important role in the economic and social life of local populations. Indeed, thuya wood is widely used for various crafts, such as marquetry, the manufacture of decorative objects, and fine carpentry. It is also used as firewood (Boudy, 1952). Unfortunately, overexploitation, diseases, pests, and environmental stresses such as drought and erosion have significantly reduced its range. Among the
approaches agreed to save this forest tree species, the improvement of silvicultural techniques would be the most effective. For this, the establishment of programs for the production of *T. articulata* mycorrhizal plants in the nursery by arbuscular mycorrhizal fungi (AMF) would be an interesting solution, knowing that this symbiosis makes it possible to improve both the nutrition of the plant and its resistance to biotic and abiotic constraints.

Also, AMF is a type of endomycorrhiza that forms a symbiotic association with plants (Redecker et al., 2000; Schübler et al., 2001). Indeed, AMF is the key component in plant-soil relationships (Van Der Heijden et al., 1998; Smith and Read, 2008). AMFs are also distinguished by the formation of a variety of structures (arbuscules, vesicles, spores, and non-specialized hyphae) (Béreau et al., 2003; Tommerup, 1984; Wipf, 2014). Although they are not specific, their effects on plants may vary between species and isolates (Királynomos, 2003). Several studies have shown that AMF plays a major role in plant productivity via the increase in water absorption capacities of plants (Meddich et al., 2015; Wu et al., 2011), the improvement of their mineral nutrition, in particular phosphorus (Wu et al., 2011), and their protection against the harmful effects of biotic (agent pathogens) (Fitter, 1991) and abiotic (drought) (Nouaïm and Chaussod, 1996; Porcel et al., 2006; Smith and Read, 2008; Simard, 2014). AMFs intervene significantly at the level of all stages of development of forest tree species (Smith and Read, 1997). The impact of mycorrhizal symbiosis on the growth of a forestry species can be assessed after the introduction of a fungal symbiont selected for its ability to stimulate the growth of the host plant under given environmental conditions (Duponnois et al., 2005) and by monitoring the mycorrhizal infectious potential (PIM) in situ (Duponnois et al., 2001).

According to Abbas et al. (2006), the *T. articulata* rhizosphere is closely associated with AMF and the growth of this essence strongly depends on the mycorrhizal symbiosis (Abbas and Duponnois, 2005). The use of mycorrhizal inoculation in the nursery could have a positive effect on the regeneration of the *T. articulata* forest by improving local ecological conditions.

Some studies were carried out on the mycorrhization of *Tetraclinis articulata* in Morocco. Abbas et al. (2013) noted that the inoculation with AMF improved the nutrition and growth of plants. However, El Khaddari et al. (2019) showed that the effect of inoculation by AMF can improve the resistance of the thuya plants to water stress.

The objective of this work is to determine the role of native arbuscular mycorrhizal fungi during the production of *T. articulata* plants in the forest nurseries (i) on the growth parameters of the aerial part and root development of the plants, and (ii) on mycorrhizal diversity in the nursery.

**MATERIALS AND METHODS**

**Inoculum Production**

The soil and root samples used for the production of the inoculum were collected from under the *T. articulata* trees of the state forest named Adrej in December 2015. Adrej is located in the biogeographical zone of the western part of the Eastern Middle Atlas massif and, more locally, to the south-west of the Bou Iblane Mountain range. It is located in the south-east of the province of Sefrou, 90 km from the centers of Almenzel and Ribat Alkhir. This forest has a total area of 1560 ha. This area is characterized by an average annual rainfall varying between 300 and 500 mm and an average annual temperature of 18.3°C. The soils of these forests are of the limestone type. In this area, the vegetation is rich and diverse, consisting of a mixture of thuya, red juniper, holm oak, and cedrus, and shrub species such as *Rosmarinus officinalis* (rosemary), *Cistus libanotis*, *Pistacia lentiscus*, and *Phillyrea latifolia*.

The soil was deposited on a healthy, disinfected platform far from any contamination. At the level of the forest nursery of Beni Souhane, the quantity of soil was estimated at around 10 kg. For the trapping of mycorrhizal fungi in thuya, we sowed the seeds of barley (*Hordeum vulgare*), which is a mycotrophic plant because it has a root system well suited to the production and multiplication of a composite inoculum of AMF. Barley seeds were disinfected with 5% sodium hypochlorite for 2 minutes. The culture was monitored for two months in daylight and at room temperature. Plant watering was carefully applied by overhead spraying to avoid loss of spores through leaching. After the development of the root system of barley plants, we proceeded to cut off its unusable aerial part and we sieved the sub with the roots, which was added to a mixture consisting of disinfected sand and rhizosphere soil containing a large number of different endomycorrhizal species.

The Production and AMF Inoculation of *T. articulata*

The seeds used for the production of *T. articulata* plants come from the Oued Beht region and are collected by the seed center of the Azrou nursery. The seeds were sown directly, without prior treatment, in April 2016.

The inoculation of the seeds was carried out in rigid containers filled with a mixture of 25% sterile forest soil from Adrej forest under holm oak, 75% sterile compost (v/v) and 10 g of mycorrhizal soil (inoculum endomycorrhizal), which contains the fragments of the mycorrhizal root of *H. vulgare*. The non-inoculated seeds were sown in sterile soil not inoculated by endomycorrhizal fungi, which we call Non-AMF. This is the witness. Sterilization is carried out in an autoclave at 250 °C for 2 hours to eliminate
mycoflora from the soil. The racks containing the inoculated and non-inoculated plants were placed in a forest nursery with daily watering and ambient temperature. The rearing containers used in our experiment consist of a rigid black polypropylene rack. It has an anti-ultraviolet treatment. Its shape is rectangular with rectangular dimensions (width = 30 cm, length = 38 cm and height = 18 cm). These racks are composed of 40 alveoli. The volume of the alveoli is 350 cc with the following dimensions: 18 cm; a wide top base, square in shape with a 5.5 cm side; a small conical lower base, whose basal opening does not allow the substrate to drain; it is 3.5 cm on the side. The internal face of the cell is equipped with an anti-chignon system, consisting of projecting vertical tabs (root guides), three per side, located in the middle and at the corners of the cell, which are continuous throughout the entire depth of the cell. The rearing racks were raised nearly 30 cm from the ground on wire mesh to promote aerial self-cerning of the roots.

**Experimental Device**

After nine months of stay in Beni Souhane's nursery, part of the *T. articulata* plants was used according to an experimental device in random blocks showing two lots of plants. The first lot (T0) contains a total of 160 non-inoculated plants aged 9 months; it's the control. The second lot (T1) contains a total of 160 plants inoculated by AMF autochthonous of Adrij (mycorrhizal plants), aged 9 months without phytosanitary treatment or the addition of fertilizers. Each lot was repeated four times. That means $40 \times 4 = 160$ plants in each uninoculated and inoculated treatment.

**Evaluation of the Growth of *T. articulata* Plants**

The plants brought back from Beni Souhane were therefore 9 months old. They were transferred to the nursery of the Center for Forest Research (CRF), (34°01’ N, 6°5’ W), located at an altitude of 135 m in a subhumid bioclimate with warm winters. The average annual rainfall is 555 mm and the average annual temperature is 17.9°C. The racks were kept for another 9 months in the CRF nursery at ambient temperature and watered daily throughout the experiment. This last period allows the plant's growth parameters to be measured. The measurements carried out are:

- Height growth.
- Measure the collar diameter with a caliper.
- The fresh weight of the aerial and root parts was measured using a digital scale.
- The aerial and root parts were separated, and the fresh weight of the aerial part and root parts was measured using a digital scale.
- The root system's length.
- The number of branches of the vegetative part was measured by counting.

The measurements were carried out at the end of the experiment (September 2017) on 3 plants per treatment. For the choice of the number of plants to evaluate, we randomly selected only 3 plants per treatment because the plants are homogeneous throughout the protocol. These plants have almost the same development and the same growth rate ("good growth throughout the protocol").

**Spore Extraction**

The wet sieving method described by Gerdemann and Nicolson (1963) and Brundrett et al. (1999) was adopted to extract spores from the soil of *T. articulata*. Thus, the soil samples used in this part of the work correspond to the content of three alveoli per treatment; these alveoli are taken randomly. The total quantity of soil sampled is 750 g at a rate of 250 g per alveolus. The identification of spores is based on microscopic observation of morphological characteristics (color, shape, size, the surface of the wall ...) (Bethenfalvay and Yoder, 1981; Schenk and Perez, 1990; Goto, 2009). This identification of spores was made according to species descriptions provided by the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM, 2017) following the classification of Redecker et al. (2013). The appearance frequency of species (A.F.S.%) represents the percentage of a morphotype compared to the other species:

$$A.F.S\% = \frac{ns}{nT} \times 100 \quad (1)$$

with:

- $ns$ ......number of isolated spores from species X,
- $nT$ ......denotes the total number of spores.

Appearance frequency of a genus (A.F.G.%) is the percentage of spores of all species of genus X that appear in relation to the total number of spores recorded.

$$A.F.G\% = \frac{100 \times nG}{nT} \quad (2)$$

with:

- $nG$ ......number of genus X spores,
- $nT$ ......denotes the total number of spores.

**Statistical Analysis**

The statistical analysis of the obtained results was performed using the SPSS 20 software. The difference between the growth parameters of the inoculated and non-inoculated plants was evaluated by analysis of the variance using a one-way ANOVA at $p < 0.05$. The Duncan ($p = .05$) test was used to separate the means.
RESULTS

Effect of Inoculation With Endomycorrhizal Fungi on Growth Parameters of Tetraclinis articulata Plants

The analysis of the results shows that inoculation with endomycorrhizal fungi significantly affects the growth parameters of T. articulata plants (Tab. I). The average collar diameter reached was 5.44 mm in mycorrhizal plants, compared to 4.13 mm in non-mycorrhizal plants (Tab. I). Also, the average number of branches is higher in mycorrhizal plants compared to that of control plants, with 27 and 24 branches per plant, respectively (Tab. I). Mycorrhizal plants then present an average higher root length, of the order of 34 cm, compared to non-mycorrhizal plants, in which this length does not exceed 30 cm. Similarly, the fresh weight of the root system in mycorrhizal plants is greater than that of non-mycorrhizal plants, with a respective 8.58 g and 7.67 g (Tab. I).

However, this symbiotic association does not have a significant effect on height and fresh weight. The results show that the height of non-mycorrhizal plants is higher than that of mycorrhizal plants, on the order of 30.5 and 19.5 cm, respectively (Tab. I). Whereas the aerial fresh weight reached 9.47 g in the non-inoculated plants versus 6.55 g in the inoculated plants (Tab. I).

Evaluation of Mycorrhizal Rate

The results of the soil test, where the T. articulata plants inoculated by native AMF were raised, clearly showed the presence of endomycorrhizal species. The average number of spores in the rhizosphere of these plants is then 139 spores/100 g of soil (Fig. 1).

The study of the morphological criteria of the AM fungi spores isolated from the rhizosphere of the inoculated plants (Tab. II and Fig. 2) made it possible to note that all of the isolated morphotypes belong to 29 species: Glomus sp., Glomus aggregatum, Rhizophagus intraradice, Acaulospora sp., Diversispora versiformis, Glomus microcarpum, Funneliformis mosseae, Acaulospora denticulata, Acaulospora scrobiculata, Septoglomus deserticola, Acaulospora kentinensis, Scutellospora nigra, Gigaspora Albida, Funneliformis monosporus, Claroideoglomus etunicatum, Gigaspora sp., Acaulospora gerrardi, Glomus spinuliferum, Acaulospora gedanensis, Septoglomus constrictum, Acaulospora laevis, Rhizophagus manihotis, Scutellospora sp., Rhizophagus clarus, Glomus albidum, Entrophospora infrequens, Acaulospora colossica, Glomus aureum, Funneliformis geosporum.

According to the classification of (Schenk and Perez, 1990; Oehl et al., 2003; Goto, 2009; Oehl et al., 2011; Redecker et al., 2013; INVAM, 2017), these species are affiliated with 10 genera (Glomus, Gigaspora, Acaulospora, Rhizophagus, Diversispora, Funneliformis, Septoglomus, Claroideoglomus, Entrophospora, and Scutellospora), 5 families (Diversisporaceae, Claroideoglomeraceae, Glomeraceae, Acaulosporaceae, and Gigasporaceae) and 2 orders (Glomerales, Diversisporales). Diversispora versiformis, Funneliformis mosseae, and Glomus micorcarpum are the most common species on the mycorrhizal roots of T. articulata plants; their frequency of occurrence (Fig. 3) is 30, 11.94, and 7.46%, respectively. We also noted the strong dominance of the genus Diversispora, which presents a percentage of 30%, followed by the genus Glomus with a percentage of 22%, and in third place, we find the genus Funneliformis and Acaulospora with a percentage of 17 and 13%, respectively. Finally, the other genera’ appearance frequencies are of the order of 7, 4, 3, 2, 1, and 1%, respectively, for Rhizophagus, Claroideoglomus, Scutellospora, Septoglomus, Entrophospora, and Gigaspora (Fig. 4).

![Graph showing the number of spores in mycorrhizal and non-mycorrhizal plants](image1)

1: Total number of spores of AMF in the soil of mycorrhizal and non-mycorrhizal plants of T. articulata
DISCUSSION

The results obtained showed the plants inoculated with AMF were better compared to the control, especially in terms of collar diameter and the development of the root system. These results are in agreement with those of Abbas et al. (2013) and those of Diaz and Honrubia, (1993). Similar results were mentioned in the cases of the argan tree (Argania spinosa) (Sellal et al., 2017) and the cases of Acacia nilotica and Acacia senegal (Laminou Manzo et al., 2009) inoculated with a mycorrhizal complex of indigenous strains.

In addition, the number of branches and the length of the root system were also higher in the mycorrhizal plants, thus confirming the results of (Sellal et al., 2017), which showed that the inoculated plants of argan had a high number of branches and their root systems were more developed and strongly branched compared to controls. These plants will undoubtedly benefit because root growth allows improved water absorption and mineral nutrition (Fester et al., 2002; Derkowska et al., 2008; Stavros et al., 2011).

Indeed, individual AMF hyphae have much smaller diameters than roots, allowing the plant to access narrower soil pores and hence increase the soil volume explored (Smith and Read, 2008; Schnepf et al., 2011), which confirms that the AM fungi increase the rooting zone (Boureima et al., 2008).

Black, (1965) mentioned that the good absorption of phosphorus can lead to the proliferation and cell elongation of plants, because phosphorus is moved...
rapidly to the roots (probably as polyphosphate), overcoming the slow diffusion that occurs in the soil solution. These factors are the major causes of increased P uptake and positive AM growth responses (Smith et al., 2011).

Several studies have demonstrated the beneficial roles of endomycorrhizal fungi for plants, including the transportation and absorption of nutrients that are not very mobile in the soil, particularly phosphorus (Duponnois et al., 2005; Lambers et al., 2008), improvement of drought tolerance (Ruiz Lozano and Azcón, 1995), and inducing morphological and physiological transformations in them to tolerate environmental constraints (Porcel et al., 2006). Furthermore, the inoculation by AMF had no significant effect on the height growth of the plants tested. The height is, in fact, higher in non-mycorrhizal plants. Our results are in agreement with those of Touati et al. (2016), who showed that the height growth of *Casuarina sp.* is greater in uninoculated plants than in inoculated plants. Thus, the mycorrhizal symbiosis first improves the development of the root. The plants will then be more robust since they will be provided with efficient means that allow them to exploit the resources of the soil. They then mobilize these means to increase their diameter at the collar, which then gives rustic plants. The latter would be better able to withstand the vagaries

<table>
<thead>
<tr>
<th>Number</th>
<th>Name</th>
<th>Color</th>
<th>Form</th>
<th>Average spore size (µm)</th>
<th>Spore surface</th>
<th>Length of hypha (µm)</th>
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of transplantation and would adapt more easily to new environmental conditions. The growth in height of non-mycorrhizal plants is obtained at the expense of the thickness of the stem and the growth of the roots, which remain poorly developed in inoculated plants. The stems of the latter may break during transplantation, and their root potential would not facilitate adaptation to the conditions on the ground. This conclusion is in agreement with that of Porras-Soriano et al. (2009), who showed that the growth response to symbiosis by AMF was more effective for root development than for shoot development. Other studies have shown that AMF has a positive effect on the growth and development of the roots of Phenix dactylifera (Sghir et al., 2014), Argania spinosa L. (Bousselmame et al., 2002) and Ceratonia siliqua Linn (Talbi et al., 2016).

On the other hand, the present work has shown that the soil of inoculated T. articulata plants was rich in spores of endomycorrhizal fungi, on the order of 139 spores/100 g of soil. This number is important compared to the number found in the soil of the T. articulata rhizosphere of the Sefrou region. This number is on the order of 124 spores/100 g of soil (El Khaddari et al., 2019). The finding of Sellal et al. (2017) allowed for the estimation of 246 spores per 100 g of soil in argan inoculated plants.

The identification work carried out on the isolated spores made it possible to draw up a list of 29 endomycorrhizal species belonging to ten genera.
These results are similar to those of Sellal et al. (2017), who also revealed the presence of 29 species from spores isolated from the soil of argan plants inoculated with an endomycorrhizal inoculum in the nursery. Similarly, Artib et al. (2017) were able to identify 23 species in the rhizosphere of inoculated *Citrus aurantium* L.

In addition, our study showed the dominance of the genus *Diversispora*, followed by the genus *Glomus*. This last is in line with previous work on the argan tree (Sellal et al., 2017), on the olive tree (Chliyeh et al., 2014), and on citrus plants (Artib et al., 2017), which also showed that the genus *Glomus* was the most dominant. According to Stutz et al. (2000), the representatives of this genus are the most adapted to fluctuations in environmental conditions. Also, Blaszkowski et al. (2002) show that most ecosystems suggest a better adaptation of the genus of *Glomus* to the most hostile conditions such as drought, salinity, and other environmental stresses.

On the other hand, our results have shown that the plants that are well branched with a thick diameter and a more developed root system will undoubtedly be more robust than fine, little branched, and long plants, as well as mycorrhizal plants, which will better resist the vagaries of transplantation and adapt more easily to the conditions of the natural environment. In addition, the long, sparsely branched and unstructured stems of the controls are unable to withstand biotic and abiotic stresses, and they are more likely to break when the plants are moved to the reforestation area. This study established the importance of native arbuscular mycorrhizal fungi in the growth of *T. articulata* plants.

**CONCLUSION**

From the whole of this study, it is clear that inoculation with native AMF has a beneficial effect on the growth parameters of *T. articulata* plants in the forest nursery as well as on the diversity of endomycorrhizal fungi in the soil where these plants were raised. The symbiosis improved plant morphological quality by promoting root development and collar diameter growth. Also, it is possible that these AMF-inoculated plants represent another approach to improving this plant's growth, which can be used as a biological means of stress tolerance. The mycorrhized plants have strong root potential and a thick stem, which are two of the criteria for rustic plants. These plants would more easily withstand the riskiness of the planting site, compared to non-mycorrhizal plants, which have a weakness in thickness and could have the risk of breaking during transport. The study, therefore, revealed the necessity of the technique of inoculation by indigenous AMF. This technique can be a solution if it is carried out in good conditions, for the success of reforestation and, subsequently, the rehabilitation of this noble species in semi-arid areas.

**Acknowledgements**

The authors express thanks to Ibn Tofail for allowing me to enroll in a doctoral degree and Mrs. Jamila Dahmani for his guidance, support, and availability during my career. We are also thankful to the Center for Forest Research for providing the laboratory for conducting this experiment.

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