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THE DESERT TRUFFLE *TERFEZIA CLAVERYI* CHATIN IMPROVES THE GROWTH OF ALEPPO PINE IN AXENIC CONDITIONS

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Abstract – The study is about Aleppo pine, which is a very important forest plant species in Algeria. We present the preliminary results of mycorrhizal synthesis under axenic conditions between an edible mushroom called terfez (*Terfezia claveryi* Chatin) and a pine species (*Pinus halepensis* Miller). The results show that mycorhization by terfez improves the growth of Aleppo pine. The presence of terfez hyphae is observed, without typical organization, in cortical cells of pine seedlings.

INTRODUCTION

Aleppo pine is an important forest species in Algeria that is used in forestry, especially in semi-arid areas. It is an introduced species and it encounters problems in natural conditions (attack by the processionary caterpillar, slow growth ....). This plant species establishes a mycorrhizal association with edible mushrooms belonging to Basidiomycetes and Ascomycetes.

The desert truffle is an ascomycete mushroom that grows particularly in the Maghreb and in the Middle East. In Algeria, it is very widespread in the steppic and northern Saharan regions as in Biskra, Tindouf, Ain sefra, Béchar (Chatin, 1892; Maire, 1906; Fortas, 1990).

In this work, we perform mycorrhizal syntheses between this mushroom and the Aleppo pine, in axenic conditions, on an inert substrate impregnated with a nutritive solution (Dib, 2002).

This study will allow us to know the role of mycorhization in improving growth of pine and on the other hand to look for the possibilities of use of the mycorhizogenic power of desert truffle, in the reforestation of desertic or degraded zones and in the protection of silvicultural species against phytopathogenic agents and against water stress.

MATERIALS AND METHODS

Biological materials

The mycelial strain of terfez used in this study belonged to the species *Terfezia claveryi* Chatin. This strain that was provided by the Laboratory : Laboratoire de Biologie des micro-organismes et Biotechnologie of Oran University1.

The Aleppo pine seeds used (*Pinus halepensis* Miller) was provided by the Agricultural Workshop of the Forestry Department of the University of Mostaganem.

Culture and conservation of the mycelial strain of terfez

Malt agar medium (1%) was distributed in Petri dishes. Each Petri dish was seeded with a 15mm^2 fungal implant, of *T. claveryi* strain. The Petri dishes are sealed with an adhesive tape and then they are incubated at 25 ° C for 30 days.

T.claveryi strain is stored at 4° C., in Petri dishes or in tubes, on malt agar medium (1%). It is transplanted every 3 months.

Obtention of sterile pine seedlings

The seedlings used came from seed germinated *in vitro*, under aseptic conditions. The interest of this technique is to control the development of the root system of the plant and the sterility of the seedlings (Strullu *et al.*, 1986).

The Aleppo pine seeds are disinfected on the surface by soaking them in hydrogen peroxide 30V for 20 minutes (Torres *et al.*, 1994) then germinated in Petri dishes on solid malt agar medium (1%) or on agar water and placed at 25 °C, in the dark, for 15 to 20 days to check for any contamination.

Mycorrhizal synthesis between *T. claveryi* and *P. halepensis* in axenic conditions

We used the method advocated by Chevalier and Pollacsek (1973). It involves contacting seedling and mycelium in the same closed flasks, on an inert substrate impregnated with nutrient solution.The cultures are carried in flasks of 500mL, containing vermiculite (inert substrate, sterilized three times at 180 °C.), impregnated with Melin and Norkans medium modified with Marx (MNM) liquid and autoclaved twice for 30 min at 120 °C with a rest of 24 hours.

The young pine seedlings of *P.halepensis* are transplanted aseptically into the flasks (1-2 seedlings per flask) when the rootlet length reaches 2 cm.

Inoculation is performed with a fragment of mycelium of *T.claveryi*, from a culture aged 30 days, applied directly in contact of the rootlets.

The inoculated and control seedlings (15 repetitions for inoculated plants and 5 for controls) are kept in the laboratory for 15 days, in natural light, to confirm the absence of contamination. Then, they are placed for 10 months in culture chamber programmed at 2000 Lux light intensity, photoperiod of 16h and temperature at 23 ±2 °C.

Evaluation of mycelial growth of *T. claveryi* strain

The assessment of mycelial growth on malt agar medium is determined by the average of diameter measurements, of its colony, taken in two perpendicular directions, each week.

Evaluation of *P. halepensis* seedling growth

Aleppo pine seedlings are examined after 10 months of culture.The aerial part of pine seedlings is composed of an axis bearing juvenile leaves. The length of the axis (measured between the neck and the terminal bud), the total number of leaves and their average length are noted (Gay, 1978). The dry weight of the aerial part is also determined (Tadja, 1996).

The root system of young pine seedlings is composed of a principal root with short insereted roots. The total length of the root system is calculated. The total number of short roots carried on the pivot and the principal root is noted; it is the total branching of the root system (Gay, 1978).

After examination, the root systems of the seedlings are preserved in the FAA, (mixture of ethanol, acetic acid and formalin with the proportions 92/2/6mL)

Observation of the mycorrhizal association between *Terfezia claveryi* and *Pinus halepensis*

The root systems of Aleppo pine are carefully washed with tap water, placed in a 10% KOH solution for 3 hours at 90 °C to empty their cell contents, rinsed with distilled water and then stained with a solution of fuschine acid at 0.1% in lactophenol for 15 min at 90 °C (Boullard, 1968). After rinsing with distilled water, 0.5cm root fragments are mounted between slide and coverslip in a drop of glycerol / lactic acid (V: V) and observed under a light microscope.

Statistical analysis

Statistical analysis (Statistica SPSS 21) was performed to compare the means of height, dry weight, root length and number of short roots of aleppo pine seedlings.

RESULTS AND DISCUSSION

Characteristics of the mycelial culture of *Terfezia claveryi*

The colony of this strain of desert truffle, on malt agar medium (1%), has a white to light brown intramatriciel mycelium with dark brown reverse. Under light microscopy, the mycelium appears branched, irregular, septate, and with numerous bulges (Fig. 1).

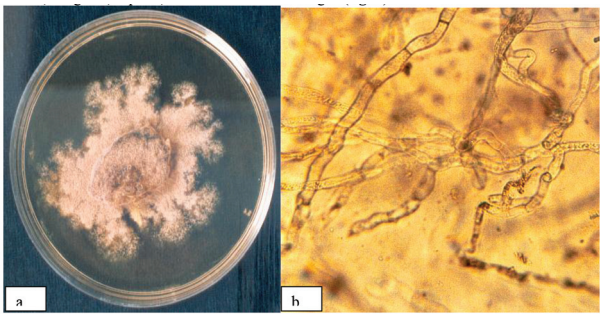

Fig. 1. Aspects of the mycelial strain of *Terfezia claveryi*: a) macrocroscopic appearance in Petri dishes and b) microscopic appearance with light microscope (Gx1000).

Description of the association between *Terfezia claveryi* and *Pinus halepensis* in axenic conditions

On the surface of the substrate containing the inoculated plantlets, many mycelial filaments growed and interpenetrated between the vermiculite leaflets. No fungal development was

observed in the control cultures, which proves the axenic character of the pine seedlings (Fig. 2).

The aerial part of the inoculated seedlings and controls showed good development (Table 1, Fig. 3).

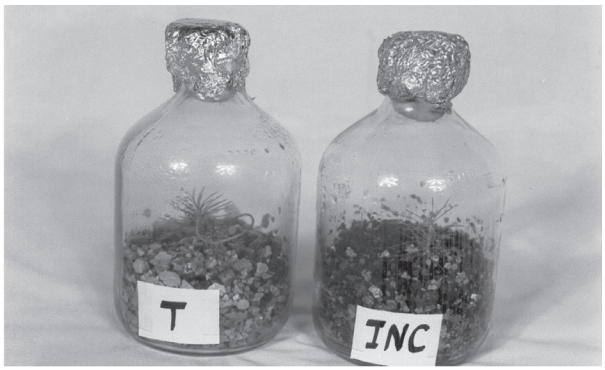

Fig. 2. Axenic culture of *Pinus halepensis* mycorrhized by *Terfezia claveryi*.

Table 1. Height of aerial parts of pine seedlings after 10 months of culture in axenic conditions

Aleppo pine seedlings	controls	Inoculated
Observed averages (cm)	8±2	16.38±3

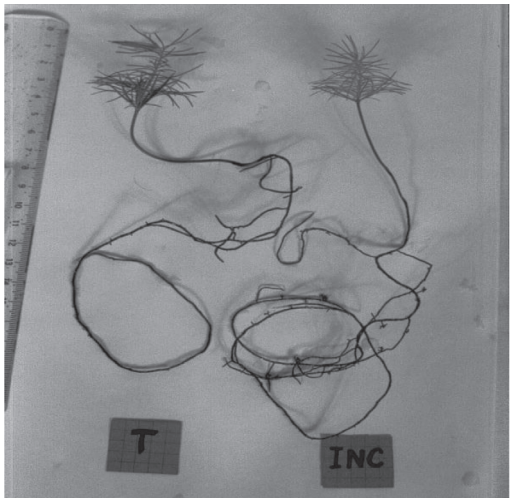

Fig. 3. Appearance of pine seedlings inoculated by *T. claveryi* (INC) and controls (T).

Statistical analysis performed on the aerial part showed that seedling inoculated with *T. claveryi* significantly improves seedling growth (Tables 2 and 3). Similar results have been obtained for several plants mycorhized with desert truffle (Fortas and Chevalier, 1992; Rovolanirina, 1986; Zitouni-Haour *et al*., 2014).

The study of the root part revealed the presence of a significant branching in the seedlings

Table 2. Dry weights of aerial parts of pine seedlings after 10 months of culture under axenic conditions

Aleppo pine seedlings	Controls	Inoculated
Observed averages (mg)	62±1	146±2

Table 3. Quantitative assessment of the development of the root system of pine seedlings after 10 months of culture in axenic conditions

Aleppo pine seedlings	Controls	Inoculated
Total root system length (cm)	56.1±2.1	172.3±2
Number of short roots (total branching of the root system	29±1	142±2

inoculated. An accentuated dichotomy is also observed on the short roots. Microscopic observations of the roots of the inoculated seedlings revealed the presence of *Terfezia claveryi* mycelial filaments crossing the cortical pine cells (Fig. 4).

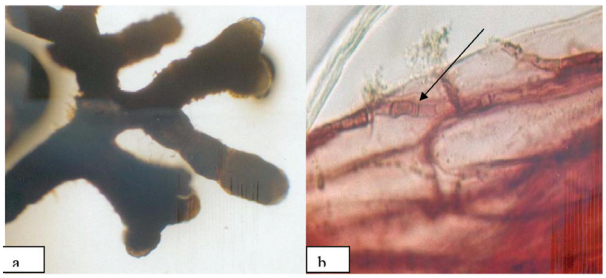

Fig. 4. Aleppo pine roots mycorrhized by *Terfezia claveryi*: a) aspect of the short root (Gx40) and b) cortical roots penetrated by the mycelial filament of *T. claveryi* (arrow) at Gx1000

CONCLUSION

The mycorrhization between *Terfezia claveryi* and *Pinus halepensis* in axenic conditions allowed us to control the axenic cultivation of Aleppo pine seedlings, on an inert substrate impregnated with nutrient solution. Also, we observed an improvement of the growth and morphological changes on the inoculated pine seedlings. On another side, we detect the presence of the *Terfezia claveryi* filaments in the cortical cells after 10 months.

The association between *Terfezia claveryi* and *Pinus halepensis* ensures the production of vigorous pine seedlings with good growth. These seedlings can be transplanted into desert or degraded zones and used in reforestation programs.

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