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La mycorhization des plantes forestières en milieu aride et semi-aride et la lutte contre la désertification dans le bassin méditerranéen

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Edible fungi adapted to arid and semi-arid areas. Molecular characterization and *in vitro* mycorrhization of micropropagated plantlets

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SUMMARY - PCR-RAPD techniques were used for characterization and identification of *Terfezia claveryi*. On the other hand, *Helianthemum almeriense* micropropagated plantlets were *in vitro* mycorrhizated with *Terfezia claveryi* on MH medium. The mycorrhization rate was about 75%. *Terfezia claveryi* formed ectendomycorrhizae with a discontinuous mantle of lax hyphae.

Key words: *Terfezia claveryi,* PCR, micropropagation, mycorrhization.

RESUME - *"Champignons comestibles adaptés aux zones arides et semi-arides. Caractérisation moléculaire et mycorhization* in vitro *de plants de micropropagation". Des techniques moléculaires (PCR-RAPD) ont été utilisées pour la caractérisation et identification de* Terfezia claveryi. *D'autre part, on résume aussi les renseignements concernant la mycorhization in vitro des* Helianthemun almeriense *avec* Terfezia claveryi, *dont le pourcentage de mycorhization a été de 75%. Finalement on décrit les ectendomycorhizes formées in vitro, caractérisées par le manteau avec une organization lâche des hyphes.*

Mots-clés *:* Terfezia claveryi, *PCR, micropropagation, mycorhization.*

Introduction

The species of the *Terfezia* genus, together with *Balsamia*, are called desert truffles or turmas (Morte *et al.*, 1994), which are very important in some of the coastal countries of the Mediterranean.

Terfezia claveryi Chatin, is a very frequent ascomycetes in marl-gypsum soils of the semi-arid western Mediterranean area, and establishes mycorrhizal symbiosis with several species of the *Helianthemum* genus, both annual and perennial (Honrubia *et al.*, 1992).

As Morte *et al.* (1994) said, the North African countries, Saudi Arabia, Kuwait, Iraq, Iran, Turkey, Egypt and Italy are the most important desert truffles consumers. In Spain, in the region between Murcia-Almería-Granada and in western Andalucía and

Extremadura, the "turmas" are also very appreciated. The culinary and the commercial value of the "turmas" does not seem to vary among the species.

The traditional criteria of macro and micromorphological characteristics of the fungi fruit bodies should be sufficient for the characterization and identification of these fungi. Contrary to this, the morphological identification of these fungi from their mycorrhizal structures is quite difficult. In the case of ectomycorrhizae, the hyphal organization of the fungal mantle can lead to the genus approximation and even the species in some cases. However, in the endo- and ectendomycorrhizas, the fungi involved do not form any type of myceliar organization that allows their definitive identification through morphological criteria. Due to this, it is necessary to resort to other work tools that allow us to discriminate between the fungi involved in the two types of the symbiosis. One way of doing this is through molecular methods (Gutiérrez *et al.*, 1995).

DNA analysis by use of polymerase chain reaction (PCR), has proved to be an instrument of great interest for mycorrhizal fungi identification from their colonized root tissues (Farmer and Sylva, 1994). Furthermore, this technique reveals the intraspecific genetic variability (Gandebouef *et al.*, 1994). Genetic sequence amplification by random amplification of polymorphic DNA (RAPD), permits the establishment of differences between species and individuals at molecular level (Cenis *et al.*, 1993; González and Ferrer, 1993).

As respects micropropagation, we think that it is necessary, because it helps us to know the plant nutritional requirement, thus letting us obtain a better mycorrhization. This mycorrhization would help plants to adapt better to the *post vitro* conditions during the acclimatization phase and later to field conditions, and, at the same time, provide a commercially interesting end-product (Morte *et al.*, 1994).

Awamed *et al.* (1979), Awamed (1981) and Chevalier *et al.* (1984) have made mycorrhizal symbiosis in semisterile conditions, whereas Fortas (1990), Fortas and Chevalier (1992), Roth-Bejerano *et al.* (1990) and Kagan-Zur (1994) have made the mycorrhizations in sterile ones (Morte *et al.*, 1994). *H. almeriense* and *T. claveryi* association has been synthesized *in vitro* on modified MMN medium and on MH medium, which is under patent (Morte and Honrubia, 1994).

Our main objective is to apply the PCR technique to study the hypogeous fungi that establish symbiosis with *Cistacea* family in southwest Spain (Gutiérrez *et al.*, 1995), and to apply the *in vitro* mycorrhization process of *Terfezia claveryi* and *Helianthemum almeriense*.

Material and methods

DNA extraction and amplification

Terfezia claveryi was the hypogeous fungi selected for these experiments. The DNA extraction was done according to the process described by Garre *et al.*, (1994), with some modifications. After that, DNA amplification by PCR-RAPD was done in two different thermalcyclers, Intelligent Heating Block model 2024 (Durviz) and DNA

Thermal Cycler model 480 (Perkin-Elmer). The amplification products were separated in agarosa gels and were seen under ultraviolet light.

Micropropagation and mycorrhization

H. almeriense plantlets were micropropagated following the procedure described by Morte and Honrubia (1992). After 4 weeks, plantlets for the experiment were collected from the rooting medium of Murashige and Skoog (MS) (1962) with salt strength 1:4, without auxins.

Culture conditions throughout the micropropagation process were 25±2ºC, 40µmol/m^2/s^1 Groulux fluorescent light 16 hour photoperiod. These conditions were also maintained for the mycorrhizal synthesis experiments.

Isolates of *T. claveryi* were obtained from fruit-body tissues. The best growth was in MMN agar medium (Marx, 1969) at pH 8.0.

According to Morte and Honrubia (1994), rooted *H. almeriense* plantlets were inoculated *in vitro* with mycelium of *Terfezia claveryi* on MH medium and on modified MMN medium.

After mycorrhization, plantlets were transferred to pots containing a mixture of peat-vermiculite-sand (1:1:1 v/v). These plantlets were acclimatized in greenhouse conditions (Morte and Honrubia,1994).

The percentage of fungal root colonization was estimated according to the gridline intersect method (Giovannetti and Mosse, 1980).

Results and discussion

With respect to DNA analysis, we are carrying out trials to verify the bands' repetitiveness. As such, we cannot provide results at the moment. In spite of this, the DNA extraction and amplification method proposed can be valid for hypogeous fungi, mycorrhizae and plants characterization and identification.

The mycelial growth in the agar allowed the perfect colonization of the entire root system of the plantlet. The mycorrhization percentage obtained varied from 61% to 75% after two months in culture (Morte and Honrubia, 1994).

As Morte and Honrubia (1994) show, there were no survival differences between the mycorrhizal and control plantlets on MH medium. On the contrary, plantlet growth on MMN medium was not satisfactory.

With respect to the morphology of the mycorrhiza formed, it was characterized by a discontinuous mantle of lax hyphae around the mycorrhizal root (Morte and Honrubia, 1994). The hyphae formed coils which occupied the whole cell lumen. The rest of the structures formed were similar to those of an ectendomycorrhiza like those described with MMN medium by Morte *et al.* (1994) and by Fortas and Chevalier (1992).

In vitro mycorrhization allowed us to control the optimum conditions of symbiosis and growth of the fungal and plant species , as well as to select the fungus strain and plant explants most appropiate to culture conditions in specific field conditions.

Although there are several works on *in vitro* mycorrhization, few have used agar as substrate (Malajczuk and Hartney, 1986; Tokin *et al.,* 1989; Roth-Bejerano *et al.*, 1990). The medium used in this experiment was designed with agar because it enables us to observe the growth of the mycelium on the root and, thus, the start of the mycorrhization. The agar also turned out to be more easily manageable than any other type of substrate (Morte and Honrubia, 1994).

Conclusions

With respect to DNA analysis by PCR-RAPD, we estimate that this is very important for quality controls on controlled mycorrhizations.

The *in vitro* system, by using the MH medium, allows us to obtain an effective, rapid, reliable and controlled mycorrhization. As a result, it has obtained a patent (Morte and Honrubia, 1994).

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