COST STSM Report

Action FP1203

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Period: From 2014-03-23 to 2014-04-06

STSM Topic: Research on desert truffles – Production Systems

COST STSM Reference Number: COST-STSM-FP1203-16555

Host: *Dr. Asunción Morte Gomez*, Micología-Mycorrhizae research group, Plant Biology Department, University of Murcia, SPAIN.

1. Background

Several desert truffles species occur in semi-arid ecosystems along the Mediterranean basin. Many of these are endemic to this region and live in symbiotic relationship with various plant species, and act as desertification and soil erosion prevention agents. Some of these desert truffles species are highly appreciated by its gastronomic value and are usually picked and sold on local markets, constituting an important economical income for many families in rural populations. Desert truffles are non-wood forest products with high gastronomic and economic interest, and even though, they do not have the intense taste and extravagant price of the true truffles (*Tuber*) they can present more interesting yields than true truffles, which makes their exploitation economically viable. The research effort on desert truffles and true truffles has always been uneven and much higher in the latter case.

Exception made for the Micología Mycorrhizae research group of the Faculty of Biology, Plant Biology Department, University of Murcia (Spain), led by Professor Mario Honrubia

and Professor Asunción Morte that have developed numerous studies on the biology, ecology and cultivation of desert truffles, particularly *Terfezia claveryi*. The advances made by them in the last two decades, have improved the ecological and economical value of this natural resource. However, it is also important to apply this knowledge to other species of desert truffles with different ecological requirements, in order to enhance the global value of this NWFP.

2. Purpose of the STSM

The main purpose of this STSM was to be acquainted with the various steps of the *Terfezia claveryi* production process (inoculum preparation, plant production, *in vitro* and *in vivo* mycorrhizal synthesis, and certification of mycorrhizal plants, establishment of the production system, production management and production limiting factors) and learn the techniques and methods for desert truffle production. Another important purpose was the establishment of a future collaboration with the Micología-Mycorrhizae research group, with the scope of broadening the existing knowledge on the taxonomy and chorology of desert truffles.

3. Description of the work carried out during the STSM

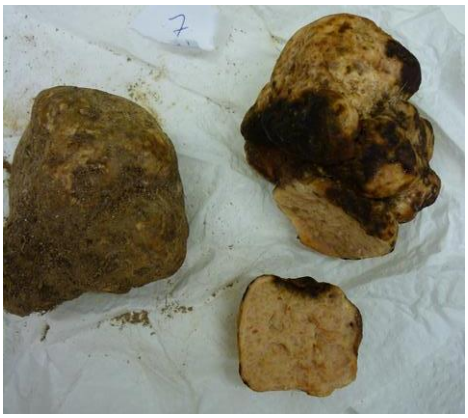
Terfezia cultivation is an extensive process with many steps, some of them requiring months, taking about 2 years from the mycorrhizal plant plantation to sporocarp collection. Since it was impossible to follow all the process sequentially, each step was observed individually using biological material supplied by the Micología Mycorrhizae research group.

The steps observed during the STSM are described below:

3.1. Pure culture isolation of *Terfezia* and production of inoculum.

Fresh *Terfezia* ascocarps were washed in tap water, brushed free of adhering soil particles and cleaned superficially with ethanol (70%). In a laminar air-flow cabinet, the ascocarps were carefully broken, opened and a small piece of glebal tissue

removed with a scalpel and placed on a Petri dish containing Modified Melin-Norkrans medium (MMN). The Petri dishes were labeled, sealed with parafilm and placed on an incubation chamber at 26ºC.



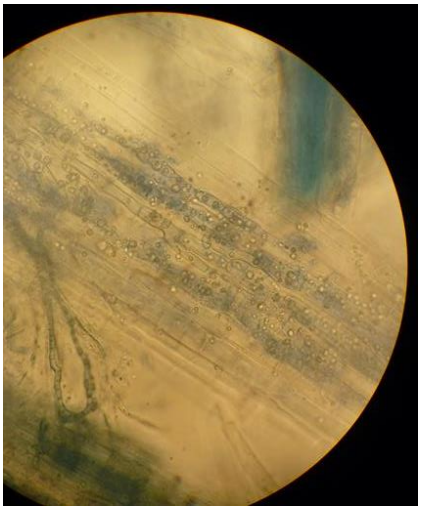
3.2. *In vivo* and *in vitro* mycorrhizal synthesis between *Helianthemum almeriense* and *Terfezia claveryi*

3.2.1. *In vivo*: Mature ascocarps were dried and scraped in to distilled water (6 g per liter) and used to inoculate *Helianthemum almeriense* plantlets obtained from germinated seedlings. Plantlets were transferred to containers with a mix substrate of sterilized soil and perlite (1:1, v/v). 15 ml of inoculum were added to each container. Plants were grown in a greenhouse.

3.2.2. *In vitro*: Mycelium pure cultures were used to inoculate *in vitro* micropropagated *Helianthemum almeriense* plantlets. Prior to inoculation, each plantlet was transferred to a test tube containing autoclave-sterilized vermiculite watered with half strength Murashige-Skoog medium nutrient solution (MS) in a laminar air-flow cabinet. Two pieces of mycelium grown on MMN medium were also transferred to each tube. Plants were grown in an incubation chamber.

3.3. Certification of *Helianthemum almeriense* plantlets colonized by *Terfezia claveryi*.

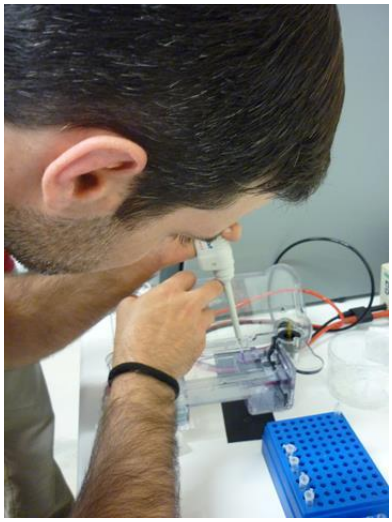
Plants were randomly chosen to examine the root system and observe the abundance and condition of *Terfezia* mycorrhizae. Root tips were washed in tap water and stained with 5 % blue ink in acetic acid (5%). A quantitative assessment of mycorrhizal formation was made by microscopic examination of root tips.

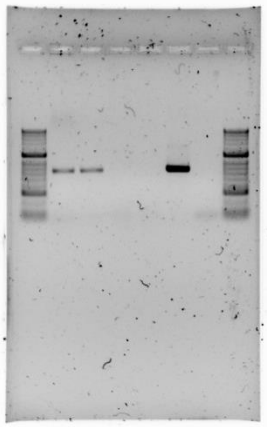


3.4. *Terfezia* DNA analysis

Genomic DNA was isolated from 150 to 200 mg of inner gleba of *Terfezia* ascocarps using the E.Z.N.A. fungal DNA kit (Omega Bio-Tek, Doraville, GA, USA). The internal transcribed spacer (ITS) region of the rDNA was amplified using ITS1f and ITS4 primers. All PCR amplifications (final volume of 25 µl) were carried out in a Mastercycler Gradiente Thermocycler. PCR products were purified using the E.Z.N.A. Cycle-Pure kit (Omega Bio-Tek, Doraville, GA, USA).

Clean PCR products were to be sequenced in both directions at Molecular Biology Service (University of Murcia).





3.5. Observation of experimental plantations for desert truffles production.

During the field trip to Calasparra it was possible to visit the experimental *Helianthemum almeriense-Terfezia claveryi* and *H. violaceum-T. claveryi* plantations. The effects of land management techniques such as: weed control (mechanical tillage versus application of glyphosphate-based herbicide); fertilization and irrigation on desert truffle yields were discussed.

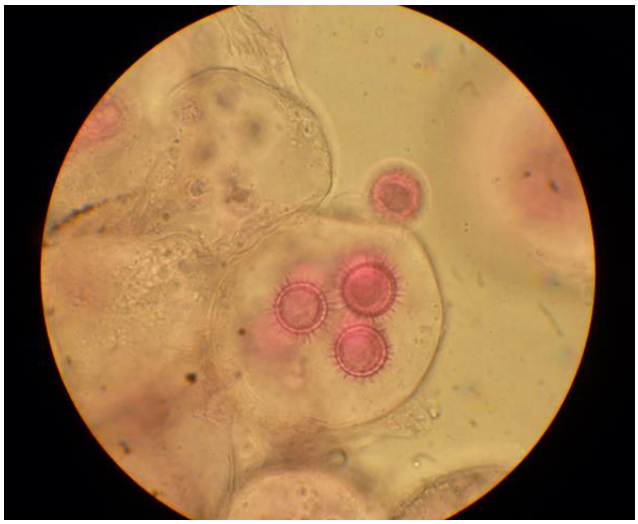
3.6. Study of *Terfezia* specimens collected in Portugal

3.6.1. *Terfezia* specimens were isolated in pure cultures and grown in 2 types of modified MMN solid media, in order to optimize mycelium growth and viability.

3.6.2. Microscopic identification of *Terfezia* specimens.

Asci and ascospores were examined using an Olympus BX51 binocular microscope equipped with a digital camera (Canon PSpro1). *Terfezia* ascospores were stained with acid fuchsine solution (0.01 % acid fuchsine in acetic acid, ethylene glycol and lactic acid, 1:1:1, v/v/v) to improve visualization and measurements.

3.6.3. DNA analysis was performed using the same methodologies mentioned in section 4.



4. Description of the main results obtained

In the Plant Biology Department facilities, under the supervision of Profª Asuncion Morte, it was possible to learn and practice the various techniques used in desert truffle cultivation. Due to the strong practical component of all the processes, this scientific mission was of the utmost importance for my research experience as a PhD student and my future professional career as a scientist.

As soon as the *Terfezia* DNA sequence are available it will be possible to compare them with other DNA sequences in GenBank and thus validate the morphological identification of the specimens brought from Portugal. This is an important result, since desert truffles are still reckoned as a poorly known NWFP.

5. Future collaboration with host institution

Due to the mutual interest in desert truffles, it is expectable that we start a more solid collaboration, namely revising desert truffles *excicata* collections in Portugal.

6. Foreseen publications/articles resulting or to result from the STSM

It is predictable that *Terfezia* DNA sequences will be published in GeneBank.