J. Mater. Environ. Sci. 5 (6) (2014) 1902-1905
ISSN : 2028-2508
CODEN: JMESCN

Study on ascospores germination of a Tunisian desert truffle, *Terfezia boudieri* Chatin

A. Slama[1*] , Z. Fortas [2], A. Boudabous [3], M. Neffati [1]

[1]*Laboratory of management and valorisation of forestry resources - National Institute for Researches on Rural Engineering, Water and Forests, INRGREF, BP.10 Ariana 2080, Tunisia*
[2] *Laboratory of Microbiology; Faculty of sciences Oran - Senia Algeria.*
[3]*Microorganisms and Actives Biomolecules Laboratory, Faculté des Sciences de Tunis, 1060 Tunisia.*
[4]*Range Ecology Laboratory, Institut des Régions Arides, 4119 Médenine Tunisia*

Received 29 June 2014; Revised 19 July 2014; Accepted 19 July 2014.
**Corresponding Author. E-mail: slamaawatef@yahoo.fr*

Abstract
Desert truffles are edible ascomycete fungi. These mushrooms are economically important and widely distributed in arid and semi-arid regions of Tunisia. This study aims to characterize the germination of *Terfezia boudieri* Chatin that would be important to isolate homokaryotic hyphae. Ascocarps of *T. boudieri* were collected from the southeast of Tunisia. Ascospores germination was tested on three culture media: Malt extract agar 1%, Melin Nokrans modified and the Potato dextrose agar. The germination was only observed on Malt extract agar 1% medium and germinating rate was in order of 4%. Isolation of germinating hyphae was successful. These hyphae derived from two material, i.e., from a single ascospore and from ascospore inside asci.

Keywords: Ascomycota, ascospore, culture media, germination, hyphae, Terfezia boudieri Chatin., Tunisia.

1. Introduction
Truffles are one of the most appreciated edible fungi in the world. From these fungi, those of desert were affiliated to *Terfeziaceae* family [1] then included in *Pezizaceae* [2]. Desert truffles are the most popular fungi in arid and semi-arid zones bordering the Mediterranean countries such as Tunisia [3-5].
These fungi establish mycorrhizal association with numerous host plant and form hypogeous fructification. Fruit bodies contain asci and ascospores whose differs among species according to their shape, size and ornamentations. Several data of truffle life cycle are lacking until now. Different studies tested mycelia isolation from gleba and spore germination with *Tuber* species [6-12]. Whereas for desert fungi, mycelium culture still weakly investigated [13]. Mycelia of several Moroccan truffle species were isolated using some glebe fragment [14]. Awameh and Alsheikh isolated mycelium from mycorrhizae [12]. According to Bonfante and Fontana [15], mycorrhzas are possible only with heterokayotic mycelium of *Tuber melanosporum* (originating from plasmogamy). Bejerano and Kagan-Zur [16], supposed that « gleba is a mixture of two intertwined types of heterokaryotic hyphae» or fertile and sterile mycelia. Isolation of homokaryotic hyphae using ascospore germination can facilitate later the survey of the different stages of growth of these fungi. It constitutes an interesting result to control desert truffle cultivation in growth room.
The objective of the current research was to examine for the first time, the germination of the ascospores of the Tunisian *Terfezia boudieri* Chatin and to isolate germinated hypha.

2. Materials and Methods
2.1. Sampling
Ascocarps of *Terfezia boudieri* Chatin were harvested in March 2009 from the region of Ben Guardene in the southeast of Tunisia (figs.1-2). They are desiccated during two months and preserved in room temperature at 30°C.

2.2. Ascospores isolation
Dried ascoscarps were firstly sterilized with alcohol 70°. Fragments from gleba were then placed in sterile water during 12 hours and mixed by a vortex agitator (CYAN CL001). 1ml of the suspension was smeared on three medium: Malt extract agar 1%, Melin-Nokrans- Modified [17] and Potato Dextrose Agar (PDA). For each ascocarp, four fragments were sampled and 10 Petri dishes were inoculated with every ascospores suspension. So in totality 40 dishes were prepared. Petri dishes were held in a growth room at 25± 2 °C and checked daily under a light microscopy (Leica DM2500) in order to mark

isolated ascospores and to characterize all germination phases. Once ascosporic germination was observed, we proceed to transfer hyphae in a new Petri dish containing the same culture media.

Figures 1-2: Harvested ascocarp location in Ben Guardan (Tunisia).

2.3. Germination rate

Under every Petri dish ascospore, location was marked by miscroscopic microscopic observation. From the 40 dishes, 100 ascospores were chosen randomly and checked every day. The sum of germinated ascopores related to percent show the germination rate.

3. Results and discussion

Among the three analysed media (Malt extract agar 1%, MNM and PDA), ascospores germination was obtained only in the Malt extract agar 1%. The percentage of germination found in this medium was 4%. Drying of the Petri dishes placed in the room culture prevents germination study for a long period.

Ascospores germination was characterized by a lag -phase (90 days) of unknown origin. This phase reflects the ability of ascospores to withstand difficult natural conditions that characterize the arid region in Tunisia. Before germination, ascospore swells and forms small globules (generally between two and four, fig. 3). Germination depends on culture medium properties [18, 19]. The medium based on malt extract proved the best medium for carrying spore germination. This medium is used for the cultivation of mushrooms [20, 21]. Bejerano and Kagan-Zur [16] announced that duplication is more significant at Fontana medium and according to them, this medium support hyphal propagation but not the germination of the spores.

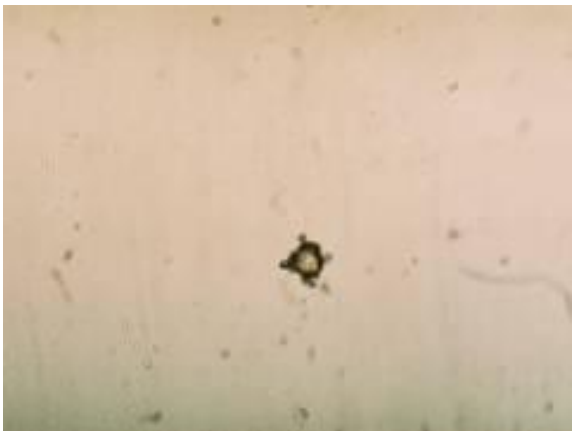
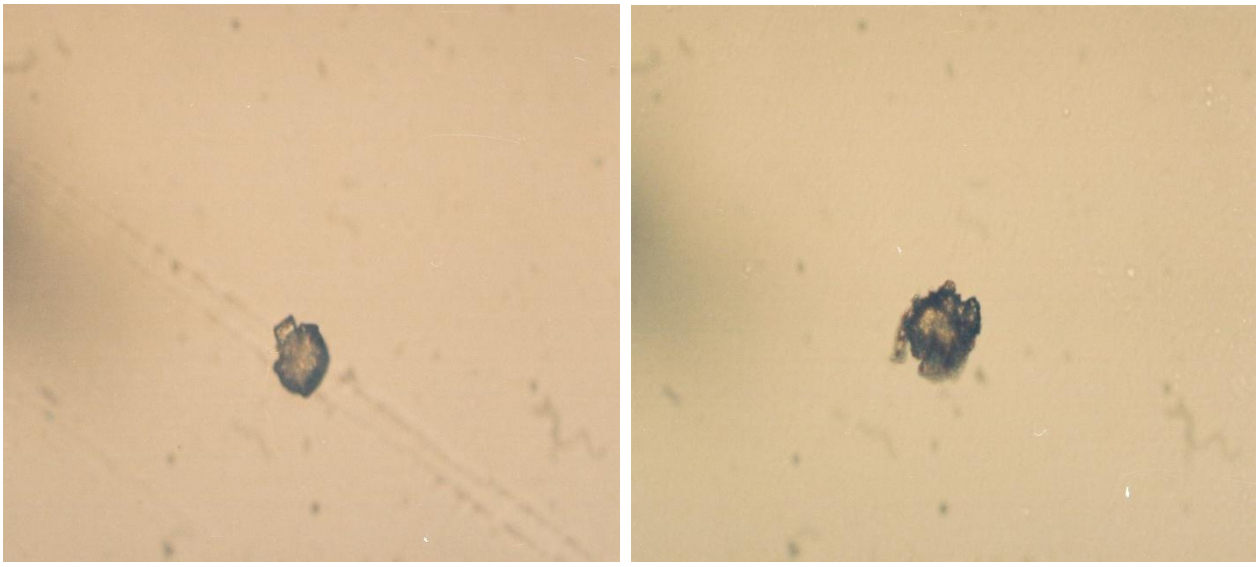


Figure 3: Microscopic observation of *Terfeia boudieri* ascossopre: small lipidic globules (10X).

T. boudieri germination was manifested by the emergence of distinct hyphae from the ascospore wall (fig.4-5). Great number of ascospores breaks up. This crump can be explained as the result of osmotic transfer between ascospore and culture media specially the important influx of water to the interior of ascospores (natural organ of resistance and dissemination).

The formed hyphae emerge without ramification or fructification and then it penetrates within the agar medium. As for our species, Glass et al. [22] indicated that in ascomycetes no clamp junctions can be found in vegetative hyphae produced after ascospore germination, opposed to the basidiomycetes hyphae. In ascomycetes, microscopic determination of homokaryotic or heterokaryotic mycelium is difficult [16] but as *Terfezia boudieri* is an ascomycete fungus we can suggest that the isolated hypha from ascospore germination is haploid. Further replicates of different strains together can facilitate the studies of diploid mycelia and fungus fructification in order to clarify some ambiguities in fungus life cycle.



Figures 4-5: Germinated ascospore of *Terfezia boudieri* on Malt extract agar 1% (20X)

Germination of *T. boudieri* ascospore was observed also inside ascus (fig.6) in the Malt extract agar 1% medium. The same lag -phase preceded this germination (90 days).

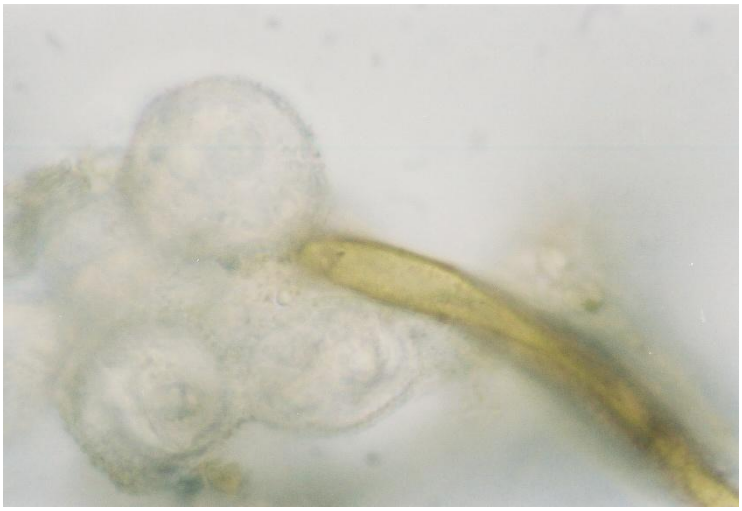


Figure 6: Microscopic observation of germination of *Terfezia boudieri* ascospore inside asci on the Malt extract agar 1% (1000X).

Germination within ascus was characterized by the access of the formed hyphae through the membrane of the ascus. According to our results, the mycelium formed after ascospore germination was colourless (fig.7). Replication of germinated ascospore showed that *Terfezia boudieri* mycelium spends a long time to grow in the Malt extract agar 1% in fact its elongation was very low.
An early contamination of bacterial and fungal origin was observed in some Petri dishes 24 hours after incubation (fig. 8). This infection is probably residual to the presence of microorganisms inside the ascocarps [23].

Figure7: Microscopic observation of germinating hypha on the Malt extract agar 1% (1000X).

Figure 8: Visible contamination 24h after incubation of Petri dishes.

Conclusion

This study allowed the characterisation of *T. boudieri* ascospores germination derived from south Tunisia and proves that germination exceeds a lag -phase for unknown reasons. Germination depends on culture medium properties and it was pronounced by the broadcast of hypha from single ascospore or from ascospore inside asci. Elongation time of germinating hypha in the Malt extract agar 1% was low according to the time.

References

1. Astier J. Truffes blanches et noires. Ed. Louis- Jean. (1998).
2. Laessoe T., Hansen K. *Mycological Research* 111(2007)1075.
3. Patouillard M. N. *Journal de Botanique* 10 (1894)181.
4. Malençon G. *Personia* 2 (1973) 261.
5. Slama A., Fortas Z., Neffati M., Khabar L., Boudabous A. *Bulletin Scoiété Mycologique France* 122 (2006) 187.
6. Melendez-howell L.M., Cailleux R. *Compte Rendue Hebdomadaire Séances Académique Scientifique* D 273 (1971) 2485.
7. Grente J., Chevalier G., Pollacsek A. *Compte Rendue Hebdomadaire Séances Académique Scientifique* D 275 (1972) 743.
8. Rouquerol T., Payre H. *Revue Mycologique* 39 (1975a) 107.
9. Rouquerol T., Payre H. *Revue Mycologique* 39 (1975b) 213.
10. Fontana A. *In*: *Atti I Congr. lntern. sul Tartuf*, Spoleto, Italy (1968)127.
11. Fontana A. *Allionia* 17 (1971) 19.
12. Chevalier G. *Compte Rendue Séances Académique Agriculture France* 58 (1972) 981.
13. Kagan-Zur V., Roth-bejerano N., Sitrit Y., Morte A. Desert truffle ed. *Springer* (2014).
14. Khabar L. Etudes pluridisciplinaires des truffes du Maroc et perspectives pour l'amélioration de production des « Terfess » de la forêt de la Mamora. Université Mohamed V Agdal University, (2002).
15. Bonfante Fasolo P. and Fontana A. 1973. Sulla nutrizione del mecelia di *Tuber melanosporum* Vitt in coltura. Atti della Academia delle Scienze di Torino 107: 713–741.
16. Bejerano N., Li Y., Kagan V. *Antonie van Leeuwenhoek* 85(2004) 165.
17. Marx D. H. *Phytopathology* 59 (1969) 153.
18. Delmas J. Les Champignons et leur culture, ed. La maison rustique (1989).
19. Awameh M.S., Alsheikh A. *Annales de Phytopathologie* 11(2) (1979a) 223.
20. Fortas Z., Chevalier G. *Cryptogamie Mycologie* 13(1) (1992) 21.
21. Crozier J., Molmes K., Graig G., Nayagam S. *Material und Organismen* 33 (4) (2000) 245.
22. Glass N.L., Jacobson D.J., Shiu P.K.T. *Annu. Rev. Genet.* 34 (2000) 65.
23. Barbieri E., Bertini L., Rossi I., Ceccaroli P., Saltarelli R., Guidi C., Zambonelli A., Stocchi V. *FEMS Microbiology Letters* 247 (1) (2005) 23.