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Evaluation of Morphology, Cytology and Mycorrhizal Relationships of Desert Truffles (*Terfezia boudieri*) in Iran

[1]Ali Ammarellou, [2]Hosein Saremi and [3]Fahretin Gucin
[1,2]University of Zanjan, Iran
[3]University of Fatih, Turkey

Abstract: Wild edible fungi are collected for food and to earn money in more than 80 countries. In one of these countries, Iran, several species of edible truffle can be found growing in Bandar-e Abbas, Tabriz, Qazvin and Zanjan provinces. They are normally harvested during mid-winter and early spring, particularly from January to March. Experinced harvesters identify the location of the truffles from crevices that appear in the surface of the soil above the truffles. A yellowish species grows solitary in the soil, is about the size of a large potato and has affirm texture. This species forms 5-10 cm below the soil surface and is yellowish inside. The truffles that had been collected from these areas were investigated in laboratories. Cytological evaluations showed that each ascus contains 8 ascospores. According to morphologic and cytological characteristics, this species was identified *Terfezia boudieri*. This species is not ectomycorrhizal with trees. Because the vegetation in the area consists of moss, lichen and perennial sedges. The field and laboratory studies showed that *Kobresia bellardii* have ectomycorrhizal associations with *Terfezia boudieri* in studied areas.

Key words: *Terfezia*, mycorrhizal associations, sedges

INTRODUCTION

Most species of fungi that produce macroscopic hypogeous (fruit below ground) sporocarps are ectomycorrhizal basidomycetes and ascomycetes (Miller *et al*., 1999) and are commonly referred to as truffles, Truffles are edible hypogeous fruit bodies produced by many genera of fungi belonging to the class Ascomycetes. Among these, *Terfezia* (Pezizaceae for merely *Terfeziaceae*) and *Tuber* (*Tuberaceae*) are classified in different taxa of the in pezizales (Norman and Egger, 1999; Percudani *et al*., 1999; Bejerano *et al*., 2004). Fungi belonging to family *Pezizaceae* (*Terfeziaceae*) form either terfezia-type ectomycorrhiza or ectendomycorrhiza, depending on the amount of phosphorous in the medium (Fortas and Chevalier, 1992), whereas those belonging to the *Tuberaceae* form ectomycorrhiza (Bonfante Fasolo and Fontana, 1973; Delmas, 1987; Pacioni, 1989). Both Tuber and Terfezia produce hypogeous ascocarps in which sterile and fertile veins were found (Ceruti, 1960; Parguey-leduc *et al*., 1990; Miranda *et al*., 1992).

Truffle spores are not spread by the wind. The truffle odour attracts animals, e.g., squirrels, mice and pigs, which feed on the fruit body and spread the spores with faeces (Bellina-Agostinone *et al*., 1987). The life-cycle of *Terfezia* and *Tuber* has not been completely elucidated, although some knowledge has indeed been accumulated (Bejerano *et al*., 2004). The early reports on mycorrhizal incidence in sedges were reviewed by Harley and Harley (1987), Tester *et al*. (1987) and Newman and Reddell (1987). Since that time, there is a lot more information about the incidence of mycorrhiza in sedges. Since 1987, information has become available for 221 sedges species, of which 88 (40%) are mycorrhizal, 24 (11%) are facultatively mycorrhizal and 109 (49%) are none-mycorrhizal. Although the effects of mycorrhiza on sedge growth are little known, the abundance and widespread occurrence of this association in sedges from many ecosystems suggests an important ecological role in the natural environment (Bejerano *et al*., 2004). Some reports exists on mycorrhiza associations of *Terfezia* with roots of members of the *Cistaceae* family such as species of *Helianthemum* (Gücin and Dülger, 1997). They form associations with other plants as well, such as *Stipagrostis* sp. (one grass species) especially in Southern Africa, where *Cistaceae* do not occur (Kiraly and Bratek, 1992).

Several species of delicious terfezia can be found growing between 27^{0N} and 39^{0N}, primarily in the Bandar-e Abbas, Tabriz, Qazvin and Zanjan provinces of Iran. They are normally harvested during mid-winter and early spring, particularly from January to March. Experinced harvesters identify the location of the truffles from crevices that appear in the surface of the soil above

Corresponding Author: Ali Ammarellou, University of Zanjan, Iran

the truffles. The research represents a study of the biological, cytological, edaphic requirements and mycorrhizal associations of these truffles.

MATERIALS AND METHODS

Location: The Tarom region is close to the cities of Foman and Manjil in the north-west of Iran to the north of Zanjan and between Gilan and Zanjan provinces. It is a particularly productive region and therefore it was chosen as the main study area. We obtained weather data from a station located in Gilvan in Abbar, Tarom, ca 25 km southwest of the study area. Between 1996 to 2005 the ecological conditions where the truffles were found, were studied; including temperature, rainfall, soil conditions and the plants with which the truffles were associated.

Climate, vegetation and soil: The Tarom region is enclosed by mountains, has a subtropical or Mediterranean climate and receives humid north air currents. In dry years the annual rainfall in only 150 mm, but in wet years it may be as high as 400 mm. Normally a high autumn rainfall induces good truffle production. The truffles were mostly found in barren, uncultivated fields in mountainous areas. The soils were generally rendzinas, chalky, alkaline, granular and well drained. About 1% of organic matter in the top soil is produced by plants.

Morphological studies: Various characteristics of the truffles ascocarps were recorded, including depth of fruiting, colour, shape, texture of terfezia ascocarps and anatomical characteristics. Because perennial sedges was regularly associated with the truffles in the field, morphology and growth of them were studied.

Cytological evaluations: The mycorrhizal plant roots that attached to base of terfezia ascocarps were used for microscopy studies. Root segment slices were mounted on glass slides after staining. The occurrence of fungal structures was assessed by using a compound light microscope. The mycorrhizal status of a plant (i.e., the presence or absence of colonization by mycorrhizal fungal structures) was determined. Also characteristics of asc and ascospore such as size and shape of them were monitored and recorded.

Vegetation and mycorrhizal relationships: The natural vegetation characteristics and kind of growing plants were studied. We evaluated associations between truffle and natural vegetation. Truffle were collected every year and zone of mycorrhizae were analysed carefully. To characterize of mycorrhizal associations, land soil samples of studied area that had crevice of truffle presence was removed about 15×15×15 cm dimensions. The soil profile was washed very slow and softly and zone of physical contact of truffle ascocarp base with roots of plants were prosecuted.

RESULTS

Features of the truffles: The species of truffle that found in Tarom (Fig. 1A) grows solitary in the soil, is about the size of a large potato, forms 5-10 cm below the soil surface, has a firm texture and is yellowish inside. This species has the best flavour. When they mature they produce telltale cracks in the soil surface that are recognized by experienced collectors (Fig. 1B and C). According to morphological and cytological characteristics this species was identified as *Terfezia boudieri*.

Cytological studies: The cytological examination showed that each ascus contains 8 ascospores. (Fig. 2A).In fresh ascocarps the spores not spotted but they form one spot on every spore in dried texture of ascocarps (Fig. 2B). Measure the diameter of ascus is about 60-80 and ascospores is about 15-20 μm.

Fig. 1: The species of desert truffle (*Terfezia boudieri J*) from Iran. The yellowish species grows solitary in the depth of soil (A). Dogs are not used to find truffles in Iran but telltale cracks in the soil betray the presence of a truffle under the surface (B). State of truffle in depth of soil after removed of surface soil (C)

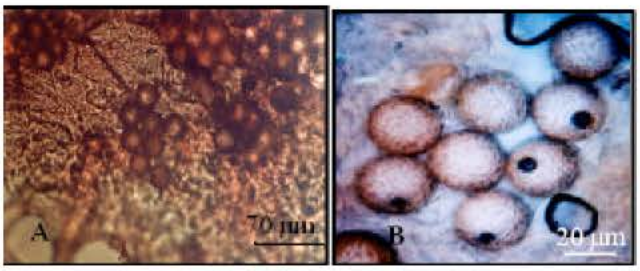

Fig. 2: Every ascus has 8 ascospores and ascus membrane disappeared after maturation (A). In fresh ascocarps (A) the spores not spotted but they form one spot on some spores in dried texture of ascocarps (B)

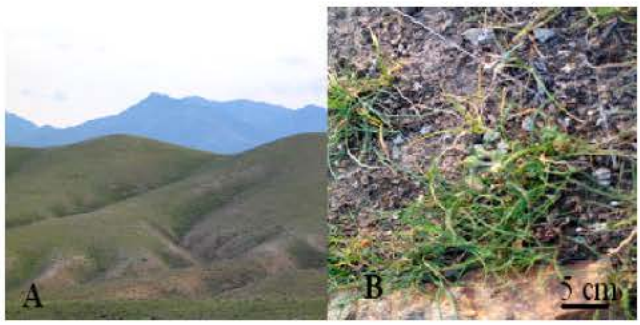

Fig. 3: The view of study area (A) and the vegetation (B)

Fig. 4: The soil profile was washed very slow and softly and zone of physical connection of truffle ascocarp base with roots of sedges were prosecuted (A,B). One truffle and sedge roots typical (C). About 60% of the rooting zone is occupied by the fungus/sedges root relationships (D). The sedges plant had survived from the previous season via rhizome (E). The penetrate of sedge root in to base of truffle ascocarp (F). The size, colour, texture and branching ectomycorrhizal roots of sedges (*Kobresia bellardii* (G). The attached mycorrhizal strands to sedge roots (H and I)

Vegetation and mycorrhizal relationships: The natural vegetation of studied areas in Tarom region is composed of moss, lichen and sedges (Fig. 3). There were no ectomycorrhizal trees in the area. About 60% of the soil volume in the rooting zone of the sedges was occupied by fungal root relationship (Fig. 4D) and so the truffles formed a symbiotic relationship with the perennial sedges (Fig. 4C).

Growth of the sedges increased during autumn when there was a high rainfall, either as a result of seeds that germinated in spring or plants that had survived from the previous season via rhizomes (Fig. 4D and E). This sedges occurred only where truffles were found and developed at the same time. We isolated the sedge roots directly from under zone of *Terfezia boudieri* (Fig. 4A, B and C). Unlike to Europeans truffles that have mycorrhizal life cycle with some trees, we identified these species appear to be associated with a perennial sedges. According to key to the species and variety of sedges, morphological and taxonomic evaluations this sedge was identified as *Kobresia bellardii*.

DISCUSSION

All the mycorrhizal types can be found in arctic and alpine areas (Vare *et al*., 1997). The ectomycorrhizal symbiosis is common in trees and shrubs. These ectomycorrhizal fungi are hosted by *Drayas octopetala*, *Betula nana*, *Salix* sp. and by herbaceous plants such as *Kobresia* sp. and *Polygonum viviparum* (Massicotte *et al*., 1998). In spite of the general consensus that the *Cyperaceae* are nonmycorrhizal, there have been numerous reports of mycorrhizal infection in certain species (e.g., Mejstrik, 1972; Haselwandter and read 1982; Gay *et al*., 1982). Kobresia Willd., a genus of about 50 species and distributed in the northern Hemisphere, especially at high altitudes in the Himalaya, China and central Asia, belongs to the subfamily *Caricoideae* of the family *Cyperaceae* (Willis, 1973; Dahlgren *et al*., 1985).

The *Caricoideae* also includes genera such as *Carex* L., *Schoenoxiphium nees* and *Uncinia pers*. The Himalayan region contains the major diversity of *Kobresia* with about 30 species. Kobresia is one of the important genera in the alpine flora of Nepal Himalaya (80°10'-88°10' E, 26°20'-30°20' N) and economically it is also an important pasture plant dominating a vast area of the alpine region where grazing animals feed on it extensively. Boeckler (1875), Clarke (1883, 1894, 1903, 1908) and Kükenthal (1903, 1909) were the pioneers who worked on Himalayan Kobresi. Ectomycorrhizas are characterized by the presence of a fungal sheath (the mantle), which adheres to the root surface and consists of aggregated hyphae. This mycelium is linked to extrametrical hyphe that explore the substrate and are responsible for the mineral nutrition and water uptake of the symbiotic tissues (Barker *et al*., 1998). Most plants with ectomycorrhiza (ECM) have roots with a modified lateral root branching pattern. This pattern, which is called heterorhizy, consists of short mycorrhizal lateral roots (called short roots) supported by a network of long roots. The restricted growth of short roots may be necessary to allow ECM fungi time to form an association, since these fungi have difficulty colonizing more rapidly growing roots (Wilcox, 1964; Chilvers and Gust, 1982). Such modifications were observed on roots of studied sedges (Fig. 4G). Some reports exists on mycorrhiza associations of *Terfezia* with roots of members of the *Cistaceae* family such as species of *Helianthemum* (Gücin and Dülger, 1997). They form associations with other plants as well, such as *Stipagrostis* sp. (one grass species) especially in Southern Africa, where *Cistaceae* do not occur (Kiraly and Bratek, 1992).

Mycorrhizal infection among sedges appears to occur in response to many factors, both environmental and phylogenetic. While some species appear to be obligately nonmycorrhizal, edaphic influences may be responsible for infection in others. Although taxonomic position does seem to be of importance in determining the mycorrhizal dependence of sedges, the pattern may be a patchwork of both mycorrhizal clades and clades that have adapted to the nonmycorrhizal state. Clearly, however, the evidence presented here and in other recent publications indicates that the coventional wisdom regarding the mycorrhizal condition of the *Cyperaceae* must be reevaluated. Further research is planned on the peculiar life cycle of these truffles. In particular we will attempt to germinate its spores *in vitro*, infect sedges and produce the truffles under controlled conditions. If successful it may be possible to produce the Iranian truffles more quickly than European species of truffles.

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