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Research Article

Mycorrhiza between *Kobresia bellardii* (All.) Degel and *Terfezia boudieri* Chatin

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Abstract: Desert truffles collected from the Tarom region in Iran were investigated in the laboratory. According to microscopic and macroscopic studies, the species was identified as *Terfezia boudieri* Chatin. Field and laboratory studies showed that *Kobresia bellardii* (All.) Degel has ectomycorrhizal associations with *Terfezia boudieri* in the study area.

Key Words: *Terfezia*, mycorrhizal associations, sedges

Introduction

Kobresia bellardii (All.) Degel is a monocotyledonous angiosperm herbaceous plant species with a wide geographical distribution, and is found in relatively snow-free habitats (Atherine et al., 1979; Massicotte et al., 1998). It is a particularly successful species in the alpine meadows of the Front Range and strongly dominates plant cover in many sites that are not extremely wind exposed, such as fell fields, or buried under snow in winter (Atherine et al., 1979). Willard (1963) noted that young *Kobresia* plants were rarely found and that some meadows may have taken as long as 1000 years to develop to their present state, usually forming up to 55% of the plant cover (Willard, 1963; Atherine et al., 1979).

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

Truffle spores are spread by animals. The truffle odour attracts animals, e.g., squirrels, mice, and pigs, which feed on the fruit body and spread the spores in their faeces (Bellina-Agostinone et al., 1987). The life-cycles of *Terfezia* and Tuber have not been completely elucidated, although some knowledge has been accumulated (Bejerano et al., 2004). Early reports of mycorrhizal incidence in sedges were reviewed by Harley & Harley (1987), Tester et al. (1987), and Newman & Reddell (1987). 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 non-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; Muthukumar et al., 2004). Some reports exist on mycorrhizal associations of *Terfezia* with the roots of species of the Cistaceae, such as Helianthemum (Gücin & Dülger, 1997). They form associations with other plants as well, such as Stipagrostis spp., especially in southern Africa, where Cistaceae do not occur (Kiraly & Bratek, 1992).

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Several species of delicious *Terfezia* can be found growing between 27° N and 39° N, 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. Experienced harvesters identify the location of the truffles from crevices that appear in the surface of the soil above the truffles. This study represents an investigation of the mycorrhizal associations of these truffles.

Materials and Methods

Study Area

The Tarom region is close to the cities Foman and Manjil in north-west Iran, north of Zanjan and between Gilan and Zanjan provinces (Figure 1). 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, 25 km south-west of the study area. Between 1996 and 2005 the environmental conditions of the area were recorded, including temperature, rainfall, and soil conditions. The Tarom region is surrounded by the Alborz Mountains, has a subtropical or Mediterranean climate, and receives humid north air currents. Annual rainfall ranges from 150 to 400 mm. Normally, abundant autumn rainfall induces good truffle production. The truffles are mostly found in barren, uncultivated meadows in mountainous areas. The soils are generally rendzina, chalky, alkaline, granular, and well drained.

Macroscopic and Microscopic Studies

Various characteristics of the truffle ascocarps were recorded, including depth of fruiting body, colour, shape, texture of ascocarps, and anatomical characteristics. The morphology and growth of perennial sedges were studied because they were regularly associated with truffles in the field.

The mycorrhizal plant roots that attached to the base of *Terfezia* ascocarps were used for microscopic studies. Root segment slices were mounted on glass slides after being stained. The occurrence of fungal structures was assessed with a compound light microscope. The mycorrhizal status of each plant (i.e. the presence or absence of colonisation by mycorrhizal fungal structures) was determined. Additionally, the size and shape characteristics of asci and ascospores were observed and recorded.

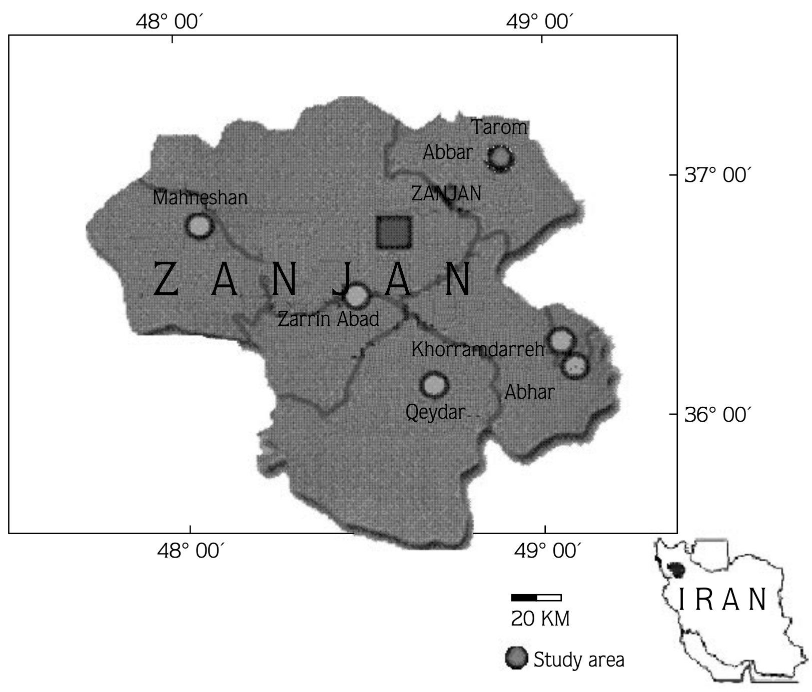

Figure 1. Map of the study area.

The characteristics of the natural vegetation growing in the study area were studied. We evaluated the associations between the truffles and natural vegetation. Truffles were collected and the zones of mycorrhizae were analysed carefully. To characterise the mycorrhizal associations, soil samples of about 15 × 15 × 15 cm were removed from the studied area where crevices indicated the presence of truffles. The soil profiles were washed very carefully and the zones of physical contact between the truffles' ascocarp bases and the roots of plants were investigated.

Results

The truffle species found in the Tarom region (Figure 2A) grows solitary in the soil, is about 3-5 cm long, forms 5-10 cm below the soil surface, has a firm texture, and is yellowish inside. When they mature, they produce telltale cracks in the soil surface that are recognised by experienced collectors (Figure 2B and C). According to macroscopic and microscopic characteristics, this species was identified as *Terfezia boudieri* Chatin. This truffle is one of the most important *Terfezia* species (Chatin, 1892; Philips, 1981).

Microscopic examination showed that each ascus contains 8 ascospores (Figure 3A). The ascus diameter is about 60-80 µm and ascospores measure about 15-20 µm (Figure 3B).

Vegetation and Mycorrhizal Relationships

The natural vegetation of the study area is composed of mosses, lichens, and sedges (Figure 4). About 60% of the soil volume in the rooting zone of the sedges was occupied by fungal root relationships (Figure 5D), the truffles forming a symbiotic relationship with the perennial sedges (Figure 5C).

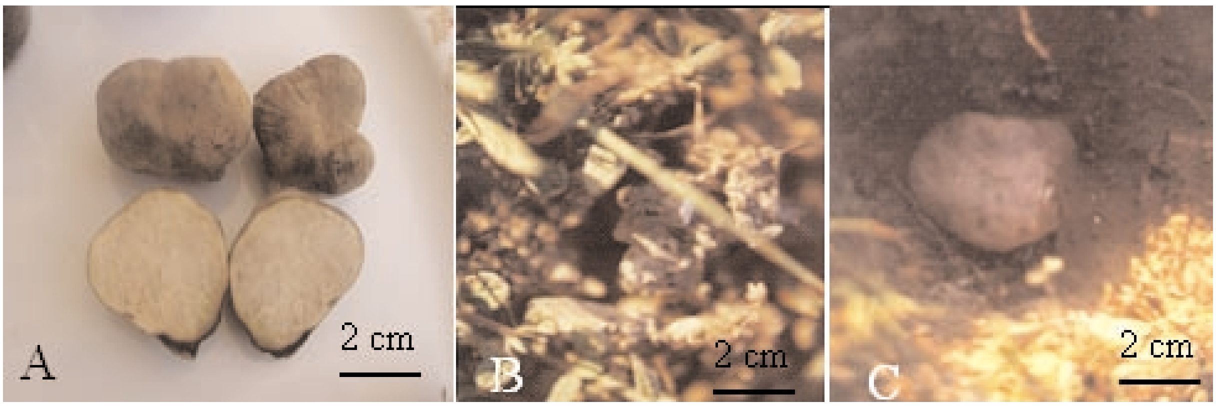

Figure 2. The desert truffle species (*Terfezia boudieri* Chatin) from Iran: A. The yellowish species grows solitary in the soil, B. Dogs are not used to find truffles in Iran, but telltale cracks in the soil betray the presence of a truffle under the surface, C. Position of a truffle after the removal of surface soil.

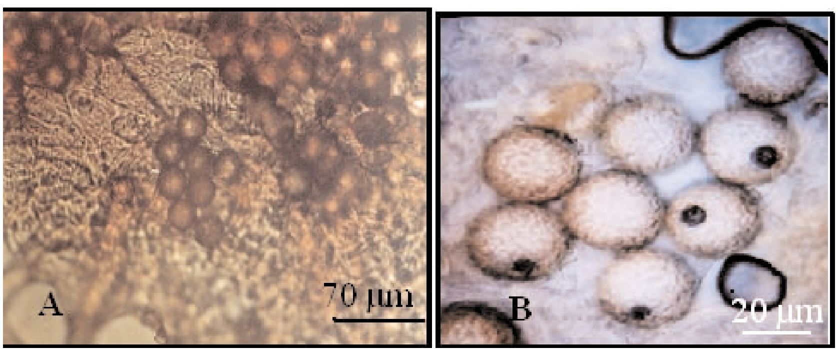

Figure 3. Every ascus has 8 ascospores and the ascus membrane disappears after maturation (A and B).

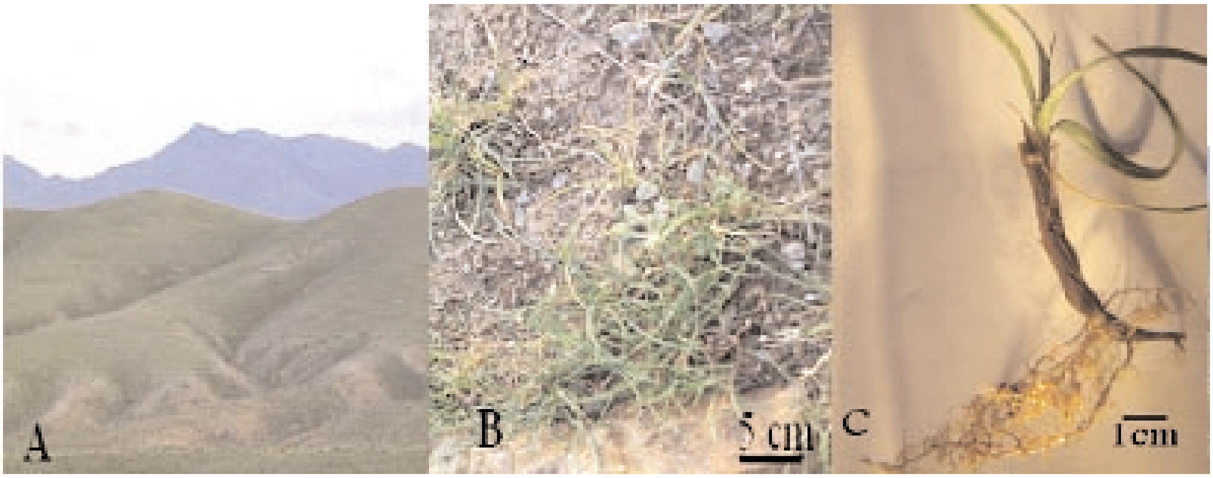

Figure 4. A. View of the study area, B. vegetation, C. typical sedge.

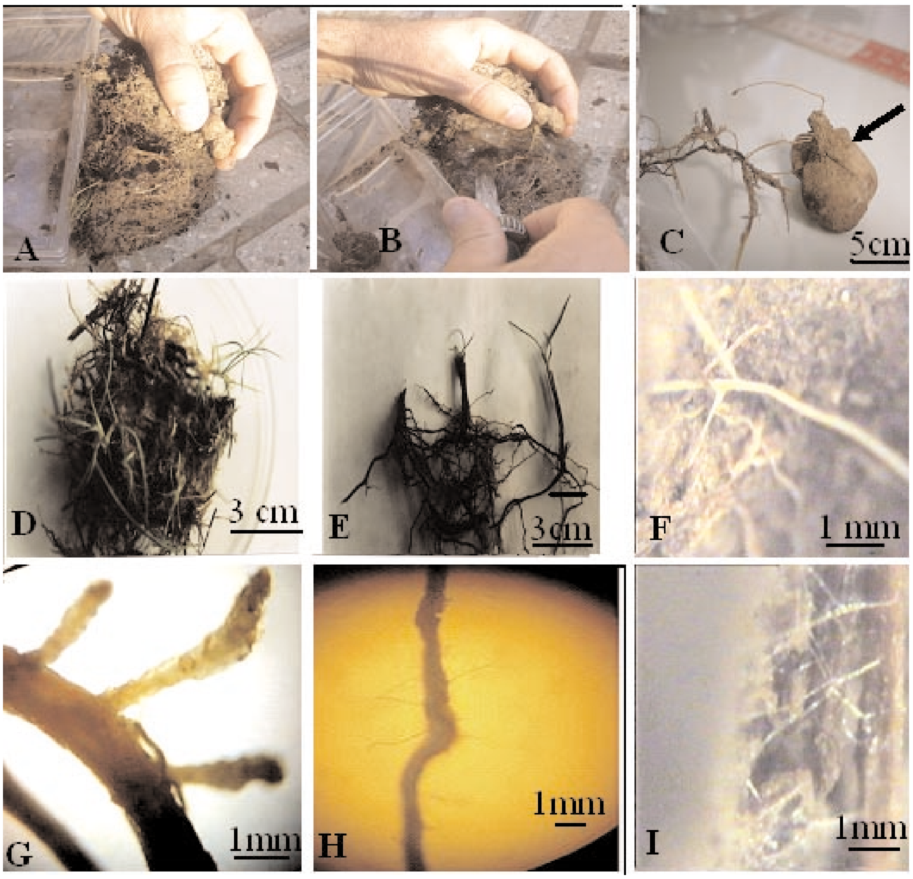

Figure 5. A-B. The soil profiles were washed very carefully and the zones of physical contact between the truffles' ascocarp bases and roots of plants were investigated, C. One typical truffle and sedge roots, D. About 60% of the rooting zone is occupied by the fungus/sedge's root relationship, E. The sedge plant survived from the previous season as a rhizome, F. The penetration of sedge root into the base of a truffle ascocarp, G. The size, colour, texture, and branching ectomycorrhizal roots of sedges (*Kobresia bellardii*, H-I. Mycorrhizal strands attached to sedge roots.

Growth of the sedges increased during autumn when there was abundant rainfall, either as a result of seeds that germinated in spring or plants that had survived from the previous season as rhizomes (Figure 4D and E). The sedges occurred only where truffles were found and developed at the same time. Unlike Europeans truffles that have a mycorrhizal life cycle with trees, we concluded that these species appear to be associated with perennial sedge. The sedge was identified as *Kobresia bellardii* (All.) Degel. Further research is planned on the peculiar life cycle of these truffles. In particular we will attempt to germinate spores in vitro, infect sedges, and produce the truffles under controlled conditions (Figure 6A and B).

Discussion

The occurrence of ectomycorrhizas on herbaceous plant genera has been known for almost a century. The herbaceous genus, *Kobresia*, in the Cyperaceae, was described by Fontana (1963) as being ectomycorrhizal (Massicotte et al., 1998). All the mycorrhizal types can be found in Arctic and alpine areas (Vare et al., 1997). Ectomycorrhizal symbiosis is common in trees and shrubs. These ectomycorrhizal fungi are hosted by host plants, including Drayas octopetala, Betula nana, and Salix spp., and herbaceous plants, such as *Kobresia* spp. and Polygonum viviparum (Massicotte et al., 1998). Despite the general consensus that the Cyperaceae are non-mycorrhizal, there have been numerous reports of mycorrhizal infection in certain species (e.g., Mejstrik, 1972; Gay et al., 1982; Haselwandter & Read, 1982). *Kobresia* Willd., a genus of about 50 species, which is distributed in the northern hemisphere, especially at high altitudes in the Himalayas, China, and central Asia, belongs to the subfamily Caricoideae of the family Cyperaceae (Willis, 1973; Dahlgren et al., 1985).

The Caricoideae also include genera, such as *Carex* L., *Schoenoxiphium* Nees, and *Uncinia* Pers. The Himalayan region contains the most diverse population of *Kobresia*, with about 30 species. *Kobresia* is an important genus in the alpine flora of the Nepalese Himalayas (lat 26°20'-30°20' N, long 80°10'-88°10' E) and economically it is also an important pasture plant, dominating a vast area of the alpine region where grazing animals feed on it extensively (Boeckeler, 1875; Clarke, 1883, 1894, 1903, 1908; Kükenthal, 1903, 1909). Ectomycorrhizae are characterised 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 extramatrical hyphae that explore the substrate and are responsible for the mineral nutrition and water uptake of the symbiotic tissues (Barker et al., 1998). Most ectomycorrhizal (ECM) plants have roots with a modified lateral root branching pattern. This pattern 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 colonising more rapidly growing roots (Wilcox, 1964; Chilvers & Gust, 1982). Such modifications were observed on the roots of sedges presently studied (Figure 5G). Mycorrhizal infection among sedges appears to occur in response to many factors, both environmental

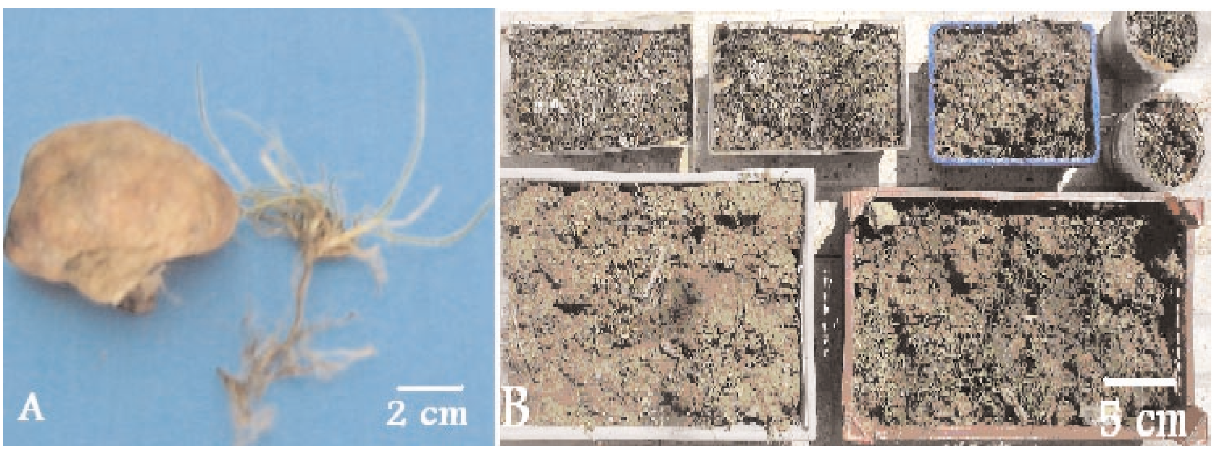

Figure 6. A. *Terfezia boudieri* and *Kobresia bellardii*, B. Culture of infected sedges in controlled conditions.

and biological. While some species appear to be obligately non-mycorrhizal, 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 non-mycorrhizal state. Clearly, however, the evidence presented herein and in other recent publications indicates that the conventional wisdom regarding the mycorrhizal condition of the Cyperaceae must be re-evaluated. The occurrence of ectomycorrhizas on *Kobresia bellardii* has been provided anatomically (Massicotte et al., 1998), but the fungal symbionts were not identified. This report of ectomycorrhizal relationships of *Kobresia bellardii* with *Terfezia* is the first of its kind.

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