

## Homokaryotic and heterokaryotic hyphae in *Terfezia*

N. Roth-Bejerano<sup>1,\*</sup>, Y.-F. Li<sup>2</sup> and V. Kagan-Zur<sup>2</sup>

<sup>1</sup>Department of Life Sciences, Ben-Gurion University of the Negev, POB 653, Beer-Sheva 84105, Israel;

<sup>2</sup>The Institutes for Applied Research, Ben-Gurion University of the Negev, Beer-Sheva 84110, Israel;

\*Author for correspondence

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### Abstract

Mycelia of *Terfezia pfeilii* (*Ascomycetes*) were obtained by two methods, i.e., from the sterile hyphae of fresh fruit bodies or by germinating ascospores. Nuclear staining revealed the existence of multinucleate cells in all mycelia. Paired nuclei were observed only in mycelia obtained from sterile hyphae proliferation, while single nuclei were found in mycelia originating from singly germinated spores. Co-cultivation of mycelia from two different ascospores apparently facilitated plasmogamy, resulting in mycelia with paired nuclei. *Terfezia boudieri* cultures originating from sterile hyphae also exhibit paired nuclei, indicating the possible existence of a long-term heterokaryon. The timing of plasmogamy and karyogamy in *Terfezia* is discussed.

### Introduction

Truffles are edible hypogeous fruit bodies produced by many genera of fungi belonging to the class *Ascomycetes*. Among these, *Terfezia* (*Pezizaceae* formerly *Terfeziaceae*) and *Tuber* (*Tuberaceae*) are positioned at opposite ends of the pezizalean tree (Norman and Egger 1999; Percudani et al. 1999). Fungi belonging to the family *Pezizaceae* (*Terfeziaceae*) form either *terfezia*-type ectomycorrhiza or ectendomycorrhiza, depending on the amount of phosphorus in the medium (Fortas and Chevalier 1992b), whereas those belonging to the *Tuberaceae* form ectomycorrhiza (Bonfante Fasolo and Brunel 1972; Delmas et al. 1981; Pacioni 1989). Both *Tuber* and *Terfezia* produce hypogeous ascocarps in which sterile and fertile veins were found (Parguey-Leduc et al. 1989; Miranda et al. 1992; Ceruti 1960; respectively).

The life-cycle of *Terfezia* and *Tuber* has not been completely elucidated, although some knowledge has indeed been accumulated. Bonfante Fasolo and Brunel (1972) showed that *Tuber melanosporum*

monosporic mycelia were not able to inoculate host plants, indicating that only heterokaryotic mycelium resulting from plasmogamy is able to form mycorrhizas. However, prevailing modern ideas, based on in depth research, doubt the long-term existence of natural vegetative heterokaryons in filamentous *Ascomycetes* (e.g. Glass et al. 2000). Fortas and Chevalier (1992a), studying *Terfezia arenaria*, detected no differences between the inoculation capacity of monosporic *versus* multisporic cultures, seemingly in support of the modern dogma.

In *ascomycetes*, microscopic determination of homokaryotic or heterokaryotic mycelium is difficult, since: (a) nuclei wander from one cell to another via the incomplete septum, resulting in varying number of nuclei per cell – between multinucleate to empty cells; (b) no clamp connections can be found in vegetative hyphae, unlike the basidiomycetes.

We have earlier discovered two different ITS (Internal Transcribed Spacer region of the rRNA genes) profiles, distributed among a population of *T. pfeilii* fruit-bodies (Kagan-Zur et al. 1999). Most fruit bodies harbored either one or the other of the two pro-

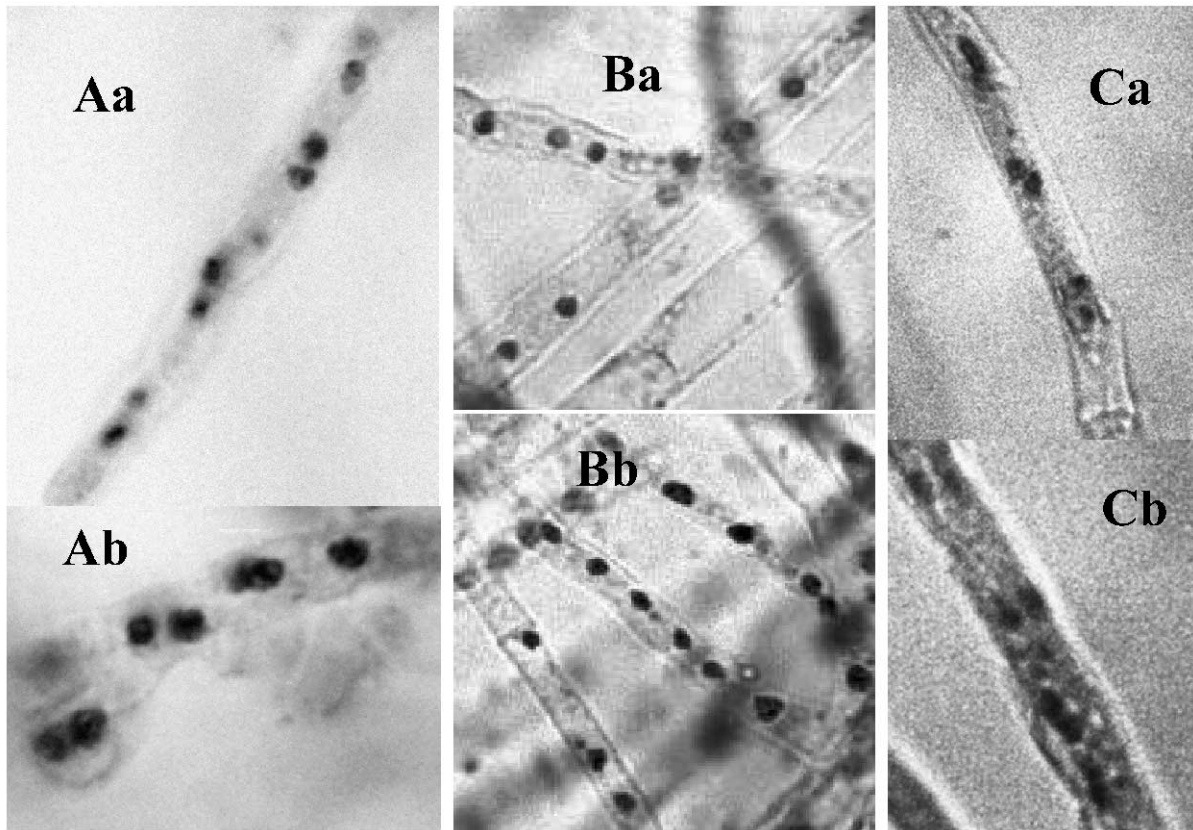


Figure 1. Hyphae of *Terfezia pfeilii* containing multinucleate cells: (a) hyphae originated from sterile fruit body veins; (b) hyphae originated from a single germinating spore; (c) hyphae obtained at the contact zone between two homokaryotic mycelia (each mycelium originated from a single ascospore). Magnification  $\times 1000$ .

files. However, two fruit bodies, each revealing a double profile, were found. When allowed to germinate all single-spore-derived cultures from these fruit bodies carried a single profile of either one or the other, never both together. The double profile observed could be explained in two ways: the gleba is a mixture of two intertwined types of hyphae; or the non-fertile hyphae are heterokaryotic. The present study was undertaken to resolve this question, and thus elucidate an aspect of the *Terfezia* life cycle.

### Materials and methods

Ascocarps of *Terfezia pfeilii* were collected in the Kalahari, and those of *Terfezia boudieri* were purchased in the local market in Beer-Sheva, the capital of the Negev Desert of Israel. Fresh fruit bodies were sterilized, and small pieces were put onto a synthetic Fontana medium (Bonfante Fasolo and

Fontana 1973) held in a growth room at  $25 \pm 2$  °C. This medium facilitates proliferation of the hyphae, but not germination of the spores. Spore germination was obtained at  $25 \pm 2$  °C when dried sterilized ascocarps were ground up in sterile water, and the suspension smeared on K.I.S.R medium (Fortas and Chevalier 1992a). Each germinated spore was transferred to a separate petri dish containing Fontana medium to facilitate further development of the mycelium.

Isolates originating from two different spores were placed together on Fontana medium. From the contact zone, where the heterokaryon should have been produced, small mycelium containing agar pieces were cut out and transferred to a fresh medium. To prevent penetration of hyphae into the growth medium, the pieces were held on top of a cellophane cover (jam cover leaves, Spontex, Paris), pre-cut to fit the inside of a Petri dish, boiled in 1 mM EDTA for 10 min, washed twice with water, autoclaved, and

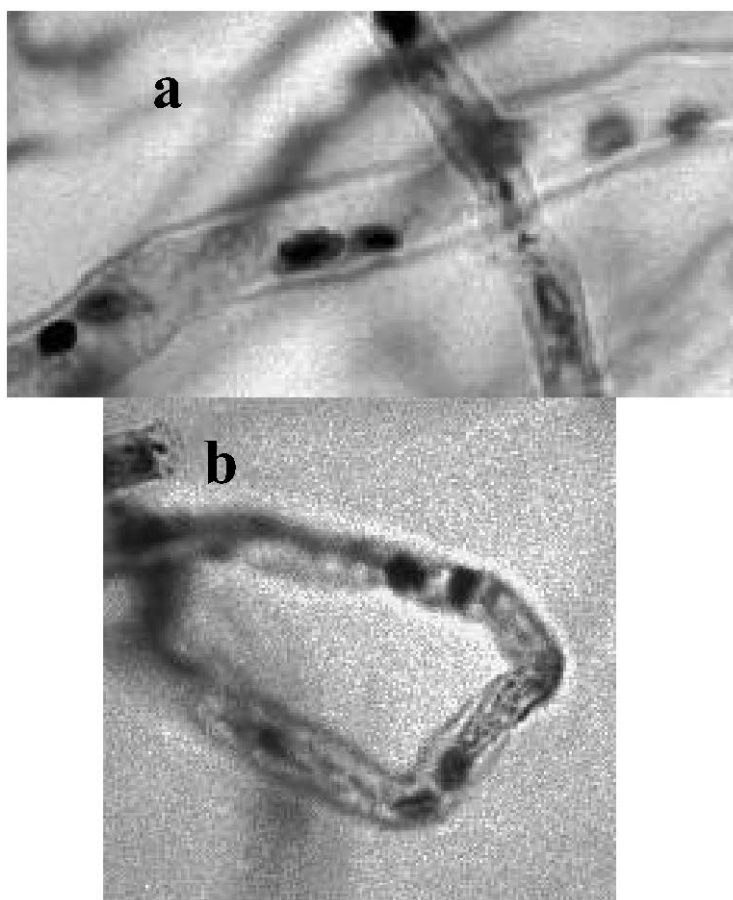


Figure 2. Hyphae of *Terfezia boudieri* culture developed from sterile glebal veins. Multinucleate cells with paired nuclei are evident. Magnification  $\times 1000$ .

laid carefully over agar-solidified Fontana medium avoiding formation of air bubbles between the cellophane and the medium.

Mycelia originating from the sterile hyphae of the gleba, from single spore germination, and from hyphae that had developed after the two homokaryotic mycelia had come into contact were stained using Giemsa stain (Wilson 1992) and viewed under a light microscope (Zeiss standard 20).

### Results and discussion

Figure 1a represents *Terfezia pfeilii* hyphae originating from glebal sterile hyphae proliferation. Multinucleate cells with paired nuclei are evident. Paired nuclei have also been found in mycelia obtained from fruit body tissues of the basidiomycete *Tricholoma robustum* (Iwase 1990), indicating that nuclei of dif-

ferent origins appear in pairs. The multinucleate cells found in hyphae originating from single ascospore germination did not exhibit nuclei pairing (Figure 1b) though anastomoses could be detected between hyphae in culture. Similar non-paired nuclei have been found in monokaryons, the primary mycelia of *Tricholoma robustum*, (Iwase 1990) and of *Morchella* (Volk and Leonard 1990). Hyphae formed at the contact zone of two primary mycelia each originating from single ascospore showed multinucleate cells with paired nuclei (Figure 1c), similar to mycelial cultures obtained from sterile glebal veins. It appears that the two compatible primary *Terfezia pfeilii* mycelia formed a heterokaryotic secondary mycelium. A similar arrangement of nuclei has been described in the secondary heterokaryotic mycelium of *Morchella* following plasmogamy of compatible primary mycelia (Volk and Leonard 1990). Mycelia of various isolates developed from the sterile hyphae of *Terfezia boudieri*

fruit bodies, kept for years in our laboratory, also contained multinucleate cells with paired nuclei (Figure 2).

From this study, it appears that the life cycle of *Terfezia* species is made up of the following stages: ascospores germinate to give the primary homokaryotic mycelium. This mycelium develops in the soil, where it either inoculates host plants or produces a secondary mycelium after plasmogamy, before inoculation takes place. It is not known at what exact time plasmogamy takes place in the case of inoculation by a primary mycelium, but fruit bodies are evidently formed by heterokaryotic hyphae, as indicated by paired nuclei. Meiosis, most likely, takes place within the fruit body primordia before ascospore creation, as is the case for *Tuber melanosporum*, whose young fruit bodies contain ascosporophytic dikaryotic hyphae with ascogenous croziers (Parguey-Leduc et al. 1990).

Our results support, therefore, the existence of long-term heterokaryons in *Terfezia* species, as was previously conjectured for two other Ascomycetous fungi, *Tuber* (Bonfante Fasolo and Brunel 1972) and *Morchella* (Volk and Leonard 1990).

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