

# Morphology and Ultrastructure of Root-Fungus Association of *Rhododendron formosanum* Hemsl. in Sanlinchi Forest of Central Taiwan

## 中台灣杉林溪森林中台灣杜鵑根菌結合體之形態及微細構造

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### Abstract

This paper describes mycorrhizal character of the Formosan azalea (*Rhododendron formosanum* Hemsl.), an indigenous ericoid plant in the Sanlinchi forest of Central Taiwan. The root-fungus associations were categorized into 6 morphological types with light microscopy. The root ultrastructure examined with scanning electron microscopy revealed that hyphal complex and hyphal coil structures were present in cortical cells. The isolated fungal strains had slow-growing septate hyphae. The results suggested that the root-fungus association of the Formosan azalea belong to ericoid mycorrhiza.

## 摘要

本研究探討中台灣杉林溪森林中台灣杜鵑之菌根特徵。台灣杜鵑根系之根菌結合體可區分為6種形態。其根部組織之微細構造顯示，皮層細胞內有菌絲複合體及菌絲圈之構造。自根系分離純化培養之菌株具有生長緩慢之隔膜菌絲。研究結果證實，杉林溪台灣杜鵑根系之根菌結合體屬於杜鵑類菌根。

**Key words:** *Rhododendron formosanum*, ericoid mycorrhiza, morphology, ultrastructure

**關鍵詞：**台灣杜鵑、杜鵑類菌根、形態、微細構造

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## Introduction

Harley (1969) categorizes mycorrhizae of Ericales into ericoid mycorrhizae and arbutoid mycorrhizae. Ericoid mycorrhizae belong to endomycorrhizae that have septate hyphae, intracellular hyphae and hyphal coils without arbuscular hyphae, mantle, Hartig net and vesicles. Arbutoid mycorrhizae belong to ectendomycorrhizae that have endomycorrhizal features of septate hyphae, intracellular hyphae, hyphal coils, ectomycorrhizal features of mantle, and Hartig net.

Ericoid mycorrhizae are present in many species of Ericales, belonging to the genera *Calluna* (Nieuwdrop 1969; Bonfante-Fasolo and Gianinazzi-Pearson 1979), *Erica*, *Vaccinium* (Nieuwdrop 1969; Bonfante-Fasolo *et al.* 1981), and *Rhododendron* (Peterson *et al.* 1980).

In respect to the microscopic structure of ericoid mycorrhizae, Rayner (1927) points out that the characteristic of ericoid mycorrhizae is

that the development of hyphae in their cells leads to the formation of characteristic hyphal coils which fill the whole cells, and the invasion of a mycorrhiza into the surface cells gives rise to generate intracellular hyphal coils which grow spirally in the epidermal cells. Therefore, fungi infect epidermal cells, spreading and ramifying over the root surface but seldom extending from a cell to another (Bonfante-Fasolo and Gianinazzi-Pearson 1979; Duddridge and Read 1982). With the advancement of instruments, Peterson *et al.* (2004) point out that within an ericoid mycorrhiza, there might be a narrow hypha that can penetrate into adjacent cells.

Although many studies have been carried out on flora of Ericales in Taiwan (Lee 2000; Tseng and Lu 2003), they were limited to physiology or ecology; there was no study on their underground mycorrhizae. Therefore, this study was intended to examine the morphology and structure of root systems of the Formosan azalea to explore if the

structural characteristics of ericoid mycorrhizae are present.

## Materials and Methods

### Sampling of root soil

Formosan azalea endemic to Taiwan is an evergreen shrub with herbaceous leaves, canescent of verso, and corolla white or pink in color (Liu *et al.* 1994). Sixty soil-core samples (5 cm in diameter) containing roots of the Formosan azalea were randomly collected in Spring 2006 from a Formosan azalea forest (120°47'31.35" E longitude and 23°38'9.85" N latitude; altitude 1700m) with stems up to 15 cm in dbh in the Sanlinchi Recreational Park in Chushang Township, Nantou County, Taiwan. The soil samples were brought to the laboratory.

### Soil chemistry

Each of the soil samples was mixed in distilled water at a soil water ratio of 1:10 and the pH was measured. Cation-exchange capacity (CEC) of the sample was determined by the conventional  $\text{NH}_4\text{OAc}$  method (Gillman 1979).

### Morphology of root association

The soil samples collected were rinsed with running tap water for 3 min, and cleaned with a ultrasonic oscillator. Morphology of root-fungus association was examined under a stereo microscope (Usuki and Narisawa 2005).

### Isolation and purification of hyphae

Swollen root segments with hyphae complex were sterilized with 30%  $\text{H}_2\text{O}_2$  for 1 min (Berch *et al.* 2002), sectioned and cultured on 1% water agar culture medium (Xiao and Berch 1992), and

incubated for 14-21 days at 20°C under dark condition. Hyphae grew slowly from edge of the root section. They were isolated and transferred into 1% MEA (malt extract agar) culture media for purification (Johansson 2001). The isolated fungal strains were assigned the number of Rf as for *R. formosanum*.

### Morphology and growth of fungus colonies

The purified hyphae were transferred to 2% MEA (malt extract agar) culture media and cultivated at 20°C in a dark incubator. The growth rate of the colony and morphological characters of the hyphae were recorded at scheduled intervals daily (Hutton *et al.* 1994; Hambleton and Currah 1997; Tsai 2003).

### Scanning electron microscopy of root samples

Root samples were fixed in 2.5% glutaraldehyde fixative at room temperature for a day. Then, they were rinsed with 0.1M phosphate buffer solution 3 times, each for 15 min, and then, followed by serial dehydration in 10, 30, 50, 70, 90, 95, 100% ethanol and 100% acetone. They were critical-point dried in a critical point dryer using liquid carbon dioxide. The dried samples were mounted on an aluminum stub, coated with gold, and observed under a scanning electron microscope (Tsai 2003).

## Results and Discussion

### Soil and litter characteristic at site

Most roots of the *Rhododendron formosanum* grew on O (organic) and OA (mixing of organism and mineral) horizons. The results of this study indicated that the soil reactions were strongly acidic (O: pH 3.267, OA: 3.30). The values of

cation-exchange capacities (CEC) were 70.51 cmol/kg (O) and 80.00 cmol/kg (OA). The organic

matters were 62.69% (O) and 52.93% (OA) (Table 1).

**Table 1.** The soil characters (means  $\pm$  standard deviation,  $n = 2$ ) of the root-fungus associations of *Rhododendron formosanum*

Horizons	pH*	Organic matters (%)	CEC (cmol/kg)
O	3.267	62.69 $\pm$ 2.66	70.51 $\pm$ 7.75
OA	3.300	52.93 $\pm$ 6.44	80.00 $\pm$ 13.26

\* Water: soil volume ratio, 1:10.

O: organic horizon. OA: mixing horizon of organism and mineral.

### Morphology of root-fungus association

The swollen root-fungus associations in the root samples of Formosan azalea were classified into six types according to the morphological characters. Type 1 was stalked, cylindrical, 0.3 mm in diameter, and creasy. Upper cylinder was golden yellow in color, fading upon aging (Fig. 1A). Type 2 was sessile, cylindrical, and yellowish brown at its initial stage (Fig. 1B). Type 3 was sessile, bi-segmented, the anterior segment conical, yellowish brown, and the anterior segment cylindrical and golden yellow (Fig. 1C). Type 4 was short and stout, corresponding to the initial stage of mycorrhizal formation, yellow in color and sparsely covered with setae (Fig. 1D). Type 5 was monilioid golden yellow at the initial stage of mycorrhizal formation, brown when aged, and sparsely covered with setae (Fig. 1E). Type 6 was bi-segmented cylindrical, growing from the lateral side of the main root. It can be subdivided into Subtype 1 with anterior segment longer than posterior segment and Subtype 2 with anterior segment shorter than posterior segment; golden yellow at the initial stage, becoming yellowish brown when mature, and surface densely covered with setae, with a peltate end (Fig. 1F).

### Characters of colonies and hyphae

Fungal strains isolated and cultured from swollen root associations are described as follows:

Colonies of Rf 9, incarnate with flocculent edge (Fig. 2A), yellow pigments appeared, growth rate  $0.82 \pm 0.05$  mm/day. Hyphae septate, 2.5-1.25  $\mu$ m in width (Fig. 2B). Colonies of Rf 28, dark green in color (Fig. 2C), growth rate  $1.0 \pm 0.6$  mm/day. Hyphae dark green, septate, 2.5-5  $\mu$ m in width (Fig. 2D). Colonies of Rf 32, brown in color (Fig. 2E), growth rate  $1.0 \pm 0.05$  mm/day. Hyphae septate, 2.5-1.25  $\mu$ m in width (Fig. 2F). Colonies of Rf-3, gray in color (Fig. 2G), growth rate  $0.54 \pm 0.02$  mm/day. Hyphae septate, 2.5-1.25  $\mu$ m in width (Fig. 2H).

### Ultra-structure of root-fungus association

The ultrastructures of root fungus associations were divided into two categories: the hyphae complex with microsclerotia or hyphal ring (Duclos *et al.* 1983; Perotto *et al.* 1995; Massicotte *et al.* 2005), and loose arrangement of branching hyphae (Massicotte *et al.* 2005).

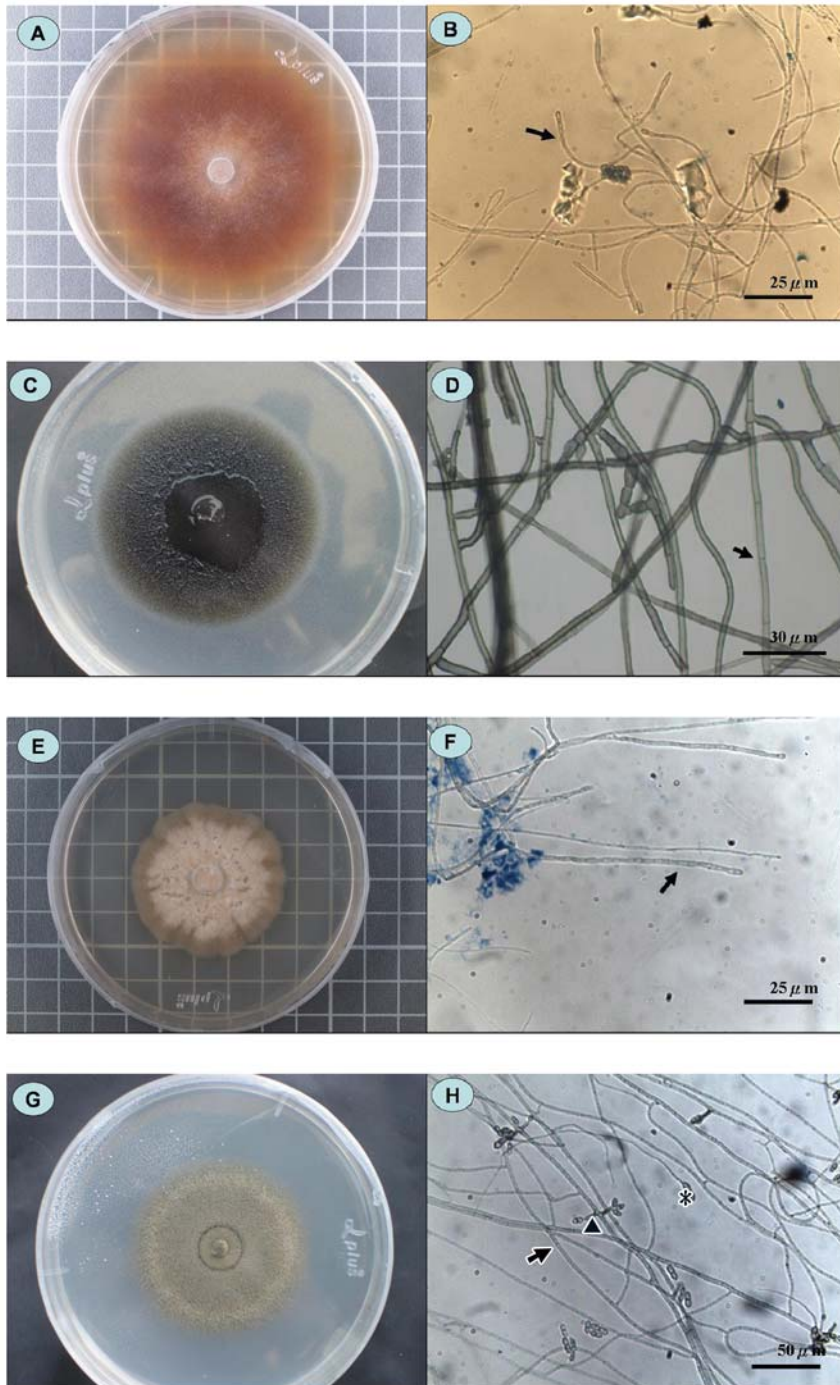
There were hyphae sparsely attached on the surface of root systems (Fig. 3A). The invading point of a hypha with a width of 0.7  $\mu$ m was also found (Fig. 3B). Hyphal complexes (Fig. 3C-3F)

and branching hyphae were observed. Most of the hyphal complexes in the root tissues were

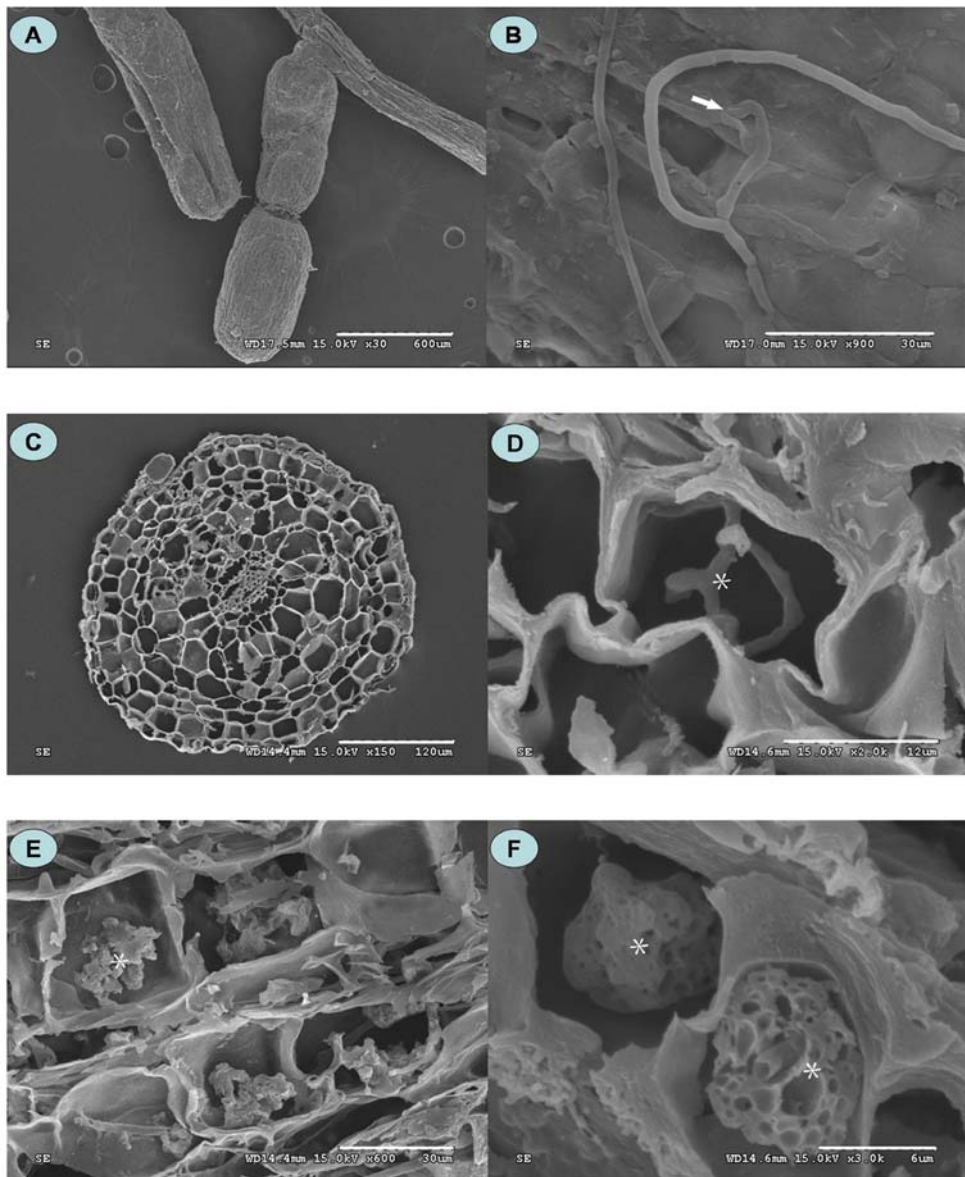
microsclerotia that were distributed in the epidermal cells (Fig. 3C).



**Fig. 1.** Types of mycorrhizae of the *Rhododendron formosanum*. A. type 1; B. type 2; C. type 3; D. type 4; E. type 5; F. type 6.



**Fig. 2.** Endophytes of the *Rhododendron formosanum*. Colony (A) and hyphae (B) of strain Rf 9; colony (C) and hyphae (D) of strain Rf 28; colony (E) and hyphae (F) of strain Rf 32; colony (G) and hyphae (H) of strain Rf-3; arrows, septate; arrowheads, conidiophore; \*, conidium.



**Fig. 3.** Ultrastructure of mycorrhiza of the *Rhododendron formosanum*. A. surface of hairy root covered with hyphae; B. hyphae within intercellular spaces (arrow); C. a cross section of mycorrhiza; D. branching hyphae (\*); E. breakdown microscerotia (\*) and F. microscerotia (\*).

## Conclusions

It is estimated that there are approximately 850 species of *Rhododendron* in the world, among

which about 500 to 600 species are in tropical regions of East Asia and Southeast Asia. Henry (1896) recorded 2 species: *R. oldhamii* Maxim. and *R. formosanum* Hemsl. from Taiwan. Recently

Tseng and Lu (2003) studied the wild *Rhododendron* flora of Taiwan and found 15 indigenous species of which 11 were endemic. Although there are many endemic species of *Rhododendron* in Taiwan, there has been no study on ericoid mycorrhizae. In contrast to Taiwan, the studies on ericoid mycorrhizae have a relatively long history in other countries. It has been found that in a stress environment with extreme climate conditions and/or poor soil conditions, *Rhododendron* has been frequently found to become dominant species after forming mycorrhizae with ericoid mycorrhizal fungi.

The results of this study showed that ericoid mycorrhizae structures such as hyphae complex and branching hyphae existed in root epidermal cells of the Formosan azalea. The endophytes had slow-growth and septate hyphae coincided with the characteristics of ericoid mycorrhizal fungi. Therefore, the root association of the Formosan azalea in the Sanlinchi forest belonged to ericoid mycorrhiza.

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