Research paper

The Ability of the Rf32 Strain (*Cryptosporiopsis*, Helotiales) to Form Ericoid Mycorrhiza Symbioses with *Rhododendron* Species

Lei-Chen Lin[1,2)]

【Summary】

The Rf32 strain isolated from *Rhododendorn formosanum* was demonstrated to be a species of *Cryptosporiopsis* (Dermateaceae, Helotiales) of ericoid mycorrhizal fungi (ERMF), and has the ability to decompose organic matter. This study further documented the symbiotic ability of the Rf32 fungal strain in association with 5 *Rhododendron* species. Inoculation results revealed that *Cryptosporiopsis* sp. Rf32-inoculated *Rhododendron* seedlings all grew vigorously, and the hyphal complex was found in cortical cells of root associations. This study demonstrated that *Cryptosporiopsis* sp. had symbiotic ability with *R. mucronatum* cv. Akemono, *R. scabrum*, *R. kanehirai*, *R. ellipticum*, and *R. rubropilosum* Hayata var. *rubropilosum*, and could form ericoid mycorrhiza *in vitro* with these *Rhododendron* seedlings.

Key words: Ericoid mycorrhiza, Ericoid mycorrhizal fungus, *Rhododendron* spp.

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[1)] Department of Forestry and Natural Resources, National Chiayi University, 300 Xuefu Rd., Luliao Vilage, Chiayi 60004, Taiwan. 國立嘉義大學森林暨自然資源學系，60004嘉義市鹿寮里學府路300號。

[2)] Corresponding author, e-mail:linerm@mail.ncyu.edu.tw 通訊作者。

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研究報告

Rf32菌株(*Cryptosporiopsis*, Helotiales)與杜鵑屬植物形成杜鵑類菌根之研究

林瑞進[1,2)]

摘　要

本研究室已成功證實自台灣杜鵑根系中分離之Rf32菌株係屬柔膜菌目，擬隱孢子屬之杜鵑類菌根菌，亦證實此菌株菌具有分解枯落物的能力。本研究更深入探討此杜鵑類菌根菌是否能與其他杜鵑屬植物共生形成菌根。試驗結果顯示，此Rf32菌株皆能成功與2種平戶杜鵑及3種台灣原生種杜鵑共生，且於植株根細皮層細胞中都可發現菌絲複合體之杜鵑類菌根構造；因此，本研究證實Rf32菌株能與平戶杜鵑(粉白杜鵑及大紅杜鵑)及台灣原生種杜鵑(紅毛杜鵑、烏來杜鵑及西施花)共生，並與其根系形成杜鵑類菌根。

關鍵詞：杜鵑類菌根、杜鵑類菌根菌、杜鵑屬植物。

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INTRODUCTION

Rhododendron is the largest genus of the family Ericaceae (Yang et al. 1999, Wu et al. 2003), and over 1000 horticultural hybrids have been bred by artificial hybridization (Bean 1976). Tseng and Lu (2003) reported that there are 15 native *Rhododendron* species in Taiwan. Among them, 11 species are endemic, comprising 73% of these native species. Although there are many studies of the indigenous *Rhododendron* of Taiwan, most are limited to physiological ecology and phylogenetic relationships of the genus (Lee 2000, Hwang and Hsu 2001, Tseng and Lu 2003, Tsai and Huang 2004), with little attention given to mycorrhizal associations.

Ericoid mycorrhiza (ERM) belongs to endomycorrhizas that have intracellular, septate hyphae and hyphal coils, but without arbuscular hyphae, a mantle, a Hartig net, or vesicles (Peterson et al. 2004, Smith and Read 2008). Currently, known ericoid mycorrhizal fungi (ERMF) are divided into 2 groups: ascomycetes (*Pezoloma ericae*) and hyphomycetes (*Cryptosporiopsis ericae*, *Oidiodendron maius*, and *Phialocephala fortinii*) (Hambleton and Currah 1997, Hambleton et al. 1998, Sigler et al. 2005, Baral and Krieglsteiner 2006). Much research indicated that ERMF positively influence the growth, survival, and competitiveness of host species by enhancing nutrient uptake (Read 1996, Read and Perez-Moreno 2003, Vohník et al. 2005, Lin et al. 2010a, b, 2011a, b), alleviating heavy metal toxicity (Perotto et al. 2002), and secreting enzymes for saprobic ability (Rice and Currah 2001, Piercey et al. 2002, Lin et al. 2011a). *Rhododendron* hosts of ERM include species of *Calluna* (van der Wal et al. 2009), *Rhododendron* (Usuki and Narisawa 2005, Vohník et al. 2007, Lin et al. 2010a, b, c, 2011a, b), *Erica* (Straker et al. 1989), and *Vaccinium* (Dalpé 1986, Vohník et al. 2007).

The Rf32 strain isolated from Formosan rhododendron (*Rhododendorn formosanum* Hemsl.) was identified as a species of *Crytosporiopsis* of ERMF (Lin et al. 2011a, b), and it has the ability to decompose organic matter (Lin et al. 2011a). This study tested the sexual reproduction and development of *Rhododendron* seedlings through studying the symbiotic ability between *Crytosporiopsis* sp. Rf32 and some *Rhododendron* seedlings.

MATERIALS AND METHODS

Fungal strain

The Rf32 strain was isolated from roots of Formosan rhododendron, located in the Sanlinchi Recreational Park, Chushang Township, Nantou County, Taiwan (120°47′31.35″E, 23°38′9.85″N) (Lin et al. 2010c, 2011b). A specimen of Rf32 was deposited at the Forest Mycobiont Laboratory of National Chiayi Univ. and also at the Bioresource Collection and Research Centre (BCRC) (BCRC 34763), and its internal transcribed spacer (ITS) genomic sequence was deposited in GenBank (HQ260955) (Lin et al. 2011a, b).

Rhododendron species

Mature seed capsules of 5 *Rhododendron* species, including the exotic Hirado azaleas (*R. mucronatum* cv. Akemono and *R. scabrum*), the endemic Taiwanese rhododendron, Kanehira azalea (*R. kanehirai* Wilson), and red-hairy rhododendron (*R. rubropilosum* Hayata var. *rubropilosum*), and the native Taiwanese rhododendron, Taiwan rhododendron (*R. ellipticum* Maxim.), were collected from native habitats. Seeds of Hirado azaleas were collected from the campus of National Taiwan University (NTU; Taipei, Taiwan; 121°32′01″E, 25°01′01″N, elev. 25 m). Seeds of Kanehira azalea were collected from the Sitou area (Nantou County, central Taiwan; 120°47′49″E, 23°40′22″N, elev. 1125 m). Seeds of red-hairy rhododendron were collected from the Mt. Hohuan area (Nantou County, central Taiwan; 121°18′02″E, 24°12′42″N, elev. 2538 m). Seeds of Taiwan rhododendron were collected from the Yushan working circle of a national forest (Chiayi Forest District Office, west-central Taiwan; 120°50′02″E, 23°28′09″N, elev. 2300 m).

Resynthesis

Pure resynthesis was done by following the method of Dalpé (1986). After surface cleaning, seeds were sterilized with a 10% sodium hypochlorite solution for 15 min, rinsed 3 times with sterilized distilled water (dH_2O), and then incubated in test tubes containing 1% agar for germination. Germinated seedlings were transplanted to modified Mitchell and Read (MMR) medium (NH_4Cl, 32 mg l^{-1}; $CaCl_2 \cdot 7H_2O$, 43.5 mg l^{-1}; $MgSO_4 \cdot 7H_2O$, 10 mg l^{-1}; KCl, 5.5 mg l^{-1}; $FeCl_3$, 3.75 mg l^{-1}; sucrose, 2 g l^{-1}; KH_2PO_4, 210 mg l^{-1}; pyridoxine, 100 μg l^{-1}; thiamine, 100 μg l^{-1}; and agar, 10 g l^{-1}). Seven days after germination, the aseptic seedlings were inoculated with the Rf32 fungal strain, and grown in a growth chamber (22℃, 65% relative humidity, and 16 h of light with a maximum illumination of 62 μmol photons m^{-2} s^{-1}).

Observation of mycorrhizal colonization of the roots of seedlings

After 2 months of culture, roots of inoculated seedlings were sampled and cleaned with water in a supersonic oscillator (Upson et al. 2007). The morphology of mycorrhizal colonization was observed using a stereomicroscope (Usuki and Narisawa 2005).

For observation of mycorrhizal colonization, root samples were cleared in 10% KOH for 24 h, washed in dH_2O, acidified in

10% HCl for 1 h, and transferred to 0.05% aniline blue (0.25 g aniline blue, 25 ml dH_2O, and 475 ml lactic acid) for 1 h and then to a destaining solution (25 ml dH_2O, and 475 ml lactic acid) for 2 h. Root segments were mounted on microscope slides in lactoglycerol (14: 1: 1, lactic acid: glycerol: dH_2O) for microscopic examination (Upson et al. 2007).

RESULTS AND DISCUSSION

Seed morphology

Seeds of *R. kanehirai* were collected in mid-November, those of *R. scabrum*, *R. ellipticum*, and *R. rubropilosum* Hayata var. *rubropilosum* in early December, and those of *R. mucronatum* cv. Akemono in mid-December, when the capsule had matured.

The morphologies of the seeds of these 5 rhododendron species are diverse. In general, they are minute, smooth, and with or without winged-like appendages on the 2 sides (Table 1, Fig. 1A~D). Among these 5 species, *R. rubropilosum* Hayata var. *rubropilosum* has the largest seed, and only the seed of *R. ellipticum* has appendages on the 2 sides.

Axenic synthesis of ericoid mycorrhiza

After 2 months of cultivation, seedlings of *R. mucronatum* cv. Akemono inoculated with the Rf32 strain were growing vigorously, and under the stereomicroscope, the mycorrhizal root system was branched, brown, and partially swollen (Fig. 2A). Under a light microscope, we discovered hyphal complexes of ERMs occupying entire cortical cells of root associations of resynthesized seedlings (Fig. 2B).

Seedlings of *R. scabrum* inoculated with the Rf32 strain were growing well. Root associations were brown and swollen under a stereomicroscope (Fig. 2C). Hyphal complexes of ERMs were found in cortical cells of root associations of inoculated seedlings under a light microscope, and they occupied the entire cell (Fig. 2D).

Seedlings of *R. kanehirai* inoculated with the Rf32 strain were growing well. Root associations were brown and swollen under a stereomicroscope (Fig. 2E). Under a light microscope, we discovered very faint hyphal complexes of ERMs occupying cortical cells of root associations of resynthesized seedlings (Fig. 2F).

Seedlings of *R. ellipticum* inoculated with the Rf32 strain were growing well. Root associations were brown and swollen under a stereomicroscope (Fig. 2G). Under a light microscope, we discovered hyphal complexes of ERMs almost occupying entire cortical cells of root associations of resynthesized seedlings (Fig. 2H).

Seedlings of *R. rubropilosum* Hayata var. *rubropilosum* inoculated with the Rf32 strain were growing well. Root associations were brown and swollen under a stereomicroscope (Fig. 2I). Features of hyphal complexes of ERMs were discovered in cortical cells of

Table 1. Seed morphology of *Rhododendron* species in this study

Species	Seed size (mm)	With appendages
R. mucronatum cv. Akemono	0.8~1.5×0.38~0.50	_
R. scabrum	0.8~1.5×0.20~0.57	_
R. kanehirai	0.8~1.5×0.30~0.50	_
R. ellipticum	1.0~1.8×0.53~0.67	+
R. rubropilosum Hayata var. *rubropilosum*	1.4~1.8×0.47~0.60	_

"+", with appendages; "_ ", without appendages.

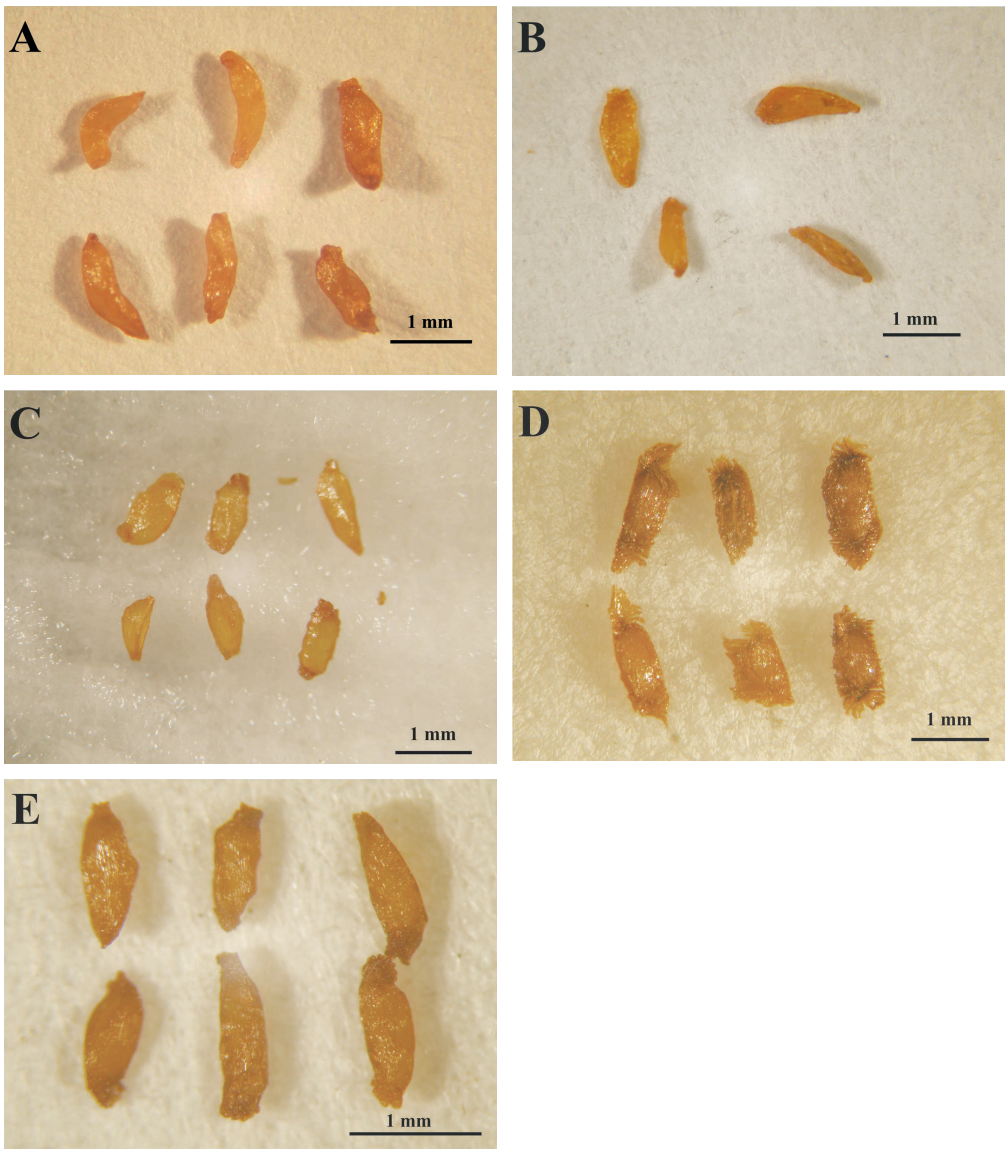

Fig. 1. Seed morphology of *Rhododendron* species. A, *R. mucronatum* cv. Akemono; B, *R. scabrum*; C, *R. kanehirai*; D, *R. ellipticum*; E, *R. rubropilosum* Hayata var. *rubropilosum*.

root associations of resynthesized seedlings under a light microscope, but they were faint (Fig. 2J).

CONCLUSIONS

These results indicated that the endophyte Rf32 is compatible with these 5 rhododendron species and forms hyphal complexes in cortical cells of root associations. This study demonstrated that this endophyte *Cryptosporiopsis* sp. Rf32 has symbiotic ability with Hirado azaleas (*R. mucronatum* cv. Akemono and *R. scabrum*), and native and endemic rhododendron species of Taiwan, such as Taiwan rhododendron (*R. ellipticum*),

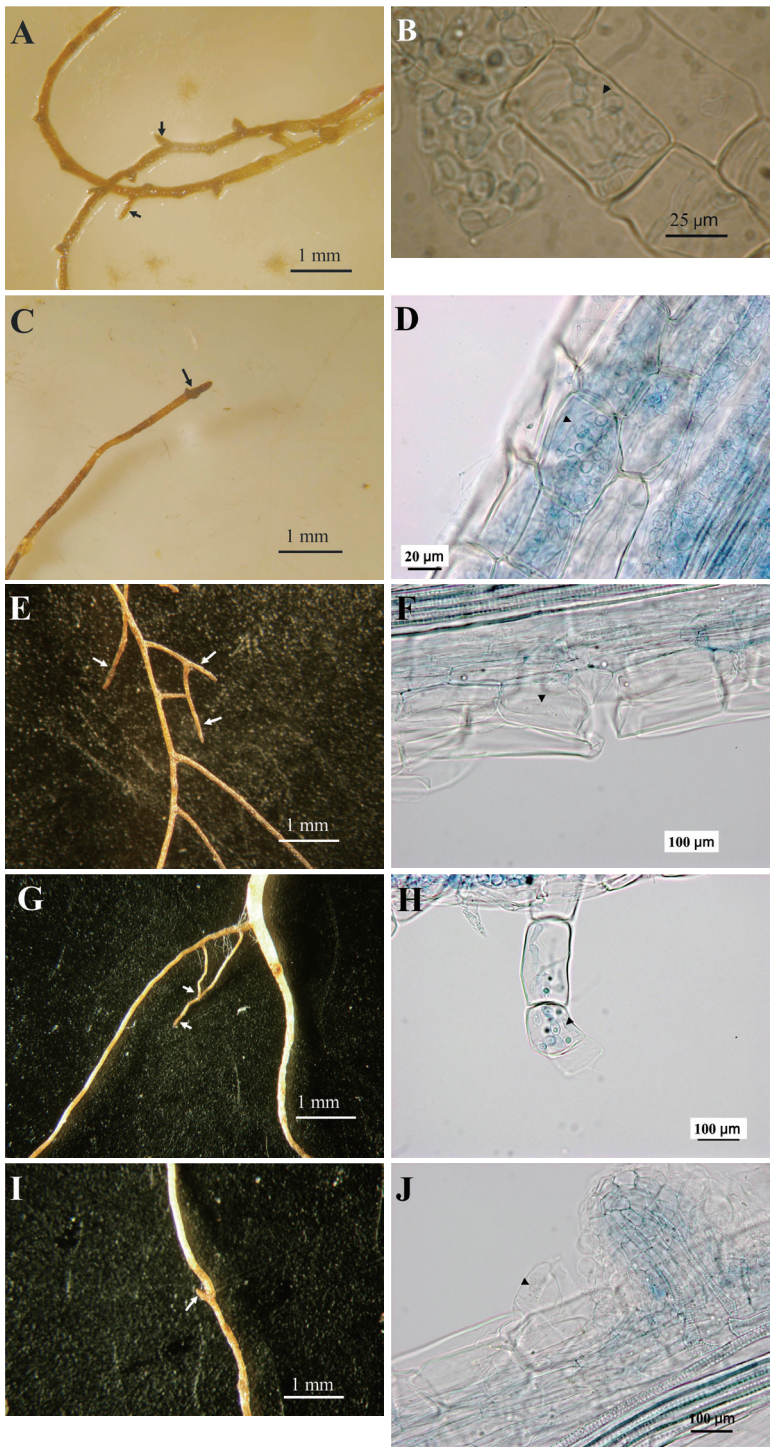

Fig. 2. Morphology of mycorrhizal synthesis. A, B, *Rhododendron mucronatum* cv. Akemono; C, D, *R. scabrum*; E, F, *R. kanehirai*; G, H, *R. ellipticum*; I, J, *R. rubropilosum* Hayata var. *rubropilosum*; root associations (arrows); hyphal complex (arrowheads).

Kanehira azalea (*R. kanehirai*), and red-hairy rhododendron (*R. rubropilosum* Hayata var. *rubropilosum*), and form ericoid mycorrhiza. Presumably, this ericoid mycorrhizal fungus (Rf32) can promote the growth of endemic *Rhododendron* species and expand their distribution ranges.

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