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Monotropoid Mycorrhizal Characteristics of *Monotropa uniflora* (Ericaceae) Collected from a Forest in Korea

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ABSTRACT : The roots of *Monotropa uniflora* were collected from a forest in Korea. Morphological characteristics of monotropid mycorrhizas of the plants were determined. Thick mantles covered the roots and fungal pegs inside the epidermal cells of the roots were observed. Fungal symbionts were identified by sequence analysis of internal transcribed spacer region. Phylogenetic analysis based on the sequences demonstrated that the fungus was the most closely related to *Russula heterophylla*. The result support the strong specificity between *M. uniflora* and *Russula* species.

KEYWORDS : Monotropoid mycorrhizas, *Monotropa uniflora*, *Russula* sp.

Monotropoideae is a subfamily of the Ericaceae, and most of the species in this subfamily are achlorophyllous, and thus, heterotrophic plants [1]. These plants are not able to fix carbon by themselves because they have very low amounts of chlorophyll-related pigments [2]. They obtain fixed carbon from photosynthetic plants through mycorrhizal hyphae; plants exhibiting this relationship are referred to as mycoheterotrophic plants [3, 4]. The mycorrhizal relationship between Monotropoideae plants and fungi is referred to as monotropoid mycorrhiza. Such relationships are associated only with certain genera of fungi, including *Lactarius*, *Rhizopogon*, *Russula*, and *Tricholoma,* which also form ectomycorrhizas with surrounding photosynthetic plants [5-8]. In particular, *Lactarius* and *Russula* are well known as relatively dominant ectomycorrhizal fungi in forest ecosystems [9, 10].

Structures of monotropoid mycorrhizas have been studied in species of several Monotropoideae plants [11-13]. The mycorrhizal structure has typical ectomycorrhizal characteristics, including a hyphal mantle covering the roots and Hartig nets between cortex cells of the roots. In addition, fungal hyphae penetrate and produce fungal pegs inside the epidermal cells of plant roots, which is the characteristic structure of monotropoid mycorrhizas. Fungal pegs have been known as structures that translocate photosynthetic carbon compounds from the fungi to photosynthetic plants.

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Monotropoideae consists of 15 species belonging to 10 genera [1], and many species of Monotropoideae are endangered because of habitat destruction. It is important to conduct studies on Monotropoideae to protect the declining populations of these plants, as well as to provide important clues regarding translocation of nutrients through the hyphal networks [14-16].

Monotropa spp. are distributed in the northern hemisphere. In particular, *M. uniflora* are often found in shaded and wet areas of old forests in Korea. The mycorrhizal status and fungal symbionts of *M. uniflora* have not yet been determined in Korea. The purpose of this study was to investigate the symbiotic fungi in the roots of *M. uniflora* collected from a forest in Korea.

A total of seven individual *M. uniflora* were collected from a mixed forest in Gangwon-do, Korea (37°43'29" N, 128°35'53" W). The roots along with the soil were collected and taken to the laboratory. These were then washed under running tap water to remove the attached soil particles. The root balls were observed under a stereomicroscope (Olympus SZX9, Olympus, Japan). Root tips were removed and sectioned using a microtome (CM1850, Leica, Germany) to examine the morphological characteristics of monotropoid mycorrhizas.

To examine the molecular characteristics of the fungi, the individual root tips were removed and washed several times with sterile water, then, the genomic DNA was extracted. The fungal specific primers ITS1F and ITS4 were used for PCR to amplify the internal transcribed spacer (ITS) region of fungal rDNA [17]. Subsequently, about 650 bp of amplified products was confirmed from 1.5% agarose gel electrophoresis and sequenced at Macrogen (Seoul, Korea). Sequences were compared with similar sequences obtained using BLAST in NCBI; further, Bayesian phylogenetic analysis was performed using MrBayes [18].

Morphological characteristics of monotropoid mycorrhizas of *M. uniflora* were examined under a microscope. The roots of all seven plants were colonized by mycorrhizal fungi, forming root balls (Fig. 1A). The mycorrhizal root tips were short and unbranched, and the shape of mycorrhizal tips observed in the roots was identical in all the individual plants. In cross-sections of the root tips, a thick hyphal mantle covered the roots (Fig. 1B), and cystidia were observed outside the mantle (Fig. 1C). In particular, the structure of the mantle covered with the cystidia is the typical characteristic of mycorrhiza between *Russula* spp. and *M. uniflora*, which has been supported by several studies, including the results of this study [19-21]. Moreover, fungal pegs were observed inside the epidermal cell of the mycorrhizal root tips (Fig. 1D), which is another typical characteristics of monotropoid mycorrhizas, and form the location of nutrient exchange between the plants and fungi.

Sequence analysis of the fungal ITS region amplified from the root tips of *M. uniflora* showed that all of the samples collected in this study were *Russula* sp. (Fig. 2). This indicates that a single species of fungus formed mycorrhizal relationship with the *M. uniflora* collected in this study. The result of BLAST search showed that *Russula heterophylla* was the most similar with the sequence from this study but the similarity was not enough to identify these fungi in species level (94~97%). Therefore, additional analyses are needed to confirm the phylogenetic identity of this fungus.

In general, it has been reported that ectomycorrhizal fungi have symbiotic relationships with various host plants [3]. However, there seems to be strong specificity with fungi among mycoheterotrophic plants. Strong specificity

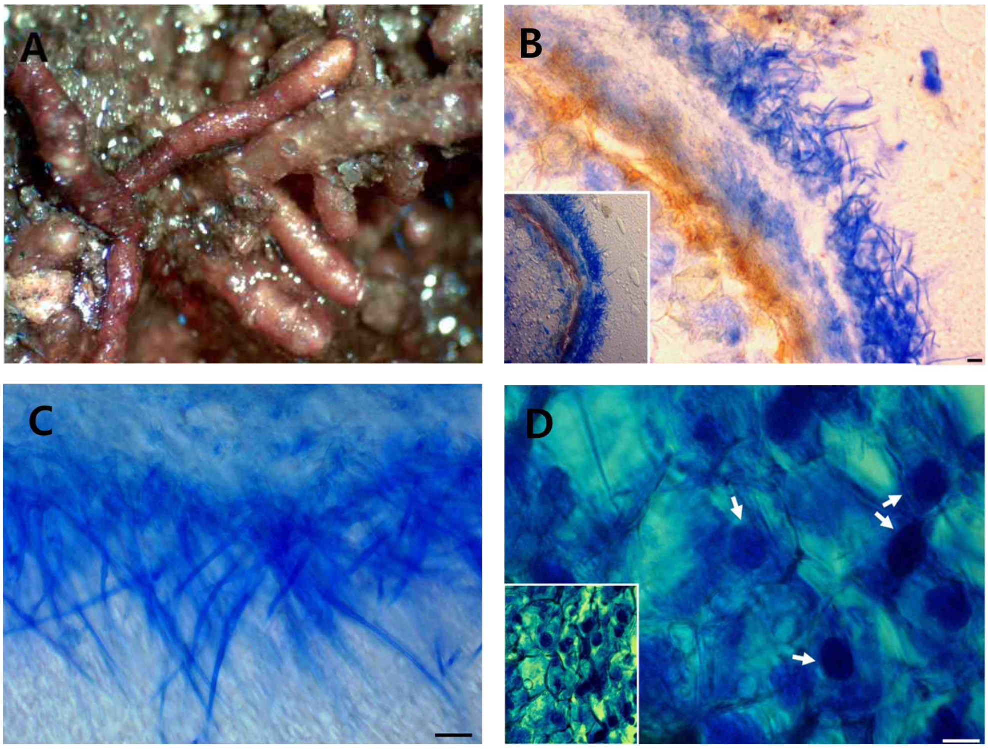

Fig. 1. Structure of monotropoid mycorrhizas of *Monotropa uniflora* collected from this study. A, fungal root tips of *M. uniflora*; B, thick mantle structure of the root tip; C, numerous cystidia on the root surface; D, fungal peg (arrows). Scale bars: 10 µm.

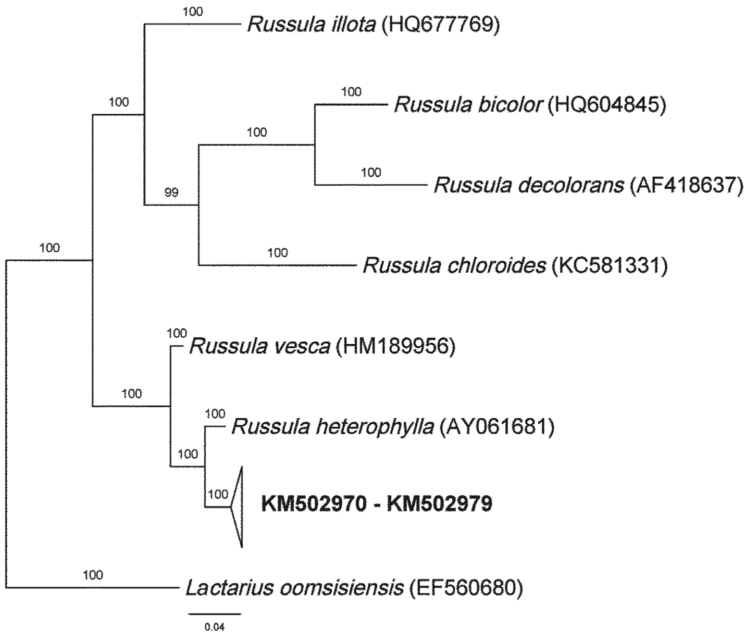

Fig. 2. Bayesian phylogenetic tree constructed using ITS sequences of the fungi associated with the *Monotropa uniflora*. *Lactarius oomsisiensis* was used as an outgroup. Sequences from this study are in bold.

of *Russula* species with *M. uniflora* has been reported in studies from Japan and China, which are geographically close to the sample sites of this study [20-22].

In previous studies, it was shown that *Monotropa* species are able to form symbiotic relationships with different species of Russulaceae depending on the area, even though they showed strong specificity with fungi at the family level [5, 21, 22]. Therefore, the symbiotic relationships between *M. uniflora* and fungi have a geographical mosaic appearance. This could be because of coevolution between plants and fungi in different ecological environments; however, strong preference of *Monotropa* to Russulaceae suggests the presence of specific recognition process between the plants and fungi.

Russula species form ectomycorrhizal relationships with various host plants in forests. In general, it is known that the mutualistic relationship has relatively weak host specificity; however, host specificity develops when one of the two interacting organisms receives relatively more benefits. Therefore, mycoheterotrophic plants such as *M. uniflora* not only provide important evidence regarding nutrient transfer through the mycorrhizal hyphal network in soil [15] but also provide important insights into understanding the coevolution between plants and fungi [16]. In addition, determining the diversity of mycorrhizal fungi associated with Monotropoideae plants provides an important basis to understand the evolutionary paths of mycoheterotrophy in Ericaceae and Orchidaceae [23].

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