

Mycorrhizal fungi associating with germination of mycoheterotrophic *Pyrola japonica*

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Purpose: A green plant of *Pyrola japonica* is supposed to be a mycoheterotrophic obtaining carbon via not only photosynthesis but also mycorrhizal fungi. Such the plant is considered to be a pre-adaptation of the evolution to a fully mycoheterotrophic plant. Mycoheterotrophic plants generally produce minute seeds called as "dust seeds". Since *P. japonica* also produces such the seeds, the plant deemed to be mycoheterotrophic. However, no information is available which fungi are involved for the germination of *P. japonica*, although mycorrhizal fungal communities associated with adults and seedlings have been elucidated. The purpose of this study was to clarify mycorrhizal communities involved in the initial growth of *P. japonica*. For this purpose, genomic DNA was extracted from both germinated seeds of *P. japonica*, and associating fungi were examined based on DNA barcoding.

Method: Seeds of *P. japonica* were put into seed packs and buried into three deciduous or evergreen forests in Tsu City, Mie, Japan in 2008 and 2014. The packs were collected in 2018 and the remaining seeds were examined under a stereomicroscope. When seeds were germinated, they were used for the extraction of DNA and applied for PCR amplification on the ITS region, TA-cloning and sequence. Successfully sequenced samples were executed for the BLAST search. The resulting sequences were compared with the previously obtained sequences of *P. japonica* seedlings and adult plants, and the mycorrhiza community was examined for changes.

Results and conclusions: Germinated seeds were confirmed and accounting for 15.2% (536/3522) in 2008 and for 0.1% (14/11021) in 2014. Based on DNA sequences obtained from germinated seeds, we discuss the succession of mycorrhizal communities with the initial stage of *P. japonica*.