

Symbiotic culture revealed different mycorrhizal specificity among coexisting three epiphytic orchids

Kento Rammitsu^{1,2}, Masaru Goto³, Yumi Yamashita⁴, Tomohisa Yukawa⁴,
Yuki Ogura-Tsujita^{1,2}

¹United Graduate School of Agricultural Sciences, Kagoshima University, Japan

²Faculty of Agriculture, Saga University, Japan

³Shizuoka Calanthe Society, Japan

⁴Tsukuba Botanical Garden, National Museum of Nature and Science, Japan

Purpose: Do coexisting orchids in natural habitats associate with distinct mycorrhizal fungi or share the same fungal partners? The purpose of this study is to reveal mycorrhizal association of coexisting three epiphytic orchids, *Oberonia japonica* (Oj), *Taeniophyllum glandulosum* (Tg) and *Thrixsperмум japonicum* (Tj), by molecular identification and symbiotic culture experiments.

Methods: In total, 108 root samples were collected from two Japanese sites, where three orchid species coexisted, and used for molecular identification of mycorrhizal fungi. The ITS sequences obtained from mycorrhizal fungi were classified into operational taxonomic units (OTUs) based on 97% sequence similarity. The main fungal OTUs associated with three orchid species were isolated from their roots, and cultured with the seeds of each orchid species.

Results: In total, 11 OTUs were identified, and Two OTUs, CE1 and TU2 belonging to the Ceratobasidiaceae and Tulasnellaceae, respectively, were dominant fungi. The frequencies of CE1 and TU2 were 4, 84, and 50% and 74, 0, and 10%, in Oj, Tg and Tj, respectively. Symbiotic culture revealed that the developments of Tg and Oj were promoted only by CE1 or TU2, respectively, whereas that of Tj was stimulated by both fungi.

Conclusions: This study clearly showed the different mycorrhizal specificity among the coexisting three orchid species. Two orchids, Tg and Oj, displayed marked high fungal specificity toward particular mycorrhizal fungi and used different mycorrhizal partners, while Tj exhibited low mycorrhizal specificity, sharing the mycorrhizal partners with coexisting other two orchids.