

Orchid and Fungal Interaction

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Introduction:

Biotic interactions between plants and fungi in the soil are essential for survival and adaptation in many plant species ^(1, 2). One of the most compelling examples of a plant-fungus interaction is the symbiosis between terrestrial orchids and orchid mycorrhizae. Unlike other plant seeds, orchid seeds do not have nutrition (endosperm) to support germination. Their germination entirely depends upon orchid mycorrhizae, which infect orchid seeds and supply nutrition necessary for the germination. This symbiotic interaction appears to be species-specific and in many cases, pathogenic fungi can be also symbiotic partners for some specific orchid species ⁽²⁾. This symbiotic relationship between orchids and orchid mycorrhizae is a very unique example of coevolution ⁽⁴⁾, providing a great opportunity to understand fundamental biological questions such as: 'how and why have obligatory symbiotic relationships evolved?' and 'what are the underlying mechanisms involved in the process?'. Furthermore, understanding the mechanism of these symbiotic relationships is essential for maintaining biodiversity and ecosystems, and is of particular importance for orchid conservation projects. The orchid family is the largest flowering plant group, and yet it is one of the most vulnerable and threatened genera, of which many species are on the verge of extinction. Understanding the molecular mechanisms underlying the interaction between orchids and orchid mycorrhizae will provide a key means of protecting orchid species from. Despite a long historical interest and applicable significance, little is currently known about the interactions between terrestrial orchids and orchid mycorrhizae.

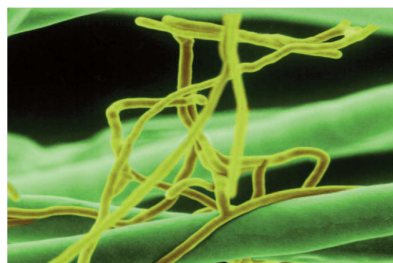


Fig. 1 Scanning Electron microscopic image showing orchid rhizoid (green), with emerging hyphae of mycorrhizal fungus (yellow) Adapted ref(3).

Project Summary

The aim of this project is to investigate the underlying molecular mechanisms involved in symbiotic interaction between orchids and orchid mycorrhizae. Two specific objectives are: 1) generating an atlas of British terrestrial orchids and their symbiotic mycorrhizae and 2) identifying and analyzing genetic and molecular components involved in the symbiotic relationships between a chosen British terrestrial orchid and mycorrhiza as a model system. Proposed work includes: 1) collecting fungal species from the soil of native British orchids in their habitats, 2) the culture and identification of the symbiotic orchid mycorrhizae, 3) RNA deep sequencing of different orchid protocorm tissues (non-germinated vs. germinated, mycorrhiza- non-infected vs. infected and different developmental stages), expression analysis of identified genetic components (qPCR and *in situ* hybridization) and 4) functional analysis of identified genetic components. For proposed work (3) the student will use a model system

which is already established in the faculty. RNA sequencing will be done in the common spotted orchid (*Dactylorhiza fuchsii*) interacting with B1 fungus (*Ceratobasidium* sp. *Anastomosis* group-C). RNA sequences will be analyzed using reference sequences from *Phaleanopsis hybrida* (Moth orchid) and also *de novo* analysis. For the functional analysis (4), the student will generate and analyze knockout and over-expresser lines in heterologous systems (*i.e.*, *Arabidopsis* and *Medicago sativa*). Upon the completion of this project, we will generate a complete list of British native terrestrial orchids and their symbiotic fungal partners and identify genetic and molecular components and understanding their role involved in orchid seed germination and fungal infection.

Proposed work includes extensive training opportunities. The student will be trained for basic field survey methods, mycology, molecular biology (manipulating DNA and RNA), histology and imaging, RNA sequencing and data analysis, plant transformation and functional analysis and phylogenetic analysis.

References

- 1) Rasmussen, H.N. (1995). Terrestrial Orchids from Seed to Mycotropic Plant. Cambridge University Press, Cambridge, UK.
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- 3) Clay, C. (2006). Orchid Mycorrhiza—In Intimate Association. *Journal of the Hardy Orchid Society*. Vol. 3 No. 4 (42)
- 4) Duttke, S. D., Zoulias, N. And Kim, M (2012). Mutant flower morphologies in the wind orchid, a novel orchid model species. *Plant Physiology* 158 (4), 1542-1547