Evolutionary study on mycoheterotrophy of Cephalanthera in Japan

論文内容要約

Evolutionary study on mycoheterotrophy of *Cephalanthera* in Japan
(日本産キンラン属における菌従属性に関する進化学的研究)

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Abstract

Background

The trophic type by which organisms obtain nutrient resources for growing on fungi is called mycoheterotrophy while that from both photosynthesis and mycorrhizal fungi is called partial mycoheterotrophy. Plants with the latter trophic type are considered to be an intermediate state in the evolutionary process from autotrophy to full mycoheterotrophy. How and when mycoheterotrophic plants originated and evolved is a one of the most important issue in evolutionary studies on mycoheterotrophy. A comparison of the characters concerning mycoheterotrophy among closely related species seems to be a valid approach to study on mycoheterotrophic evolution. *Cephalanthera* includes both partially and fully mycoheterotrophic species with wide range of dependencies on fungi, and is one of the ideal groups for understanding evolution of mycoheterotrophy.

Aim

The aim of the study is to reveal some characters associating mycoheterotrophy of putative partially mycoheterotrophic, five Japanese green *Cephalanthera* species (*C. longibracteata*, *C. falcata*, *C. erecta*, *C. longifolia* and *C. subaphylla*). I examined molecular methods, isotope analysis, chlorophyll fluorescence measurement, and morphological measurement to address the tasks. Thus, I reconstructed phylogenetic tree and ancestral characters of *Cephalanthera* species and discussed the influences of mycoheterotrophic characters to the evolution of Japanese *Cephalanthera*.

Methods

In this study, I tried to study on mycoheterotrophy of Japanese *Cephalanthera* species from some views, fungal specificity, nutrient dependency, photosynthetic ability, investment ratios to vegetative organs, and a phylogenetic relationship. First, mycorrhizal fungi of the five *Cephalanthera* species were identified by molecular methods. A total of 242 root segments were collected from 96 individuals of five *Cephalanthera* (*C. longibracteata*, *C. erecta*, *C. falcata*, *C. longifolia*, and *C. subaphylla*)species native to Japan and mycorrhizal fungi were identified based on the sequences of nuclear ribosomal internal transcribed spacer regions Second, leaf samples of the five *Cephalanthera* species were collected from five sites in 2012-2014 and carbon and nitrogen dependencies on

mycorrhizal fungi using isotopic analyses were estimated. Third, chlorophyll fluorescence was measured in leaves of *Cephalanthera* species, and degrees of light inhibitions and electron transport rate as barometer of photosynthetic ability were estimated. Fourth, dried biomass of aboveground and subterranean portion and leaf areas were measured in the five *Cephalanthera* species. In addition, leaf area per total dry weight (*LAR*; leaf area ratio), leaf mass per total dry weight (*LMR*; leaf mass ratio), underground part mass per total dry weight (*RIR*; root investment ratio), and specific leaf area per leaf mass (*SLA*; specific leaf area) were calculated for each plant to assess the ratios of investment in leaf and underground part. Lastly, to infer the phylogenetic relationships in *Cephalanthera*, phylogenetic trees were constructed by nuclear ribosomal internal transcribed spacer region.

Results

Several groups of ectomycorrhizal fungi, including Russulaceae, Sebacinaceae, and Thelephoraceae were identified from the roots of *C. longibracteata* and *C. falcata* while *C. erecta*, *C. longifolia* and *C. subaphylla* had high specificity to Thelephoraceae. The ectomycorrhizal fungi symbiotic with *C. subaphylla* were first reported here. These results suggested that *C. longibracteata* and *C. falcata* had lower fungal specificity than other *Cephalanthera* species did.

The results of isotope analyses showed that almost all individuals of *Cephalanthera* species had higher ^{13}C and ^{15}N abundances than autotrophic references had, indicating all of the Japanese *Cephalanthera* species were partial mycoheterotrophy. For carbon dependencies, no significant differences were found among four *Cephalanthera* species, *C. falcata*, *C. erecta*, *C. longibracteata*, and *C. longifolia* while significant differences were found between these four species and *C. subaphylla*. The comparison of investment ratios, *LAR*, *LMR* and *RIR*, among the *Cephalanthera* species showed some significant differences, indicating that the four species, *C. falcata*, *C. erecta*, *C. longibracteata* and *C. longifolia*, have higher investment ratio in leaf than *C. subaphylla* did. Phylogenetic analysis suggested that the Japanese *Cephalanthera* species were divided into two groups, the clade A consisted of *C. falcata* and *C. longibracteata* and the clade B consisted of *C. erecta* and *C. subaphylla*. On the other hand, *C. longifolia* was paraphyletic with respect to other four *Cephalanthera* species.

Main conclusion

As a result of mycorrhizal identifications, two tendency of fungal association were confirmed; *C. longibracteata* and *C. falcata* had broad fungal specificity, associating with the ectomycorrhizal fungi belonging to Russulaceae, Sebacinales and Thelephoraceae. On the other hand, the three *Cephalanthera* species, *C. erecta*, *C. subaphylla* and *C. longifolia* had narrow fungal specificities only to Thelephoraceae fungi as other *Cephalanthera* species. Isotope analysis showed that the nutrient dependencies on fungi of *C. subaphylla* were the strongest, and those of *C. erecta* were the second strongest, next was those of *C. falcata* and *C. longibracteata*, and that of *C. longifolia* was the least. Moreover, although *C. subaphylla* had significantly lower *ETR* and F_v/F_m than the three species, *C. falcata*, *C. erecta*, and *C. longibracteata*, which showed the same level as autotrophic plants. The results of morphometric measurements were similar with the photosynthetic characters. These results indicate that resource investment ratios are different between *C. subaphylla* and the other four *Cephalanthera* species; in particular *C. subaphylla* invested more resources in subterranean part than in leaves. Thus, Japanese *Cephalanthera* species were separately divided into two groups by mycoheterotrophic characters; *C. subaphylla* is the second stage of partial mycoheterotrophy and the four *Cephalanthera* species are the first stage of partial mycoheterotrophy.