Plasticity of nutrition strategies in mixotrophic orchids as an intermediate stage in evolution of mycoheterotrophy

Michał May

Members of Orchidaceae family exhibit three diverse nutrition strategies: autotrophy (AT) based on photosynthesis, mycoheterotrophy (MH), in which sustenance is obtained from a mycorrhizal partner – a soil fungus, and mixotrophy (MX), which involves both photosynthetic activity and reversed mycorrhizal carbon flow simultaneously. All orchid species demonstrate germination strategy that relies on fungal carbon and are therefore mycoheterotrophic in their juvenile state. After developing photosynthetic organs most of the species transition to AT and revert the direction of carbon flow to the functioning expected of common mycorrhizal relationship. Some species, however, do not undergo the switch and remain MH in adulthood as an evolutionary adaptation to low light conditions. The transition from AT to MH occurred independently multiple times in evolutionary history in multiple separate lineages of Orchidaceae. MX nutrition can be considered an intermediate state between those two strategies and is an evolutionary pre-requisite to transition into MH. Multiple MX species exhibit a high adaptation potential to revert to AT nutrition in conditions that favor photosynthesis over dependency on fungal carbon. The presented thesis encloses several studies, involved in a consistent sequence of cause-and-effect research questions and hypotheses that emerged from gathered, analyzed and discussed results. Sequencing of plastidial genomes of two AT orchid species, *P. chlorantha* and *D. majalis*, done to set a baseline template for a genetic makeup of an autotroph, revealed that both species possess intact plastomes and carry functional photosynthetic machinery. Further description of 13 new plastomes sequenced from orchid species, 9 of which were previously described as mixotrophic and 4 as autotrophic, revealed 10 cases of intact plastomes and 3 cases of reductions in plastome compositions that do not affect their photosynthetic capabilities. No correlation between degree of dependency on fungal carbon and gene loss was detected. MX plants retain genetic capability of reverting to AT nutrition, which is further observed on mixotrophic species *E. helleborine* grown in a greenhouse. The absence of trees, that contribute photosynthetic carbon to a common mycorrhizal partner and act as a primary carbon source in mycorrhizal network facilitates reverting to autotrophic nutrition, confirmed by isotopic content analysis. The analysis of MH orchid transcriptomes indicates far reaching degradation in their plastidial genomes but detects no genes unique to mycoheterotrophs; and a comparison of expression profiles between their above- and below ground organs suggests that physiology of MH plants is a result of expression reprogramming rather than novel genetic constructs. The assembly of transcriptomes for non-model species is challenging, especially among plants – without fully sequenced genome to act as a reference for guided assembly, a less accurate *de novo* assembly approach is required. In an attempt to improve its quality, a novel approach combining two sequencing strategies was evaluated. This hybrid assembly technique involves employing short reads from Illumina sequencing and long reads from MinION platform to overcome drawbacks of both technologies and deliver a transcriptome of higher quality. In addition to the exploratory study of plasticity in plant nutrition strategies, this work delivered a unified, optimized toolset for future investigation of trophic modes in Orchidaceae.