学 位 論 文 内 容 の 要 約

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Physiological and molecular mechanisms of phosphate uptake and translocation in arbuscular mycorrhizal symbiosis
（アーバスキュラー菌根共生におけるリン酸吸収および輸送の生理・分子機構）

Phosphate is an essential macronutrient in plants, but the availability in soil is often limited due to the formation of sparingly soluble salts with iron and aluminum that are abundant in the soil. Association with arbuscular mycorrhizal (AM) fungi is a distinctive strategy of most land plants for enhancing phosphate uptake. The fungi take up phosphate through hyphal networks constructed in the soil (i.e. extraradical hyphal networks) and convert to polyphosphate (polyP) that is a linear chain of three to thousands phosphate residues. The compound is the largest phosphate storage and likely to be involved in long-distance translocation of phosphate in the fungi, but the physiological and molecular mechanisms underlying have yet to be elucidated.

1. Transcriptome response that leads to synchronous and equivalent uptake of inorganic cations during polyphosphate accumulation

Phosphate application to phosphate-starved extraradical hyphae of the fungi triggers rapid and massive accumulation of polyP, which results in accumulation of a large amount of negative charge in the cell, leading to the hypothesis that there is a regulatory mechanism for maintaining cellular charge neutrality. An AM fungus *Rhizophagus clarus* HR1 (MAFF520076) was grown in association with *Lotus japonicus* under phosphate-starved conditions, and extraradical mycelia were harvested prior to and after phosphate application. Levels of polyP, inorganic cations, and amino acids were measured, and transcriptome analysis was performed on the Illumina platform. Phosphate application triggered not only polyP accumulation but also near-synchronous and -equivalent uptake of sodium, potassium, calcium, and magnesium, whereas no distinct changes in the levels of basic (cationic) amino acids were observed. During

polyP accumulation, genes responsible for phosphate and cation uptake, polyP and nitrogen metabolisms, and the maintenance of pH homeostasis were significantly up-regulated. These results provide evidence that inorganic cations play a major role in neutralizing the negative charge of polyP and the processes are achieved by the orchestrated regulation of gene expression.

2. Fungal water channel mediates transpiration-driven long-distance translocation of polyphosphate towards the host

PolyP translocation through AM fungal hyphae has so far been interpreted by simple diffusion and/or motor protein-driven organelle transport in random directions, and no mechanism for directed translocation towards the host has been proposed. I tested the hypothesis that water flow through hyphae towards the roots, which might be created by osmotic gradients between the fungal and root cells through host transpiration, would drive directed translocation of polyP. Transpiration of *L. japonicus* grown in association with *R. clarus* HR1 was suppressed by either of abscisic acid application, dark treatment, or shoot removal, and the effect of the suppression on polyP translocation was evaluated. One of the three AM fungal genes encoding water channel (aquaporin), *RcAQP3*, was found to be highly expressed in hyphae in the roots by transcriptome analysis and knocked down by the virus-induced gene silencing (VIGS) technique to examine the involvement of the aquaporin in polyP translocation via mediating water flow to root tissue. Suppression of transpiration decelerated polyP translocation towards the roots in proportion to the levels of suppression. *RcAQP3* expression was successfully knocked down by VIGS, in which polyP translocation towards the roots was positively correlated with the expression levels, supporting the hypothesis. These results provide the first evidence for the directional translocation of polyP towards roots in the associations.

The present study unveiled the mechanisms underlying phosphate acquisition and delivery in AM symbiosis at the physiological and molecular levels. The study also provides a technical breakthrough in manipulating gene expression in the obligate biotrophic fungi that cannot be transformed by conventional methods, which will enhance future research in this field.