# 学　位　論　文　審　査　の　要　旨

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## 学　位　論　文　題　名

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）

This thesis consists of 29 figures, 14 tables, 127 references, General introduction, two Chapters, and General discussion in a total of 115 pages with two accompanying publications.

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. Association with arbuscular mycorrhizal (AM) fungi is a distinctive strategy for enhancing phosphate uptake in most land plants. The fungi take up phosphate through hyphal networks constructed in the soil (i.e. extraradical hyphal networks) and accumulate as polyphosphate 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 through hyphae. However, the physiological and molecular mechanisms underlying polyphosphate accumulation and translocation have yet to be elucidated.

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

Phosphate-starved hyphae of the fungi are capable of accumulating a massive amount of polyphosphate, which results in accumulation of a large amount of negative charge in the cell. The study addressed the hypothesis that there is a regulatory mechanism for maintaining cellular charge neutrality. The AM fungus *Rhizophagus clarus* HR1 was

grown in association with *Lotus japonicus* under phosphate-starved conditions, and extraradical mycelia were harvested prior to and after phosphate application. Levels of polyphosphate, inorganic cations, and amino acids were measured, and transcriptome analysis was performed on the Illumina platform. Phosphate application triggered not only polyphosphate 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 polyphosphate accumulation, genes responsible for phosphate and cation uptake, polyphosphate and nitrogen metabolisms, and the maintenance of pH homeostasis were up-regulated. These results indicate that inorganic cations play a major role in neutralizing the negative charge of polyphosphate and these 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

Polyphosphate translocation through AM fungal hyphae has so far been interpreted by simple diffusion and/or motor protein-driven organelle transport in random directions. No mechanism for directed translocation towards the host, however, has been proposed. The study hypothesized that water flow through hyphae towards the roots, which is created by osmotic gradients between the fungal and root cells through host transpiration, would drive directed translocation of polyphosphate. Suppression of transpiration decelerated polyphosphate translocation through hyphae towards the roots in proportion to the levels of suppression. A water channel (aquaporin) gene *RcAQP3* that is expressed in intraradical hyphae was identified in *R. clarus* HR1 and successfully knocked down by virus-induced gene silencing. The knockdown of *RcAQP3* decelerated polyphosphate translocation towards the roots in proportion to the expression levels. These results indicated that the aquaporin mediates polyphosphate translocation through hyphae, which is primarily driven by host transpiration, providing the first evidence for the directional translocation of polyphosphate 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, which will enhance future research in this field.

Therefore, we acknowledge that the author is qualified to be granted the Degree of Doctor of Philosophy in Agriculture from Hokkaido University.