# 学位論文の要旨

論文題目　Physiological Responses and Variation of Rhizobacterial Community to Phosphorus Deficiency in Distinct Root Architectures of Lupins (異なる形態の根を持つルーピンにおけるリン欠乏への生理応答と根圏細菌群集の変動)

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Phosphorus (P), an essential element for plants, plays a crucially important role in various biochemical cycles and serves as a crucial component of nucleic acids and cell membranes. Moreover, P can limit crop growth and productivity because it tends to become fixed by soil mineral particles, ultimately converting into legacy P forms over time. Organic P, generally accounts for 30% to 65% of the total P in soils, typically derived from plant residues, microbial biomass, and animal excreta. Po primarily exists in stable forms in the soil, such as inositol phosphates, which cannot be directly utilized by plants. Typically, crops absorb only about 20–30% of applied inorganic phosphate (Pi) fertilizer. The ongoing increase in Pi fertilizer usage for high yields has led to elevated residual P level in the soil. Under conditions of P deprivation, plants have therefore evolved a multitude of nutrient acquisition strategies structurally and physiologically to adapt to P deficiency stress. Under P-deficient condition, the morphological adjustment exhibited by plants including the increase in the root-to-shoot ratio, root length promotion and root hair proliferation. Moreover, some plants exhibit specific changes in root architecture such as the formation of cluster root. From a physiological perspective, plants secrete organic acids or protons from their roots under P stress to solubilize insoluble P. They also increase phosphatase activity to effectively mineralize soil organic P. For instance, phytase produced by plants or soil microorganisms can effectively mineralize phytate-P.

White lupin (*Lupinus albus*), blue lupin (*L.angustifolius*), and yellow lupin (*L.luteus*) are among the 11 Old-World lupin species. Blue lupin and yellow lupin are classified under clade B based on the modified phylogenetic relationships and clade separation, while white lupin is classified under clade D. White lupin stands as a prominent model plant for studying plant adaptation mechanisms amidst P deficiency because it can develop dense rootlets on secondary roots to amplify the root absorptive surface area. Additionally, extensive research has substantiated the enhancement of secretion of organic acids and the increase in phosphatase activity in cluster roots from a physiological perspective. In contrast to the white lupin, which can produce massive cluster roots or proteoid roots, yellow lupin has longer lateral roots. Under a P-deficient situation, cluster-like root structures are notably visible in yellow lupin, whereas blue lupin cannot form these root structures even under extreme P scarcity. How do distinct lupin species with different root architectures respond to P deficiency, and what adaptation mechanisms do they develop? Does the unique cluster root structure produced by white lupin help itself more efficiently mobilize and utilize P in the soil solution? A thorough understanding of the ultimate differences in P utilization across three lupin species necessitates a comprehensive investigation of the P mineralization processes mediated by root secretions and soil microbes.

To study the influence of P deficiency on various lupin varieties with different root architectures, including the growth analysis of lupins and the comparison of P distribution differences in different organs under distinct P treatments, as well as the adaptive strategies developed in response to P-deficient stress. Based on these experimental objectives, we first adopted the hydroponic method, which allows for better control of environmental factors and more accurate measurements of the physiological adaptation strategies such as the composition of exudation. The adaptation in this research focuses on the morphological variation of root system, biomass and P allocation in diverse organs of lupin seedlings, secretion of root exudates, and the enhancement of enzyme activities. In the hydroponic study, plants were grown with (64 μM $NaH_2PO_4$) or without P for 31 days. Under P limitation, more biomass was allocated to roots to improve P absorption. Blue lupin was found to be most sensitive to the absence of P: it showed the strongest reduction in RGR. Under +P conditions, the P content increased in lupins, especially in blue lupin and yellow lupin. Yellow lupin accumulated the highest amount of P in its leaves for high photosynthetic efficiency under P omission condition. These differences among the three lupin species underscore the variation in their responses to P supply. To elucidate the responses of lupins via the perspective of absorption kinetics and secretion analysis, blue and yellow lupins were confirmed to have stronger affinity and absorption capacity for orthophosphate after P deprivation cultivation, whereas white lupin and yellow lupin had greater ability to secrete organic acids. The exudation of blue lupin had higher acid phosphatase activity. This study elucidated that blue lupin was more sensitive to P scarcity stress and yellow lupin had the greater tolerance of P-deficient condition than either of the other two lupin species. In conclusion, compared to white and blue lupins, we speculate that yellow lupin may exhibit higher adaptability under P-deficient stress conditions. However, in the practical situation, root exudates assist plants under P scarcity via solubilizing soil-bound insoluble P. Furthermore, the rhizosphere microorganisms play a crucial role in the mineralization of highly abundant organic-P in the soils that requires further validation using plant–soil–microbe systems.

Considering the activation effect of soil microorganisms on unavailable P and the mineralization effect of lupin root exudates on soil organic P components, we designed a pot experiment. In this pot experiment, three lupin species were subjected to three P treatments (-P, +Pi and +Po). After two months of cultivation, biomass allocation, P content in lupin varieties; Hedley-P fractions, exudation composition, and bacterial diversity in the rhizosheath under distinct treatments were determined. The results indicated that white lupin accumulated high P content under both -P and +Po treatments, and there was no significant difference in P content for yellow lupin between +Po and +Pi treatments. Additionally, when compared to sufficient Pi application, white lupin exhibited higher citrate secretion, ALPase, and PHYase activities under -P condition, as well as greater PHYase and β-Glu activities under +Po condition in the rhizosheath, thereby facilitating the maintenance of plant P content. Meanwhile, rhizobacteria played an essential role in the phytate activation process for white lupin and yellow lupin exposed to the +Po condition. Under phytate supplement condition, the enrichment of *Burkholderia* and *Pseudomonas* in the white lupin rhizosheath was associated with improving phytase activity. Yellow lupin exposed to phytate supplementation condition showed more abundant rhizobacterial diversity compared to other species. Moreover, it is assumed that *Methylacidiphilum*, Acidobacteria_Subgroup_3, and *Mucilaginibacter* played crucial role in the mobilization of organic P. Particularly, the *Segetibacter*, *Granulicell*, *Candidatus_Methylacidiphilum*, and *Bryobacter* genera were simultaneously present in the rhizosheath of white lupin and yellow lupin, with higher abundance observed only under +Po treatment suggesting their involvement in the phytate mobilization process. This study systematically elucidated the physiological responses of three lupin species and the differences in rhizosheath bacterial communities under P-deficient and phytate supplementation, focusing on the rhizosheath-lupin-bacteria interactions. The findings emphasized the necessity for further research to uncover the role of rhizosheath bacteria in white lupin and yellow lupin regarding phytate activation in the future.