DOCTORAL THESIS

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

(Summary)

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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. It participates in numerous vital physiological and biochemical processes in plants. However, despite its importance, the availability of P in the soil is often limited because it tends to become fixed by soil mineral particles, ultimately converting into legacy P forms over time. Moreover, P losing from the soil accounts for 5-10% of the total soil phosphorus, which, through sedimentation, transforms into phosphate rock and ultimately returns to the soil as phosphate fertilizer. Compared to labile P, which is available to crops, organic P (Po) generally constitutes around 80% of the total P content in soil. Po primarily exists in stable forms in the soil, such as inositol phosphates, which cannot be directly utilized by plants. The mineralization of phytate in the soil primarily relies on phytase, which hydrolyzes phytate into inositol, inorganic phosphate, and other trace elements for plant uptake. The main sources of phytase are microorganisms, including bacteria, fungi, and yeast. This means that microorganisms play a crucial role in the soil organic phosphorus cycle. 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. The mineralization of organic P and the adsorption-desorption processes of inorganic P are influenced by various environmental factors.

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. Carboxylates are released via an anion channel and the exact way in which phosphatases are released is not known. Carboxylates mobilize both inorganic P, organic P and some of the cations that bind P. Some of these cations (especially Fe) move to the root surface for uptake by the roots, others move down the soil profile, where give rise to the formation of precipitates. Moreover, phosphatases can hydrolyze organic P compounds, once these have been mobilized by carboxylates.

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. Compared to *L. albus*, both *L. angustifolius* and *L. luteus* are shown to be monophyletic based on Internal Transcribed Spacer (ITS) sequencing results, indicating that they may be more closely related from a phylogenetic perspective. 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 36 days. Different lupin species with the distinct root architectures under P omitted cultivation, white lupin can produce massive cluster roots, yellow lupin has dense root hairs and blue lupin cannot form these root structures even under extreme P scarcity. Under P limitation, more biomass was allocated to roots to improve P capture capacity whereas lupins allocated more biomass to shoot that facilitate overall plant development. Blue lupin was found to be most sensitive to the absence of P: it showed the strongest reduction in relative growth rate. 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 in the 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 (phytate). 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. Similarly, the total biomass in blue lupin was reduced significantly under P scarcity cultivation. 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, alkaline phosphatase, and phytase activities under -P condition, as well as greater phytase and β-glucosidase activities under +Po condition in the rhizosheath, thereby facilitating the maintenance of plant P content. Rhizosphere microorganisms, especially in a rhizosheath directly surrounding plant roots, exhibit higher plant growth-promoting activity and richness than in bulk soil. Dynamic interactions between the root systems of different plant species and their secretions with microbial communities shape the colonization of distinct microbial taxa. After cultivating by phytate, the bacterial community alpha diversity was greater for yellow lupin than blue lupin and white lupin. Though the comparison and composition of soil bacterial communities in rhizosheath, P treatment has the bigger impact on the composition of bacteria in the rhizosheath compared with the lupin species. Particularly, the abundance of *Flavisolibacter, Sphingomonas*, *Flavobacteriaceae*, and *Dyadobacter* (Plant growth promoting rhizobacteria, PGPR) increased in the rhizosheath of white lupin under -P. Under phytate supplement condition, the enrichment of the genera *Burkholderia* and *Massilia* in the white lupin rhizosheath was associated with improving phytase activity. Moreover, it is assumed that *Methylacidiphilum*, Acidobacteria_Subgroup_3, and *Mucilaginibacter*, the three dominant biomarkers in the yellow lupin rhizosheath after supplying Po, played crucial role in the mobilization of phytate. On the other hand, the addition of phytate not only serves as a P supply but also as a C source for the rhizosheath bacteria, enabling them to synthesize sufficient ATP to sustain high-energy metabolic activities, such as the degradation of fatty acids, phenylalanine, and lysine. In addition, 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, the functional gene prediction analysis of the four strains shared for white and yellow lupins rhizosheath showed that the enhancement expression of phosphate regulon sensor histidine kinase PhoR gene and the alkaline phosphatase synthesis response regulator PhoP gene, which 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 mobilization in the future.

To sum up, both in the hydroponic and pot experiments, blue lupin showed significant inhibition under P-deficient conditions, indicating that blue lupin has the worst tolerance to P omission compared to the other two species. After hydroponic P-deficient cultivation, yellow and blue lupins have greater affinity and absorption capacity for Pi than white lupin from the absorption kinetic perspective. In terms of the exudation composition, young cluster roots (36 days in hydroponic experiment) primarily secrete malate, while mature cluster roots (more than 2 months in pot experiment) predominantly secrete citrate. White lupin and yellow lupin can secrete more organic acids under -P hydroponic cultivation, while blue lupin can elevate phosphatase activities to cope with P deficiency. Therefore, the formation of cluster roots is positively correlated with the release of organic acids but is not related to the phosphatase activity. In addition, increased bacterial richness in the white and yellow lupins rhizosheath can promote the mineralization of phytate in the soil.