

**Doctoral Thesis**

**Root Growth Plasticity and Phosphorus Remobilization in Rice as Adaptive  
Mechanisms to Phosphorus Deficiency**

**Abstract**

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**Title:**

Root Growth Plasticity and Phosphorus Remobilization in Rice as Adaptive Mechanisms to Phosphorus Deficiency

**Introduction:**

Among the cereal crops growing today, rice (*Oryza sativa* L.) is regarded as the most important, feeding a large share of the world's population. Rice cultivars with enhanced phosphorus (P)-use efficiency are increasingly important for sustainable food production, as P is a prominent nutritional constraint to global rice production. Alternative and sustainable approaches are needed to decrease agriculture's overdependence on P fertilizers, and include manipulating crops by: (i) enhancing the ability of its roots to acquire limiting inorganic P (Pi) from the soil (i.e. increased P-acquisition efficiency), and/or (ii) increasing the total biomass/yield that is produced per molecule of P assimilated from the soil (i.e. increased P-use efficiency or PUE).

**Research Objectives:**

Aims of the study were to examine (1) low-P tolerance mechanisms exhibited by rice plant under P-deficient conditions, (2) molecular mechanisms that drive P remobilization in rice, (3) partitioning of acquired P among different vegetative and reproductive organs by low-P tolerant and sensitive rice genotypes, (4) genotypic differences of yield components, grain yield, and grain P loading of low-P tolerant and sensitive rice genotypes, and (5) genomic regions associated with low-P tolerance of Japonica rice

**Research Methodology:**

The entire study comprised of two experiments with Akamai (low-P tolerant) and Koshihikari (low-P sensitive) cultivars done in pots with Regosol soil and another experiment with F5 mapping population originated from crossing Akamai with Koshihikari. Supporting hydroponic experiment was done. For experiments in Regosol, two P fertilizer treatments: P0 (no P supply) and P100 (P supply to a rate of 100 mg P kg<sup>-1</sup> soil Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>•H<sub>2</sub>O). Supporting experiment was done in nutrient solutions either with (64 μM/+P) or without (0 μM/-P) P. In first experiment with Regosol, Plants were harvested at three consecutive sampling times; 28 days after transplanting (DAT), 49 DAT, and at the start of the panicle initiation. Shoots were partitioned into bottom, middle, and top leaves. Biomass and P accumulation in these different tissues, P remobilization from mature/senescing leaves to upper young leaves were measured. In second soil experiment, plants were harvested at physiological maturity and shoots were partitioned into stems, fully senesced, partly senesced, and green leaves, and panicles. Soil column in the pot was divided into two equal portions (13 cm each) and were considered as upper and lower soil layers in which root dry weight (DW) and length were measured in each layer. Grain yield and yield components were also determined. In supporting experiment, plants were sampled at four-leaf stage in which bottom two leaves were considered as lower leaves and top two leaves as upper leaves. Membrane lipid components of different leaves were separated by two-dimensional thin-layer chromatography (2D TLC). To identify the genomic regions associated with low-P tolerance of Japonica rice, F5 mapping population was grown in Andosol without P addition for 23 days. Top 10 plants and bottom 10 plants in terms of shoot length were selected as low-P tolerance and low-P sensitive sets, respectively. DNA extracted from young leaves of each set was used to perform quantitative trait loci (QTL) – sequence.

## **Results and Discussion:**

### Plasticity of root growth and shoot growth response:

Low-P tolerant Akamai is capable of acquiring more P and producing higher plant biomass than low-P sensitive Koshihikari. At all harvesting times, Akamai grown under P0 could produce biomass similar to that of Koshihikari in a P-supplied condition. Akamai shares this similarity with Koshihikari in terms of the total P uptake highlighting the ability of Akamai for growth and strong establishment under P-deprived conditions in soil. The specific adaptation associated with below ground part of Akamai to P-deficiency is the more plastic nature of root growth. Akamai exhibited more explorative root growth behavior (enhanced root DW and length). It attained approximately 3-fold greater root DW and length than those of Koshihikari under P0. This probably helps to explore greater volumes of soil to acquire more P under P-deficient conditions.

### Membrane lipid remodeling based P remobilization:

Results confirm that Akamai grown under P-deprived conditions possess an efficient P remobilization that supports redistribution of acquired P based on the demand of the different segments within the plant. The P remobilization efficiency of Akamai shifted from 56% to 72% during the period from 28 DAT to panicle initiation. At maturity, the value shifted to 85% and Akamai in P0 achieved similar green leaf P concentration to that grown in P100 condition. Akamai utilizes acquired P more efficiently through investing lesser amount of P to lower senescing leaves and markedly greater proportion to upper young leaves and panicles under P-limitation driven by its efficient P remobilization. Supporting experiment reveals that efficient P remobilization of Akamai under P-deficiency is partly related to membrane lipid remodeling (replacement of phospholipids with lipids that do not contain P). Among the two rice cultivars, only Akamai made this replacement strongly only in lower leaves when it is grown without external P supply whereas under same conditions, Koshihikari did not alter phospholipid content among lower and upper leaves. In lower leaves of Akamai, phospholipids were mainly replaced by galactolipids. However, phospholipids in upper leaves of Akamai between treatments with and without P addition did not differ. From these results, it is evident that, under P-deficient conditions, Akamai maintains lower level of phospholipid pool and use lipids that do not contain P instead in lower leaves while investing more phospholipids to upper region of the plant where leaf emergence and expansion occur.

### Grain yield, yield components, and loading of P into grains:

Both cultivars tended to show higher grain yield when they were grown under P100 and this happening was more prominent with Koshihikari. The highest grain yield was produced by Koshihikari in P100 whereas the lowest was recorded when it is grown in P0. The reduction of grain yield of plants grown in P0 compared to those in P100 was 20% for Akamai and 45% for Koshihikari. Under P100 condition, Koshihikari showed 20% more grain yield than that of Akamai. In contrast, Akamai recorded 22% and 59% greater grain yield and grain P concentration, respectively, than those of Koshihikari under P0 condition. The number of filled grains per panicle appeared to be the key yield component determining the grain yield difference of two cultivars under two P treatments where under P100 conditions, this parameter was higher in Koshihikari. Under P0 conditions, Akamai had a higher value. For that reason, P-deficiency had a stronger impact on grain filling of Koshihikari where the filled grain percentage of Koshihikari under P0 was lower by 29% than that under P100. However, for Akamai, it was only 11% lower.

### Genomic regions associated with low-P tolerance of Japonica rice:

Genomic region associated with low-P tolerance of Akamai rice cultivar was identified in chromosome 12 in the region from 23.6 Mb to 27.5 Mb (lower end) and was named as *QTL for Low-P Tolerance 1* (*qLPT1*). Novel genes responsible for low-P tolerance could exist in *qLPT1* and further molecular examination of *qLPT1* would clarify those novel genes associated with the low-P tolerance of Akamai.

### **Conclusions:**

In response to P-deficient conditions, low-P tolerant Akamai rice cultivar develops an extensive root system and explores greater volumes of soil to acquire more soil P than low-P sensitive Koshihikari. This development and exploration helps Akamai to support its enhanced shoot growth. Akamai starts to remobilize part of the P in lower mature leaves to upper younger leaves starting from early growth stage and at maturity, it allocates remarkably lower P concentrations to roots, fully and partly senesced leaves, and stems while investing a greater amounts to more active green leaves and panicles. Efficient leaf P remobilization of Akamai is partly related to lipid remodeling in lower mature leaves in which phospholipids were mainly replaced with galactolipids. Akamai could attain the yield advantage over Koshihikari under P-deficient conditions and the key yield component determining the grain yield difference between two cultivars is the number of filled grains per panicle. Low P-tolerance trait of Akamai is attributed by *QTL for Low-P Tolerance 1* (*qLPT1*) located in chromosome 12. P efficient rice genotypes could be achieved by: (i) developing cultivars having explorative root growth to acquire more soil P under P-deficiency, and (ii) producing high-yielding plants having overall lower P concentrations or by increasing the redistribution of P within the plant so as to maximize growth and biomass allocation to the developing organs. Significant reductions in rice P pools may be achieved by replacing membrane phospholipids with galacto- and sulfolipids, which do not contain P. Improved PUE would also be achieved by increasing P remobilization from senescing tissues to young, expanding organs, including the developing grains.