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Does OsPHR2, central Pi-signaling regulator, regulate some unknown factors crucial for plant growth?

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OsPHR2*, the homolog of *AtPHR1*, is a central Pi-signaling regulator. The Pi-signaling pathway downstream of AtPHR1, similarly of OsPHR2,[1,2] involves a noncoding RNA which targets mimicry of *miR399*. miRNA399 mediates cleavage of PHO2.[3,4] The regulating pathway downstream of OsPHR2 is negatively regulated by the Pi-signaling responsive gene *OsSPX1*.[5,6] Overexpression of *AtPHR1* and *OsPHR2* leads to an increased concentration of Pi in the shoot tissues with leaf toxic symptom and growth retardation similar as the phenotype of *pho2* mutant, especially under Pi abundant conditions.[2,6,7] It has been known that the low affinity Pi transporter OsPT2 mainly contributes to the shoot Pi accumulation mediated by OsPHR2, and overexpression of *OsPT2* results in shoot Pi accumulation and leaf toxic symptom and growth retardation under Pi abundant conditions.[6] Two curious questions are emerging from the reported results: How Os SPX1 functions on the negative regulation of the pathway and what mechanism of the growth retardation mediated by OsPHR2. For the second question, our favored hypothesis is that the growth inhibition mediated by overexpression of OsPHR2 is caused by toxic physiological effects due to excessive Pi accumulation in shoots (Pi toxicity). In fact, the toxic symptoms become diminished with decreased Pi levels in growth medium. However, the plant growth retardation mediated by overexpression of *OsPHR2* may be caused by some unknown genetic factor(s) regulated by OsPHR2.

Plant Growth Inhibition Mediated by OsPHR2 is not Restricted to Shoot Pi Concentration

To determine whether the plant growth inhibition mediated by OsPHR2 is restricted to shoot Pi concentration, we performed a experiment with several Pi levels in a solution culture to found a condition under which the shoot Pi concentration in *PHR2(O)* plants is similar as in shoots of the wild type plants at 10 mg Pi/L level. At the supplied Pi level of 0.5 mg Pi/L, the shoot Pi concentration in *OsPHR2(O)* plants is similar as that in the shoots of wild type plants at the supplied Pi level of 10 mg Pi/L (**Fig. 1A**), that the shoot Pi concentration in *PHR2(O)* is not toxic level in term of physiology, while the biomass of *PHR2(O)* plants is still much lower compared with that of the wild type (**Fig. 1B**).

Isolation of Mutant with Rescue of Plant Growth Inhibition Under Overexpression of *OsPHR2*

Because the plant growth inhibition mediated by OsPHR2 is not restricted to shoot Pi concentration, we reasoned that there should be some genetic factor(s) under the control of OsPHR2. The upregulation or repression of the factor(s) by OsPHR2 may limit the plant growth. In fact, some mutants with rescued growth under Pi-supplied condition were isolated from an EMS-generated mutant library of seeds under background of overexpression of *OsPHR2* (**Fig. 2A**). To confirm the background of the isolated mutants, the expression patterns of *OsPHR2* and the

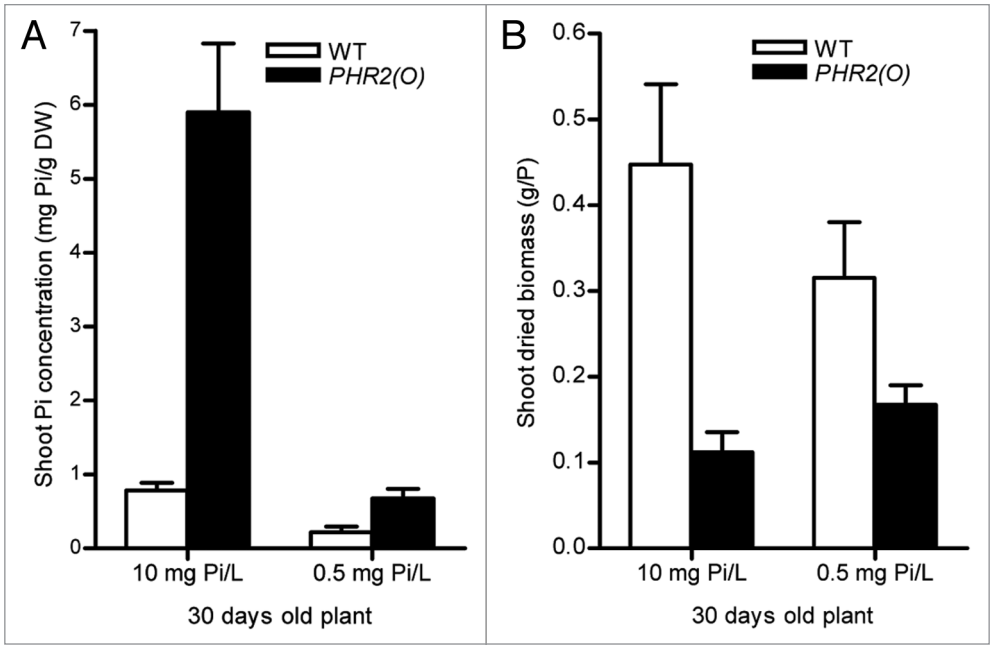

Figure 1. Shoot Pi concentration (A) and shoot biomass of 30 d-old plants of wild type (WT) and the transgenic plants (B) with overexpression of *OsPHR2* under solution culture with abundant supplied Pi (10 mg Pi/L) and low Pi level (0.5 mg Pi/L). The solution pH is 5.5 and each plant occupied one liter of culture solution. The culture solution was replaced every two days.

PSI (Pi-starvation induced) genes downstream of OsPHR2 were investigated. The results indicate that the recovery of mutant plant growth is under background of overexpression of *OsPHR2* (**Fig. 2B**). The Pi concentration analysis also showed that the shoot Pi accumulation mediated by OsPHR2 is remarkably reduced in the mutant (**Fig. 2C**). The present results provide the evidence that OsPHR2 plays multiple functions on Pi-signaling, Pi uptake and translocation and the factor(s) which may negatively regulate plant growth.

Genetic Factor(s) Involved in the OsPHR2-Mediated Plant Growth Inhibition May be Unknown Factor(s)

Several SPX domain (SYG1/PHO81/XPR1) genes in plants were found to be involved in responses to environmental cues or internal regulation of nutrition homeostasis in plants.[8-12] It has been demonstrated that OsSPX1 is a negative regulator of OsPHR2 and involved in the feedback Pi-signaling network in roots defined by OsPHR2 and OsPHO2.[6] At least six genes with an exclusive SPX domain in rice have been based on the present rice genome database. Among them, overexpression of *OsSPX3* inhibits plant growth and OsSPX1 positively regulates *OsSPX3* in shoots.[13] To determine whether OsSPX3 is involved in the rescue of plant growth in the mutant under background of *OsPHR2(O)*, the expression pattern of *OsSPX3* in the mutant was compared with that in the wild type and *OsPHR2(O)* plants. qRT-PCR analysis showed upregulation of *OsSPX3* in the mutant as in *PHR2(O)* plants (**Fig. 2B**). The result indicates that the rescue of the mutant plant growth under background of *PHR2(O)* may be independent of OsSPX3.

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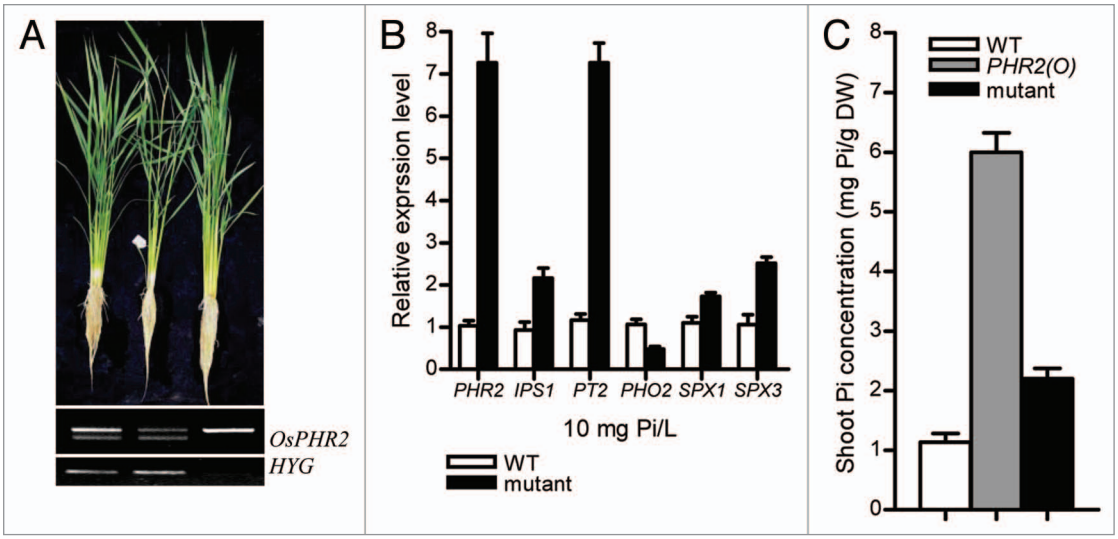

Figure 2. Mutants showed rescue of plant growth under background of overexpression of *OsPHR2*. (A) Ninety days old plants under solution culture with Pi-supplied condition (10 mg Pi/L). PCR analysis using the primers flanking 3, 4, 5 introns of *OsPHR2* and *HYG* (hygromycin gene) (below). (B) Relative expression levels of *OsPHR2* and the PSI (Pi-starvation induced) genes downstream of OsPHR2. (C) Shoot Pi concentration in the wild type, transgenic plants with overexpression of *OsPHR2* and the mutant.