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Toward deciphering the genome-wide transcriptional responses of rice to phosphate starvation and recovery

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Phosphate (Pi) limitation is one of the major factors negatively impacting crop yield worldwide. Next generation sequencing (NGS) was used to profile the transcriptomes of rice (*Oryza sativa*) roots and shoots after phosphate starvation and recovery, shedding further light on the complex and dynamic mechanisms involved in Pi homeostasis. The use of NGS also enabled the identification of previously not annotated loci and novel isoforms of genes that are specifically induced by Pi starvation. Furthermore, phosphate re-feeding was observed to have a unique response with a variety of transcription factors and kinases induced in a transient manner. Expression profiles of miRNAs were also assessed upon long-term Pi starvation in roots and shoots revealing several novel miRNAs associated with Pi starvation. Altogether, this study provides key findings regarding Pi homeostasis in plants that will provide a valuable resource for research aimed at generating crops with increased Pi acquisition/use efficiency.

Keywords: phosphate starvation, transcriptome, RNAseq, rice, nutrient starvation

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Phosphate (Pi) availability is a major factor determining plant growth and development, thus directly affecting crop productivity. Because of its low mobility and high sorption capacity in the rhizosphere, Pi is one of the least available macronutrient in the soil.[1] To overcome this constraint, heavy fertilization has long been used, ultimately leading to environmental pollution.[2] In addition, the declining availability of phosphate rock, the main source of Pi fertilizer, has considerably increased the price of Pi fertilizers and thus strengthens the need to develop crops with greater phosphorus efficiency, i.e., crops that can maintain their growth and yield in soils with reduced phosphorus availability. Thus, it is critical to improve our knowledge on the complex mechanisms involved in Pi homeostasis. While previous studies had already examined the effects of Pi starvation on the transcriptome in various species,[3-11] recent technologies such as next generation sequencing, now allows capturing the dynamic mechanisms involved in these processes by being able to profile numerous conditions and time points.

In our recent study, RNA sequencing technology (RNA-seq) was used to profile the transcriptional responses of rice roots and shoots to short- and long-term Pi deprivation as well as to Pi recovery. Covering an extensive period of Pi stress consisting of 9 time points over more than three weeks provided a comprehensive overview of the complex and dynamic mechanisms involved in Pi homeostasis. Interestingly, our data showed that during the first 3 d of Pi deprivation, very little modifications of the root transcriptomes could be observed, with only 101 genes being upregulated after 3 d of Pi starvation (**Fig. 1**). Indeed, only after a week of Pi deprivation did the number of differentially regulated genes in the roots start to increase, before reaching a plateau after 21 d of Pi deprivation. Among the well characterized phosphate starvation-induced genes (PSI) such as the *SPXs* genes and high affinity phosphate transporters genes, several highly induced genes previously not associated to Pi homeostasis were also detected. Among these, LOC_Os06g44220, encoding a putative low-temperature and salt-responsive gene

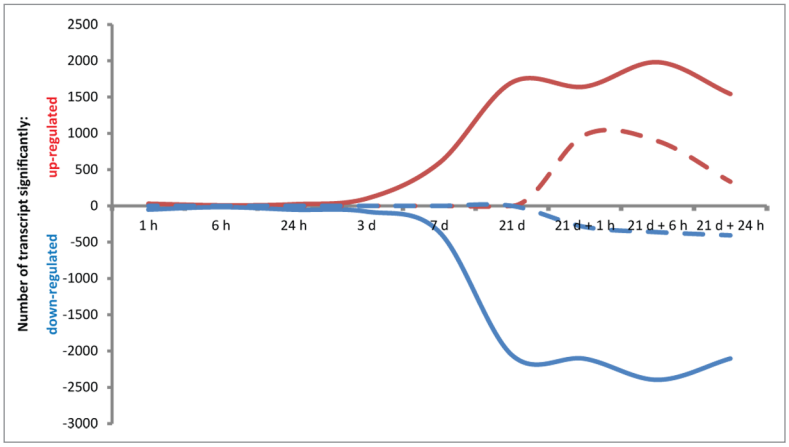

Figure 1. Number of differentially regulated genes upon Pi deprivation and re-supply at discrete time points in rice roots (FDR < 0.05). Solid line represents differentially expressed genes in response to Pi deprivation while dashed line represent differentially expressed genes in response to Pi resupply. Lines in red represent upregulated genes, while blue lines represent genes downregulated by the various Pi treatments.

not expressed under normal conditions (< 2 FPKM), showed some of the highest induction upon Pi deprivation, reaching more than 40 FPKM in the roots after 21 d of Pi starvation. Yet, very little is known regarding the function of this gene, probably exacerbated by the fact that it is not present on the widely used rice Affymetrix microarrays chip. As a consequence, this study not only provides detailed kinetics of the molecular responses to Pi stress, but also identifies potential novel candidate genes essential for plant responses to Pi starvation. In addition, this work also focused on the less studied effects of short-term Pi re-supply to Pi starved plants, resulting in the identification of numerous genes that are transiently differentially expressed, many of which were transcription factors and kinases. Genes coding for the cupin domain containing proteins (LOC_Os12g05870 and LOC_Os12g05880) were among the most highly induced upon Pi re-supply. Cupins are extremely diverse and include catalytically inactive seed storage proteins, sugar-binding metal-independent epimerases, and metal-dependent enzymes and possess a wide range of activities.[12] The *Arabidopsis* ortholog of these cupins is a germin-like protein (At4g14630), previously shown to be induced in response to NaCl stress.[13] Altogether, the identification of these rapid and ephemeral transcriptional modifications provides new elements to further identify regulators of Pi homeostasis.

While this study generated a detailed overview of the transcriptional responses induced by Pi starvation, it also assessed how conserved these responses were when compared with those observed in *Arabidopsis*. To do so, a set of conserved *Arabidopsis* root PSI genes was identified from previous studies.[3,4,8] While it is known that the experimental design can greatly affect the transcriptomes of Pi starved plants with limited conservation of PSI genes between studies, we identified a set of 130 core PSI genes in *Arabidopsis* roots. This set of conserved root PSI genes was then compared with the rice root PSI orthologs, resulting in the identification of 76 genes that were induced by Pi starvation in both rice and *Arabidopsis* roots. While this result strengthens the fact that the core molecular mechanisms involved in response to Pi starvation are highly conserved in plants, subtle differences exist as exemplified with *PHO2*. Our work has identified a novel *PHO2* isoform, referred to as *PHO2.2*, that, unlike the original *PHO2* isoform, is specifically and highly induced by Pi starvation in roots and shoots. In addition, it was shown that *PHO2.2* was more actively translated into protein than the original isoform, thus providing an extra level of complexity in the regulation of one of the key regulators of Pi homeostasis.

Due to increasing evidence of the role of small RNAs in growth, development, stress adaptation and nutrient signaling,[14] we generated the first report of miRNAs expression levels upon long-term Pi starvation (21 d) in rice roots and shoots. While this study, confirmed the induction of the well characterized miR399 and miR827, involved in negatively regulating the expression levels of *PHO2*[15] and *SPX-MFS1*[16], respectively, this work also identified numerous miRNAs previously not associated with Pi starvation such as miR6250, a miRNA previously shown to be induced by arsenite.[17] The identification of novel Pi-starvation induced miRNAs, which are potentially also involved in the response to other stresses, e.g., arsenite, may help decipher the complex crosstalks between nutrients, such as the one observed for at-miR827 involved in maintaining nitrate-dependent Pi homeostasis.[18]

Altogether this work presents a high-resolution genome-wide transcriptome analysis of the responses of rice to Pi starvation and recovery in roots and shoots. In addition, this analysis also identifies numerous potential candidate genes to improve our understanding of Pi homeostasis in plants. (**Fig. 2**)

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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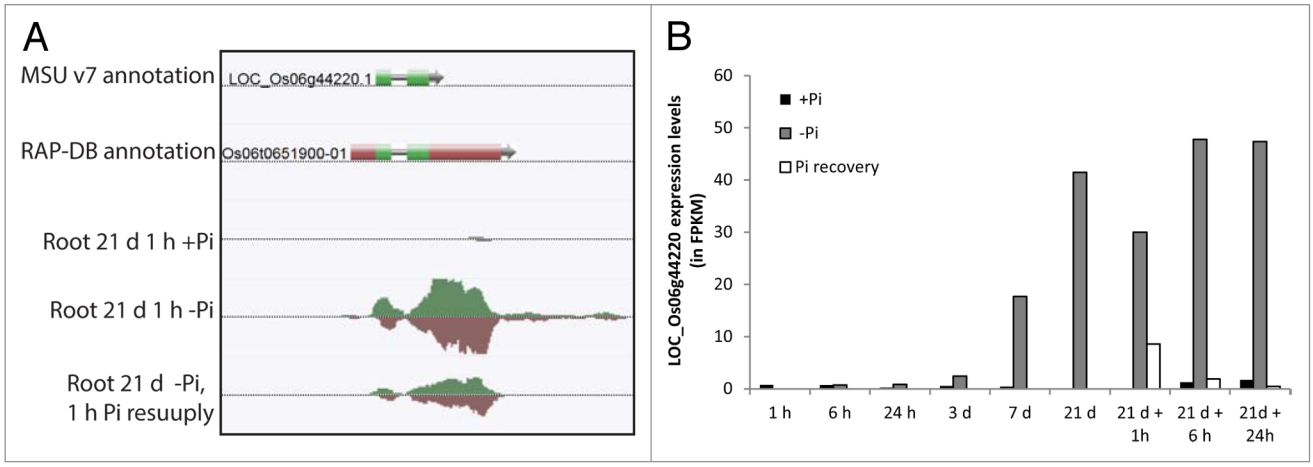

Figure 2. Identification of novel potential candidate genes involved in Pi homeostasis. (**A**) Graphical representation of LOC_Os06 g44220, a gene previously not associated with Pi starvation, but highly induced by Pi deprivation. Screen capture is made from the AnnoJ genome browser (http://www.plantenergy.uwa.edu.au/annoj/Secco_2013.html), representing gene annotations from MSU v7 and the RAP-DB IRGSP-1.00 assemblies as well as RNA expression tracks. (**B**) Graphical representation of the expression level of LOC_Os06 g44220 during Pi deprivation and Pi re-supply. Expression level is shown in FPKM (for fragment per kilobase of exon per million fragments mapped).

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