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A Re-Evaluation of the Importance of Protein Kinases in the Response of Arabidopsis Roots to Phosphate Starvation and Iron Deficiency

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ABSTRACT

Phosphorus (P) and iron (Fe) are essential mineral nutrients for plant growth, and deficiencies in these elements alter many developmental programs. Our previous genome-wide co-expression analysis has predicted that protein kinase (PK) plays a key role in the regulation of phosphate (Pi) deficiency-induced root hair remodeling in Arabidopsis. In this study, the RNA-seq data were re-mined to dissect the potential functions of PKs in Arabidopsis roots under Pi and Fe deficiency. Our results showed that 33 PKs were identified as changed expression at transcript level and a subset of 18 genes was detected with uniquely matched peptides at protein level. Some of the 18 corresponding proteins were found to be also involved in the responses to multiple abiotic stresses. The importance of PKs in the responses to nutritional stress was discussed.

Phosphorus (P) is a critical component of many metabolites and macromolecules in plants. However, the inorganic phosphate (Pi, the major form of soluble P which can be absorbed by plants) present in the soil is often at low levels and P thus is a growth-limiting factor. Protein kinases (PK) play a significant role in sensing phosphate availability and regulating the specific responses of plants. Genome-wide co-expression analysis has identified 173 Pi-response genes that encode protein kinases in Arabidopsis roots^[1]. The 173 PK were differentially expressed under Pi-deficient conditions and putative functional modules were constructed based on co-expression analysis using the MACCU toolbox^[2]. The largest sub-module named PKPP1 was composed of 21 PK genes (and 1 PP genes) and gene ontology analysis revealed that these genes are mainly involved in root hair development and growth. Further co-expression analysis based on 208 root epidermal core genes and PKPP1 genes identified 46 fished genes that may be associated with PKs under Pi starvation in Arabidopsis roots^[3]. The genome-wide level analysis of PK genes in Arabidopsis roots emphasized the value of protein kinases in Pi-response and also provided useful guidelines for further studies by reverse genetic methods.

Iron (Fe) is also one of the most common factors stunting plant growth and development because of its tendency to form complexes of low solubility. Lan et al. have analyzed the Fe deficiency-induced changes in the protein profile of Arabidopsis roots using iTRAQ^[4]. However, the changes in protein profiles upon Fe deficiency show low congruency with previously reported alterations in transcript levels. As iron was one of the metals exhibiting the highest level of accumulation in plants when Pi was removed from the medium, the overlapped genes regulated by iron deficiency and phosphate starvation in Arabidopsis roots were analyzed at a genome-wide level^[5,6]. Analysis showed a total of 579 overlapping genes that are responsive to both Fe deficiency and Pi starvation in Arabidopsis roots. In this study, we re-analyzed the relationship between protein kinases and phosphate/iron homeostasis in Arabidopsis roots, aimed to uncover the specific and general effects of protein kinases on the regulation of roots in response to abiotic stresses (**Figure 1**).

RESULTS AND DISCUSSION

A comparison of the 173 Pi-response PK genes with the 579 overlapping genes that respond to both Fe and Pi deficiency resulted in 33 PK genes which were differentially expressed under both Pi- and Fe-deficient conditions in Arabidopsis roots. However, only one gene *At2g29000*, out of the 33 PK genes, belongs to the PKPP1 sub-module comprising 21 PK genes^[1]. In

addition, genes in this module have been suggested to be involved in the processes of root hair development and growth under Pi deficiency [4]. Moreover, a genome-wide survey of the overlapping genes between phosphate and potassium/nitrate data in Arabidopsis roots showed no different expression of these 21 PK genes in the module PKPP1. Taken together, these results suggested that these 21 PK genes are specifically regulated under Pi starvation rather than other nutritional deficiency examined, and are associated with Pi deficiency-induced root hair remodeling.

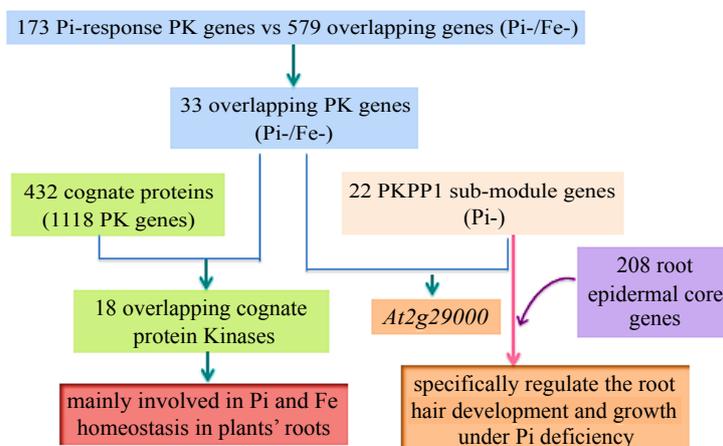


Figure 1. An overview of re-analysis of protein kinases in Arabidopsis roots.

For the 33 PK genes, 18 cognate proteins were identified with at least one uniquely matched peptide at protein level, indicating these protein kinases are biologically functional in response to abiotic stress. Next, the transcriptional changes of these 33 overlapping PK genes were examined in the microarray data sets of potassium/nitrate treated Arabidopsis roots. Except one genes *CIPK23* (*At1g30270*), a positive regulator of the potassium transporter *AKT1* by directly phosphorylating *AKT1* [7], all other 32 PK genes were not detected in roots under potassium deficiency in two previous reports and only five out of 33 PK genes were shown changed in gene expression under nitrate limitation [8-10]. These results suggested that these 33 overlapping PK genes might be mainly involved in Pi and Fe homeostasis in Arabidopsis roots and the crosstalk between phosphorus and iron might be tighter than that of others.

In summary, the re-analysis in this study sharply narrowed down the follow-up research on the roles of protein kinases in root remodeling, and the identified PK genes that maybe participate in Pi- and Fe-response are worthy further study.

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