Transcription factor MePTF1 positively mediates low phosphate starvation response in Cassava

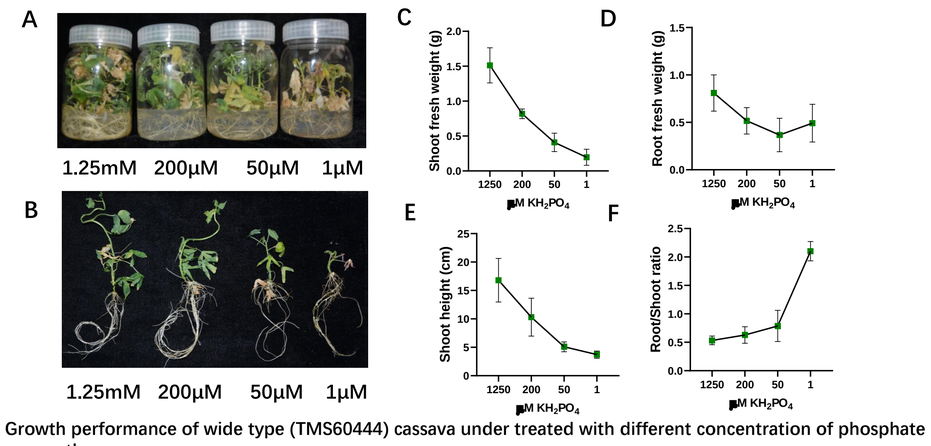
Weijing Tong[1,2], Qiuxiang Ma[1,2], Xinlu Lu[1,2], Peng Zhang[1,2,*]

[1]CAS Center for Excellent in Molecular Plant Science, Institute of Plant Physiology and Ecology, Chinese Academy of Science, Shanghai, 200032, China
[2]University of Chinese Academy of Sciences, Beijing 100049, China
E-mail: tongweijing@cemps.ac.cn

Summary Phosphorus is an essential macronutrient required for plant growth and development and is a limiting factor threatening agricultural production. Cassava (*Manihot esculenta* Crantz) is an important root crop to provide major dietary carbohydrates for human in the tropics. In present study, we found low phosphate conditions resulted in stunted growth of cassava plants, RNA-seq data and qPCR analysis revealed several low-Pi induced genes are up-regulated by low-Pi stress. A bHLH transcription factor, *MePTF1* whose expression is upregulated under low-Pi stress. qPCR analysis and GUS staining assay indicates that MePTF1 mainly express in the xylem tissues of stems, petioles and roots in cassava plants. Overexpression of *MePTF1* enhance tolerance to low-Pi stress and show higher phosphate content than that of wide type under low-Pi condition. RNA-seq data indicated that expression of genes related to carbon metabolism and flavonoid biosynthesis were significantly altered in overexpression transgenic plants under low-Pi condition. Moreover, we found MePHR1, an essential factor regulating Pi starvation response (PSR) in plant can target *MePTF1* and activates its expression. Our results revealed a novel transcription factor positively regulating low Pi response in cassava, providing a candidate gene for improving crop tolerance to low-Pi stress.

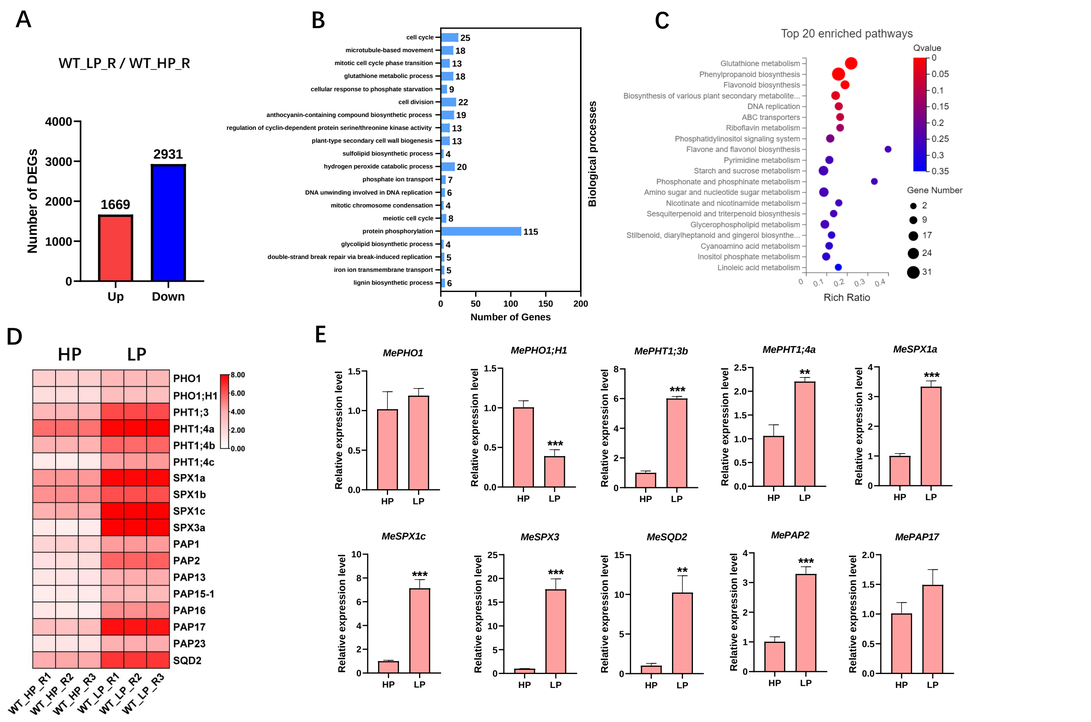
Results

1. Low phosphate stress led to stunted growth in cassava



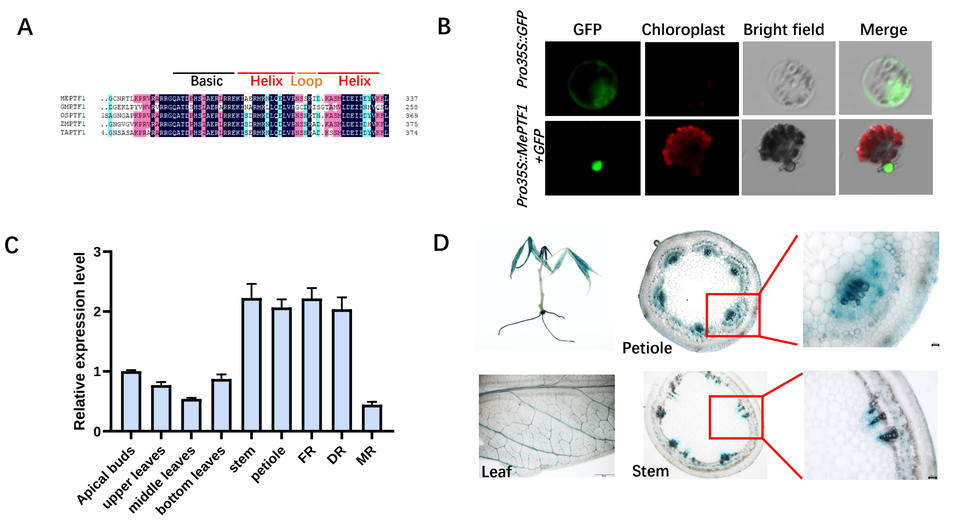
A-B. Growth performance of wide type (TMS60444) cassava under treated with different concentration of phosphate for two months.
C-F. Statistics of shoot fresh wight(C), root fresh weight(D), shoot length(E) and root/shoot ratio(F) of WT treated with different concentration of phosphate.

2. RNA-seq analysis of cassava under low phosphate treatment



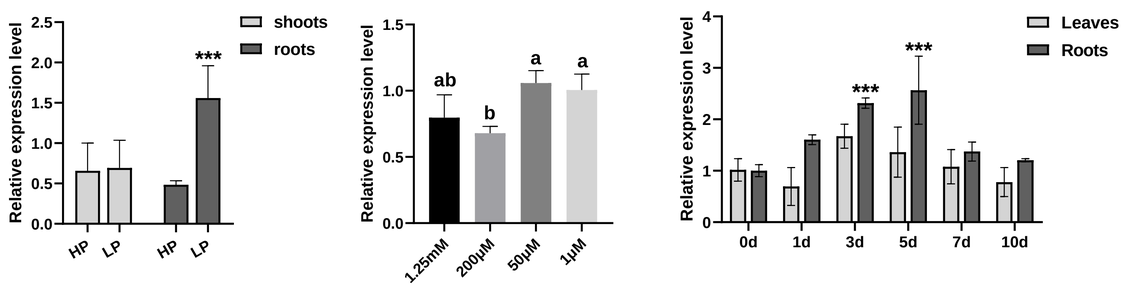
A. Statistics number of differential expressed gene in wide type before and after treatment.
B. GO analysis of enrichment of up-regulated genes in terms of biological process.
C. The top 20 enriched KEGG pathways among the up-regulated DEGs.
D. Heat map of several PSI gene expression under normal and low-Pi condition.
E. RT-qPCR analysis of spatio-temporal expression patterns of PSI genes in roots

3. MePTF1 is a nucleus-localized bHLH transcription factor and mainly expressed in vascular tissues



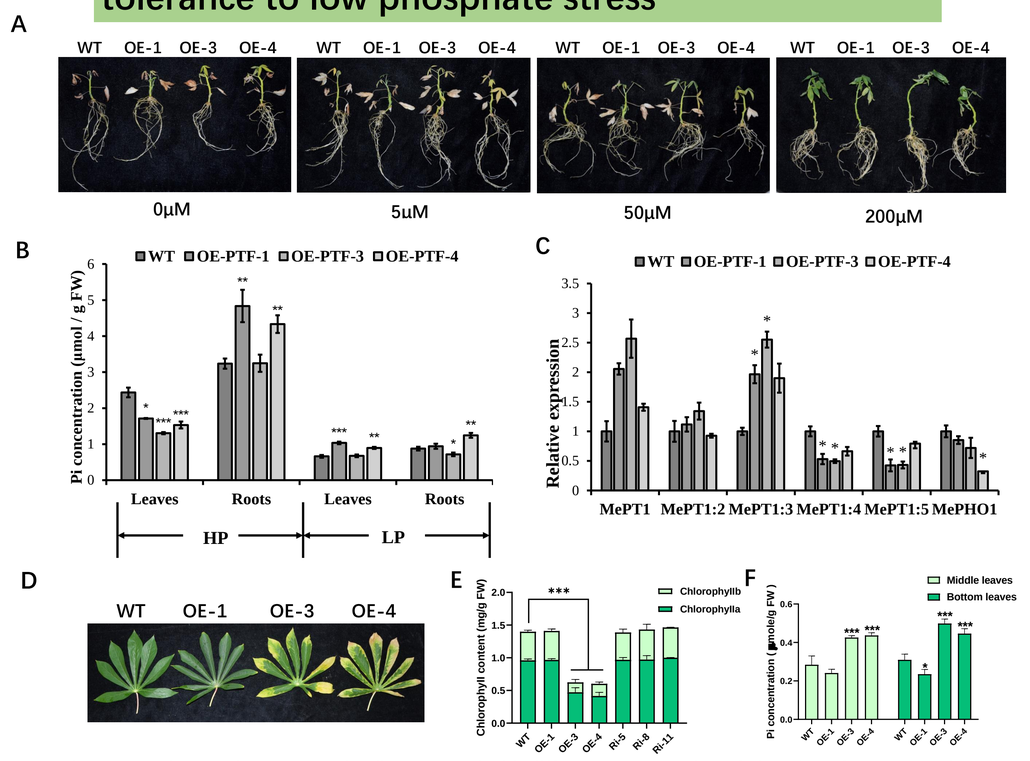
A. Multiple alignments of the bHLH domains of MePTF1 and PTF1s from other plants
B. Subcellular localization analysis of MePTF1-GFP fusion protein in Cassava leaf protoplasts.
C. Relative expression of *MePTF1* in different tissues of Cassava.
D. GUS staining assay showing the specific expression pattern of MePTF1

4. *MePTF1* is responsive to Pi starvation



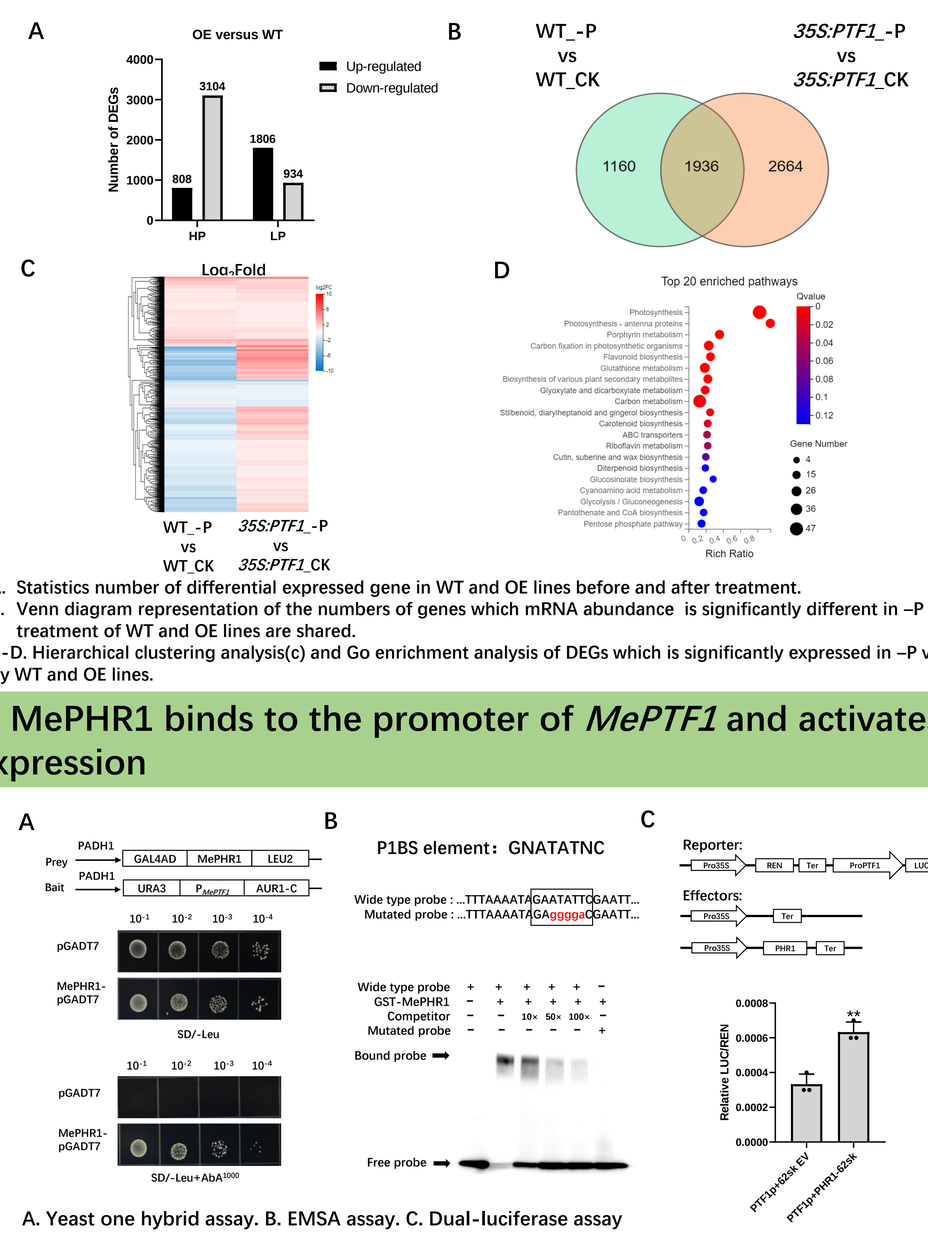
A. Relative expression of *MePTF1* in response to low phosphate stress based on RNA-seq data.
B. Relative expression of *MePTF1* under different concentration of phosphate.
C. Relative expression of *MePTF1* in response to low-Pi treatment.

5. Overexpression of *MePTF1* in cassava enhance tolerance to low phosphate stress



A. Phenotype of WT and overexpression lines treated with different concentration of phosphate for two months.
B. Inorganic phosphate content of WT and overexpression lines under normal and low phosphate condition.
C. PSI gene expression in WT and overexpression lines under low-Pi condition.
D. Leaf phenotype of WT and overexpression lines grown in the field.
E. Chlorophyll content. F. Inorganic phosphate content.

6. Genes related to carbon metabolism, flavonoid biosynthesis and glutathione metabolism are altered in the MePTF1-overexpressing lines under low-Pi condition

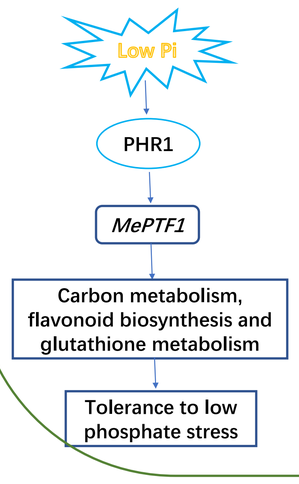


A. Statistics number of differential expressed gene in WT and OE lines before and after treatment.
B. Venn diagram representation of the numbers of genes which mRNA abundance is significantly different in –P versus +P treatment of WT and OE lines are shared.
C-D. Hierarchical clustering analysis(c) and Go enrichment analysis of DEGs which is significantly expressed in –P versus +P shared by WT and OE lines.

7. MePHR1 binds to the promoter of *MePTF1* and activates its expression

A. Yeast one hybrid assay. B. EMSA assay. C. Dual-luciferase assay

8. Conclusion and perspectives



Low phosphate condition leads to stunted growth in cassava. We proposed a working model deciphering the major role of *MePTF1* in low Pi starvation response in cassava. When facing low-Pi stress, MePHR1 activates *MePTF1* expression. Through carbon metabolism, flavonoid biosynthesis and glutathione metabolism, overexpression of *MePTF1* enhanced tolerance to low-Pi stress of transgenic plants. We will use Chip-Seq methods combining RNA-seq data to find the direct target gene of MePTF1 during PSR pathway and get more evidence of the link between PSR and these metabolism pathways.