

An R2R3-type MYB transcription factor MYB103 is involved in phosphate remobilization in *Arabidopsis thaliana*

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Abstract

Background The MYB transcription factor (MYB TF) family has been reported to be involved in the regulation of biotic and abiotic stresses in plants. However, the involvement of MYB TF in phosphate remobilization under phosphate deficiency remains largely unexplored. Results Here, we showed that an R2R3 type MYB transcription factor, MYB103, was involved in the tolerance to P deficiency in *Arabidopsis thaliana*. AtMYB103 was induced by P deficiency, and loss function of AtMYB103 significantly enhanced sensitivity to P deficiency, as root and shoot biomass and soluble P content in the *myb103* mutant were significantly lower than those in wild-type (WT) plants under the P-deficient condition. Furthermore, the expression of Pi deficiency -responsive genes was more profound in *myb103* than in WT. In addition, AtMYB103 may also be involved in the cell wall-based P reutilization, as less P was released from the cell wall in *myb103* than in WT, which was in company with a reduction of the ethylene production.

Conclusions These findings uncover the role of MYB103 in the P remobilization, presumably through ethylene signaling.

Background

Inorganic phosphate (Pi) is the only form of phosphorus (P) that can be assimilated by plants. Pi, however essential for plant growth and development, typically falls short to meet the requirement of most crops due to its active chemical property and microbial activity [1, 2]. Limited P availability in the soil solution results in lower crop yields and higher demand for P fertilizers. However, the large use of the P fertilizers that is based on the possible finite resource-phosphate rocks has dramatically affected our ecosystems [3]. Therefore, breeding new crop varieties with higher P-acquisition and -utilization efficiencies is an imperative goal now. An integrated view of the mechanism underlying plant response to P deficiency will be helpful to achieve that goal.

Plants have evolved a wide range of sophisticated strategies to mount adaptive responses to P deficiency at multiple levels [4–7]. At physiological and biochemical levels, this strategy involves remodeling of root architecture, enhanced secretion of organic acids, symbiosis with arbuscular mycorrhizal fungi [7]. At molecular level, numerous transcription factors (TFs) have been characterized that are involved in the transcriptional reprogramming of plant responses to P deficiency [8–10]. Recently, many efforts have been devoted to the elucidation of the role of the MYB TFs in P deficiency, which comprise the largest gene family in plants and harbor conserved N-terminal DNA-binding domains and a myeloblastosis (MYB)-related domain [11]. For instance, AtPHR1 [12] and AtMYB62 [13] are involved in the transcriptional response to P deficiency in *Arabidopsis*. In rice, an R2R3 MYB TF, OsMYB2P-1, plays a role in Pi starvation stress, as *OsMYB2P-1* overexpression enhanced tolerance to P starvation in rice [14]. However, whether other MYB TFs are involved in P nutrition have yet to be examined.

Besides, several MYB TFs have been shown to couple with the hormone signaling network to confront environmental stresses. Ethylene is a well-known phytohormone that is synthesized from S-

adenosylmethionine (SAM) [15]. For instance, in mangosteen fruit, ethylene directly regulates *GmMYB10* at the transcription level [16]. Overexpression of ethylene response factor *TERF2* confers cold tolerance in rice through activating the expression of *OsMYB* [17]. In fact, ethylene not only acts as an important signal in plant development such as fruit ripening [18], but also participates in the cell wall P reutilization in P deficient *Arabidopsis* and rice [19, 20]. However, the upstream regulatory mechanism underlying the ethylene mediated cell wall P reutilization mechanism still remains elusive. Here, we explore the role of a MYB gene, *MYB103*, in P deficiency in *Arabidopsis thaliana*. Additionally, we examine the involvement of ethylene in remobilizing P from the cell wall through characterization of *myb103*, a loss-of-function mutant of *MYB103* in response to P deficiency.

Methods

Plant material and culture conditions

A. thaliana ecotype Columbia (Col-0: WT) and the *myb103* mutant in the Col-0 background (SALK_083678; obtained from the Arabidopsis Biological Resource Center, Columbus, OH, USA), were used in this study. Seeds were first sterilized by 75% ethanol for 5 min, washed by sterilized water for three times. After then, some seeds were germinated on the 0.8% agar-solidified MS nutrient medium for 1 week for the petri dish treatment [19], while other seeds were germinated on the sponge supplied with the MS nutrient solution for 6 weeks for the hydroponic treatment.

For the petri dish treatment, uniform seedlings were selected and transplanted into other solid MS nutrient mediums with (+P) or without P (-P; 0.1 mM NH_4NO_3 was used instead of 0.1 mM $\text{NH}_4\text{H}_2\text{PO}_4$) for another week. The petri dishes were vertically placed in a growth chamber under a controlled environment, in which the temperature was set to 22°C and the duration of the day was 16 h with a light intensity of 140 $\mu\text{mol m}^{-2}\text{s}^{-1}$. For the hydroponic treatment, uniform seedlings were selected and transplanted into other MS nutrient mediums with (+P) or without P (-P) for another week. The treatment solution was renewed every 3 d.

Determination of soluble P content

Roots and shoots were first rinsed with distilled water, followed by determination of the biomass. Tissues were then homogenized in liquid nitrogen, and 400 μL 5 M H_2SO_4 diluted in 8 mL of distilled water was added. After standing at room temperature for 2 h, the mixture was centrifuged at 15 600 g for 5 min, and 400 μL supernatant was then collected and mixed with 200 μL ammonium molybdate, which contained 15% ascorbic acid (w/v; pH 5.0), for 30 min at 37°C, followed by measurement of the absorbance at 650 nm. The soluble P content was calculated by fresh weight.

Cell wall and pectin extraction

Cell walls were extracted as previously described [21]. Briefly, samples were first extracted with 8 mL 75% ethanol (v:v) for 20 min at 4°C after homogenized with liquid nitrogen. After centrifuged at 15 600 g for 15 min at 4°C, the pellet was washed with 8 mL acetone, 8 mL chloroform:methyl alcohol mixture (1:1), 8 mL methyl alcohol for 20 min at 4°C, in turn. Pellets that were recorded as cell walls were freeze dried, and stored at 4°C for further use.

Pectin was extracted as previously described [21]. The above cell wall material was weighed, and after incubated with 1 mL distilled water for 1 h at 100°C, the mixture was centrifuged at 15600 g for 15 min, and the supernatant was collected. The pellet was then incubated with distilled water for 1 h at 100°C for another two times as described above, and the supernatants were combined as the pectin fraction.

Measurement of pectin content

Pectin content was measured as described [22]. First, 200 μ L pectin fraction was combined with 1 mL 12.5 mM $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ that was dissolved in 98% H_2SO_4 in a 2 mL Eppendorf tube, followed by incubation at 100 °C for 5 min. After chilling, the above samples were mixed with 20 μ L 0.15% M-hydroxydiphenyl at 28 °C for 20 min, followed by measurement of the absorbance at 520 nm. Galacturonic acid (Sigma) was used as the standard.

Measurement of cell wall and pectin P content

Cell wall P content was determined as described [23]. First, about 5 mg cell wall material was weighed, and 1 mL 2 M HCl was added with occasional shaking for 72 h. After centrifuged at 15 600 g for 15 min, 0.7 mL supernatant was collected and 4.3 mL 2 N HCl was added for the P concentration measurement by ICP-MS.

Pectin P content was determined as described [23]. First, 1.5 mL pectin fraction was combined with 3.5 mL 2 M HCl, followed by measurement of the P concentration by ICP-MS.

Quantitative real-time PCR (qRT-PCR) analysis

Total root RNA was extracted using the RNeasy Pure Kit (TianGen, China). The quality of the RNA was assessed by agarose gel electrophoresis. Total RNA was then reverse transcribed to cDNA using the PrimeScript RT® reagent kit (Takara, Japan) following the manufacturer's procedure. For qRT-PCR, the reaction mixture contained SYBR Premix ExTaq (5 μ L; Takara, Dalian, China), forward primer (0.4 μ L), reverse primer (0.4 μ L), RNA-free water (2.2 μ L) and 5-fold-diluted cDNA template (2 μ L). Four replicates were used for each cDNA sample, and the relative quantification level of each gene was calculated using the $2^{-\Delta\Delta\text{CT}}$ method [24] and *UBIQUITIN 10* as the internal reference. Primers used here are listed in Table 1.

Detection of root ethylene production

Measurement of root ethylene production was conducted as described [25]. Roots were detached and then transferred to 6 mL glass vials. After 1 mL distilled water was added, the vials were immediately sealed in darkness for 3 h at 28 °C. The concentration of ethylene in the glass vials was then measured.

Data analysis and statistics

Experiments in the present study here were performed four times, and the data was analyzed by one-way ANOVA, while the mean values were compared using the Duncan's multiple range test.

Results

***MYB103* is upregulated in response to P deficiency**

First, qRT-PCR was conducted to investigate whether expression of *MYB103* is responsive to P deficiency. After exposure to P deprivation for 7 days, the relative expression of *MYB103* was determined. In *A. thaliana*, the expression of *MYB103* (*AtMYB103*) was increased in response to P deficiency, albeit slightly (Fig. 1). This observation is in agreement with a previous study that expression of another MYB TF, *PHR1*, is only weakly responsive to Pi starvation [12]. The observation that P deficiency induced the expression of *MYB103* in *A. thaliana* with various degrees could be suggestive of the possible involvement of *MYB103* in responses to P deficiency.

Disruption of *MYB103* confers sensitivity to P deficiency

To further clarify the role of *MYB103* in response to P deficiency, a null mutant in Arabidopsis was chosen for further study. One major symptom of P deficiency is the inhibition of the root growth. After growth on P-deficient agar medium for 7 d, the root length of Arabidopsis WT (Col-0) was inhibited by approximately 15%, whereas more inhibition (42%) was observed in *myb103* (Fig. 2B and 2C), indicating that the disruption of *MYB103* resulted in increased sensitivity to P deficiency when compared to WT seedlings. This conclusion was further demonstrated by their significantly altered root biomass (Fig. 2A and 2D), although no remarkable variations were observed in their shoot biomass (Fig. 2E).

Disruption of *MYB103* impairs P remobilization

The P deficiency-sensitive phenotype of the *myb103* mutant was attributed to its lower levels of root and shoot soluble P than WT (Fig. 3A and 3B), suggesting that *MYB103* is involved in the P-deficiency response. All the above results correlated well with the up-regulated expression of P deficiency-responsive

genes such as *PHOSPHATE 1 (PHO1)* and *PHOSPHATE TRANSPORTER 1 (PHT1)* under P deficiency (Fig. 4).

Since there was no soluble P in the P-deficient nutrient solution, and cell wall acted as the major P source in the P-deficient plants [19, 23], the root and shoot cell wall P retention in both WT and *myb103* were thus analyzed. It is interesting that more P was retained in the *myb103* root cell wall than in WT (Fig. 5A and 5C), although no significant difference was found in the shoot cell wall P reutilization (Fig. 5B and 5D), suggesting that less P was reutilized in the *myb103* mutant root. Furthermore, as pectin contributes significantly to cell-wall P reutilization in rice [23], we tested whether pectin was involved in the low efficiency of P reutilization in the *myb103* mutant. As shown in Fig. 6, more P was adsorbed in the pectin fraction of the *myb103* root cell wall under P deficiency while similar P was adsorbed under the P-replete condition compared to WT, indicating lower P-release potential in the pectin fraction of *myb103* root cell walls (Fig. 6A). In consistent with this, a reduction of the pectin content was found in the *myb103* root cell wall (Fig. 6B), indicating that cell wall pectin contributes to the low efficient P recycling in *myb103*.

Disruption of *MYB103* leads to less ethylene accumulation

Our findings raised a question that how MYB103 interferes with the reutilization of the P from the cell wall. Recently, Yu et al. (2016) demonstrated that ethylene plays pivotal roles in the reutilization of the cell wall P in Arabidopsis; therefore, we measured the production of ethylene in roots of WT and *myb103*. As shown in Fig. 7, less ethylene production was observed in *myb103* roots under either P-deficient or P-replete conditions when compared to WT, indicating that ethylene could be involved in the *MYB103*-regulated cell wall P reutilization.

Discussion

To cope with P limitation, plants exhibit a series of physiological, biochemical, and molecular strategies such as modifying the architecture of the roots [26, 27], secreting carboxylates and acid phosphatase [28], elevating transcription of genes essential for P acquisition [29], and enhancing the remobilization of the previously stored P [19, 23, 30]. Recently, accumulating evidence has supported the participation of TFs in Pi starvation signaling [31], although much remains to achieve a full understanding of the Pi starvation signaling network, as well as its crosstalk with other pathways such as phytohormone signaling cascades. Furthermore, recent studies have reported that P stored in root cell wall can be reutilized in Arabidopsis and rice, and numerous phytohormones or signal molecules such as ethylene and nitric oxide (NO) participate in this process [20]. In this study, we for the first time reported that a MYB TF is involved in the tolerance to P deficiency in *A. thaliana*, and found that the sensitivity of P deficiency in the loss function mutant, *myb103*, also showed a clear association with the cell wall, and in particular the phytohormone ethylene.

Numerous MYB TFs were reported to be involved in plant responses to environmental stresses [32, 33]. For example, HOS10 [34], MYB14 and MYB15 [35], and MdMYB23 [36] are involved in the modulation of

cold stress response while MYB12 is involved in response to nitrogen deficiency [37]. PSR1 [38] and PHR1 [12] are two well characterized R2R3 MYB transcription factors that have been implicated in the positive regulation of Pi stress responses. Furthermore, MYB62 has been implicated to act as a negative regulator of P starvation response [13], as its expression was significantly induced during P deficiency [39], and MYB62 overexpressing lines exhibited phosphate accumulation in roots. Moreover, MYB62 also serves as a repressor of GA biosynthesis [39]. However, little is known about whether other MYB TFs take part in the P deficiency signaling. In the present study, we found that MYB103, a R2R3 MYB TF, was involved in the P deficiency response in Arabidopsis. The expression of MYB103 was induced under the P deficient condition in Arabidopsis (Fig. 1), and the Arabidopsis loss function mutant of MYB103 exhibited the P deficiency-sensitive phenotype (Fig. 2), with lower levels of shoot and root soluble P (Fig. 3).

Cell wall, which consists of cellulose, hemicelluloses, pectin and other matrix polysaccharides, acts as an important P repository in rice and Arabidopsis [19, 23, 40]. Among them, only pectin was demonstrated to be involved in the P recycling under the P deficient condition, as the carboxyl-acid groups in the cell wall pectin have strong affinity for cations, such as Fe³⁺, which lead to the release of the P [23, 41]. Our results establish the role of the P pool in cell wall pectin in WT under P deficiency, as loss function of MYB103 results in the significantly decreased P reutilization when under -P compared with +P (Fig. 5A and 5C), in company with the greatly reduced pectin content (Fig. 6B). As a result, when in comparison to WT, more P was retained in the *myb103* cell wall under the -P condition (Fig. 5), thus less soluble P was found in *myb103* roots and shoots, which rendered it more sensitive to the P deficiency (Figs. 2 and 3). In agreement with this, the expression of P deficiency-responsive genes including PHR1, PHT1, PHO1 and PHO2 was higher in *myb103* roots than in WT under P starvation (Fig. 4).

Then, how does MYB103 regulate the cell wall P reutilization? What are the downstream signals? As we know, ethylene acts as a signal molecule that not only plays important roles in various physiological processes and plant growth, but also participates in the regulation of responses to different abiotic stresses [15, 42, 43]. While the involvement of ethylene in response to P deficiency in plants has been documented [19, 20, 44, 45], the mechanism underlying the regulation of ethylene production remains largely elusive. In the present study, we found that less ethylene was produced in roots of *myb103*, when compared with WT, irrespectively of the P status (Fig. 7), indicating that ethylene production is regulated by MYB103 in response to P deficient in Arabidopsis roots.

In conclusion, our results demonstrate that a R2R3 MYB TF, MYB103, is involved in the cell wall-based P reutilization under P deficiency through regulating ethylene production. Our study, thus, not only demonstrated that a single TF could be responsible for P remobilization and phytohormone-induced reutilization of cell wall P, but also provided an entry point for dissecting the association between nutritional cues and phytohormone signaling pathways.

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Declarations

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Availability of data and materials

Plant materials are available under request to the respective owner institutions. The datasets supporting the results of this article are included within the article and its additional files.

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Contributions

JL and FY conceived the and supervised the work. FY and SW performed the experiments. FY, WZ, LY, HW analyzed the data. FY drafted the manuscript. JL and ZF revised the manuscript. All authors read and approved the final manuscript.

Ethics declarations

Ethics approval and consent to participate

Not Applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Additional information

Additional files

Additional file 1:

Table S1 List of Primers used in the current stud

Figures

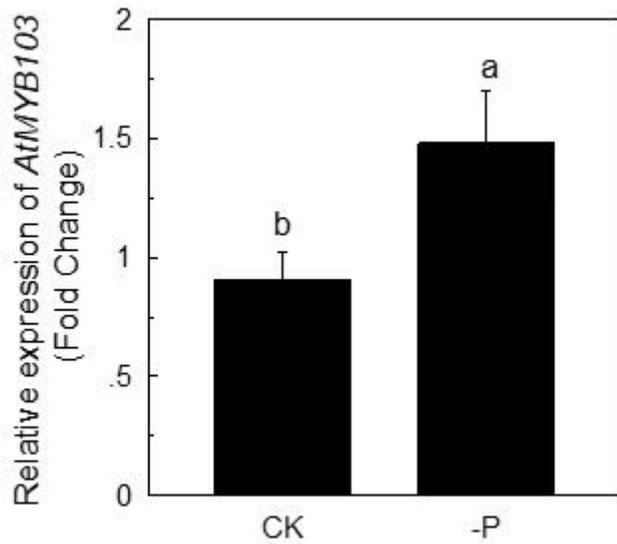


Figure 1

Relative expression of MYB103 in response to P deficiency in roots of *Arabidopsis thaliana*. Seedlings were hydroponically treated with +P or -P solution for 7 d, and the expression of MYB103 was measured in *A. thaliana*. Values are means \pm SD, $n = 4$. Different letters on the bars indicate significant differences at $p < 0.05$.

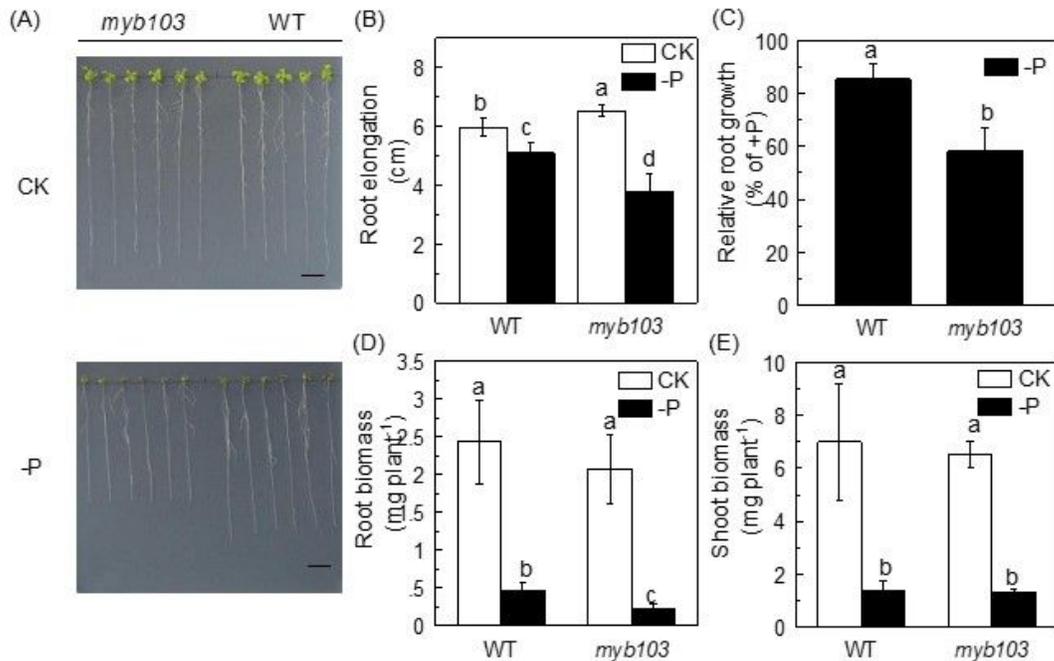


Figure 2

Phenotype of the loss function mutant of Arabidopsis MYB103. Seeds of WT and *myb103* were surface-sterilized and germinated on the complete nutrient medium, and seedlings with the root length of about 1 cm were transferred to the complete nutrient medium (+P) or medium without P (-P) for 7 d (A), and root elongation (B), relative root growth (C), ratio of root elongation in +P to root elongation in -P), root biomass (D) and shoot biomass (E) were measured. Scale bar = 1 cm. Values are means \pm SD, $n = 4$. Different letters on the bars indicate significant differences at $p < 0.05$. Scale bar= 1cm.

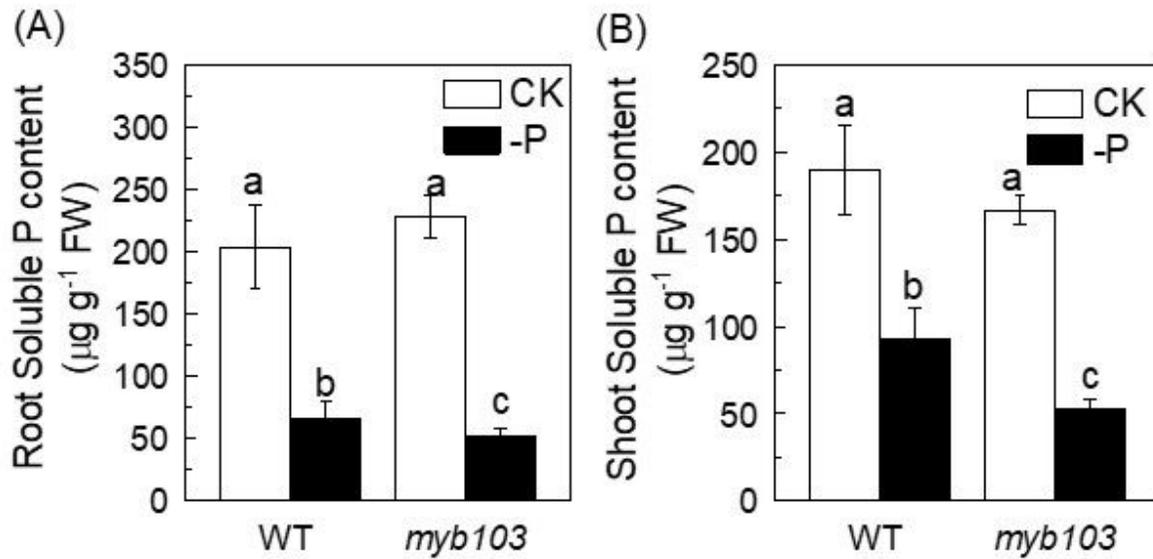


Figure 3

Root and shoot soluble P content. After 6-week-old WT and *myb103* were hydroponically treated with +P or -P solution for 7 d, soluble P content in root (A) and shoot (B) were determined. Values are means \pm SD, $n = 4$. Different letters on the bars indicate significant differences at $p < 0.05$.

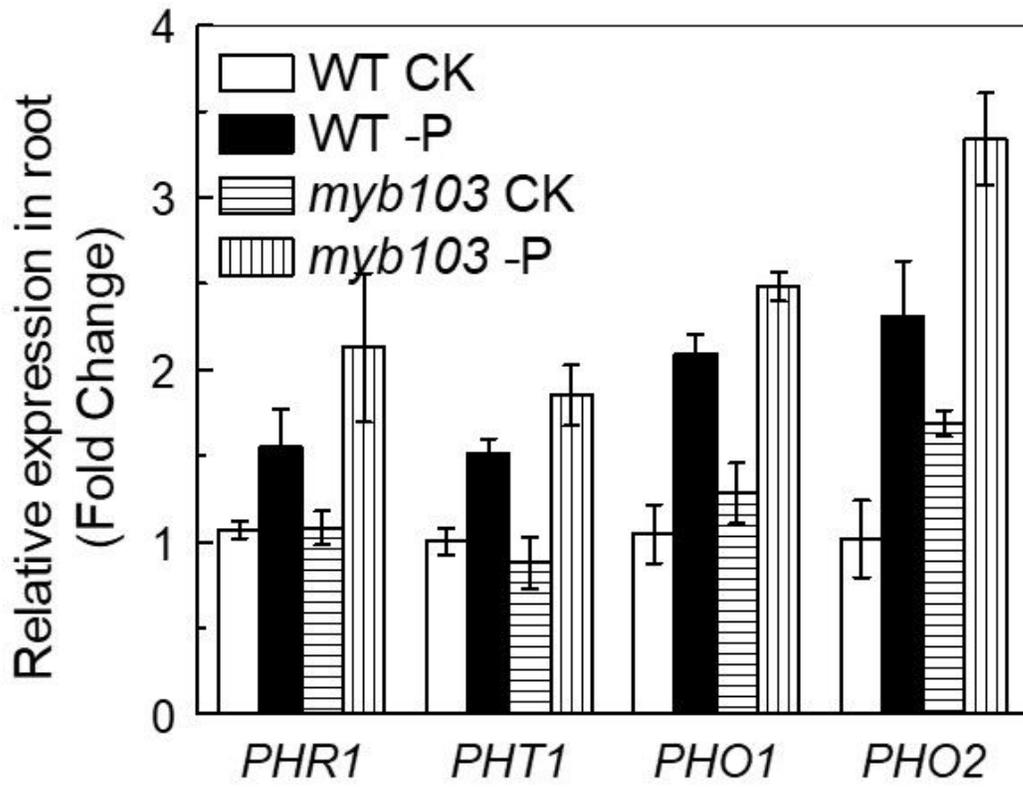


Figure 4

Relative expression of genes involved in P acquisition and allocation in roots of WT and myb103. Seedlings were hydroponically treated with +P or -P solution for 7 d, and roots were harvest for expression analysis.

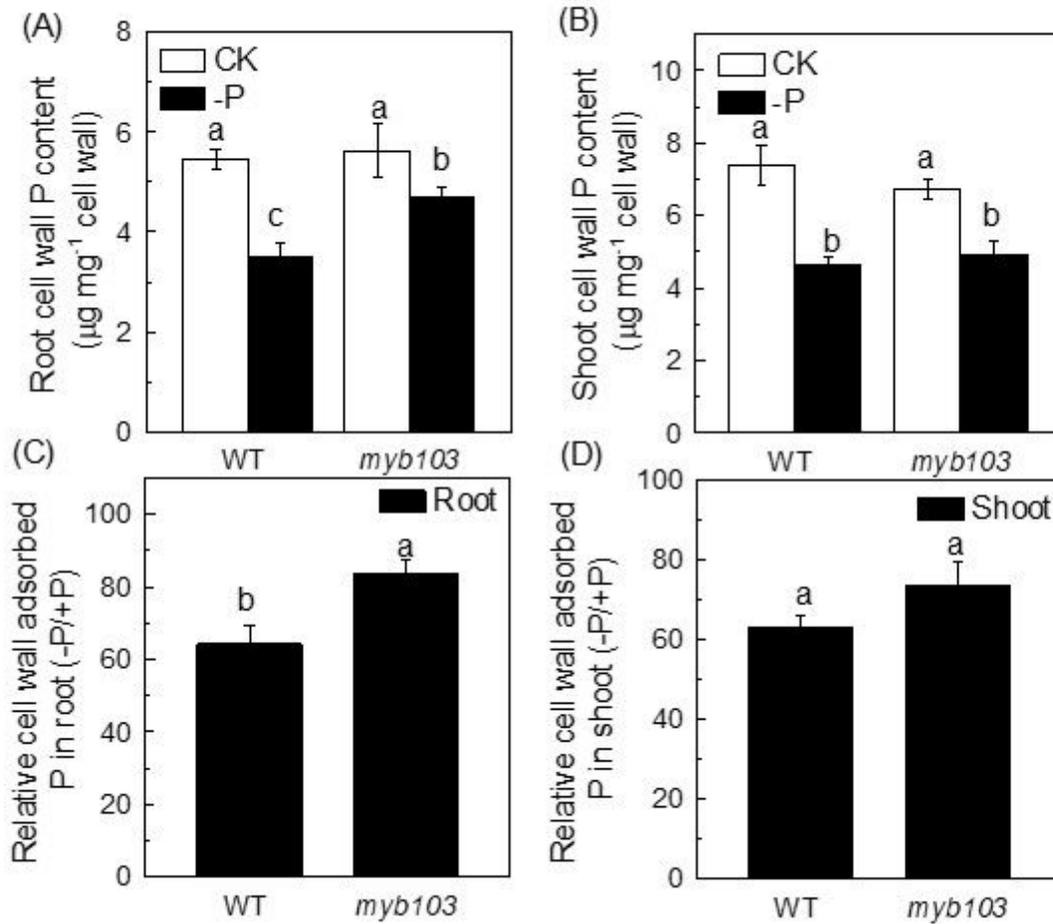


Figure 5

Cell wall P content. Seedlings were hydroponically treated with +P or -P solution for 7 d, and the P contents were measured in the root (A) and shoot (B) cell wall. The relative cell-wall-adsorbed P in the root (C) and shoot (D) was calculated from the P content in the root or shoot cell wall under -P divided by the P content in the root or shoot cell wall under +P. Values are means \pm SD, $n = 4$. Different letters on the bars indicate significant differences at $p < 0.05$.

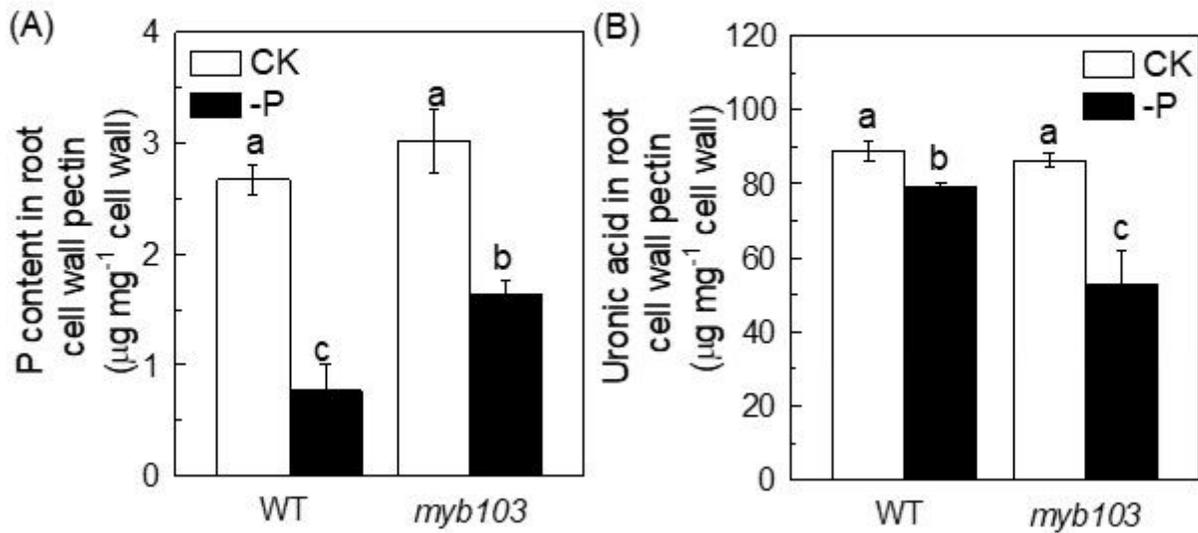


Figure 6

P content in cell wall pectin and pectin content. Seedlings were hydroponically treated with +P or -P solution for 7 d, and cell wall materials that were extracted from roots were fractionated into pectin, then the P content (A) and the uronic acid level (B) in the pectin were measured. Values are means \pm SD, $n = 4$. Different letters on the bars indicate significant differences at $p < 0.05$.

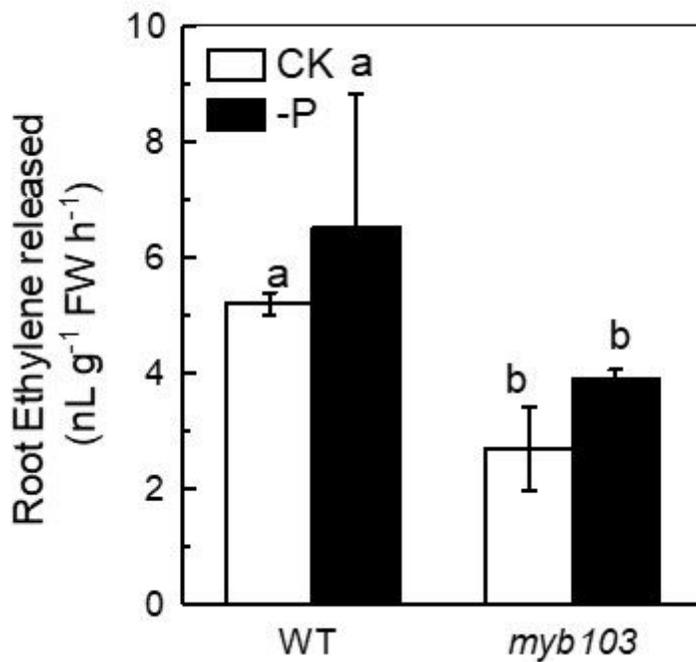


Figure 7

Production of ethylene in WT and myb103 roots. After seedlings were hydroponically treated with +P or -P solution for 7 d, the production of the ethylene was measured by GC-MS. Values are means \pm SD, n = 4. Different letters on the bars indicate significant differences at $p < 0.05$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableS1.docx](#)