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An R2R3-type myeloblastosis transcription factor MYB103 is involved in phosphorus remobilization

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Abstract

The members of myeloblastosis transcription factor (MYB TF) family are involved in the regulation of biotic and abiotic stresses in plants. However, the role of MYB TF in phosphorus remobilization remains largely unexplored. In the present study, we show that an R2R3 type MYB transcription factor, MYB103, is involved in phosphorus (P) remobilization. *MYB103* was remarkably induced by P deficiency in cabbage (*Brassica oleracea* var. *capitata* L.). As cabbage lacks the proper mutant for elucidating the mechanism of MYB103 in P deficiency, another member of the crucifer family, *Arabidopsis thaliana* was chosen for further study. The transcript of its homologue *AtMYB103* was also elevated in response to P deficiency in *A. thaliana*, while disruption of *AtMYB103* (*myb103*) exhibited increased sensitivity to P deficiency, accompanied with decreased tissue biomass and soluble P concentration. Furthermore, *AtMYB103* was involved in the P reutilization from cell wall, as less P was released from the cell wall in *myb103* than in wildtype, coinciding with the reduction of ethylene production. Taken together, our results uncover an important role of MYB103 in the P remobilization, presumably through ethylene signaling.

Keywords: *Brassica oleracea* var. *capitata* L., *Arabidopsis thaliana*, Cell wall, Ethylene, Phosphorus, MYB103

Introduction

Phosphorus (P) is an important part of the adenylates and nucleotides, that is also involved in a wide range of cellular processes in all organisms (Lauer et al. 1989). The main source of P taken up by plants is inorganic phosphate (H_2PO_4^- and HPO_4^{2-}), but this form typically falls short to meet the requirement of most crops due to its active chemical property and microbial activity (Schachtman et al. 1998; Vance et al. 2003). Limited P availability in the soil results in higher input of P fertilizers to ensure crop yield. However, this practice is neither sustainable nor environmental-friendly. Therefore, study on how plants utilize P will be helpful to breed new crop varieties with a higher P-acquisition and P-utilization efficiency.

Plants have evolved a wide range of sophisticated strategies to mount adaptive responses to P deficiency at multiple levels (Hammond et al. 2003; Lopez-Arredondo et al. 2014; Morcuende et al. 2007; Gutierrez-Alanis et al. 2018). In higher plants, excess P is primarily stored in vacuole under P sufficiency and transported from vacuole under P deficiency. At the whole-plant level, stored P in older leaves is remobilized and transported to younger growing leaves and other active sinks under P deficiency (Mimura 1995). At physiological and biochemical levels, this strategy involves remodeling of root architecture, enhanced secretion of organic acids, and symbiosis with arbuscular mycorrhizal fungi (Lopez-Arredondo et al. 2014; Hans et al. 2006). At the molecular level, several transcription factors (TFs) have been implicated in the transcriptional reprogramming of plant responses to P deficiency (Baker et al. 2015; Franco-Zorrilla et al. 2004; Liang et al. 2014; Su et al. 2015; Wendrich et al. 2020). Recently, many efforts have been devoted to the elucidation of the role of

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the myeloblastosis transcription factors (MYB TFs) in P deficiency, which constitute one of the largest gene families in plants. For instance, AtPHR1 (Rubio et al. 2001) and AtMYB62 (Devaiah et al. 2009) are involved in the transcriptional response to P deficiency in Arabidopsis. In rice, an R2R3 MYB TF, OsMYB2P-1, plays a role in P starvation, as *OsMYB2P-1* overexpression enhances tolerance to P starvation in rice (Dai et al. 2012). However, given that the MYB TFs are large in number and diverse in function (Dubos et al. 2010), our knowledge has yet to be expanded in terms of the roles of other MYB TFs in P nutrition.

Besides, several MYB TFs have been shown to couple with the hormone signaling network to confront environmental stresses. For instance, in mangosteen fruit, ethylene directly regulates *GmMYB10* at the transcription level (Palapol et al. 2009). Overexpression of ethylene response factor *TERF2* confers cold tolerance in rice through activating the expression of *OsMYB* (Tian et al. 2011). In fact, ethylene is not only involved in plant development such as fruit ripening (Schellingen et al. 2014), but also implicated in the cell wall P reutilization in P-deficient Arabidopsis and rice (Yu et al. 2016; Zhu et al. 2016). Here, we explored the role of *MYB103*, in P deficiency in *Arabidopsis thaliana*. Additionally, we examined the involvement of ethylene in remobilizing P from the cell wall through characterization of *myb103*, a loss-of-function mutant of *MYB103*.

Materials and methods

Plant material and culture conditions

A. thaliana wildtype (WT, Col-0) and the *myb103* mutant in the Col-0 background (SALK_083678; obtained from the Arabidopsis Biological Resource Center, USA), cabbage (*Brassica oleracea* var. *capitata* L.) cultivar Sukan107 were used in this study. The MS nutrient solution contains the following macronutrients in mM: $\text{Ca}(\text{NO}_3)_2$ 4.0, MgSO_4 1, KNO_3 6.0 and $\text{NH}_4\text{H}_2\text{PO}_4$ 0.1, and the following micronutrients in μM : CuSO_4 0.5, MnSO_4 1, H_3BO_3 12.5, ZnSO_4 1, H_2MoO_4 0.1, NiSO_4 0.1 and Fe(III)-EDTA 50.

Seeds were sterilized in 75% ethanol (v/v) for 5 min, and washed by sterilized water for three times. Then seeds were either grown on the 0.8% agar-solidified MS nutrient medium for 1 week before the petri dish assay, or grown on the sponge supplied with the MS nutrient solution for 6 weeks before the hydroponic assay. For the petri dish assay, uniform seedlings were selected and transferred onto solid MS nutrient medium with (+P; complete medium as described above) or without P (-P; 0.1 mM $\text{NH}_4\text{H}_2\text{PO}_4$ was replaced by 0.1 mM NH_4NO_3) for another week. The petri dishes were vertically placed in a growth chamber with a temperature of $23^\circ\text{C} \pm 1^\circ\text{C}$, a light intensity of $140 \mu\text{mol m}^{-2} \text{s}^{-1}$ and a photoperiod

of 16 h of light and 8 h of dark. For the hydroponic assay, uniform seedlings were selected and transplanted into MS nutrient solution with (+P) or without P (-P) for another week. The solution was renewed every 3 d.

Determination of soluble P concentration

Fresh weights of root and leaves of Arabidopsis were determined, followed by homogenization in liquid nitrogen with mortar and pestle. The finely ground tissue powder was used for determination of soluble P as previously described (Wang et al. 2018).

Cell wall and pectin extraction

Cell wall preparation was conducted as previously described (Zhong and Lauchli 1993). Briefly, finely ground samples were added with 8 mL 75% ethanol (v/v) and incubated at 4°C for 20 min. After centrifugation at 8000 rpm for 15 min at 4°C , the pellet was sequentially washed with 8 mL acetone, 8 mL chloroform-methanol mixture (1:1, v/v), 8 mL methanol, each for 20 min at 4°C . Cell wall materials were freeze dried, and kept at 4°C for further use.

Pectin was extracted as previously described (Zhong and Lauchli 1993). The above cell wall materials were weighed, incubated with 1 mL distilled water for 1 h at 100°C , followed by centrifugation at 12000 rpm for 15 min to collect the supernatant. 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 (Blumenkrantz and Asboe-Hansen 1973). Firstly, 200 μL pectin fraction was combined with 1 mL 12.5 mM $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ (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 μL 0.15% M-hydroxy-diphenyl (Sigma, Cat.: 26, 225-0) and kept at room temperature for 20 min, followed by measurement of the absorbance at 520 nm. Galacturonic acid (Sigma) was used as the standard.

Measurement of P concentration in cell wall and pectin

Cell wall P concentration was determined as described (Zhu et al. 2015). Firstly, about 5 mg cell wall material was weighed, and 1 mL 2 M HCl was added with occasional shaking for 72 h. After centrifugation (16, 363 g for 15 min), 0.7 mL supernatant was collected and 4.3 mL HCl (2 M) was added for the P concentration measurement by inductively coupled plasma-mass spectrometry (ICP-MS).

Pectin P concentration was determined as described (Zhu et al. 2015). Firstly, 1.5 mL pectin fraction was combined with 3.5 mL HCl (2 M), followed by measurement of the P concentration by ICP-MS.

Quantitative real-time PCR (qRT-PCR) analysis

Tissue RNA was extracted using the RNAprep Pure Kit (TianGen, China). Total RNA was then reverse transcribed to cDNA using the PrimeScript RT[®] reagent kit (Takara) following the manufacturer's procedure. For qRT-PCR, the reaction mixture contained SYBR Premix ExTaq (5 μ L; Takara), 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 $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001) and *UBIQUITIN 10* as the internal reference. Primers used here were listed in Table S1.

Detection of root ethylene production

Measurement of root ethylene production was conducted as described (Wu et al. 2011). Roots were detached and then transferred to 6 mL glass vials. After adding 1 mL distilled water, 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

All experiments were independently conducted at least three times. Student's *t* test was performed to compare two groups of data. For multiple comparisons, the data were analyzed by One-way ANOVA, followed by post hoc analysis with Duncan's multiple range test. Different letters indicate significant differences at $P < 0.05$.

Results

MYB103 is upregulated in response to P deficiency

Firstly, qRT-PCR was conducted to investigate whether expression of *MYB103* is responsive to P deficiency. After exposure to P deprivation for 7 d, the relative expression of *MYB103* was determined. In *B. oleracea* var.

capitata L., the expression of *MYB103* (*BoMYB103*) was dramatically increased in response to P deficiency (Fig. 1a). We are interested to see whether it is also true in any other member of crucifer family like *A. thaliana*. As expected, the expression of *MYB103* (*AtMYB103*) was also increased under P deficiency, albeit to a lesser degree (Fig. 1b). The observation that P deficiency induces the expression of *MYB103* in both *A. thaliana* and *B. oleracea*, albeit to different extents, can be suggestive of the possible involvement of *MYB103* in responses to P deficiency. As cabbage lacks the proper mutant for elucidating the mechanism of *MYB103* in P deficiency, the homologue of *MYB103* in *A. thaliana* was chosen for further study.

Disruption of *MYB103* causes increased sensitivity to P deficiency

To further clarify the role of *MYB103* in response to P deficiency, a T-DNA insertion line (SALK_083678) was selected for further study. The T-DNA was inserted into the third exon of *AtMYB103* (Supplemental Figure 1A), thereby disrupting *AtMYB103* expression in the homozygous line (Supplemental Figure 1B). One major symptom of P deficiency is the inhibition of root growth. After growth on P-deficient medium for 7 d, the root length of WT was inhibited by approximately 15%, whereas more dramatic inhibition (42%) was observed in *myb103* (Fig. 2a, b and c). In addition, *myb103* exhibited decreased root biomass compared to WT (Fig. 2d), although no remarkable variations were observed in their shoot biomass (Fig. 2e). These results together indicate that the disruption of *MYB103* resulted in increased sensitivity to P deficiency.

Disruption of *MYB103* impairs P remobilization

The sensitivity to P deficiency in *myb103* mutant was attributed to its lower levels of root and shoot soluble P

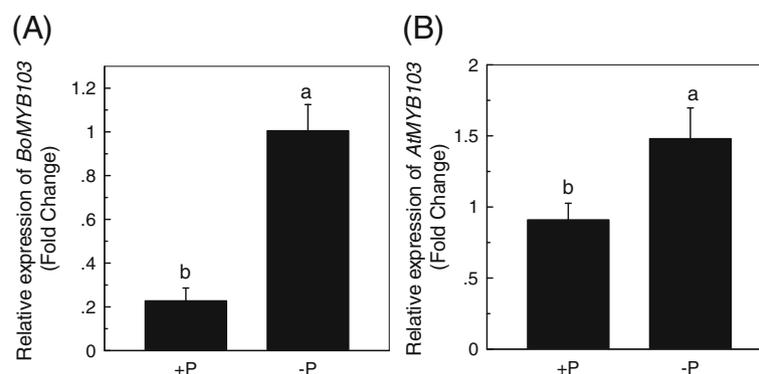


Fig. 1 Relative expression of *MYB103* in response to P deficiency in *Arabidopsis thaliana* and *Brassica oleracea*. Seedlings were hydroponically treated with +P or -P solution for 7 d, and the expression of *MYB103* was measured in *B. oleracea* (a) and *A. thaliana* (b) respectively. Values are means \pm SD, $n = 4$. Different letters on the bars indicate significant differences at $p < 0.05$

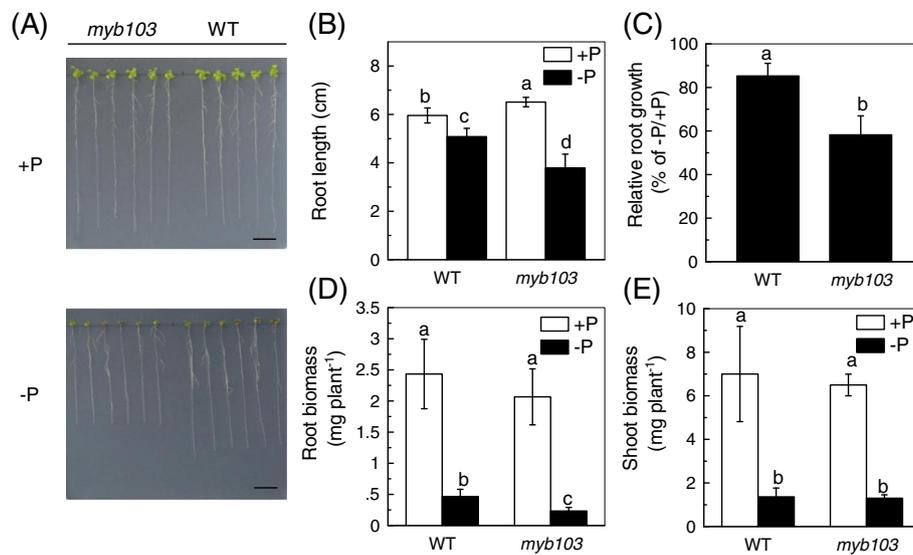


Fig. 2 Phenotype of *myb103* mutant. Seeds of WT and *myb103* were surface-sterilized and germinated on the complete nutrient medium, and seedlings with the root length of about 1 cm were transferred to the complete nutrient medium (+P) or medium without P (-P) for 7 d (a), and root length (b), relative root growth (c), ratio of root length in +P to root length 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 $p < 0.05$

than WT (Fig. 3a and b), also, less P was translocated to the shoots (Fig. 3c), suggesting that MYB103 was involved in response to P deficiency. 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 is no soluble P in the P-deficient nutrient solution, and cell wall can function as an important P source when external P is not available (Yu et al. 2016; Zhu et al. 2015), the root and shoot cell wall P retention in both WT and *myb103* were thus analyzed. Interestingly, more P was retained in the *myb103* root cell wall than in WT (Fig. 5a and c), although no significant difference was found in the shoot cell wall P reutilization (Fig. 5b and d), suggesting that less P was reutilized in the *myb103* root. Furthermore, as pectin dramatically contributes

to cell wall P reutilization in rice (Zhu et al. 2015), we tested whether pectin content was affected 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 P sufficiency when 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). Reduced pectin content in *myb103* meant that there were fewer carboxyl groups available, which in turn led to reduced binding of cations like Fe^{3+} , and thereby less P was released from the root cell wall in *myb103*.

Disruption of MYB103 leads to less ethylene production

Our findings raises a question that how MYB103 interferes with the reutilization of the P from the cell wall.

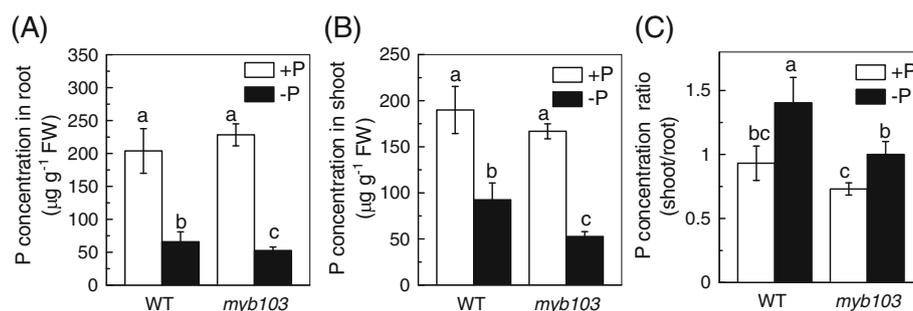


Fig. 3 Disruption of MYB103 affects root-to-shoot P translocation. WT and *myb103* seedlings were grown under +P or -P conditions for 7 d, root (a) and shoot (b) P concentration was measured. The ratio of shoot P concentration to root P concentration (c) was calculated. Values are means \pm SD, $n = 4$. Different letters represent a significant difference at $P < 0.05$

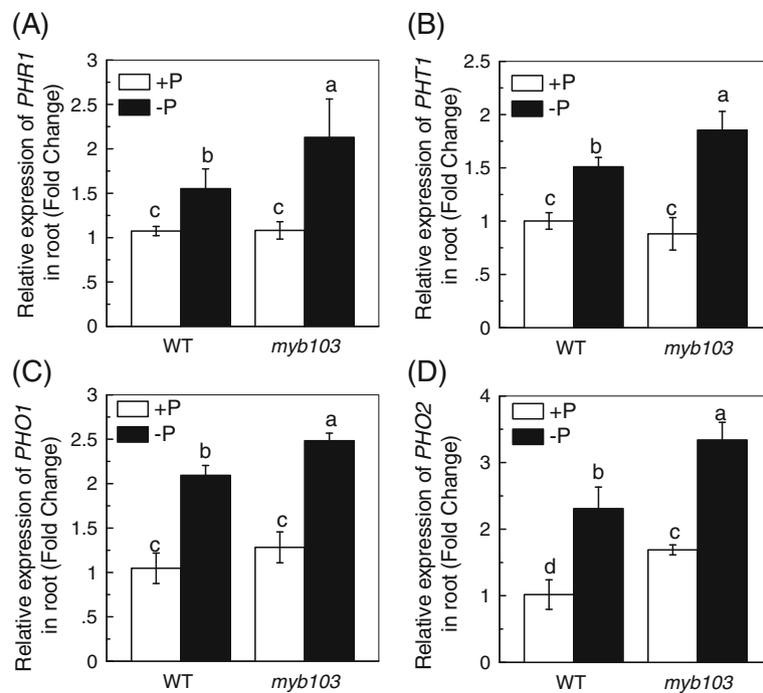


Fig. 4 Relative expression of genes involved in P acquisition and allocation in roots of WT and *myb103*. Seedlings were hydroponically treated with +P or -P solution for 7 d, and roots were harvested for expression analysis

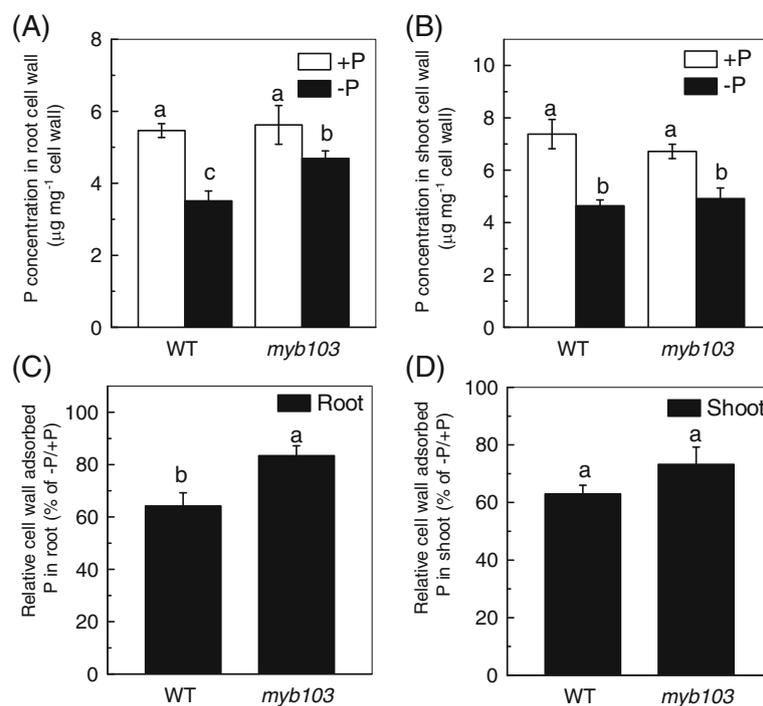


Fig. 5 Cell wall P concentration. Seedlings were hydroponically treated with +P or -P solution for 7 d, and the P concentrations were measured in the root (a) and shoot (b) cell wall. The relative cell-wall-adsorbed P in the root (c) and shoot (d) was calculated from the P concentration in the root or shoot cell wall under -P / the P concentration 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 $p < 0.05$

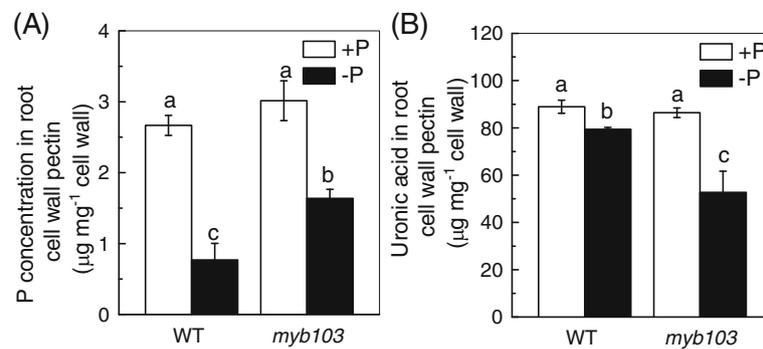


Fig. 6 P concentration in cell wall pectin and pectin content. Seedlings were hydroponically treated with +P or -P solution for 7 d, and cell wall materials extracted from roots were fractionated into pectin, then the P concentration (a) and the uronic acid level (b) in the pectin were measured. Values are means \pm SD, $n = 4$. Different letters on the bars indicate significant differences at $p < 0.05$

Recently, ethylene has been shown to play pivotal roles in the reutilization of the cell wall P in Arabidopsis (Yu et al. 2016); 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 an important player in the MYB103-regulated cell wall P reutilization.

Discussion

To cope with P limitation, plants deploy a series of physiological, biochemical, and molecular strategies such as modifying root architecture (Steingrobe 2001; Wang et al. 2010), secreting carboxylates and acid phosphatase

(Baldwin et al. 2001), elevating transcription of genes essential for P acquisition (Zhou et al. 2008), and enhancing the remobilization of the previously stored P (Kuga et al. 2008; Yu et al. 2016; Zhu et al. 2015). Transcriptional reprogramming, regulated by TFs, is the key to these adaptive responses to P deficiency (Gu et al. 2016; Wendrich et al. 2020), although many details remain to be unveiled. In this study, we show that a MYB TF, MYB103 is involved in P remobilization.

MYB TFs are implicated in a variety of plant's responses to environmental stimuli (Li et al. 2015; Stracke et al. 2001). For example, HOS10 (Zhu et al. 2005), MYB14 and MYB15 (Agarwal et al. 2006), MdMYB23 (An et al. 2018) and MdMYB308L (An et al. 2020) are involved in the modulation of cold stress response. MYB111 modulates salt stress (Li et al. 2019) while MYB12 is involved in response to nitrogen deficiency (Lea et al. 2007). PSR1 (Wykoff et al. 1999) and PHR1 (Rubio et al. 2001) are two well characterized R2R3 MYB transcription factors that have been implicated in the positive regulation of P stress responses. In addition, MYB62 plays an important role in the regulation of phosphate starvation responses via changes in gibberellic acid metabolism and signaling (Devaiah et al. 2009). In the present study, we found that MYB103, an R2R3 MYB TF, was involved in the P deficiency response in Arabidopsis. The expression of *MYB103* was induced under P-deficient condition in Arabidopsis (Fig. 1), and disruption of *MYB103* exhibited sensitivity to P deficiency (Fig. 2), due to lower levels of shoot and root soluble P (Fig. 3).

Cell wall, consisting of cellulose, hemicelluloses, pectin and other matrix polysaccharides, acts as an important P repository in rice and Arabidopsis (Cosgrove 2005; Yu et al. 2016; Zhu et al. 2015). Among them, only pectin has been shown to be involved in the P recycling under P-deficient condition, as the carboxyl groups in the cell wall pectin have strong affinity for cations, such as Fe^{3+} , which lead to the release of the P (Gessa et al. 1997; Zhu

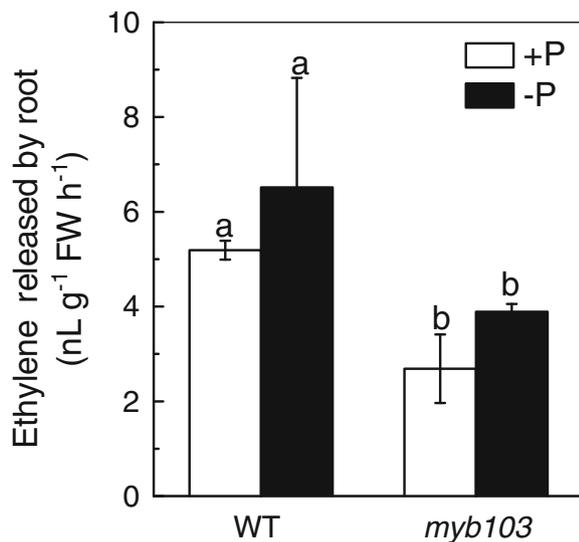


Fig. 7 Ethylene production in WT and *myb103* roots. After seedlings were hydroponically treated with +P or -P solution for 7 d, the production of the ethylene was measured in a gas chromatograph. Values are means \pm SD, $n = 4$. Different letters on the bars indicate significant differences at $p 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

et al. 2015). Our results once again confirmed the role of the P pool in cell wall pectin in WT under P deficiency, as *myb103* was remarkably compromised in P reutilization when subjected to P deficiency (Fig. 5a and c), in company with the greatly reduced pectin content (Fig. 6b). As a result, more P was retained in the *myb103* cell wall under P-deficient condition (Fig. 5), thus less soluble P was available in *myb103* roots and shoots, rendering it more sensitive to the P deficiency (Figs. 2 and 3). In agreement with this, the expression of P deficiency-responsive genes including *Phosphate starvation response 1 (PHR1)*, *PHO1*, *Phosphate 2 (PHO2)* and *PHT1*, 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? Ethylene is known to act 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 (Johnson and Ecker 1998; Kazan 2015). While the role of ethylene in P deficiency in plants has been extensively examined (Nagarajan et al. 2011; Song and Liu 2015; Yu et al. 2016; Zhu et al. 2016), the regulators of ethylene production are largely elusive. MYB103 is presumably an important regulator of ethylene production. In the present study, we found that less ethylene was accumulated in roots of *myb103*, when compared with WT, irrespective of the P status (Fig. 7), indicating that less ethylene production was associated with increased sensitivity to P deficiency in *myb103*. However, it has yet to be investigated as to the molecular mechanism underlying regulation of ethylene production by MYB103.

Conclusion

Our results show that an R2R3 MYB TF, MYB103, is involved in the cell wall-based P reutilization under P deficiency through regulating ethylene production. Our study, thus, supports the importance of P remobilization from the plant cell wall under P deficiency, reveals an important regulator in this process, and furnishes new evidence on the essential role of ethylene during the important and prevalent macronutritional stress of P deficiency.

Supplementary information

Supplementary information accompanies this paper at <https://doi.org/10.1186/s43014-020-00038-6>.

Additional file 1 Table S1. List of Primers used in the current study.

Additional file 2 Figure S1. Identification of the *myb103* mutant. (A) Schematic structure of the *myb103* mutant carrying a single copy of the T-DNA insert in the third exon. The gray box represents exon while the black bold line represents intron. (B) Quantitative RT-PCR analysis of *MYB103* expression in the roots of *Arabidopsis*. Values are means \pm SD, $n=4$. Different letters on the bars indicate significant differences at $p < 0.05$.

Abbreviations

MYB TF: Myeloblastosis transcription factor; WT: Wildtype; P: Phosphorus; ICP-MS: Inductively coupled plasma-mass spectrometry; NO: Nitric oxide

Acknowledgements

Not applicable.

Authors' contributions

JL and FY conceived 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.

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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.

Ethics approval and consent to participate

Not Applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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