

# The Chloroplast Protease AMOS1/EGY1 Affects Phosphate Homeostasis under Phosphate Stress<sup>1</sup>

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Plastid intramembrane proteases in *Arabidopsis* (*Arabidopsis thaliana*) are involved in jasmonic acid biosynthesis, chloroplast development, and flower morphology. Here, we show that Ammonium-Overly-Sensitive1 (AMOS1), a member of the family of plastid intramembrane proteases, plays an important role in the maintenance of phosphate (P) homeostasis under P stress. Loss of function of *AMOS1* revealed a striking resistance to P starvation. *amos1* plants displayed retarded root growth and reduced P accumulation in the root compared to wild type (Col-0) under P-replete control conditions, but remained largely unaffected by P starvation, displaying comparable P accumulation and root and shoot growth under P-deficient conditions. Further analysis revealed that, under P-deficient conditions, the cell wall, especially the pectin fraction of *amos1*, released more P than that of wild type, accompanied by a reduction of the abscisic acid (ABA) level and an increase in ethylene production. By using an ABA-insensitive mutant, *abi4*, and applying ABA and ACC exogenously, we found that ABA inhibits cell wall P remobilization while ethylene facilitates P remobilization from the cell wall by increasing the pectin concentration, suggesting ABA can counteract the effect of ethylene. Furthermore, the elevated ABA level and the lower ethylene production also correlated well with the mimicked P deficiency in *amos1*. Thus, our study uncovers the role of AMOS1 in the maintenance of P homeostasis through ABA-antagonized ethylene signaling.

Phosphorus (P) is indispensable for all living organisms. P functions in a variety of central cellular processes, including the biosynthesis of organic compounds, bio-energy generation, and cell signaling (Poirier and Bucher, 2002). P, however essential, is highly dilute and immobile in soils due to its unique chemical properties. To survive P limitation, plants employ sophisticated mechanisms to facilitate P acquisition and mobilization, which include remodeling of root architecture, enhancing organic acid excretion, and increasing the expression of transporters and acid phosphatases (Fang et al., 2009). A key role of ethylene in the morphological and physiological responses to P deficiency has also been established (Lopez-Bucio et al., 2003; Zhang et al., 2003). Although knowledge of P acquisition and the P-signaling system has increased dramatically in recent

years (Baek et al., 2013; Chandrika et al., 2013; Lv et al., 2014; Wang et al., 2014a, 2014b; Ayadi et al., 2015; Chen et al., 2015; Lambers et al., 2015), the regulatory networks underlying plant adaptation to P deficiency still remain to be clarified.

The site-2 protease (S2P) is a membrane-targeting protease (Rawson et al., 1997). In mammals, S2Ps function in signaling pathways involved in fundamental cellular processes, such as lipid metabolism and the endoplasmic-reticulum (ER) stress response through proteolytic activation of downstream transcription factors such as SREBPs and AFT6, releasing them from the membrane (Rawson, 2013). These processes are essential under stress. Since their discovery in mammals, S2P-like proteins have also been found in bacteria, fungi, and plants (Rudner et al., 1999; Bolter et al., 2006; Bien et al., 2009). The *Arabidopsis* (*Arabidopsis thaliana*) genome harbors four S2P homologs: AtS2P1/AraSP (At2g32480), AtS2P2 (At1g05140), AtAMOS1/AtEGY1 (At5g35220), and AtEGY2 (At5g05740; Adam, 2015). Although their substrates have yet to be determined, the S2P homologs in *Arabidopsis* have been implicated in jasmonic acid biosynthesis, chloroplast development, and flower morphology (Adam, 2015). However, whether S2Ps may be involved in P nutrition has not been examined. Among the four S2P homologs, AtAMOS1/AtEGY1 is of particular interest. In a previous study, AtAMOS1 was demonstrated to recruit abscisic acid signaling to regulate global gene expression under ammonium stress (Li et al., 2012), suggesting a central role for the S2P homolog AtAMOS1 in macronutrient physiology. Here, we explore the role of *amos1*, an ammonium-hypersensitive

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mutant, in response to P deficiency. Additionally, we examine the involvement of the phytohormones abscisic acid (ABA) and ethylene in remobilizing P from the cell wall during the P-deficiency response. Our findings unravel the critical role of the plastid metalloprotease AMOS1 in the maintenance of P homeostasis under P stress, through recruitment of ABA-antagonized ethylene signaling.

## RESULTS

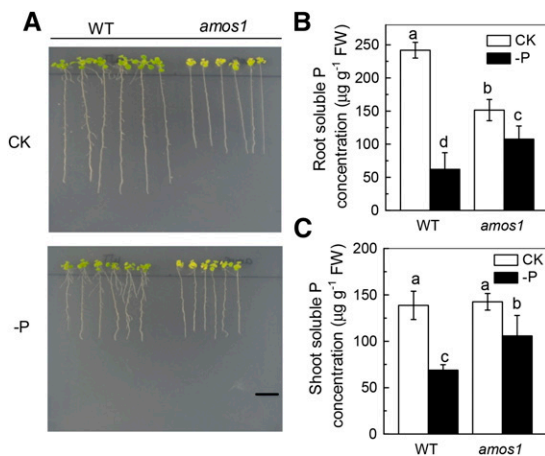
### Loss of Function of AMOS1 Confers Resistance to P Deficiency

To investigate the response of the Arabidopsis ammonium-hypersensitive mutant *amos1* to P deficiency, wild type and *amos1* plants were grown in either P-replete or deficient media for 7 d. As shown in Figure 1A and Supplemental Figure S1, P deficiency led to reduced root and shoot growth of wild type while there was essentially no influence on the growth of *amos1*, indicating that *amos1* was more resistant to P deficiency than wild type. However, as *amos1* showed reduced root growth under P-replete condition compared to wild type (Fig. 1A), another question was whether the relatively superior response of *amos1* under P-deficient conditions was due to the mutant's already poorer growth (and presumably P acquisition) when grown under P-replete conditions. For this reason, the response of *amos1* to nitrogen (N) and potassium (K) deficiency was also tested, and, rather than showing

resistance, *amos1* still grew poorly both under N-deficient and K-deficient conditions, as seen in root length and biomass (Supplemental Fig. S2), underscoring that *amos1* is uniquely resistant to P deficiency. Determination of soluble P concentration in the root showed that *amos1* accumulated less P in the root than wild type when grown under control conditions; however, upon P starvation, soluble P in both root and shoot in *amos1* was higher than that in wild type (Fig. 1, B and C), indicating that *amos1* can reutilize P more efficiently.

### Cell Wall P in Roots Contributes to P Recycling in *amos1*

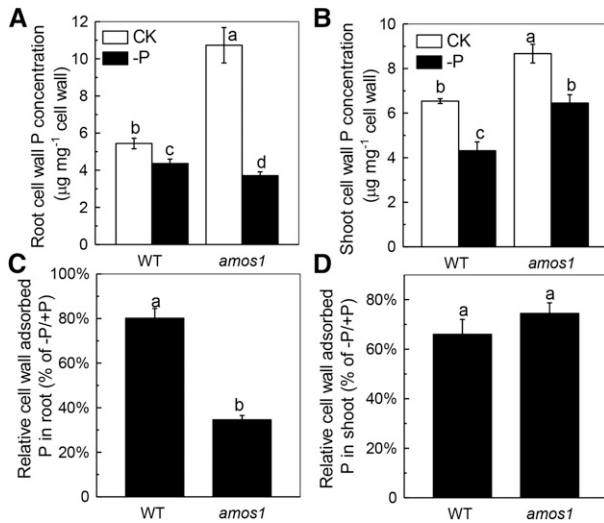
We next set out to answer the question where the P originated in the *amos1* root or shoot when grown under P-deficient conditions. It was previously demonstrated that nearly 50% of total P is retained in the cell wall and that the cell wall can contribute greatly to cellular P reutilization in rice (*Oryza sativa*; Zhu et al., 2015). To verify whether accessing of the P reservoir in the cell wall was associated with the resistance of *amos1* to P deficiency, the size of the P pool adsorbed in the cell wall of wild type and *amos1* was determined under both +P and -P conditions. Interestingly, although P concentration was much higher in *amos1* shoots than roots under P deficiency, there was almost no alteration in shoot P reutilization as indicated by shoot cell wall P (Fig. 2, B and D). Thus, the elevated P in *amos1* shoots under P deficiency was most likely due to P translocation from the roots, and *amos1* roots may act as the P source. As expected, a greater quantity of P was adsorbed in the cell wall fraction of *amos1* roots under P-replete conditions, while a significantly smaller P pool was found in the root cell walls of *amos1* under P-deficient conditions compared to wild type (Fig. 2A). Thus, the ratio of P retained (-P/+P) in the *amos1* root cell wall was much lower than in the wild type (Fig. 2C), indicating that more P was released from the root cell wall of *amos1*, allowing for sustained growth of *amos1* under P deficiency.



**Figure 1.** Impact of phosphate withdrawal on wild type and *amos1*. The seeds of wild type and *amos1* were surface-sterilized and germinated on complete nutrient medium, then seedlings of identical root length (1 cm) were transferred to either complete nutrient medium (CK) or medium without P (-P) for 7 d (A). For determination of soluble P, 6-week-old wild-type and *amos1* seedlings were subjected to P-sufficient or -deficient solution for 7 d, prior to determination of the soluble P concentration in the root (B) and shoot (C). Scale bar = 1 cm. Values are means  $\pm$  SD (SD),  $n = 4$ . Significant differences ( $P < 0.05$ ) are denoted by different letters on the bars.

### Cell Wall Pectin Contributes to Efficient P Recycling in *amos1*

Previous studies showed that P deficiency decreases pectin concentration in the root cell wall of Arabidopsis (Zhu et al., 2012) and that pectin contributes greatly to cell wall P reutilization in rice (Zhu et al., 2015). Since the cell wall matrix acts as an important repository for P and a potential source for P reutilization, we tested whether pectin plays a role in the P-deficiency response in Arabidopsis. As expected, more P was adsorbed in the pectin fraction of the *amos1* root cell wall under P-replete conditions while less P was adsorbed under P-deficient conditions compared to wild type, indicating greater P-release potential in the pectin fraction of the *amos1* root cell walls (Fig. 3A). In agreement with this, an increase in the pectin concentration of the *amos1* root



**Figure 2.** P retention in the cell wall. Seedlings were grown hydroponically under P-replete or P-deficient conditions for 7 d, and cell wall-adsorbed P was measured in the root (A) and shoot (B). 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 deficiency divided by the P concentration in the root or shoot cell wall under P-replete conditions, respectively. Values are means  $\pm$  SD,  $n = 4$ . Significant differences ( $P < 0.05$ ) are denoted by different letters on the bars.

cell wall was found under P-deficient versus P-replete conditions, while the pectin concentration was decreased in the wild-type root cell wall under P-deficient conditions (Fig. 3C). However, the almost complete absence of any change in the shoot cell wall pectin concentration and its P retention in both the wild type and *amos1* (-P versus +P), again, excludes the involvement of the shoot in the greater degree of P reutilization observed in *amos1* (Fig. 3, B and D). Thus, we focused on roots instead of shoots in following examinations.

**Expression of Genes Involved in P Uptake, Translocation, and Allocation in P-Deficient Plants**

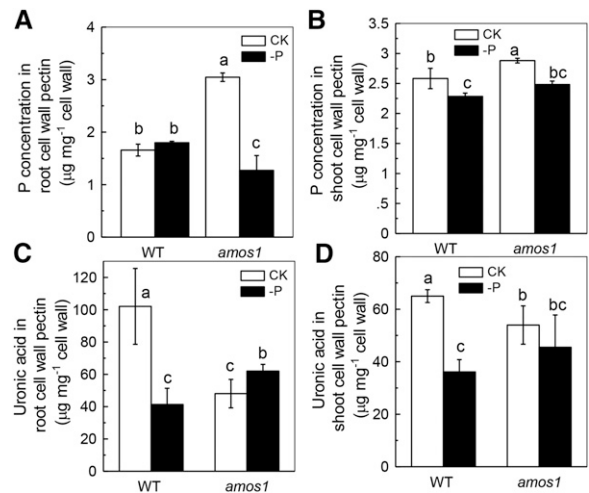
To determine the transcriptional response to P starvation, quantitative real-time PCR (RT-PCR) was subsequently performed following P starvation for 7 d. As revealed by RT-PCR, the wild-type root showed increased transcription of *PHR1*, *PHT1*, *PHO1*, and *PHO2* after P starvation (Fig. 4), although the expression of all these genes was consistently higher in *amos1*. To our surprise, *amos1* roots already displayed elevated transcription of *PHR1*, *PHT1*, *PHO1*, and *PHO2*, even in the absence of P starvation (Fig. 4), suggesting that loss of function of *AMOS1* itself mimicked the stress imposed by P deficiency, consistent with the retarded root growth under P-replete control conditions that was indistinguishable from that under P-deficient conditions (Fig. 1A).

**Involvement of ABA in P Recycling from the Cell Wall in *amos1***

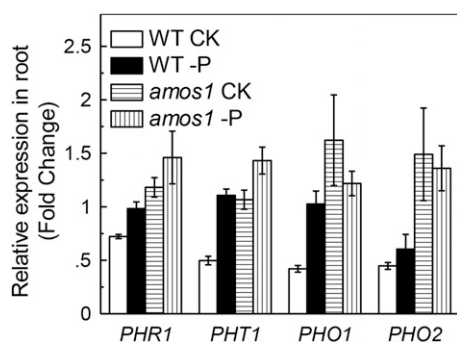
ABA was shown to function downstream of *AMOS1* in its response to ammonium hypersensitivity (Li et al., 2012). We now ask whether ABA was also involved in the response to P deficiency in *amos1*. As shown in Figure 5, P deficiency significantly reduced the root ABA concentration of *amos1*, while there was almost no influence on wild type, indicating that ABA may be involved in the resistance to P deficiency of *amos1*. Examination of a loss-of-function mutant in a key transcription factor for ABA signaling, *ABSCISIC ACID INSENSITIVE4* (*abi4*), and exogenous application of ABA confirmed this hypothesis; *abi4* phenocopied *amos1*, showed improved growth (Fig. 6A), significantly greater root length, higher root and shoot biomass (Supplemental Fig. S3), significantly higher soluble P pools in roots and shoots (Fig. 6, B and C), and less root cell wall P retention under P-deficient conditions (Fig. 6, D and E), while exogenous ABA application retarded root and shoot growth (Fig. 7A; Supplemental Fig. S4), decreased soluble P in roots and shoots (Fig. 7, B and C), and increased cell wall-adsorbed P under the -P treatment (Fig. 7D).

**ABA Antagonizes Ethylene Signaling in P Recycling from the Cell Wall in *amos1***

Lastly, we asked the question how ABA interferes with P remobilization from the cell wall in *Arabidopsis*. A recent study showed that the transcription of *ACS4*



**Figure 3.** The concentration of P in cell wall pectin and pectin. Seedlings (after germination) were subjected to P-sufficient and -deficient nutrient solutions for 7 d, and cell wall polysaccharides from roots and shoots were fractionated into pectin, then cell wall P concentration was deposited in pectin from the root (A) or shoot (B), and uronic acid in the root (C) or shoot (D) was determined accordingly. Values are means  $\pm$  SD,  $n = 4$ . Significant differences ( $P < 0.05$ ) are denoted by different letters on the bars.



**Figure 4.** Relative transcription of genes involved in P acquisition, and allocation in roots of *amos1* and wild type. Seedlings were grown in P-replete or -deficient solution for 7 d.

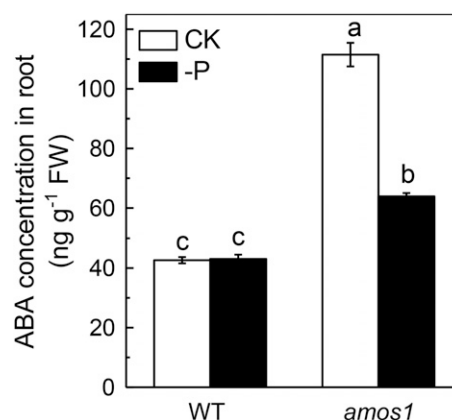
and *ACS8* genes, involved in ethylene biosynthesis, is inhibited by *ABI4* (Dong et al., 2016), and more recently, Zhu et al. (2016) found that ethylene acts upstream of cell wall pectin to increase P remobilization from the cell wall in rice; therefore, we examined ethylene production in wild type and *amos1* root. As expected, significantly enhanced ethylene production was observed in *amos1* roots under P-deficient versus P-replete conditions compared to wild type (Fig. 8), indicating that ABA antagonizes ethylene signaling in the P-remobilization mechanism from the cell wall, which was furthermore confirmed by the exogenous application of the ethylene precursor ACC. As shown in Supplemental Figure S5, after ACC was applied exogenously under the P-deficient and P-replete conditions, production of ethylene was enhanced, accompanied by an increase in the cell wall pectin concentration; thus, more P was released from the cell wall pectin fraction, and, as a result a higher root-soluble P concentration was available, which was in agreement with Zhu et al. (2016). All the above results indicate that ABA counteracts the effect of ethylene in remobilizing the root cell wall P, and this conclusion was further supported by the fact that the high ABA level and the low ethylene production correlated well with the lower cell wall P reutilization in the *amos1* mutant under P-replete conditions.

## DISCUSSION

To overcome low P availability, plants have adopted a series of biochemical, physiological, and molecular strategies, including enhanced acid phosphatase (APase) activity and elevated transcription of genes essential for P acquisition (Goldstein et al., 1988; Devaiah et al., 2007; Zhou et al., 2008; Niu et al., 2013; Wang et al., 2014c). In addition, many nonspecific, shock-induced, gene-expression changes are also classically observed under P deficiency (Hammond et al., 2003). In addition to improved exploitation of P resources in the soil, plants can also remobilize previously acquired and internally sequestered P during periods of P deficiency (Raghothama and Karthikeyan, 2005). This can involve the plant-

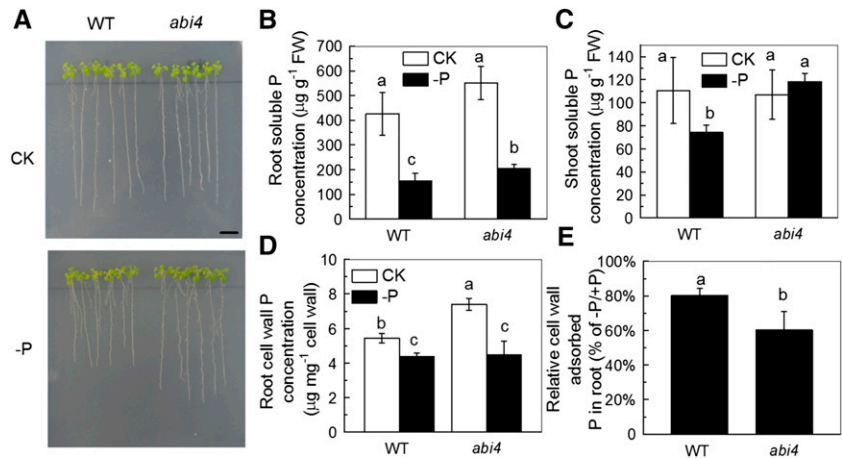
internal engagement of acid phosphatases, ribonucleases, and phosphohydrolases (Yun and Kaeppler, 2001; Gong et al., 2011). A recently uncovered mechanism engaged under P starvation in rice is associated with remobilization of P from storage pools in the root cell wall, and more specifically, the cell wall pectin fraction (Zhu et al., 2015). In the current study in Arabidopsis, resistance of P starvation also shows clear association with the cell wall, and in particular the pectin fraction, in the loss-of-function mutant *amos1*. To elucidate the possible mechanisms underlying cell wall-based P recycling in *amos1*, we examined the involvement of the phytohormones ABA and ethylene and show them to be fundamental to plant resistance to P starvation and the accessing of the P pool in the root system. Our work, thus, provides an important mechanistic extension to the earlier study in rice, in establishing not only the importance of cell wall P remobilization, but its regulation through phytohormone signaling.

The mature plant cell wall consists largely of cellulose and matrix polysaccharides, including pectin and hemicelluloses (Cosgrove, 2005). Cell wall polysaccharides of groundnut roots have been shown to exhibit P-solubilizing activity after growth on low P substrates (Ae et al., 1990). Polygalacturonic acids easily form complexes with metals, such as  $\text{Fe}^{3+}$  or  $\text{Al}^{3+}$ , via phosphate ligand exchange, facilitating the desorption of P from soil minerals (Nagarajah et al., 1970). The carboxyl-acid groups, in particular of the D-GalUA component of cell wall pectin, have a strong affinity for  $\text{Fe}^{3+}$ , and such metal binding has been linked to P release (Gessa et al., 1997). More recently, P deficiency was shown to lead to a decrease in the pectin and hemicellulose 1 fractions of cell walls in Arabidopsis in the context of cadmium exposure, and pectin was shown to enhance P remobilization in rice under P deficiency (Zhu et al., 2012, 2015, 2016). These studies indicate that cell wall polysaccharides become involved



**Figure 5.** ABA accumulation in roots of seedlings of wild-type and *amos1* under P-sufficient or -deficient conditions. Values are means  $\pm$  sd,  $n = 4$ . Significant differences ( $P < 0.05$ ) are denoted by different letters on the bars.

**Figure 6.** Effect of phosphate depletion on soluble P and P retention in the cell wall in wild-type and *abi4* seedlings. Seeds of wild type and *abi4* were surface-sterilized and germinated on solid complete medium, then seedlings of identical root length (1 cm) were transferred to either solid complete medium (CK) or medium without P (–P) for 7 d (A). For determination of soluble P, 6-week-old wild type and *abi4* seedlings were subjected to P-sufficient or -deficient solution for 7 d, prior to determination of the soluble P concentration in the root (B) and shoot (C). The relative cell wall-adsorbed P in the root (D) and shoot (E) were calculated as described in Figure 3. Scale bar = 1 cm. Values are means ± SD, n = 4. Significant differences (P < 0.05) are denoted by different letters on the bars.

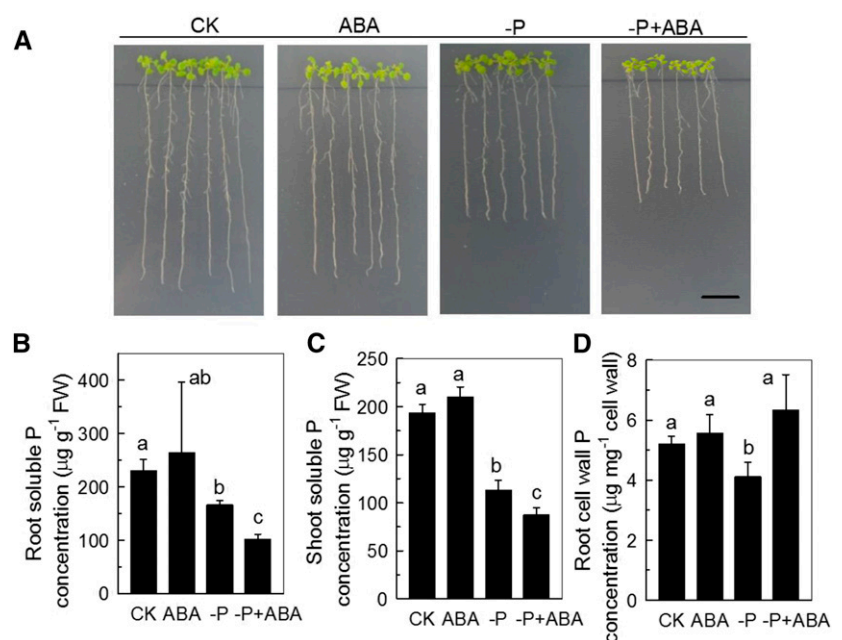


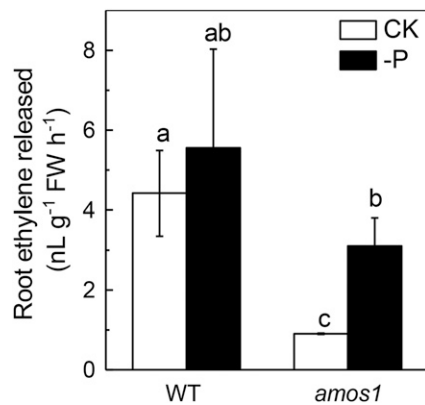
in the recycling of P deposited in the plant cell wall when external P becomes scarce. Our study establishes the role of the P repository in cell wall pectin in *amos1* under P deficiency, with greatly enhanced P release from the pectin fraction in the cell walls of *amos1* under P deficiency compared to P-replete conditions (Fig. 3A), accompanied by significant increases in the pectin concentration of the *amos1* root cell wall (Fig. 3B). As a result, less P was retained in the *amos1* cell wall under P deficiency when compared with wild type (Fig. 2), with elevated soluble P concentration in both roots and shoots of *amos1*, rendering it more resistant to P deficiency than wild type (Fig. 1; Supplemental Fig. S1). It is interesting that the expression of *PHR1*, *PHT1*, *PHO1*, and *PHO2* was consistently higher in *amos1* root compared with wild type in the absence of P starvation (Fig. 4), further indicating that the loss of function of AMOS1 itself mimicks the stress imposed by P deficiency; as a

result, *amos1* displayed retarded growth under P-replete (control) conditions (Fig. 1; Supplemental Fig. S1). However, *amos1* also showed reduced root and shoot growth under –K and –N conditions, which is consistent with the retarded growth under P-replete conditions (Fig. 1; Supplemental Figs. S1 and S2), indicating that *amos1* is uniquely resistant to P deficiency.

The finding that *amos1* accumulates more ABA under P-replete than under P-deficient conditions (Fig. 5) led us to hypothesize that ABA may be involved in the response to P deficiency in *amos1*. Our results here demonstrate that ABA is a negative regulator in the response to P deficiency, as exogenous application of ABA resulted in higher sensitivity while the loss-of-function mutant in a key transcription factor for ABA signaling, *abi4*, exhibited higher resistance to P deficiency (Figs. 6 and 7). This is in accordance with earlier reports that ABA is engaged negatively in the response

**Figure 7.** The effect of ABA on soluble P and on cell wall-retained P. A, Seedlings of WT were subjected to P-replete or -deficient solid medium, with or without 0.05 µM ABA for 7 d. For determination of soluble P concentration and cell wall P, seedlings were subjected to P-replete or -deficient solution, with or without 0.05 µM ABA for 7 d. B and C denote soluble P concentration in root and shoot respectively. D indicates cell wall-retained P in the root. Scale bar = 1 cm. Values are means ± SD, n = 4. Significant differences (P < 0.05) are denoted by different letters on the bars.





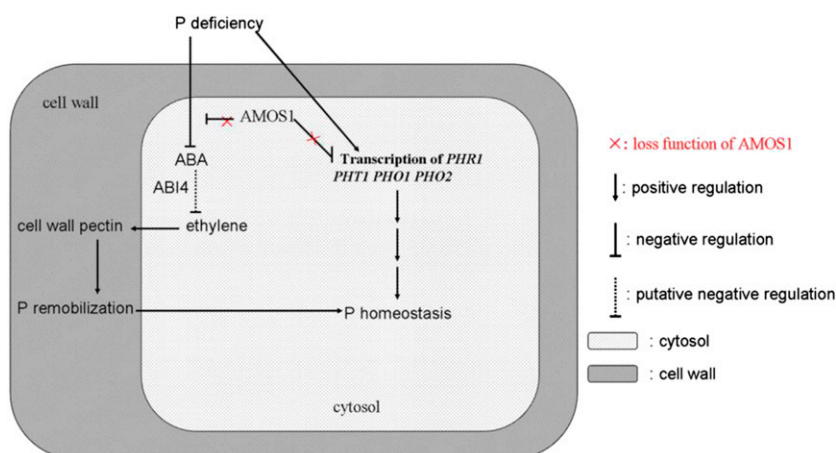
**Figure 8.** Ethylene production in roots of *amos1* and wild-type seedlings. Seedlings of *amos1* and wild type were grown hydroponically under P-replete or -deficient conditions for 7 d, followed by determination of ethylene using GC-MS. Values are means  $\pm$  SD,  $n = 4$ . Significant differences ( $P < 0.05$ ) are denoted by different letters on the bars.

to P deficiency as evidenced in the repressed transcription of several At4 family members, a group of highly conserved P-deficiency-induced riboregulators in Arabidopsis (Shin et al., 2006). In addition, Arabidopsis mutants exhibiting lowered sensitivity to ABA or impaired ABA biosynthesis, *abi2-1* and *aba1*, respectively, displayed reduced P-starvation response (PSR) gene expression when grown under low P supply (Trull et al., 1997; Ciereszko and Kleczkowski, 2002). However, defects in ABA signaling in these mutants did not affect other aspects typically related to P deficiency, such as enhanced phosphatase activity or altered root-to-shoot biomass ratio (Trull et al., 1997). Together, these data indicate that ABA is involved as a negative regulator in discrete, but not all, branches of the signaling responses engaged under P deficiency. The role of ABA in P deficiency is therefore complex, and the underlying physiological and molecular mechanisms remain elusive. Here, we demonstrate that

ABA can exacerbate sensitivity to P deficiency in Arabidopsis by reducing P remobilization from the cell wall, as seen in *amos1*, *abi4*, and wild type supplied with exogenous ABA. This negative role of ABA in P remobilization from plant-internal P repositories, thus, appears to constitute one of the branch points at which ABA affects plant response to P deficiency.

Several studies have also established the role of the gaseous hormone ethylene in the response to P starvation (Nagarajan and Smith, 2012). Several genomic studies have demonstrated that P deficiency alters expression of genes involved in both ethylene biosynthesis and signaling in the root (Thibaud et al., 2010; Chacon-Lopez et al., 2011). By means of modification of the cell wall, ethylene was shown to mediate resistance against fungal colonization (Lloyd et al., 2011), and, in a previous study, ethylene was shown to be a likely signal in P remobilization from the cell wall (Zhu et al., 2016). Thus, the fact that *amos1* accumulated more ABA (Fig. 5) and less ethylene compared to wild type under normal conditions (Fig. 8) suggests ABA may act against ethylene in the context of P deficiency. More recently, ABA was also found to inhibit ethylene biosynthesis via ABI4 (Dong et al., 2016). In agreement with these data, less ethylene was detected in the ABA-accumulating mutant *amos1* in our current study (Fig. 8), and this was furthermore consistent with enhanced P retention in the root cell wall, indicating that ABA may play a negative role in P remobilization from the cell wall.

The fact that the ammonium-responsive AMOS1 is also involved in the maintenance of cellular P homeostasis raised the possibility that the N-signaling pathway engages in crosstalk with P signaling. Indeed, several lines of evidence suggest the existence of such crosstalk: First, an E3 ubiquitin ligase, nitrogen limitation adaptation (NLA), was reported to serve as a regulator of both N and P homeostasis in Arabidopsis (Kant et al., 2011). Second, transcriptomic analysis has shown that several P-starvation-responsive genes identified previously (Müller et al., 2007), *MYB75*, *NF-YA10*, and *Phosphoenolpyruvate Carboxylase Kinase (PPCK)*, are



**Figure 9.** Schematic representation of P remobilization in *amos1*. Solid arrows and solid lines with perpendicular lines indicate positive and negative regulation patterns, respectively. Dashed lines indicate connections as verified by another study (Dong et al., 2016). P deficiency suppresses ABA production and increases transcription of genes responsible for P acquisition and transportation, which are negatively regulated by AMOS1. Ethylene biosynthesis was relieved of transcriptional repression mediated by ABI4, a downstream element of ABA signaling, due to a reduction in ABA levels. Enhanced ethylene production promotes cell wall pectin formation, which enables the release of P from the root cell wall, thus maintaining P homeostasis in *amos1* under P deficiency.

down-regulated in *amos1* by excessive ammonium treatment (Li et al., 2012). Third, Li et al. (2012) demonstrated that ABA signaling is recruited by AMOS1 under ammonium stress, while, in the current study, ABA signaling was found to be employed by AMOS1 under P deficiency. However, it has yet to be determined whether ethylene is involved under ammonium stress and whether N-acquisition-related genes alter their expression in response to P deficiency.

In conclusion, we demonstrate here, for the first time, that the plastid intramembrane protease AMOS1 is involved in the regulation of P remobilization from the root cell wall under P deficiency via recruitment of ABA-antagonized ethylene signaling in Arabidopsis (Fig. 9). Our study, thus, supports the importance of P remobilization from the plant cell wall under P deficiency, reveals a novel regulatory player in this process, and furnishes new evidence on the distinct roles of ABA and ethylene signaling during the important and widespread macronutritional stress of P deficiency.

## MATERIALS AND METHODS

### Plant Materials and Growth Conditions

Arabidopsis (*Arabidopsis thaliana*) wild type (Columbia ecotype, Col-0) and the mutants *amos1* and *abi4* in the Col-0 background were used in the current study. The nutrient solution was composed of macronutrients as follows, in mM: KNO<sub>3</sub>, 6.0; Ca(NO<sub>3</sub>)<sub>2</sub>, 4.0; MgSO<sub>4</sub>, 1; NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>, 0.1; and micronutrients as follows, in μM: Fe(III)-EDTA, 50; H<sub>3</sub>BO<sub>3</sub>, 12.5; MnSO<sub>4</sub>, 1; CuSO<sub>4</sub>, 0.5; ZnSO<sub>4</sub>, 1; H<sub>2</sub>MoO<sub>4</sub>, 0.1; NiSO<sub>4</sub>, 0.1, according to Murashige and Skoog (1962). Seeds were stored in an eppendorf tube in the dark at 4°C for 2 to 3 d, followed by surface-sterilization and sowing on solid complete medium (nutrient solution supplemented with 8% agar). For the P-deficiency (–P) assay on solid culture, NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> was replaced by the same concentration of NH<sub>4</sub>NO<sub>3</sub>. For the potassium-deficiency (–K) assay on solid culture, KNO<sub>3</sub> was omitted. For the nitrogen-deficiency assay (–N) on solid culture, 4 mM CaCl<sub>2</sub> and 3 mM K<sub>2</sub>HPO<sub>4</sub> were used. The same solid medium containing all nutrient elements as described above served as the control in –P, –K, and –N assays, respectively. Seedlings were maintained in a growth chamber at 23°C ± 1°C under a light intensity of 140 μmol m<sup>–2</sup> s<sup>–1</sup> and a photoperiod of 16 h of light and 8 h of dark.

For hydroponic culture, seedlings were first planted on the above solid Murashige and Skoog medium for 2 weeks, and the young plantlets were placed on vermiculite for an additional 3 weeks in controlled-environment chambers. Seedlings of similar rosette diameter were then transferred to the nutrient solutions containing the above-described Murashige and Skoog salts for another week. Then, plants were subjected to the following treatments: +P (complete nutrient solution), –P (P withdrawn from the control solution), and +P and –P with or without ABA and ACC (0.05 μM ABA or 1 μM ACC were added where indicated).

### Determination of Soluble P Concentration

Fresh weights of root and leaves of Arabidopsis were determined, followed by tissue homogenization with mortar and pestle in liquid nitrogen. Inorganic phosphate was extracted with 4 mL of 5% (v/v) sulphuric acid (5 M) solution. After centrifugation at 12,000 g, 400 μL of supernatant was transferred and mixed with 200 μL of 15% (w/v) freshly prepared ascorbic acid (pH 5.0) dissolved in ammonium molybdate. The mixture was incubated at 37°C for 30 min, and the A<sub>650</sub> was recorded. P concentration was calculated by normalization to fresh weight.

### Cell Wall Extraction and Fractionation, and Pectin Measurement

Extraction of crude cell wall materials from root and shoot, and subsequent fractionation of cell wall components, were carried out as previously described (Zhu et al., 2015).

Uronic acid concentration in pectin was determined using GalUA (Sigma) as a standard (Blumenkratz and Asboe-Hansen, 1973). Briefly, 200 μL pectin extracts were treated with 1 mL 98% H<sub>2</sub>SO<sub>4</sub> (containing 0.0125 M Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O) and incubated at 100°C for 5 min. After chilling, 20 μL m-hydroxy-diphenyl (0.15%) was applied to the solution and kept for 20 min at room temperature, followed by measuring the A<sub>520</sub> with spectrophotometry.

### P Retention in the Cell Wall

A total of 5 mg cell wall material was placed into a 2-mL eppendorf tube to which 2 N HCl had been added. That solution was shaken on a rotary shaker for 3 d. Subsequently, supernatant was collected by centrifugation at 23,000 g and determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES; Fisons ARL Accuris, Ecublens, Switzerland). The P-adsorption ability of cell wall material was assessed according to the P concentration in the –P plants compared to that in the +P plants.

### Quantitative Real-Time PCR Analysis

Total RNA was isolated from root using TRIzol reagent (Invitrogen). 1 μg of total RNA served as a template, and cDNA was reversely transcribed using the PrimeScript RT reagent kit (Takara). For the quantitative analysis of gene expression, 1 μL of 10-fold-diluted cDNA was used as the template, performed with SYBR Premix ExTaq (TaKaRa) using the following primer pairs: for *TUBULIN*, forward: 5'-AAGTCTCTGGGAAGTGGTT-3' and reverse: 5'-CTCCCAATGAGT-GACAAA-3'; *AtPHR1*, forward: 5'-ACAGCAATAACGGAACCGGCAAG-3' and reverse: 5'-GCTCTTCTACTACCGCAAGACTG-3'; *AtPHT1*, forward: 5'-GCCAAGGTAGACGCAGGATA-3' and reverse: 5'-AACCTCAG CTCAC-CAGAGA-3'; *AtPHO1*, forward: 5'-TACGCGAGAGAAA ACAACGA-3' and reverse: 5'-TTCCGGAGAACCAAATTGTC-3'; *AtPHO2*, forward: 5'-TTTACACAAGCCACCAAAGC-3' and reverse: 5'-TCACGAGCATGTCCAACAA-3'. Each cDNA sample was run in triplicate. Relative quantification values for each target gene were calculated by the 2<sup>–ΔΔCT</sup> method (Livak and Schmittgen, 2001) using *TUBULIN* as a reference gene.

### Measurement of Ethylene and ABA Production

Determination of ethylene production was conducted as previously described (Li et al., 2013). Endogenous ABA accumulation was quantified by the ELISA as described in Yang et al. (2001).

### Data Analysis

All histograms in “Results” are shown as the mean ± SD for one set of representative data from four independent replicates. Data were subjected to one-way ANOVA, and the means were compared by Student's *t* test. Bars with different letters mark statistical significance at the *P* < 0.05 level.

### Supplemental Data

The following supplemental materials are available.

**Supplemental Figure S1.** Effect of phosphate bioavailability on whole-plant growth in Arabidopsis.

**Supplemental Figure S2.** Effect of potassium and nitrogen bioavailability on whole-plant growth in Arabidopsis.

**Supplemental Figure S3.** Effect of phosphate bioavailability on whole-plant growth of wild-type and *abi4*.

**Supplemental Figure S4.** The influence of ABA on whole-plant growth.

**Supplemental Figure S5.** The influence of ACC on whole-plant growth.

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