Plant Signaling & Behavior 10:7, e1035851; July 2015; © 2015 Taylor & Francis Group, LLC

Arabidopsis phosphatase under-producer mutants *pup1* and *pup3* contain mutations in the *AtPAP10* and *AtPAP26* genes

Ye Zhang, Xiaoyue Wang, and Dong Liu*

MOE Laboratory of Bioinformatics; Center for Plant Biology; School of Life Sciences; Tsinghua University; Beijing, China

Keywords: acid phosphatase, *AtPAP10*, *AtPAP26*, phosphatase under-producer mutant, Phosphate deficiency response, *pup1*, *pup3*

Production and secretion of acid phosphatases (APases) is a hallmark adaptive response of plants to phosphate (Pi) deprivation. Researchers have long hypothesized that Pi starvation-induced APases are involved in internal Pi recycling and remobilization as well as in external Pi utilization. Two phosphatase under-producer (*pup*) mutants, *pup1* and *pup3*, were previously isolated in *Arabidopsis*. Characterization of these 2 *pup* mutants provided the first genetic evidence for the above hypothesis. To date, however, the molecular lesions in these 2 *pup* mutants remain unknown. In this work, we demonstrate that *pup1* and *pup3* contain point mutations in the *Arabidopsis* purple acid phosphatase gene *AtPAP10* and *AtPAP26*, respectively. Our results answer a long-standing question about the molecular identity of the *PUP1* and *PUP3* genes and corroborate the conclusions from previous studies regarding the function of AtPAP10 and AtPAP26 in plant acclimation to Pi deprivation.

Phosphorus (P) is an essential macronutrient required for plant growth and development. Inorganic phosphate (Pi) is the major form of P that plants uptake from soil. In most soils, however, the majority of P exists in organic form,[1] and the deficiency of Pi in soil has become an important limiting factor for agriculture production.

When grown under Pi deficiency, plants produce and secret acid phosphatases (APases).[1] These enzymes catalyze the hydrolysis of Pi from various Pi monoesters and anhydrides in the acidic pH range. Researchers have long proposed that the intracellular APases are involved in the recycling and remobilization of internal Pi, while external or secreted APases play a dominant role in the utilization of organic P from the rhizosphere. The genetic evidence supporting this hypothesis, however, came only after the isolation of 2 *Arabidopsis* (*Arabidopsis thaliana*) phosphatase under-producer (*pup*) mutants, *pup1* and *pup3*.[2,3] These 2 mutants have reduced APase activity on their root surface as revealed by histochemical staining. In addition, *pup1* and *pup3* lack or have reduced production of certain APase isoforms. When grown in soil in which organic P is the major source of P, both *pup1* and *pup3* accumulate less Pi in their shoots and roots than the wild type (WT).[3] Although 10 y have past since these 2 mutants were genetically and physiologically characterized, the molecular lesions in *pup1* and *pup3* remain unidentified.

Arabidopsis contains 29 purple acid phosphatase (AtPAP) genes.[4] In this *AtPAP* gene family, *AtPAP10, AtPAP12*, and *AtPAP26* form a subgroup based on their protein sequences. AtPAP12 and AtPAP26 are major intracellular and secreted APases in *Arabidopsis*[5-7] while AtPAP10 is a major secreted APase and is predominantly associated with the root surface.[8] The transcription of *AtPAP10* and *AtPAP12* genes is Pi starvation-inducible whereas the transcription of *AtPAP26* is not Pi-dependent.[8,9] Furthermore, we found that sucrose and ethylene are 2 positive regulators of root-associated AtPAP10 APase activity.[10,11] And interestingly, the effect of ethylene on the induction of root-associated AtPAP10 activity depends on sucrose, but that the effect of sucrose does not depend on ethylene.[12] Knockout of *AtPAP10, AtPAP12*, or *AtPA26* reduces plant growth when the major source of P in the growth medium is in organic form.[8,9,13,14] In contrast, overexpression of either of these 3 APase genes causes plants to grow better in the same medium.[7,8] Because the *pup1 and pup3* mutants display similar phenotypes as *atpap10* and *atpap26*, we speculated that these 2 *pup* mutants might have mutations in the *AtPAP10* and *AtPAP26* genes.

To identify the molecular lesions in *pup1* and *pup3*, we obtained the *pup1* and *pup3* mutants that were originally described by Trull & Deikman[2] and Tomscha et al.[3] from the Arabidopsis Biological Resource Center. Root surface-associated APase activity can be detected by applying a substrate of APase, BCIP (5-bromo-4-chloro-3-indolyl-phosphate), to the root surface. The cleavage of BCIP by APases produces a blue precipitate. The root surface of *pup1* mutant (in the Wassilewskija (WS) ecotype background) completely lacked BCIP staining (**Fig. 1A**). This was similar to that of the *Arabidopsis nop1* mutant (in the Columbia (Col) ecotype background) that contains a mutation in the *AtPAP10* gene.[8] Under our experimental conditions,

*Correspondence to: Dong Liu; Email: dongliu64@yahoo.com
Submitted: 02/20/2015; Revised: 03/19/2015; Accepted: 03/23/2015
http://dx.doi.org/10.1080/15592324.2015.1035851

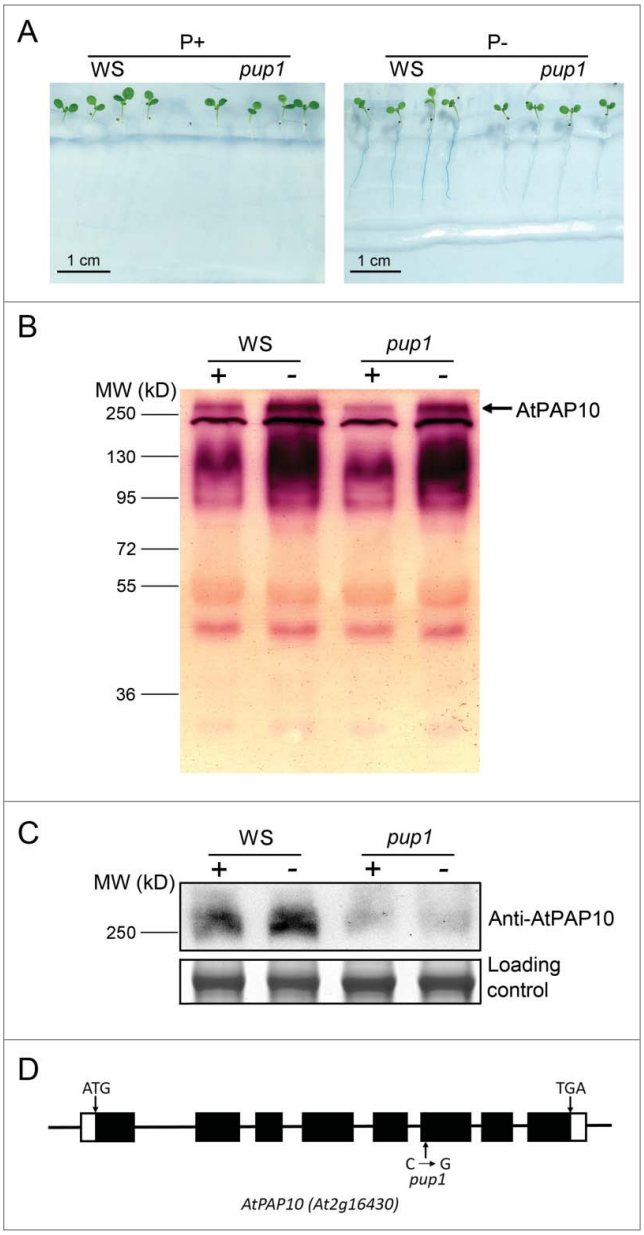

Figure 1. Characterization of *pup1*. (**A**) Histochemical staining of root-surface APase activity of 8-day-old *Arabidopsis* seedlings using BCIP as the APase substrate; (**B**) Analysis of APase profiles of 8-day-old *Arabidopsis* seedlings using an SDS-denatured in-gel assay; (**C**) Western blot of the proteins displayed in (**B**) using anti-AtPAP10 antibodies; In (**A**), (**B**), and (**C**), P+: Pi sufficiency, P-: Pi deficiency; in (**B**) and (**C**), molecular weight (MW) is indicated on the left. (**D**) A diagram showing the position of the point mutation in the *AtPAP10* gene. Boxes: exons; the single line between the boxes: intron; Empty boxes: untranslated regions; Black boxes: coding sequences; ATG: start codon; TGA: stop codon. The AGI code of the *APAP10* gene is shown in parenthesis.

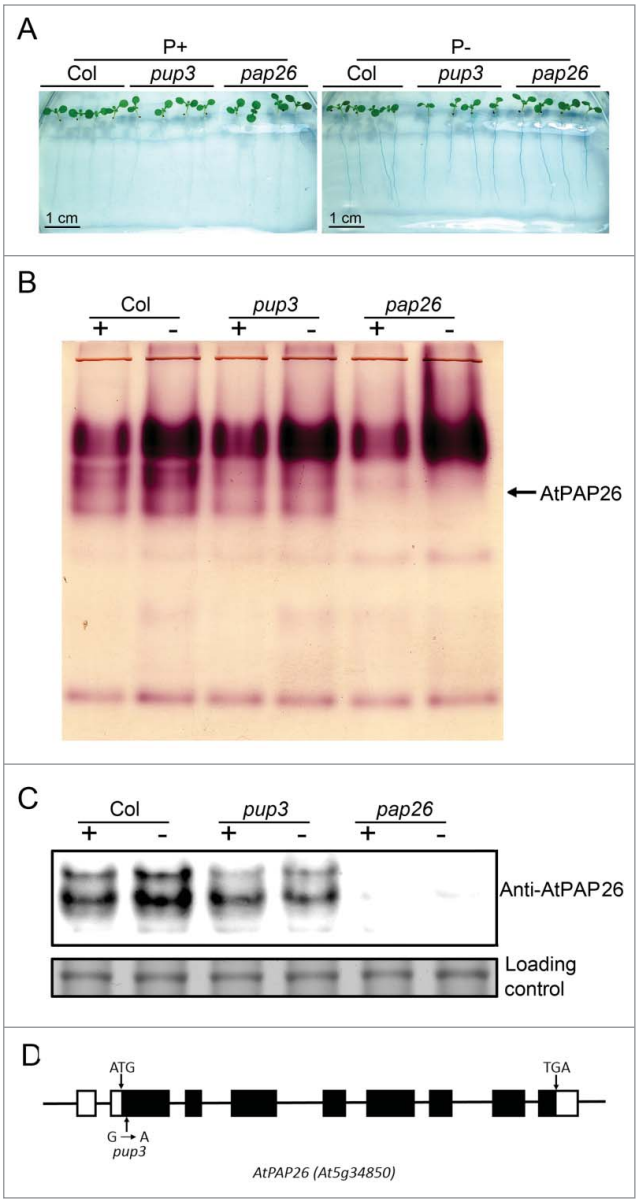

Figure 2. Characterization of *pup3*. (**A**) Histochemical staining of root-surface APase activity of 8-day-old *Arabidopsis* seedlings using BCIP as the APase substrate; (**B**) Analysis of APase profiles of 8-day-old *Arabidopsis* seedlings using a non-denatured in-gel assay; (**C**) Western blot of the proteins displayed in (**B**) using anti-AtPAP26 antibodies. In (**A**), (**B**), and (**C**), P+: Pi sufficiency, P-: Pi deficiency. (**D**) A diagram showing the position of the point mutation in the *AtPAP26* gene. Boxes: exons; the single line between the boxes: intron; Empty boxes: untranslated regions; Black boxes: coding sequences; ATG: start codon; TGA: stop codon. The AGI code of the *APAP26* gene is shown in parenthesis.

however, we did not observe the absence of an APase isoform in the in-gel assay as reported by Trull & Deikman.[2] Instead, we observed a reduction in the intensity of the top band in the denatured in-gel assay (**Fig. 1B**). This result was consistent with our observations of the *nop1* mutant.[7] In our previous studies, we showed that this top band contained a mixture of AtPAP10 and AtPAP12 proteins.[7] The remaining activity in this top band in *pup1* probably represented the activity of AtPAP12. To determine whether the reduction of the activity in the top band was due to the defect in AtPAP10 production, we transferred the proteins displayed in the in-gel assay to a PVDF membrane and performed a Western blot using a monoclonal anti-AtPAP10

antibody. For the WT plant with the WS ecotype background, there was a basal level accumulation of AtPAP10 proteins at the position corresponding to the top band shown in the in-gel assay (**Fig. 1C**). When grown under Pi deficiency, the level of AtPAP10 in the WT was increased whereas the AtPAP10 protein in *pup1* was not detectable under both Pi-sufficiency and -deficiency conditions. In *pup1*, the level of *AtPAP10* mRNA was also decreased to the half of that of the WT under P- condition (**Fig. S1A**). Finally, we sequenced the *AtPAP10* gene in *pup1* and found a point mutation in its sixth exon. This C to G mutation led to a conversion of a glutamine to a glutamate at amino acid position 294 (**Fig. 1D**). The above results indicated that this mutation might decreases the stability of both AtPAP10 mRNA and protein. It was interesting that such a small change in the structure of an amino acid completely eliminated the accumulation of AtPAP10 proteins. This is probably due to a change of charge in an amino acid which affects the protein stability.

Under our experimental conditions, the *pup3* mutant did not exhibit an obviously reduced BCIP staining on its root surface as reported by Tomscha et al.[3] (**Fig. 2A**); but we did observed that 2 APase isoforms were less abundant in the *pup3* mutant than in the WT in a non-denatured in-gel assay (**Fig. 2B**), which was consistent with that reported by Tomscha et al.[3]. In the *atpap26* T-DNA knockout mutant (SALK_152821), these 2 isoforms were not detected, indicating that the 2 reduced isoforms corresponded to AtPAP26. The molecular identity of these 2 isoforms was confirmed by Western blot using anti-AtPAP26 antibodies (**Fig. 2C**). Tomscha et al.[3] also found that these 2 reduced APase isoforms could react with anti-AtPAP12 antibodies. This was not surprising because, as previously demonstrated, the anti-AtPAP12 antibodies that they used cross-reacted with AtPAP26.[6,9] In *pup3*, the expression level of *AtPAP26* mRNA was similar to that of the WT (**Fig. S1B**). Finally, we sequenced the *AtPAP26* gene in *pup3* and found a point mutation in the second exon immediately after the start codon (**Fig. 2D**). This G to A mutation caused a conversion of a glycine to a glutamate at the amino acid position 20. The above results indicated that this point mutation did not affect the stability of *AtPAP26* mRNA but reduced the accumulation of AtPAP26 proteins.

Conclusions

In summary, we demonstrated that the previously reported *Arabidopsis* phosphatase under-producer mutants *pup1* and *pup3* contain mutations in the *AtPAP10* and *AtPAP26* genes, respectively. These results answer long-standing questions about the identity of the *PUP1* and *PUP3* genes and add to the genetic evidence that these 2 APases function in plant adaptation to Pi deprivation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank the Arabidopsis Biological Resource Center for providing the seed stocks of the *pup1* and *pup3* mutants and the *AtPAP26* T-DNA insertion line (SALK_152821). We also thank Prof. William Plaxton of Queen's University (Canada) for providing anti-AtPAP26 antibodies.

Funding

This work was supported by funds from the National Natural Science Foundation of China (grant no. 31370290) and the Ministry of Agriculture of China (grant no. 2014ZX0800932B).

Supplemental Material

Supplemental data for this article can be accessed on the publisher's website.

References

1. Tran HT, Hurley BA, Plaxton WC. Feeding hungry plants: The role of purple acid phosphatases in phosphate nutrition. Plant Sci 2012; 179:14-27; http://dx.doi.org/10.1016/j.plantsci.2010.04.005
2. Trull MC, Deikman D. An Arabidopsis mutant missing one acid phosphatase isoform. Planta 1998; 206:544-50; PMID:9821687; http://dx.doi.org/10.1007/s004250050431
3. Tomscha JL, Trull MC, Deikman J, Lynch JP, Guiltinan MJ. Phosphatase under-producer mutants have altered phosphorus relations. Plant Physiol 2004; 135:334-45; PMID:15122033; http://dx.doi.org/10.1104/pp.103.036459
4. Li D, Zhu H, Liu K, Liu X, Leggewie G, Udvardi M, and Wang D. Purple acid Phosphatases of *Arabidopsis thaliana* – comparative analysis and differential regulation by phosphate deprivation. J Biol Chem 2002; 277:27772-81; PMID:12021284; http://dx.doi.org/10.1074/jbc.M204183200
5. Veljanovski V, Vanderbeld B, Knowles VL, Snedden WA, Plaxton WC. Biochemical and molecular characterization of AtPAP26, a vacuole purple acid phosphatase up-regulated in phosphate-deprived *Arabidopsis* suspension cells and seedlings. Plant Physiol 2006; 142:1282-93; PMID:16963519; http://dx.doi.org/10.1104/pp.106.087171
6. Tran HT, Qian WQ, Hurley BA, She Y, Wang D, Plaxton WC. Biochemical and molecular characterization of AtPAP12 and AtPAP26: the predominant purple acid phosphatase isozymes secreted by phosphate-starved *Arabidopsis thaliana*. Plant Cell Environ 2012; 33:1789-803; http://dx.doi.org/10.1111/j.1365-3040.2010.02184.x
7. Wang L, Lu S, Zhang Y, Li Z, Du X, Liu D. Comparative genetic analysis of Arabidopsis purple acid phosphatases AtPAP10, AtPAP12, and AtPAP26 provides new insights into their roles in plant adaptation to phosphate deprivation. J Integr Plant Biol 2014; 56:315-32; PMID:24325391; http://dx.doi.org/10.1111/jipb.12144
8. Wang L, Li Z, Qian W, Guo W, Gao X, Huang L, Wang H, Zhu H, Wu J, Wang D, Liu D. The Arabidopsis purple acid phosphatase AtPAP10 is predominantly associated with the root surface and plays an important role in plant tolerance to phosphate limitation. Plant Physiol 2011; 157:1283-99; PMID:21941000; http://dx.doi.org/10.1104/pp.111.183723
9. Robinson WD, Park J, Tran HT, Del Vecchio HA, Ying S, Zins JL, Patel K, McKnight TD, Plaxton WC. The secreted purple acid phosphatase isozymes AtPAP12 and AtPAP26 play a pivotal role in extracellular phosphate scavenging by *Arabidopsis thaliana*. J Exp Bot 2012; 63:6531-42; PMID:23125358; http://dx.doi.org/10.1093/jxb/ers309
10. Lei M, Liu Y, Zhang B, Zhao Y, Wang X, Zhou Y, Raghothama K, Liu D. Genetic and genomic evidence that sucrose is a global regulator of plant responses to phosphate starvation in Arabidopsis. Plant Physiol 2011; 156:1116-30; PMID:21346170; http://dx.doi.org/10.1104/pp.110.171736
11. Lei M, Zhu C, Liu Y, Karthikeyan A, Bressan R, Raghothama K, Liu D. Ethylene signalling is involved in regulation of phosphate starvation-induced gene expression and production of acid phosphatases and anthocyanin in Arabidopsis. New Phytol 2011; 189:1084-95; PMID:21118263; http://dx.doi.org/10.1111/j.1469-8137.2010.03555.x
12. Zhang Y, Wang X, Lu Shan, Liu D. A major root-associated acid phosphatase in Arabidopsis, AtPAP10, is regulated by both local and systemic signals under phosphate

starvation. J Exp Bot 2014; 65: 6577-88; PMID:25246445; http://dx.doi.org/10.1093/jxb/eru377
13. Wang L, Liu D. Arabidopsis purple acid phosphatase 10 is a component of plant adaptive mechanism to phosphate limitation. Plant Signal Behav 2012; 7:306-10; PMID:22476468; http://dx.doi.org/10.4161/psb.19019
14. Hurley BA, Tran HT, Marty NJ, Park J, Snedden WA, Mullen RT, Plaxton WC. The dual-targeted purple acid phosphatase isozyme AtPAP26 is essential for efficient acclimation of Arabidopsis to nutritional phosphate deprivation. Plant Physiol 2010; 153:1112-22; PMID:20348213; http://dx.doi.org/10.1104/pp.110.153270