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The secreted purple acid phosphatase isozymes AtPAP12 and AtPAP26 play a pivotal role in extracellular phosphate-scavenging by *Arabidopsis thaliana*

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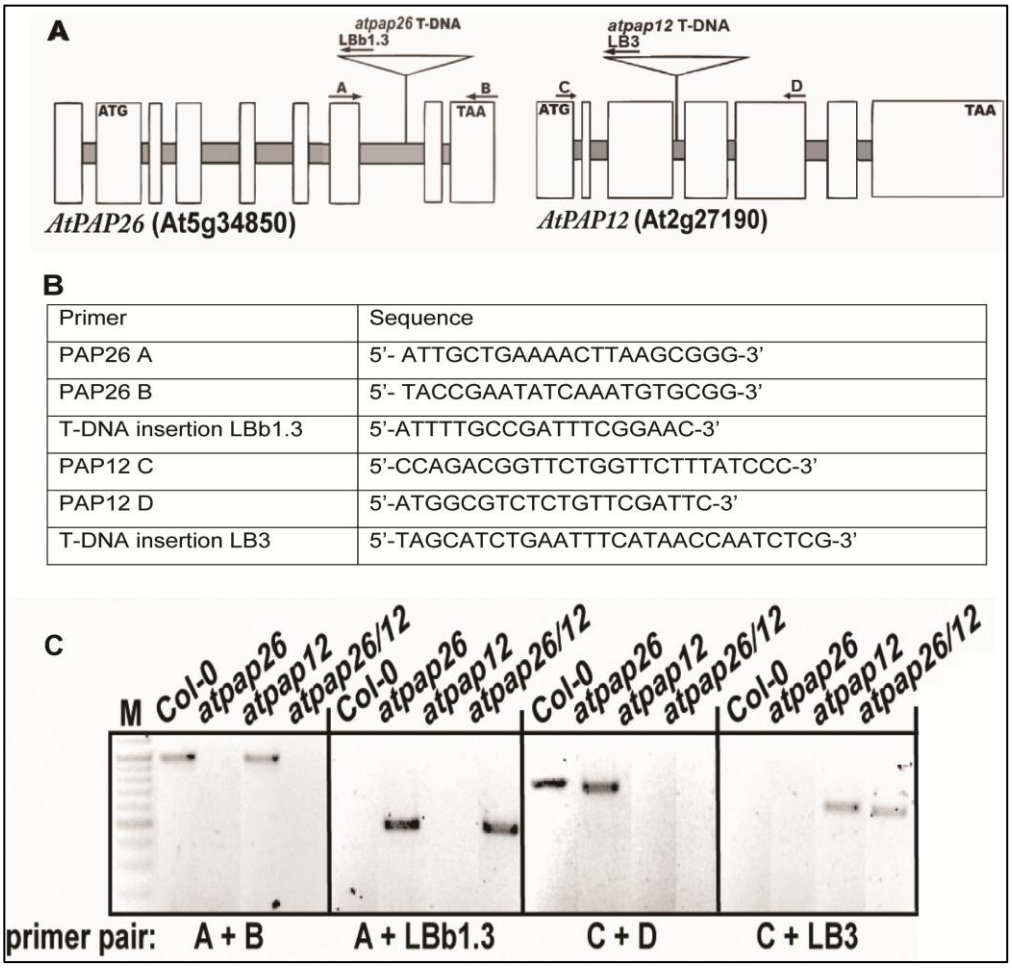
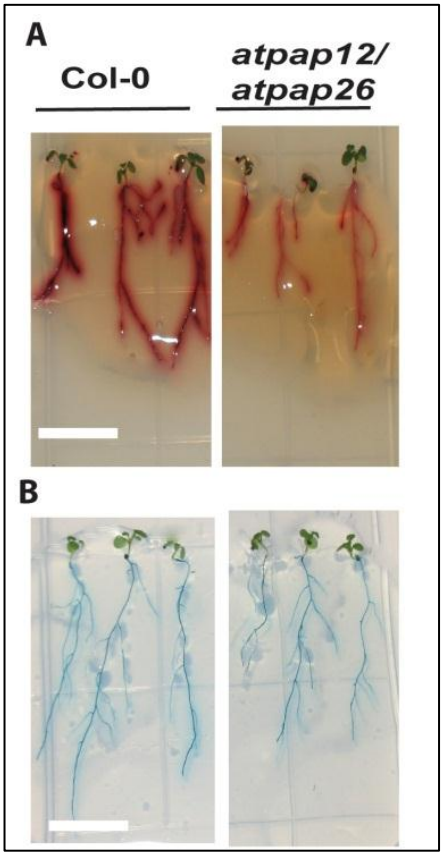
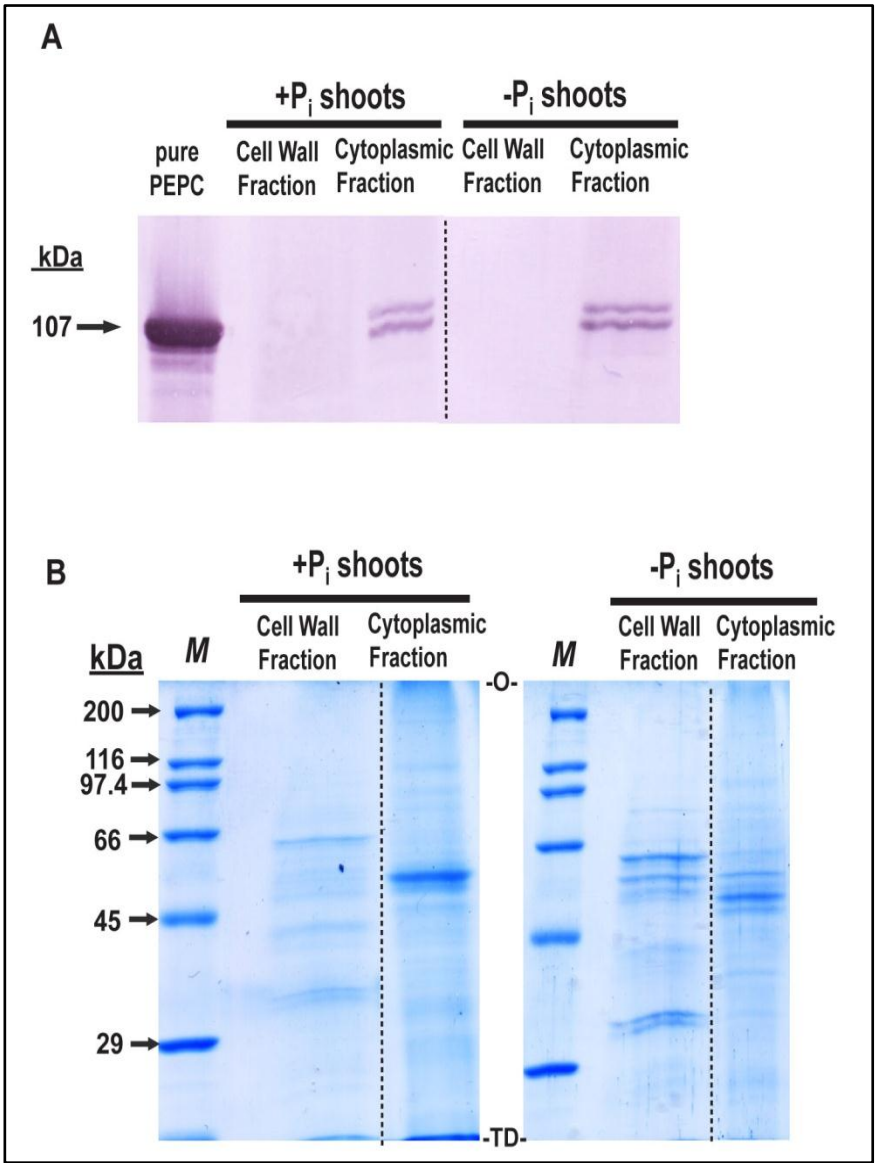
Supplementary Fig. S1. Confirmation of T-DNA insert location and loss of *AtPAP12* and/or *AtPAP26* gene expression in *atpap12*, *atpap26*, and *atpap12/atpap26* mutants. A. Schematic representation of the *AtPAP26* and *AtPAP12* genes; white boxes and solid lines represent exons and introns, respectively. T-DNA insertion locations are indicated by *atpap12* and *atpap26* T-DNA, while arrows represent primers (B) used for RT-PCR and genotyping. C, Assessment of T-DNA location and homozygosity of mutants via PCR-based screening of gDNA template isolated from leaves of +P_i soil grown seedlings. PCR products were amplified using *AtPAP26*-specific primers (primer pair A+B) and *AtPAP12*-specific primers (primer pair C+D) from the Col-0, *atpap26*, *atpap12,* and *atpap26/atpap12* gDNA. The lack of the *AtPAP26* DNA band in *atpap26* and *atpap26/atpap12* demonstrates that the mutant is homozygous. Comparable results were obtained with the *atpap12* mutant, where the *AtPAP12* DNA band was absent in both the *atpap12* and *atpap26/atpap12* mutants. T-DNA insertion positions were confirmed using a left-border primer (LBb1.3 and LB3) and gene specific primers (A and C) respectively for *atpap26* and *atpap12* mutants.



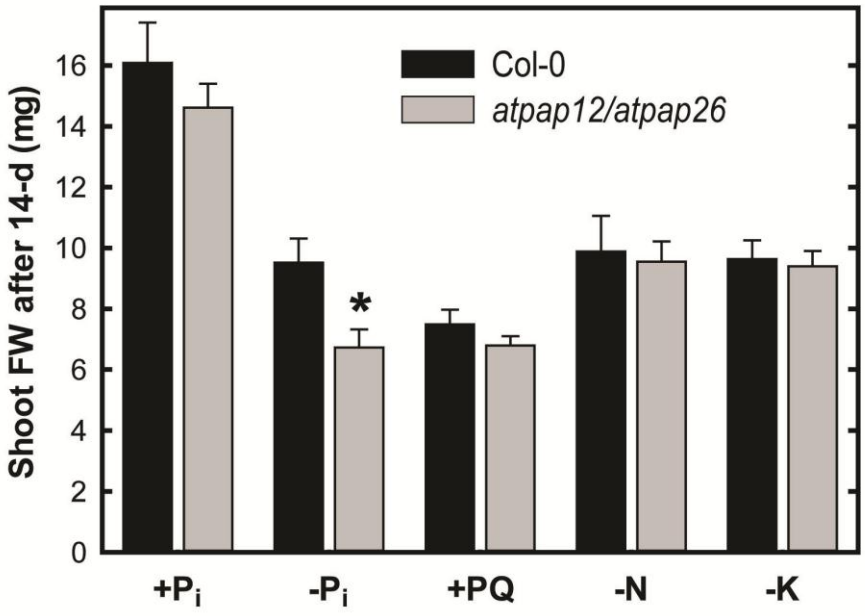
Supplementary Fig. S2. Histochemical staining of root surface APase activity in Col-0 and *atpap12/atpap26* seedlings using ß-naphthyl-P or BCIP. Plants were cultivated for 2 weeks on vertically oriented agar plates containing 0.5x MS media, 1% (w/v) sucrose, and 50 µM Pi before staining. A. Roots were rinsed in sodium acetate buffer (0.1 mM, pH 5.0) and covered with an agarose substrate mixture containing 200 mM acetate buffer, pH 5.0, 0.2% (w/v) β-naphthyl-P, and 0.2% (w/v) diazo blue B (O-dianisidine tetrazotized) (Gilbert *et al*., 1999); a dark purple/red color indicates APase activity. B. Root segments were embedded in an agarose substrate mixture containing 0.01% (w/v) BCIP (Wang et al., 2011); the blue color indicates APase activity. Images shown in panel A and B are representative of at least three biological replicates; scale bar = 1 cm.



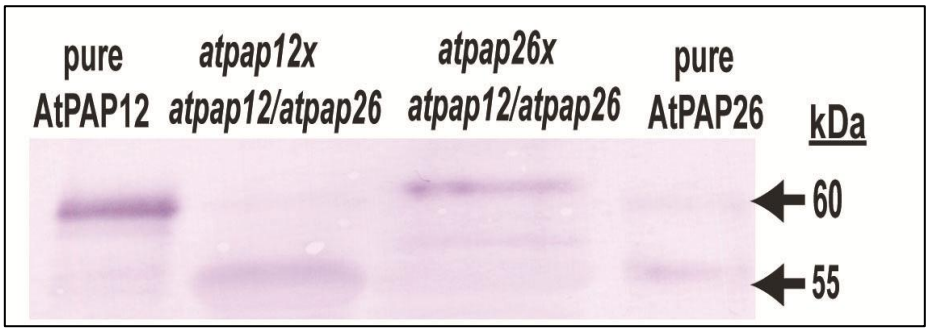
Supplementary Fig. S3. Immunoblot and SDS-PAGE analysis of cytoplasmic and cell wall extracts isolated from shoots of $+P_i$ versus $-P_i$ Col-0 Arabidopsis seedlings. Seedlings were cultivated for 7-d in liquid 0.5x MS media containing 0.2 mM Pi before being transferred into 2.5 or 0 mM Pi ($+P_i$ and $-P_i$, respectively) for an additional 7-d. Soluble cytoplasmic and concentrated cell wall proteins extracted from rosette leaves of the $-P_i$ and $+P_i$ seedlings were resolved using 10% SDS-PAGE mini-gels (30 µg protein/lane) and (A) subjected to immunoblot analysis using affinity-purified rabbit anti-(castor bean PEPC)-IgG, or (B) stained with Coomassie Blue R-250 to visualize total protein; *M*, molecular mass standards; TD, tracking dye front. The immunoreactive PEPC polypeptides shown in panel A were detected as described in the Materials and Methods; 'Pure PEPC' represents 100 ng of homogeneous PEPC isolated from endosperm of developing castor beans.



Supplementary Fig. S4. Influence of nutrient deprivation or oxidative stress on growth of *atpap12/atpap26* and Col-0 seedlings. Shoot fresh weight of seedlings cultivated on horizontal agar plates containing 1.5 mM P_i for 7-d, then grown for an additional 7-d on media containing 1.5 mM or 50 µM P_i (+P_i and -P_i, respectively), or on +P_i media lacking nitrogen (-N) or potassium (-K), or containing 1 µM paraquat (PQ). All values represent means ±SE of *n* = 12 different seedlings from four different plates; asterisks denote values that are significantly different from Col-0 (*P* < 0.01).



Supplementary Fig. S5. Immunoblot analysis of AtPAP12 and AtPAP26 polypeptides in clarified rosette extracts of 21-d-old Arabidopsis plants cultivated in -P_i soil. The *atpap12/atpap26* double mutant was backcrossed with *atpap12* and *atpap26* single mutants to restore the expression of AtPAP26 and AtPAP12, respectively (for details see text and legend for Fig. 5). Protein extracts (5 µg/lane) as well as homogeneous native AtPAP12 and AtPAP26 (50 ng each) (Tran *et al*., 2010a) were subjected to immunoblotting using anti-AtPAP12 as described in the Materials and Methods.



Supplementary Fig. S6. A classification scheme for Arabidopsis PAPs based on clustering analysis of amino acid sequences. The main groups (groups I, II, and III) are further divided to yield the 8 subgroups (second column). The bootstrap values for the three main groups are boxed, whereas those for the 8 subgroups are indicated by arrows. The predicted molecular masses of the deduced polypeptides are listed in the third column. Figure modified from Li *et al.* (2002).