Supporting Information

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SI Text

Evaluation of GMPase Protein Levels. Total RNA samples were extracted from the desired *Arabidopsis* tissues using the TriZol reagent (Gibco-BRL). The oligonucleotide primer pairs for amplifying GMPase transcripts were 5′-CGCTGGTCCTCTG-GCTCTA-3′ and 5′-AGTCTCAAACCCGTTATGTAGTCA-3′. The amplification of actin transcripts was used as internal controls for normalizing cDNA samples and monitoring the dynamics of thermoamplification during PCR. The primer pairs for amplifying actin transcripts were 5′-TCTCTATGCCAGTG-GTCGTA-3′ and 5′-CCTCAGGACAACGGAATC-3′. The WT *Arabidopsis* GMPase-coding sequence, amplified by RT-PCR using the primers 5′-CTCATATGATGAAGGCACT CATTC-3′ and 5′-CTAAGCTTCATCACTATCTCTGG-3′ (the underlined nucleotides form the restriction sites for NdeI and HindIII, respectively), was cleaved with NdeI and HindIII, followed by cloning into pET30a. The resulted construct was used to express recombinant GMPase in the bacterial cells. The bacterially expressed GMPase was purified, verified, and used to prepare a polyclonal antiserum specific for *Arabidopsis* GMPase as described above. For comparing GMPase protein levels by immunoblotting with the GMPase-specific polyclonal antibody, total proteins were extracted from *Arabidopsis* tissues, separated by 10% SDS/PAGE, and electroblotted onto nitrocellulose membrane as above. The immunodetection was accomplished as detailed (1).

1. Qian W, *et al.* (2007) Molecular and functional analysis of phosphomannomutase (PMM) from higher plants and genetic evidence for the involvement of PMM is ascorbic acid biosynthesis in *Arabidopsis* and *Nicotiana benthamiana*. *Plant J* 49:399–413.

Effect of Higher pH on *in Planta* GMPase Activity. To investigate whether higher pH in the growth medium may affect GMPase activity *in planta*, WT *Arabidopsis*, *hsn1*, and *vtc1* seedlings (8 DAG, germinated on NO_3^- medium, pH 5.7) were transferred onto 3 different growth media, respectively. The 3 media were NO_3^- + NH_4^+ (pH 5.7), NO_3^- (pH 5.7) and NO_3^- (pH 6.7). At 24 h after the transfer, the shoots and roots were separately harvested for extracting total proteins, which were then used for GMPase activity assay and protein blot analysis, respectively. The GMPase activity assay was conducted as described in *Materials and Methods*. Protein blot analysis was carried out as described above.

AsA Assay. Total AsA concentration in *Arabidopsis* tissues (μmol/g fresh weight) was determined by using the ascorbate oxidase assay as described (1).

Data Collection and Analysis. Replication experiments were designed and conducted to ensure the accuracy and reproducibility for each of the data sets obtained. The photographic data presented are generally representative of at least three sets of independent experiments. For the numerical data, the means and error bars were calculated by using the data from at least three sets of independent experiments. Statistic analyses of the quantitative data were carried out with the software program SPSS for Windows (SPSS; www.spss.com) with appropriate confidence intervals.

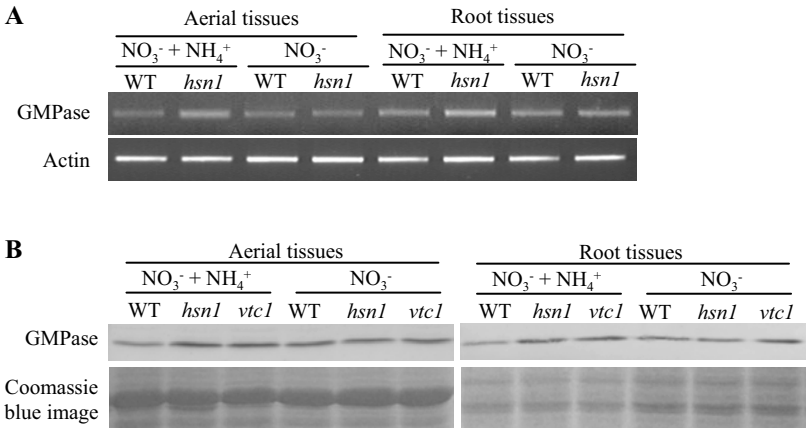

Fig. S1. Comparisons of the relative transcript and protein levels of GMPase in WT *Arabidopsis* and GMPase mutants (*hsn1*, *vtc1*). The data displayed are all representative of 3 independent sets of experiments. (*A*) GMPase transcript levels in the aerial and root tissues of WT *Arabidopsis* and *hsn1* seedlings (10 DAG) culture on $NO_3^- + NH_4^+$ or NO_3^- medium. The transcript levels shown were revealed using semiquantitative RT-PCR with normalized cDNA templates derived from total RNA samples. The amplification of actin transcripts was used as an internal control for RT-PCR. No major differences in the GMPase transcript level were found between WT *Arabidopsis* and *hsn1* tissues or among WT *Arabidopsis*, *hsn1*, and *vtc1* tissues (data not shown). (*B*) GMPase protein levels in the aerial and root tissues of WT *Arabidopsis*, *hsn1* and *vtc1* seedlings (10 DAG) culture on $NO_3^- + NH_4^+$ or NO_3^- medium. The GMPase levels displayed were resolved by protein blotting, conducted using total protein samples (30 μg each) and a polyclonal antibody specific for *Arabidopsis* GMPase. The Coomassis blue-stained gel images illustrate equal loading of total protein samples during SDS/PAGE.

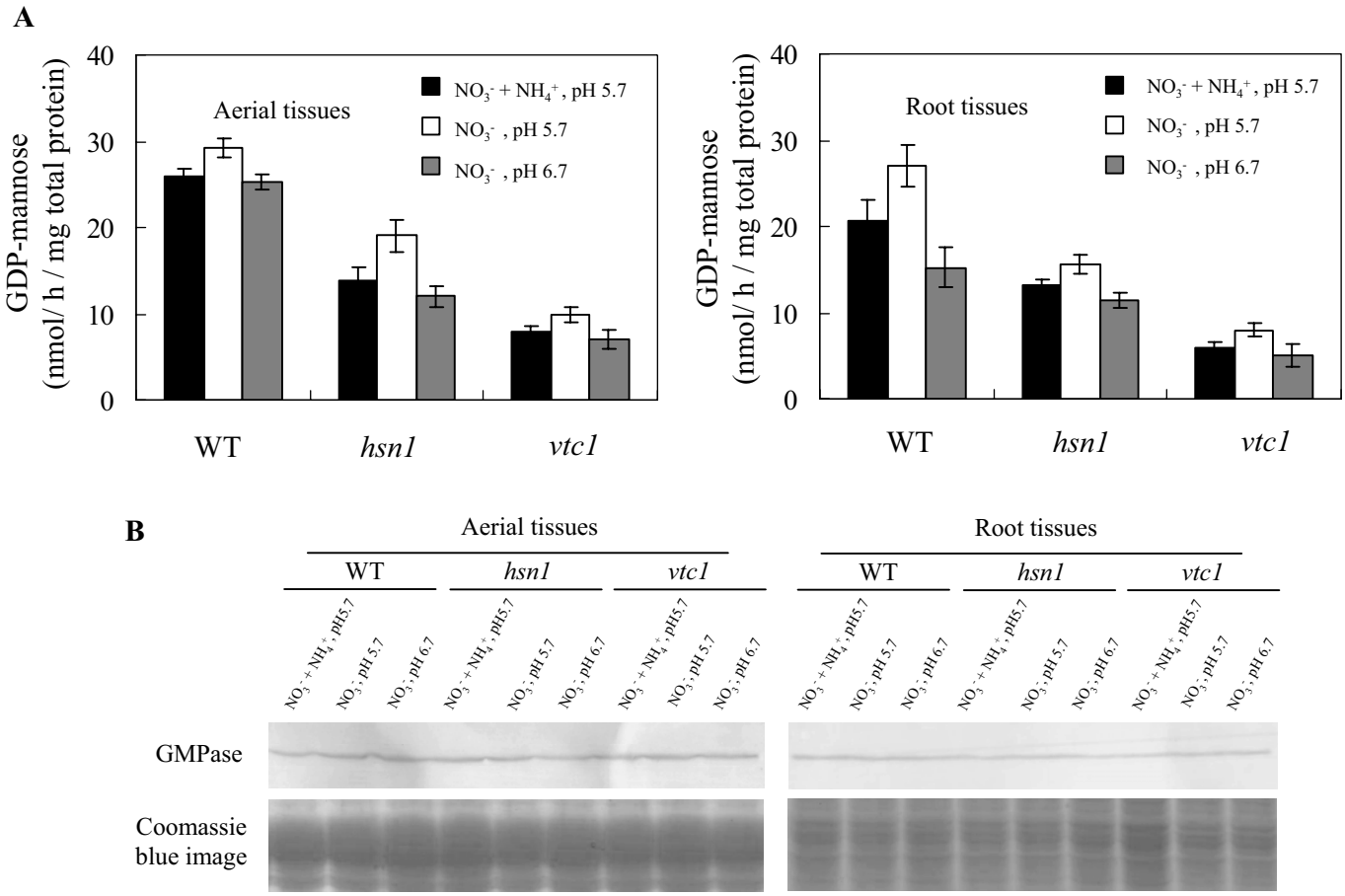

Fig. S2. Effect of higher pH on *in planta* GMPase activity in WT *Arabidopsis* and GMPase mutant (*hsn1*, *vtc1*). WT *Arabidopsis*, *hsn1* and *vtc1* seedlings (8 DAG, germinated on NO_3^- medium, pH 5.7) were transferred onto 3 different growth media, respectively. At 24 h after the transfer, the shoots and roots were separately harvested for extracting total proteins for GMPase activity assay and protein blot analysis, respectively. (*A*) The *in planta* GMPase activity levels in the arterial and root tissues of WT *Arabidopsis* and 2 GMPase mutant (*hsn1*, *vtc1*) seedlings cultured on 3 different growth media, NO_3^- + NH_4^+ (pH 5.7), NO_3^- (pH 5.7) and NO_3^- (pH 6.7). (*B*) Accumulation of GMPase protein in the aerial and root tissue samples that were assayed for GMPase activity levels in *A*. Total proteins (30 μg per sample) were resolved using SDS/PAGE. Protein blot analysis was conducted using a polyclonal antibody specific for *Arabidopsis* GMPase. The Coomassie blue-stained gel images show equal loading of total protein samples during SDS/PAGE.

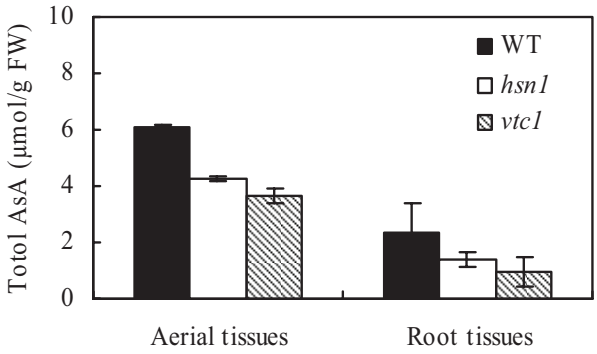

Fig. S3. Total AsA contents in the aerial and root tissues of WT *Arabidopsis*, *hsn1*, and *vtc1* seedlings (10 DAG) cultured on $NO_3^- + NH_4^+$ medium. The data points are each calculated using the measurements from 3 separate sets of assays. In either the aerial or root tissues, the total AsA contents of *hsn1* and *vtc1* were significantly lower than that of WT *Arabidopsis* ($P < 0.01$). The total AsA contents in the aerial and root tissues of *hsn1* and *vtc1* seedlings were also significantly lower than those of WT *Arabidopsis* controls when grown on NO_3^+ medium (data not shown).