Regulation of Arbuscular Mycorrhizal Phosphate Transporter Genes in Acidic Forest Soils.

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Background

- Many temperate hardwood forests of the Northeastern U.S. experience chronic acid deposition, which can dramatically lower soil pH. When soil pH is below 5, aluminum becomes soluble and can bind to inorganic phosphorus (P_i), rendering much of this P unavailable for plants.
- An association with mycorrhizal fungi may help forest plants overcome P limitation in acidic soils due to fungal hyphae acting as extensions to plants' root systems.
- The mechanisms by which mycorrhizal symbioses benefit plants in P-limited systems, however, remain largely unknown.

Hypothesis

We hypothesize that the activity of phosphate transporters, which are specific to arbuscular mycorrhizal fungi (AMF), is a primary mechanism by which forest plants can overcome P limitation in acidic soil conditions.

Rationale

- AMF possess high affinity inorganic phosphate transporters (PTs) in their hyphae, which transport P from the soil into the fungal cells (Figure 1).
- Under greenhouse and laboratory conditions, gene expression of AMF-specific PTs are altered by P concentrations, suggesting that acidic soil conditions can indirectly affect PTs.
- There is evidence to suggest that AMF-specific PTs are proton-coupled (Figure 2) and that soil acidity can affect their activity.

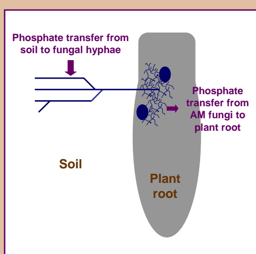


Figure 1. Diagram of phosphate transport in plants that form AM fungal associations. Fungal tissues are represented in blue with the thick lines of hyphae connecting the vesicles and arbuscules to the outside soil. Diagram is not to scale and is modified from Karandashov and Bucher (2005).

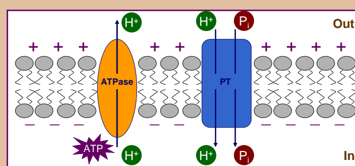
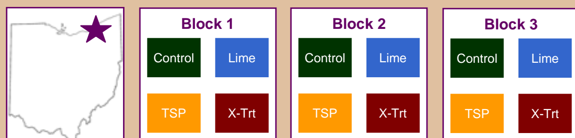


Figure 2. Diagram showing the mechanism of phosphate transport across the plasma membrane. ATPases actively transport H⁺ ions outside the cell, which creates a proton concentration gradient across the cell membrane. The membrane potential then allows for the symport of Pi and H⁺ ions into the cell via the PTs. Diagram is modified from Karandashov and Bucher (2005).

Study Sites



Plots: 20m x 40m (800 m²) plots are located in a mixed-mesophytic forest dominated by maple, oak, and beech. Soils are silt loams and are Alfisols (Endoaqualfs) with an ambient pH of around 4.

Treatments:

Control - ambient soil conditions

Elevated pH - lime amendment (11.5 Mg ha^{-1})

- High calcium lime was applied in October 2009 and 2010. Target soil pH = 5.5.

Elevated P - TSP amendment (4.2 g P m^{-1} y^{-2})

- Triple super phosphate (TSP) was added October 2009, March 2010, and May 2011.

X-Treatment - lime & TSP amendment Elevated pH & Elevated P

Methods

At four time points in the spring of 2011 (Table 1), four soil cores were sampled within each plot 1 m from the base of a randomly selected maple tree (*Acer* spp.). Maple trees were selected because they form associations with AMF. Soil cores were sieved to 4mm to separate roots from bulk soil. Roots were transferred to a cutting board and homogenized with a knife (Figure 3). Adhering rhizosphere soil was not washed from the roots. The uncleaned root samples were flash frozen with liquid N, transferred to the lab on dry ice, and stored at -70°C.

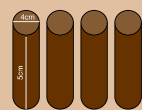


Figure 3. Sieving and homogenizing root samples in the field.

Table 1. Description of sampling times.

Sample Dates	Description
5/23/2011	Prior to TSP application in 2011 (TSP applied 5/24/2011)
6/1/2011	7 days post TSP application in 2011
6/8/2011	14 days post TSP application in 2011
6/22/2011	28 days post TSP application in 2011

Total nucleic acids were extracted from the uncleaned root samples with a standard phenol/chloroform procedure that used two replicate root samples from each plot. The extraction was partitioned into two extracted DNA samples that were combined and treated with DNase for one resulting RNA sample. cDNA was made from the extracted RNA using Superscript II Reverse Transcriptase (Life Technologies) and random primers (Figure 4). The quantity of PT genes found in the cDNA samples were analyzed with quantitative PCR using previously published primers (Sokolski *et al.*, 2011). cDNA samples were also analyzed with quantitative PCR using AMF-specific18S primers as a control.

M – Low mass ladder (Life Technologies)

DNA – PCR product using extracted nucleic acids as template

RNA – PCR product using DNase-treated nucleic acids as template

cDNA – PCR product using reverse-transcribed total RNA as template

Figure 4. PCR using the products of RNA extraction and reverse transcription as template and PT-specific primers on two samples.

Results

Chemical analyses on the bulk soil indicated significantly higher inorganic P in the TSP and X-Trt plots and significantly higher pH in the Lime and X-Trt plots (Figures 5 and 6).

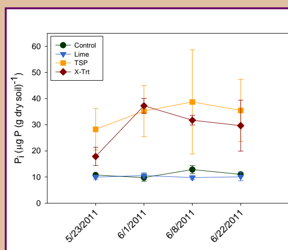


Figure 5. Inorganic P (mean ± SE) at each sampling point measured with a bicarbonate P extraction.

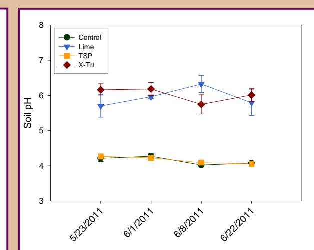


Figure 6. Soil pH (mean ± SE) at each sampling point measured with a pH-H_2O protocol.

Results, cont'd

Of the 48 samples that were collected, the PT gene was detected with QPCR in only nine of the cDNA samples. (In comparison, the AMF-specific 18S gene was detected in all 48 cDNA samples.) There was no relationship between PT gene copy number and P_i concentration in our nine samples (Figure 7) or between PT gene copy number and AMF-specific 18S gene copy number (Figure 8).

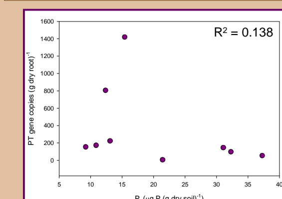


Figure 7. The relationship between PT gene copy number (determined by QPCR on cDNA samples from uncleaned roots) and inorganic P (measured with a bicarbonate P extraction on rhizosphere soil).

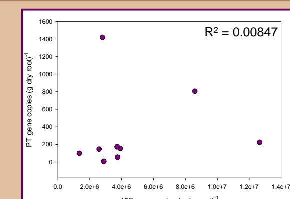


Figure 8. The relationship between PT gene copy number and AMF-specific 18S gene copy number (determined by QPCR on cDNA samples from uncleaned roots).

Of the nine cDNA samples where PT genes were detected, seven of them occurred in plots that received lime (Figure 9). Similarly, in the extracted DNA samples, the frequency of PT gene detection was higher in Lime and X-Trt plots (18 samples from plots receiving lime compared to three in Control and TSP plots) (Figure 10).

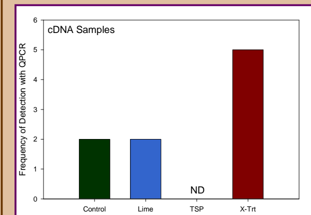


Figure 9. The frequency of PT gene detection in cDNA samples (determined with QPCR). ND = not detected.

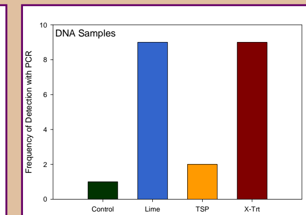


Figure 10. The frequency of PT gene that were PCR-amplified from DNA samples extracted with a phenol/chloroform protocol.

A clone library of PCR products using the same PT-specific primers was constructed using DNA extracts from AMF root samples collected in August and September of 2010. The top matches on Genbank for all 25 clones with a blastx search were to the same five AMF-specific phosphate transporter protein sequences (Table 2).

Table 2. Genbank matches of clones from test samples.

Genbank Match	Accession Number	Average % Identity	Average E-value
Rhizophagus intraradices phosphate transporter	AEK70396.1	92%	4.17e-23
Rhizophagus intraradices phosphate transporter	AAK72559.1	92%	2.08e-21
Rhizophagus intraradices phosphate transporter	AAL37552.1	92%	3.33e-21
Funneliformis mosseae phosphate transporter	AAZ22389.1	90%	8.33e-21
Glomus versiforme phosphate transporter	AAC49132.1	87%	3.75e-19

Conclusions

- The frequency of PT gene detection was higher in plots with elevated pH. This suggests either an increased level of detection due to higher AMF colonization or a possible community shift for AMF. Clone libraries on these samples will help to determine if the higher pH soils in the Lime and X-Trt plots supported different AMF taxa compared to Control and TSP plots.
- Though PT genes were successfully quantified in only nine cDNA samples, our data suggest no relationship between PT activity and P_i concentration, which is contrary to a number of laboratory studies. However, the highest frequency of PT gene detection for cDNA samples was in the X-Trt plots, suggesting that pH and P availability together may have played a role in PT gene regulation.

Acknowledgments

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