Conclusion

We have developed a protocol for isolation of root plastids from *Arabidopsis thaliana*. Localization to the root plastids was made possible by antibody specific Western Blotting. In-vitro radioactive transport experiments corroborate ANTR-3 activity to be dependent on a gradient either from H^+ or Na^+ ions playing a pivotal role in Pi transport. Incredibly large mutants with reduced starch levels in roots indicate disturbance in source/sink allocation of Pi.

Acknowledgement

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Localization, functional analysis and physiological role of Anion Transporter 3 (*Arabidopsis thaliana*)

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Background: Even though Phosphorus is a pivotal macronutrient for plant growth, it is least accessible in the soil. So plants have evolved an array of molecular and morphological strategies to cope up with this restraint which include inorganic phosphate(Pi) acquisition and recycling. This is made possible by specific Pi transporters one of with is the focus of this study—Anion Transporter-3 (ANTR-3) alias PHT4;2.

ANTR-3 was reported to be expressed in roots and their transcript levels were found to be decreased by 80 % during light phase (previous studies).

Hypothesis: ANTR-3 is present in the envelope of non-green root plastids and is responsible for the cycling of Pi and hence crucial for many biochemical pathways like starch, amino acid and fatty acid biosynthesis.

Aim: The goal of this study was to screen out mutants, standardize a protocol for isolation of root plastids from *Arabidopsis thaliana*, localize ANTR-3 and verify its function as a phosphate transporter in radioactive assays.

M & M: The model organism *Arabidopsis thaliana*, both wild-type (wt) and mutants was cultivated hydroponically.

Reverse genetics were applied with the help of bioinformatics and previous studies provided vital inchoate clues. Screening of homozygotes proceeded standardization of a protocol for isolation of root plastids. The presence of this protein was checked by antibody specific Western blotting followed by functional analysis and characterization using radioactive transport experiments.

Results: Mutants were found to grow 30 % more profusely and had reduced starch levels when compared to wt (fig.1). Development of a protocol for isolation of root plastids with high yields were successful and their intactness were checked using phase contrast microscopy (fig.2). Antibody specific Western blotting revealed a single band around 45 KDa in wt plants but absent in the mutants.

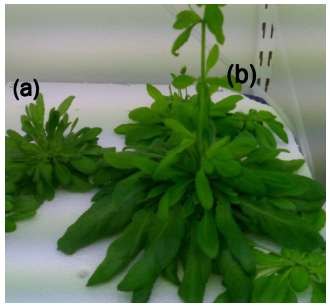

Fig 1: *Arabidopsis thaliana* wt(a) and mutant plants(b).

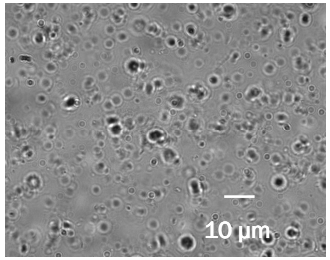

Fig 2: Root plastid preparation under Phase contrast microscope.

Functional characterization in radioactive transport experiments revealed higher activity in wt plants at a pH of 7.5 in presence of Na^+(fig. 4). Back exchange experiments measured the highest rate of export at pH 6.5 Na^+ to 7.5 Na^+ (fig. 5).

Fig 3: Western blot of root plastids.wt and *antr*1 (another Pi transport protein) mutant in lane 1 and 2. Lanes 3-5 has *antr-i,* and *p*.

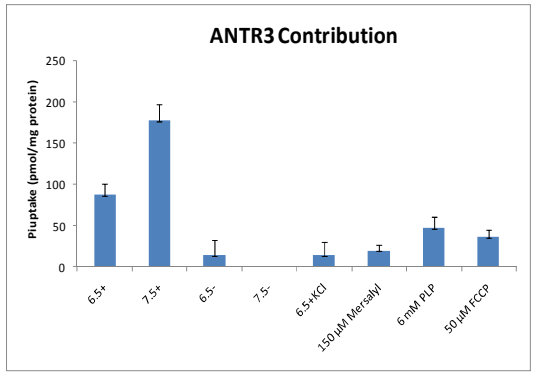

Fig 4: Bar graph plotted with different conditions and the total ANTR3 contribution during transport experiments.

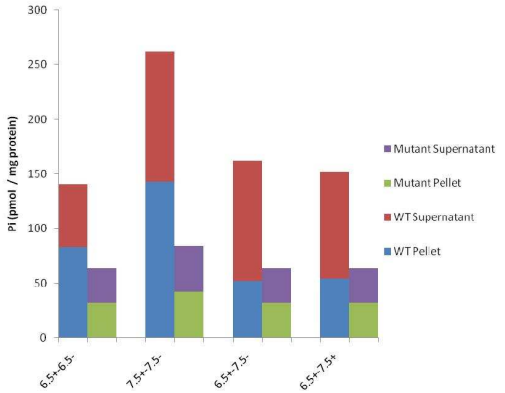

Fig 5: Bar graph plotted against ratio of iP in pellet and supernatant during back exchange.