Analysis of a Polyol Transporter Family from Arabidopsis thaliana



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1. Introduction

AtPLTs, putative polyol transporters, are members of the monosaccharide transporterlike superfamily from Arabidopsis thaliana comprising 53 sugar transport proteins in total. The 6 members of the AtPLT subfamily show significant homology to transporters for the linear polyols sorbitol and mannitol identified in celery and plantain, two plant species that translocate polyols within their vascular tissue. Furthermore, these transporters are discussed to be essential for the salt tolerance of these species. Both of these properties are not characteristic for *Arabidopsis* as neither sugar alcohol transport nor salt stress tolerance has been observed so far.

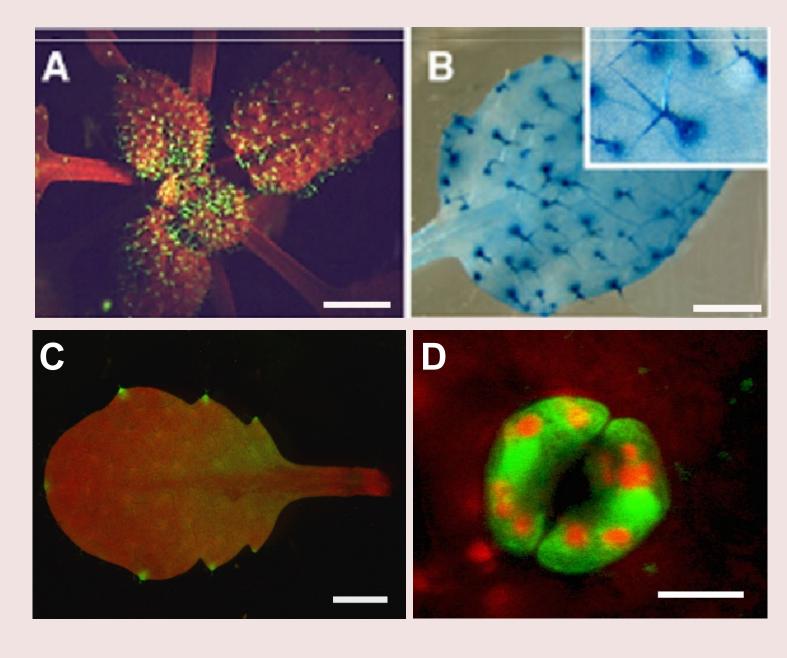
Heterologous expression in yeast revealed that AtPLT1, 2, and 5 are capable of translocating a variety of short chain polyols and sugars like xylitol, sorbitol, fructose or glucose, whereas mannitol is no substrate for these AtPLTs.

In Xenopus oocytes it was shown that AtPLT5 is a H⁺- symporter and also expression in yeast showed that AtPLT5 is localized to the plasma membrane. Analysis of the gene expression shows a mainly sink tissue specific expression pattern. To determine the subcellular localization of the AtPLT family, the GUS or GFP reportergene was fused to ~1500bp promoter regions of AtPLT4, 5 and 6. AtPLT4 is basically localized in trichomes and root tip, AtPLT5 showed expression in the vascular tissue and in shoot below the flower, whereas AtPLT6 was detectable in guard cells and in hydathodes.

2. Aims: The functional and physiological characterization of the proteins encoded by the AtPLT subfamily

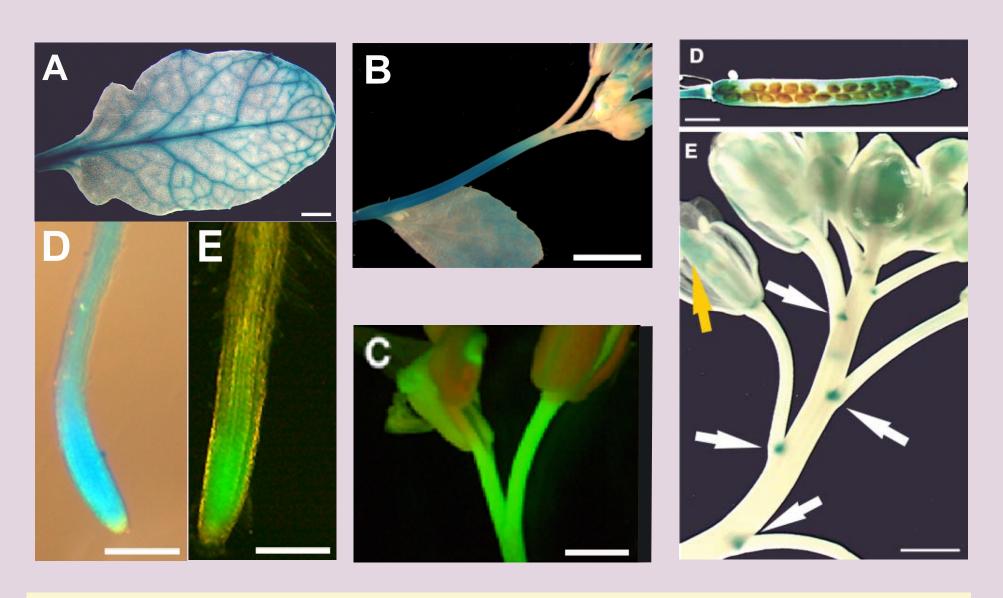
3. Localization of the AtPLTs

The expression pattern was analyzed in promoter:: GFP or promoter:: GUS plants expressing the reporter gene under the control of the AtPLT promoters

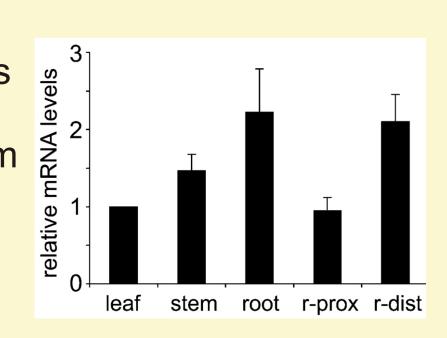


AtPLT4 is strongly expressed in trichomes (A+B). The cutout in **B** shows a single stained trichom of an AtPLT4-promoter::GUS plant.

C+D show AtPLT6-promoter::GFP plants. GFP fluorescence was observed hydathodes (C) and in guard cells of siliques (D).



Real-Time RT-PCR with different Arabidopsis tissues shows the relative *AtPLT5* mRNA levels from leaf, stem and root preparations. AtPLT5 expression was primarily found in the distal regions of the root.

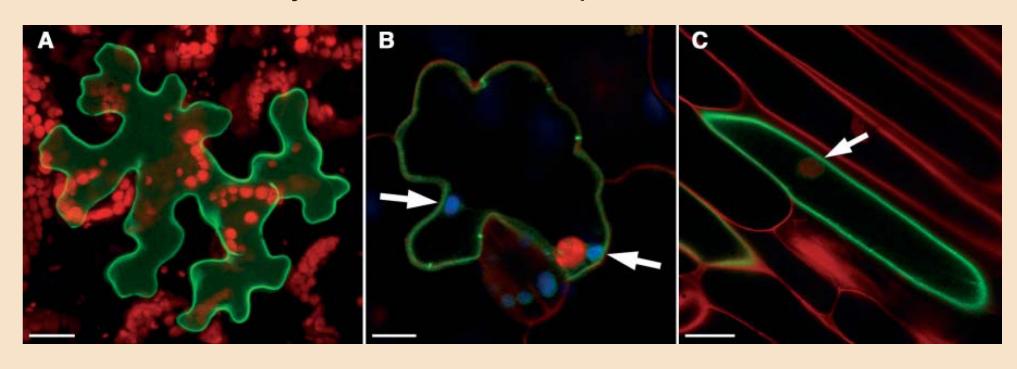


AtPLT5 is localized in the vascular tissue (A). **B+C** show the expression of *AtPLT5* in shoot below the flower.

The main localization spot is the root tip both in AtPLT5promoter::GUS (**D**) and::GFP plants (**E**).

AtPLT5 was also detectable in mature siliques (D) and in the vascular strands of the sepals and in the ovary (E).

Transient expression of an *AtPLT5-GFP* fusion construct in Arabidopsis and onion epidermal cells show that AtPLT5 is clearly localized to the plasma membrane.



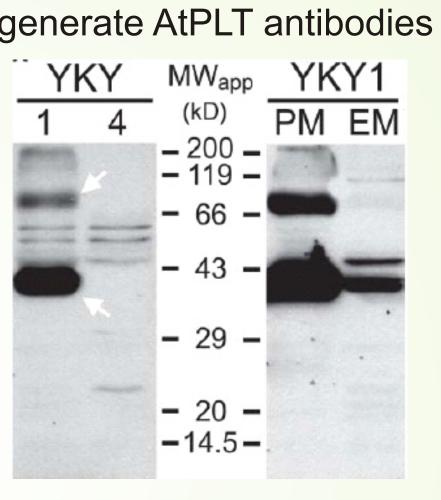
4. Immunocytological Analysis

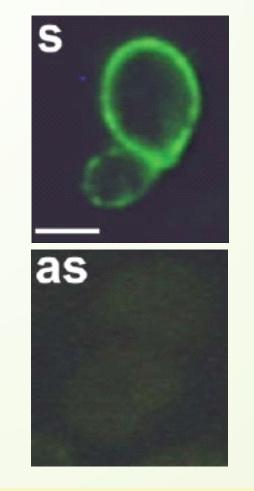
C-terminal oligopeptides were used to generate AtPLT antibodies

Western analyses from yeast cells expressing AtPLT5 and control cells. The anti-AtPLT5 antisera recognize protein bands at ~48 kDa that are not seen in total membranes from control cells (1+4).

The AtPLT5 signal is strongly enriched in the plasma membranes (PM), whereas only a weak signal is visible in the endomembrane fraction (EM).

Cross sections of the AtPLT5expressing yeasts show the localization of the protein in the plasmamembrane (s), whereas control yeasts do not show any signal (as). The same localization was observed in AtPLT1 & 2 expressing yeast cells (data not shown).



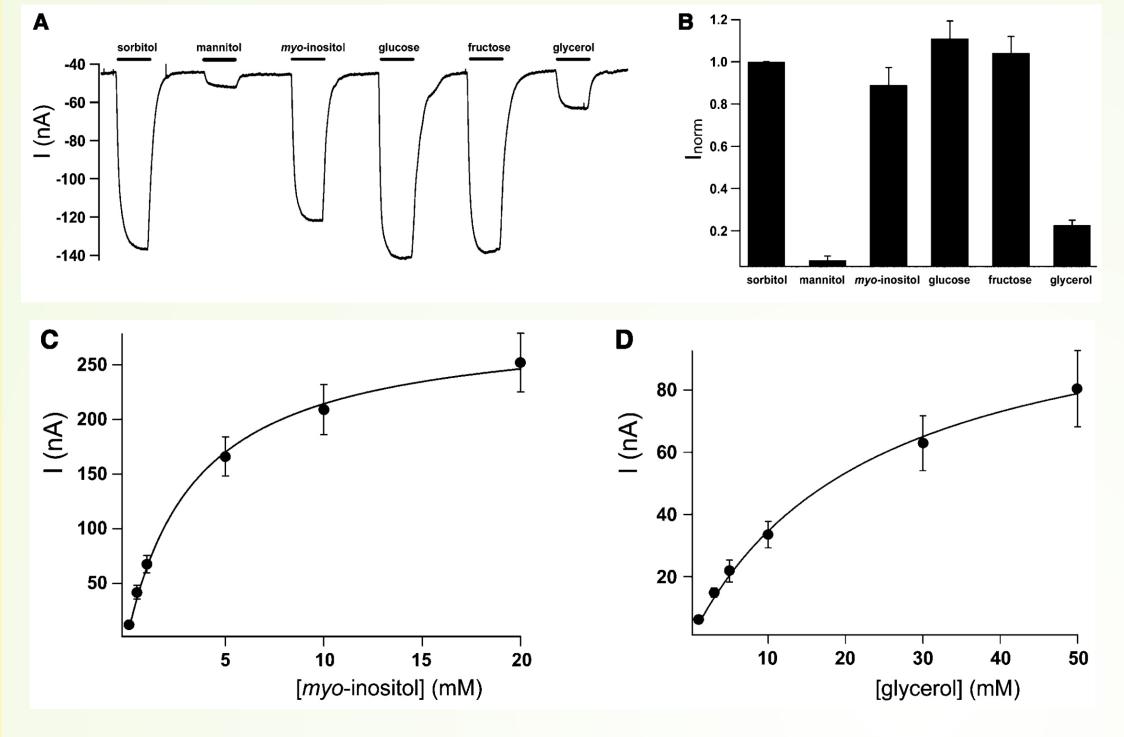


These data show that in *Arabidopsis*:

AtPLTs are low affinity transporter for sugars and short chain polyols Is xylitol the physiological substrate? AtPLTs are primarily localized in sinks AtPLT5 is a H⁺Cotransporter that is localized to the plasma membrane

5. Physiological Characterization

Heterologous expression of the AtPLT5 cDNA Xenopus laevis oocytes



A: AtPLT5-mediated H⁺ currents in response to various sugars and polyols (3mM) at a membrane potential of -60mV. Substrates were added for 20 s.

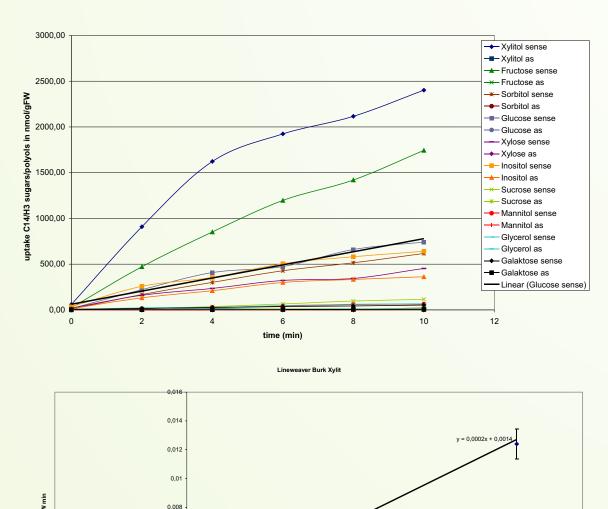
B shows the normalized currents in response for sorbitol from three independent experiments

C: Saturation curve for myo-inositol induced H[†] currents. Km value for myo-inositol: 3.5mM

D: Saturation curve for glycerol induced H⁺ currents. Km value for glycerol: 23.4mM

Km value for sorbitol is 0.50mM (not shown) Km value for glucose is 1.5mM (not shown)

Heterologous expression of the AtPLT1 cDNA baker's yeast



Transport of 14C-labeled polyols and sugars in AtPLT1 expressing yeast cells (sense orientation) in comparison to the negative control (antisense orientation).

The concentration for each substrate is 0.1mM.

AtPLT1 is not only a transporter for polyols like xylitol and sorbitol, but also a transporter for pentoses and hexoses like xylose, ribose and glucose.

A further substrate for this group of transport proteins is fructose. Surprisingly mannitol, which differs from sorbitol in the orientation of only one hydroxylgroup, is transported at very low rates.

Km value for AtPLT1 for xylitol is 0.14mM (not shown) Km value for AtPLT2 for xylitol is 0.18mM (not shown)