

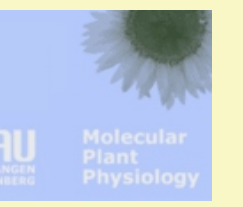
Analysis of a Polyol Transporter Family from *Arabidopsis thaliana*

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1. Introduction

AtPLTs, putative polyol transporters, are members of the monosaccharide transporter-like 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.

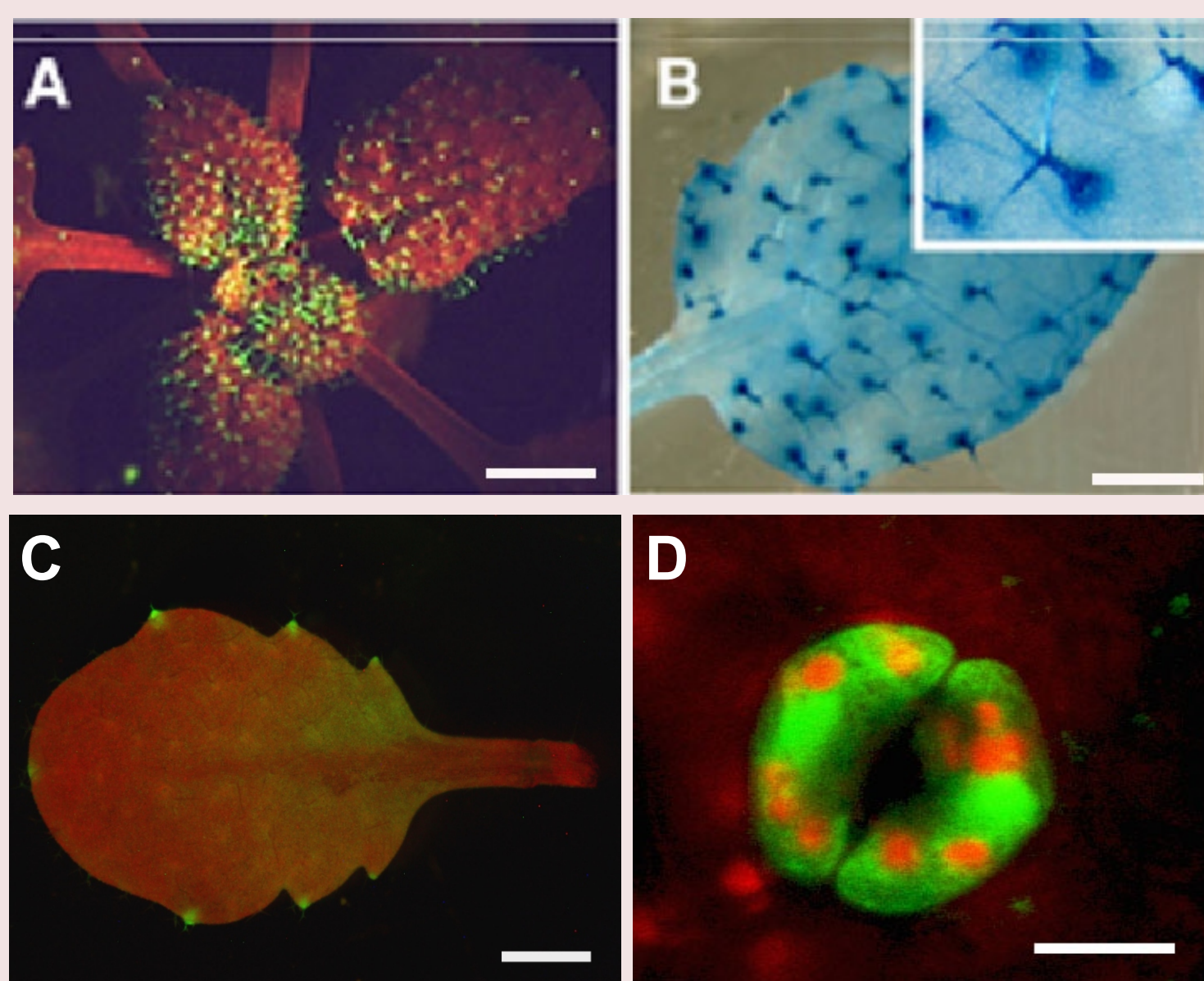
Heterologous expression in yeast revealed that AtPLT1, 2, and 5 are capable of translocating a variety of short chain polyols and sugars like xylitol, sorbitol, fructose or glucose, whereas mannitol is no substrate for these AtPLTs.

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* reporter gene 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.

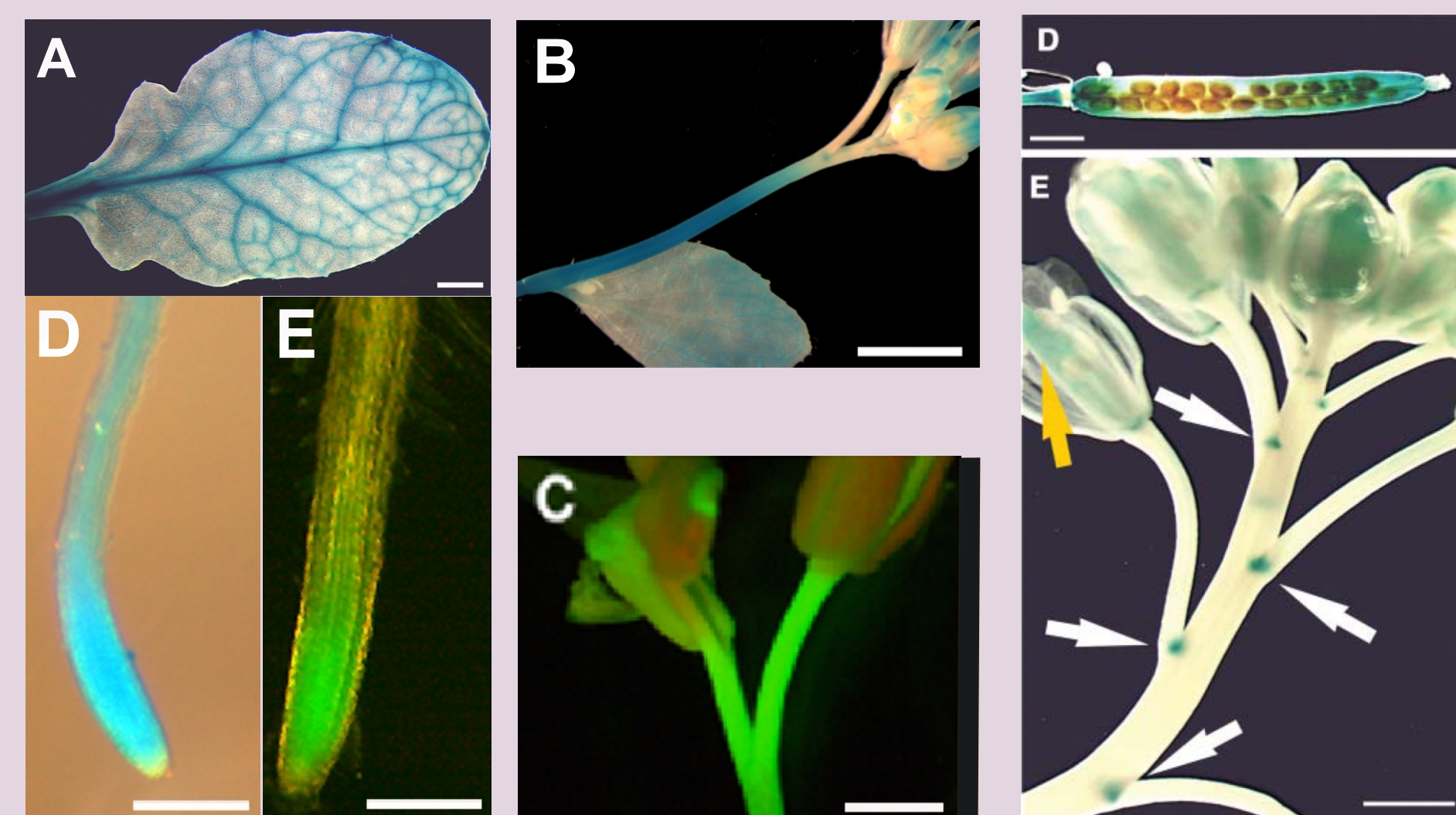
2. Aims: The functional and physiological characterization of the proteins encoded by the AtPLT subfamily

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

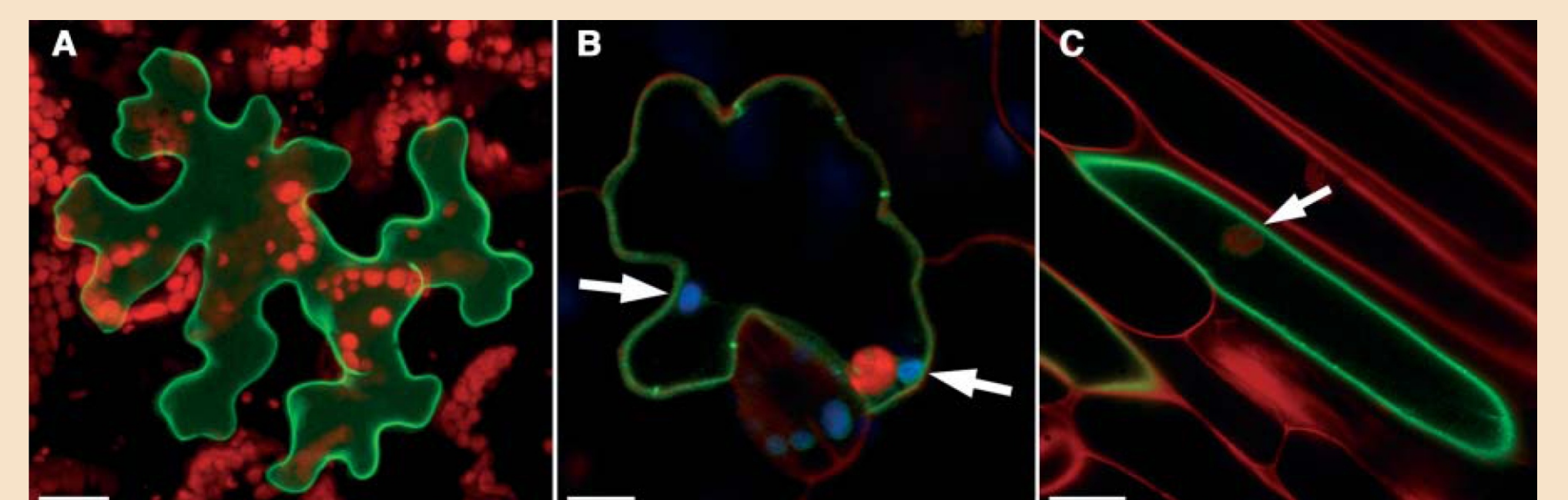


AtPLT4 is strongly expressed in trichomes (A+B). The cutout in B shows a single stained trichome of an AtPLT4-promoter::*GUS* plant. C+D show AtPLT6-promoter::*GFP* plants. GFP fluorescence was observed hydathodes (C) and in guard cells of siliques (D).

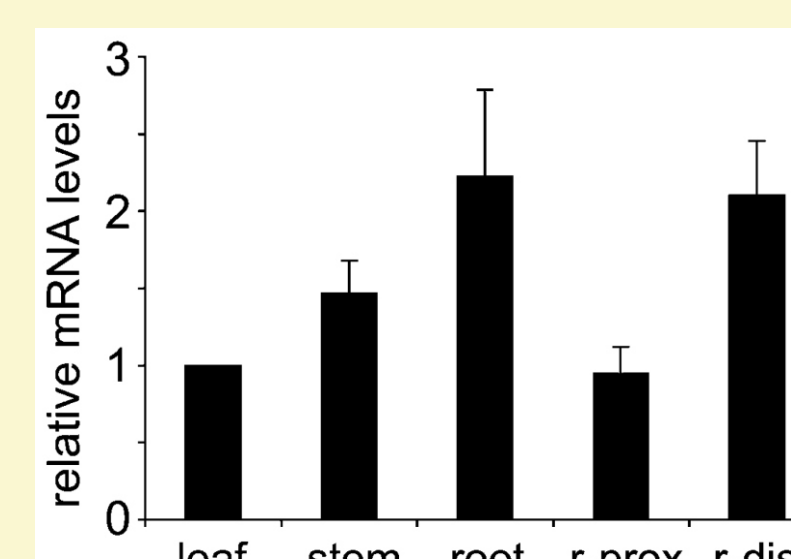


AtPLT5 is localized in the vascular tissue (A). B+C show the expression of AtPLT5 in shoot below the flower. The main localization spot is the root tip both in AtPLT5-promoter::*GUS* (D) and ::*GFP* plants (E). AtPLT5 was also detectable in mature siliques (D) and in the vascular strands of the sepals 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.



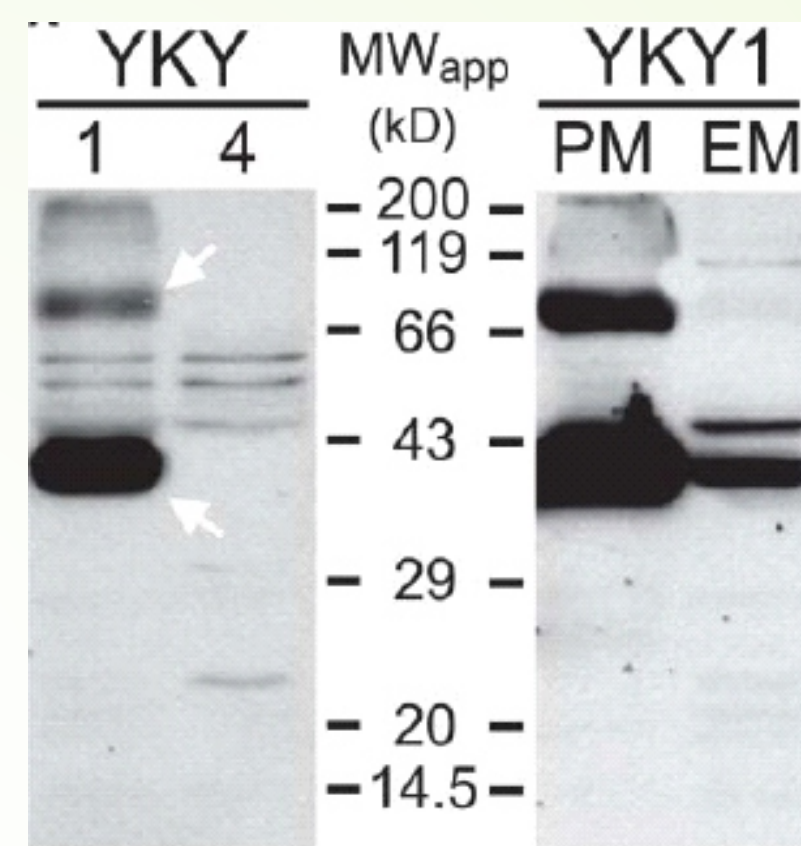
Real-Time RT-PCR with different Arabidopsis tissues shows the relative AtPLT5 mRNA levels from leaf, stem and root preparations. AtPLT5 expression was primarily found in the distal regions of the root.



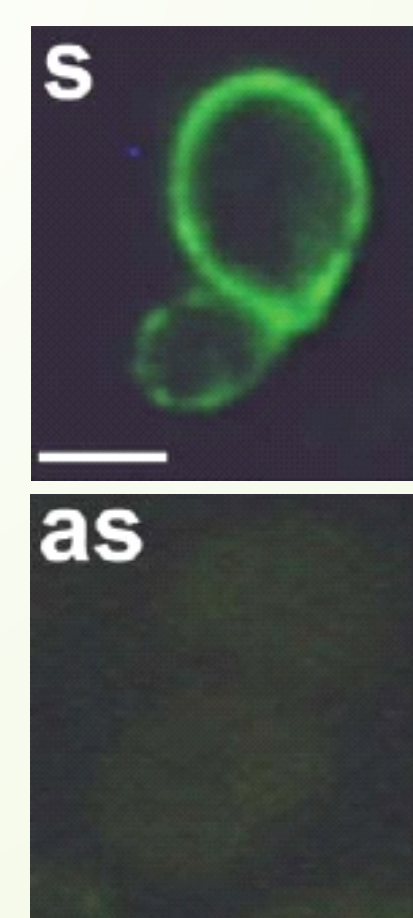
4. Immunocytological Analysis

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 AtPLT5-expressing 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).

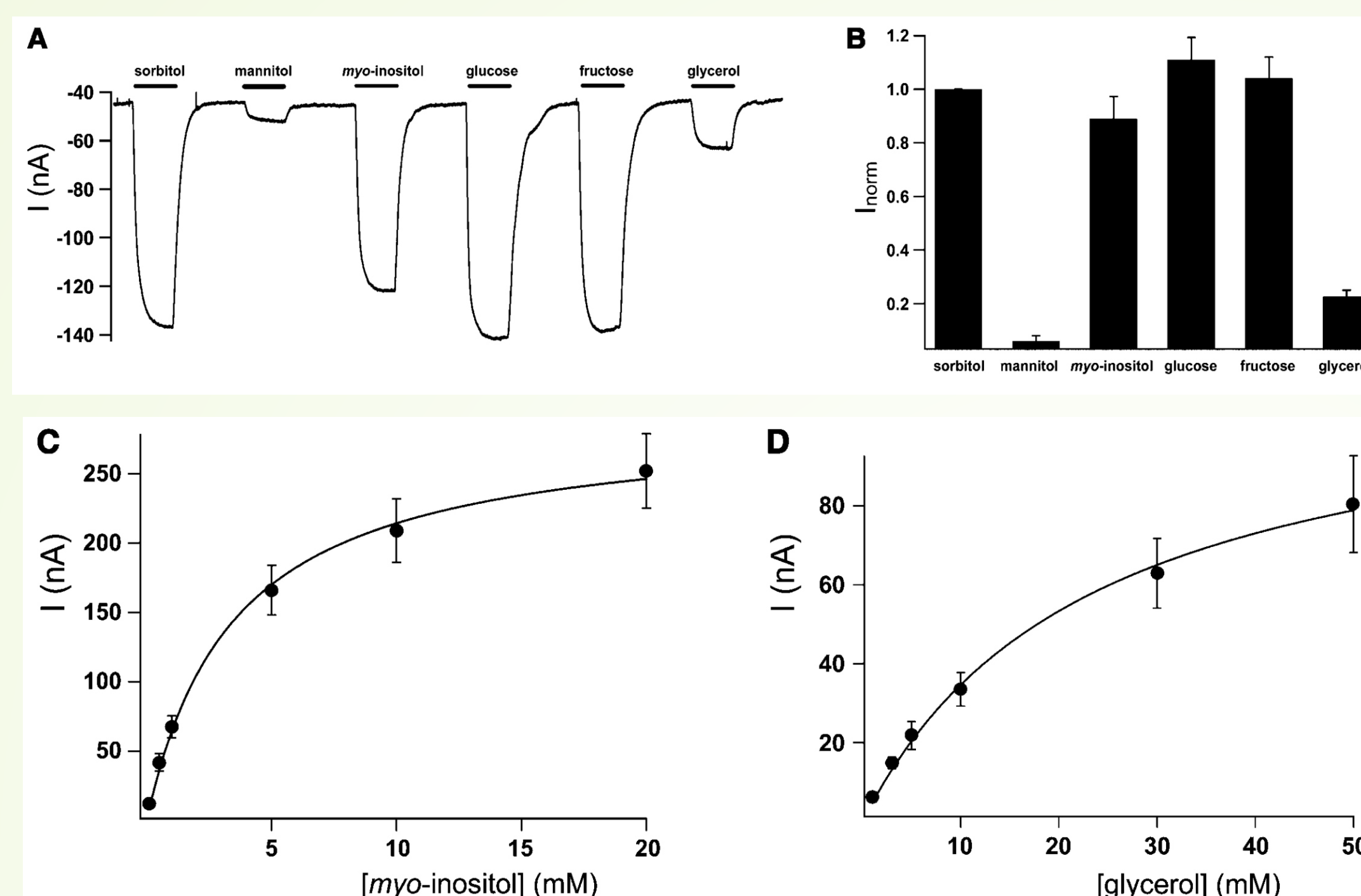


These data show that in *Arabidopsis*:

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



Heterologous expression of the AtPLT1 cDNA baker's yeast

