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SHORT COMMUNICATION

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Contribution of sucrose transporters to phloem unloading within *Sorghum bicolor* stem internodes

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ABSTRACT

Sucrose produced in source leaves is loaded into collection phloem, transported to sinks and unloaded for utilization or storage. In the context of long distance transport, sucrose transporters (SUTs) can function to load sucrose into collection phloem, retrieve leaked sucrose during long distance transport, and load sucrose into sink cells. SUTs have also been proposed to efflux sucrose under conditions of low proton motive force and low extracellular sucrose. The involvement of sucrose transporters in phloem unloading in a representative monocot stem, *Sorghum bicolor*, was evaluated during different stages of internode development. Transcript levels and functional properties of selected key transporters were measured, with both cellular and subcellular localization determined.

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Fixed carbon is transported in the phloem from sites of photosynthesis to heterotrophic sink tissues. Sucrose transporters (SUTs) play key roles during long distance transport, namely, loading sucrose into the phloem, sucrose retrieval and loading sucrose into storage cells. SUTs may also efflux sucrose from the developing phloem (protophloem) in the absence of companion cells that generate a proton motive force (*pmf*) to energise secondary active transport of sucrose by SUTs. Compared with eudicot species, understanding of the roles of SUTs in monocots that accumulate high concentrations of sucrose is lacking, especially their involvement in phloem unloading.

Our recent study examined sucrose transporter function with respect to phloem unloading and sucrose accumulation in sweet *Sorghum bicolor* stems of the cultivar Rio.[1] Aspects of transporter function, gene expression, as well as cellular and subcellular localization were investigated. Transporter functional properties were tested by heterologous expression in both yeast (*S. cerevisiae* strain EBY4000) and *Xenopus laevis* oocytes. SbSUT1 and SbSUT5 were functional in both systems, whereas SbSUT4 was not. SbSUT1 and SbSUT5 had $K_{0.5}$ values for sucrose of 6.3 ± 0.7 and 2.4 ± 0.5 mM, respectively, when expressed in oocytes and they responded differently to changes in pH and membrane potential. Sucrose affinity of SbSUT1 was dependent on pH and membrane potential whereas SbSUT5 sucrose affinity was not. Expression of *SbSUT* genes was quantified by qPCR within developmental zones of an elongating and a fully-elongated internode. *SbSUT1* was preferentially expressed later during internode development. In contrast, *SbSUT5* exhibited a peak in expression within the recently elongated and transition internodal zones.

Interestingly, the tonoplast sugar transporters (*SbTSTs*, previously named *TMTs*) were highly expressed during later stages of internode development when unloading follows a symplasmic route.[1] Accordingly, TSTs may be principal contributors to stem sugar storage in *Sorghum*, as they are predicted to transport sucrose across the tonoplast into the vacuole. This interpretation is supported by the observation of higher *SbTST* expression in sweet (cv. Rio) as compared with grain *Sorghum* stems[2] (cv. BTx623) that correlates with sucrose concentrations of 400 mM[3] and 100 mM respectively in the maturing zone of fully-elongated Internode 4 at anthesis. The Arabidopsis and sugar beet (*Beta vulgaris*) TST orthologues were capable of sucrose transport, so it is likely SbTSTs also share this capability.[4,5]

Subcellular SbSUT localization was determined by transient expression of *SbSUT-GFP* fusion constructs in tobacco mesophyll protoplasts. As expected, SbSUT1 and SbSUT5 were both localized to the plasma membrane, whereas SbSUT4 was localized to the tonoplast. This observation could account for the lack of detectable transport activity by SbSUT4 in yeast and oocytes. Cellular SUT localization was investigated in internode developmental zones using the PEP2 antiserum, raised against a conserved peptide of the potato StSUT1 transporter.[6] This antiserum has been clearly demonstrated to react against SbSUT1, SbSUT4 and SbSUT5,[1] but in view of the very high homology to the conserved motif in the other SbSUTs, it would be expected to detect these also. The immunolocalization studies showed that SbSUTs were localized to sieve elements but not companion cells in all developmental zones examined. Within the elongating and recently elongated zones of

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elongating Internode 10, SUTs were also observed on the surface of storage parenchyma cells. Sieve element localization of SUTs has also been reported in wheat internodes.[7]

In addition to these published results, *Xenopus* oocytes were also used to test the functional properties of the grain *Sorghum* cv. BTx623 SUT5 variant (SbSUT5G; Sb04g023860) which differed by 9 amino acids from the sweet *Sorghum* cv. Rio SUT5[8] (GenBank Accession KY287233). Expression of *SbSUT5* was higher in the sweet versus grain *Sorghum* Internode 5 during vegetative growth and in the flag internode at anthesis.[8] Hence

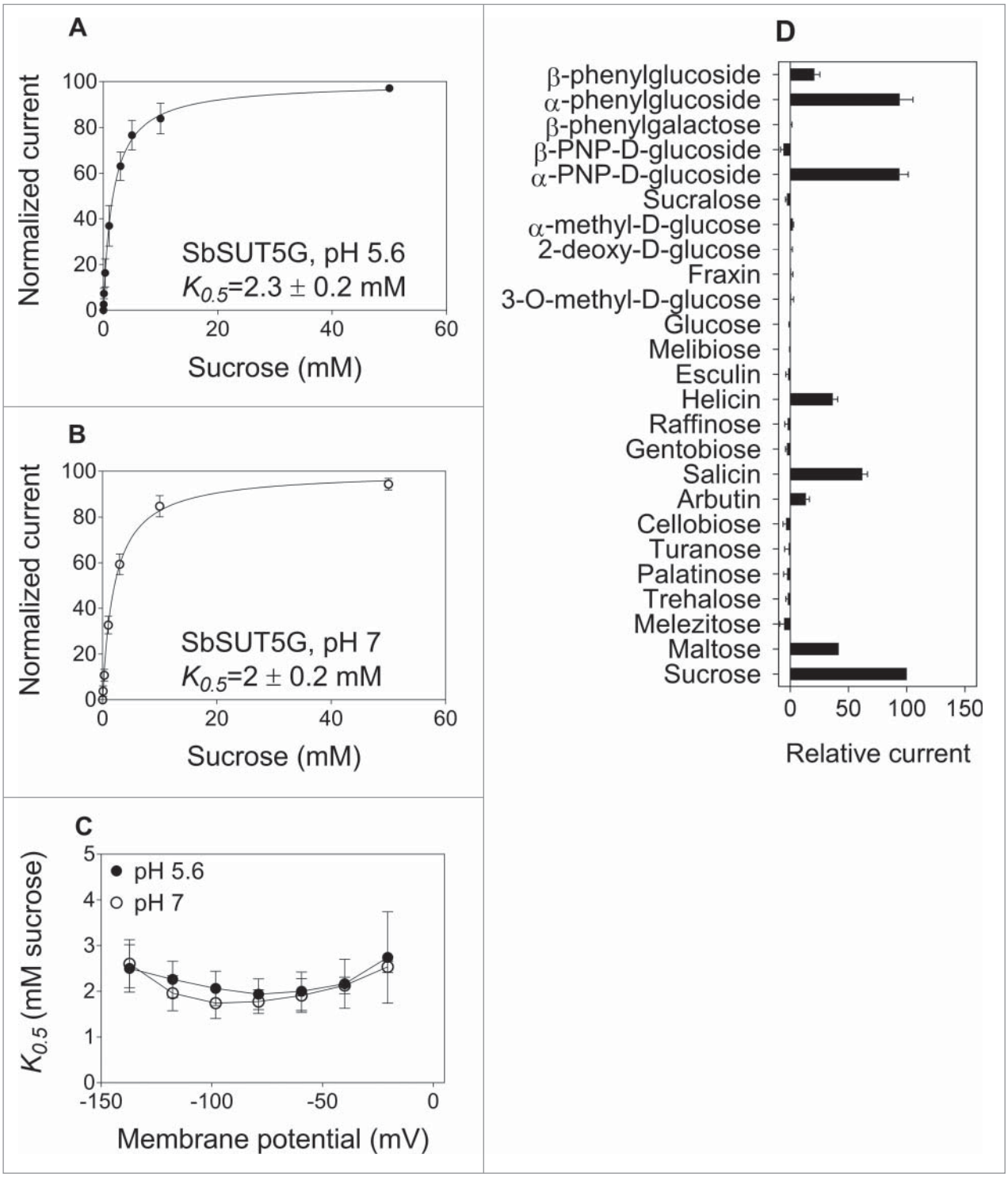

Figure 1. Functional properties of SbSUT5G – pH and voltage dependence of sucrose affinity, and substrate specificity. *SbSUT5G* was expressed in *Xenopus* oocytes and recordings made by two-electrode voltage clamping. (A, B) Concentration-dependent sucrose transport at pH 5.6 (A) and pH 7 (B). (C) SbSUT5G sucrose affinities at membrane potentials from −137 mV to −20 mV. (D) Substrate-dependent currents measured at a membrane potential of −117 mV. Substrates were supplied at a concentration of 10 mM in modified Na-Ringer solution, pH 5.6 except for fraxin (1 mM) and esculin (5 mM) which were added at their limits of solubility. Currents were normalized to currents observed for 10 mM sucrose, to eliminate the influence of expression level differences between oocytes. Mean ± SD of 3 to 5 oocytes.

we evaluated whether amino acid differences between SbSUT5 and SbSUT5G resulted in different transport properties. Sucrose affinities at pH 5.6 and pH 7 were 2.3 ± 0.2 and 2.0 ± 0.2 mM sucrose respectively (Fig. 1A, B). Just like for SbSUT5,[1] sucrose affinity of SbSUT5G was not voltage dependent (Fig. 1C) and SbSUT5G was highly selective for sucrose (Fig. 1D). Therefore, it appears that differences in SUT5 expression levels, rather than transport properties, may contribute to higher sucrose accumulation in the sweet cultivar.

In summary, during early internode development, SUTs present in protophloem sieve elements may efflux sucrose from the phloem under conditions of low *pmf*. In the later phases of internode development, SUTs other than SbSUT4, are likely to function in sucrose retrieval, for the purpose of maintaining turgor homeostasis, driving symplasmic unloading by bulk flow to the storage parenchyma. Sucrose storage within the vacuole is likely to be driven by tonoplast localized TSTs.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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