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Letter to the Editor

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# Disruption of the Sugar Transporters *AtSWEET11* and *AtSWEET12* Affects Vascular Development and Freezing Tolerance in *Arabidopsis*

Dear Editor,

In plants, the transport of sugars from the site of biosynthesis to the site of utilization or storage relies on sugar transporters located in different subcellular compartments and different vascular cell types. The SWEET sugar transporter family is one such class and is represented by 17 members in *Arabidopsis*. AtSWEET1 was the first plant SWEET transporter to be characterized; it acts as a glucose uniporter in multiple systems (Chen et al., 2010). AtSWEET11 and AtSWEET12 were subsequently identified as key players in sucrose efflux from phloem parenchyma cells, a prerequisite for phloem loading by import into the sieve element–companion cell complex (Chen et al., 2012), whereas AtSWEET16 and AtSWEET17 probably export fructose out of the vacuole and contribute significantly to the regulation of fructose levels in *Arabidopsis* (Chardon et al., 2013; Klemens et al., 2013; Guo et al., 2014). The identification of the SWEET proteins as sucrose transporters has raised a number of questions. In particular, since *AtSWEET11* and *AtSWEET12* are expressed in most *Arabidopsis* organs (Chen et al., 2012), what roles might they play beyond phloem loading? The AtSWEET11 and AtSWEET12 proteins were shown to localize to the plasma membrane and to be expressed in a subset of leaf phloem parenchyma cells (Chen et al., 2012). By using transcriptional fusions and performing *in situ* hybridization experiments on flower stem sections, we confirmed the expression of these genes in the phloem tissues of *Arabidopsis* flower stems (Figure 1A–1D), but we also demonstrated that both genes are expressed in cells associated with the xylem vessels, and that *SWEET11* is also in xylem cells close or adjacent to the cambium region (Figure 1A–1D), suggesting that they have some other role in addition to being the missing link in sucrose phloem loading as suggested by Chen et al. (2012). This is supported by the fact that under our conditions, the AtSWEET11 and AtSWEET12 proteins were both capable of transporting glucose and fructose as well as sucrose. Their substrate flexibility was demonstrated by heterologous expression in *Xenopus laevis* oocytes (Figure 1E) and supported by the complementation of the yeast strain EBY.VW4000 (Supplemental Figure 1), which is deficient in hexose transport.

Since cold treatment is known to induce sugar accumulation, we analyzed the behavior of the different mutant lines after a week at 4°C. Interestingly, under long-day conditions, the single *sweet11-1* accumulated twice as much glucose and four times more fructose than the wild-type after cold treatment (Figure 1F). This result supports a role in hexose transport, as has been observed for other SWEET proteins such as AtSWEET1, AtSWEET16, and AtSWEET17 (Chen et al., 2010; Chardon et al., 2013; Klemens et al., 2013; Guo et al., 2014). Under long-day conditions, the glucose and fructose contents of the wild-type doubled when the temperature was reduced to 4°C, whereas those of the double mutant did not increase in response to the same cooling (Figure 1F and Supplemental Figure 2A). This is probably because the double mutant had already high levels of cytosolic monosaccharides at 22°C (Supplemental Figure 2A), which were sensed by the cells and prevented further sugar accumulation. However, under short-day conditions, the double mutant accumulated much greater levels of all three sugars than the wild-type in response to cold treatment (Figure 1G and Supplemental Figure 2B), suggesting that cold acclimation is differentially regulated under short- and long-day conditions. Soluble sugars and sucrose in particular are known to act as osmoprotectants and can thus help to maintain cellular integrity and function (Ruan et al., 2010). Therefore, we sought to determine whether the enhanced sugar accumulation had any effect on the freezing tolerance of the mutants and we measured the electrical conductivity of the plants' leaves, which is a highly reproducible way of quantifying the freezing tolerance of individual plant lines (Nagele and Heyer, 2013). The double mutant *sweet11-1sweet12-1* released only 28% of its total electrolytes, whereas the wild-type released 43%; no significant difference was observed between the wild-type and the single mutants (Figure 1H). Thus, the double mutant exhibited greater freezing tolerance than the wild-type and both single mutants. The cold-related phenotype is supported by the down-regulation of *SWEET11* and *SWEET12* expression by cold treatment as shown in Supplemental Figure 2C.

Interestingly, when the plants were grown under long-day conditions, the flower stem of the *sweet11-1* single mutant was slightly but significantly thinner than that of the wild-type (Figure 1I). Although the *sweet12-1* single mutant was not distinguishable from the wild-type, the double mutant had a substantially thinner flower stem (Figure 1I). The reduction in stem diameter observed for the *sweet11-1* and *sweet11-1sweet12-1* mutants were coupled with a reduction in the phloem and xylem areas (Figure 1J and 1K), which was proportional in that the phloem/xylem ratio remained constant (Supplemental Figures 3 and 4A and 4B). The reduction in phloem and xylem poles could be explained by a reduced number of cells (Figure 1L and 1M and Supplemental Figure 4C). For *sweet11-1*, the number of cells per phloem pole as well as the number of certain xylem cell categories were slightly but not significantly reduced compared with wild-type (Figure 1L and Supplemental Figure 4C). In the double mutant, we also observed a reduced circular diameter of xylem vessels, which likely contributed to reducing the xylem

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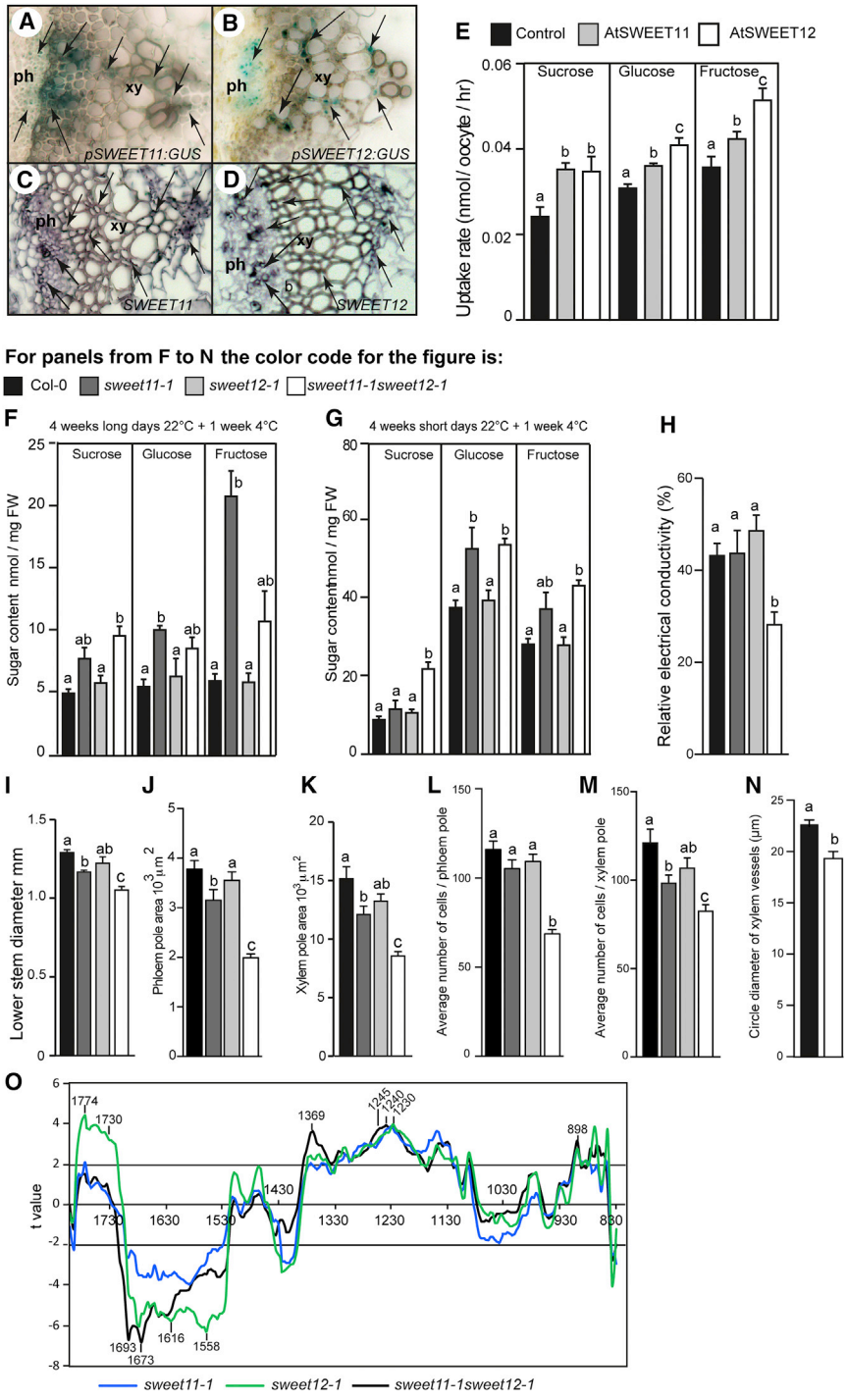


**Figure 1. The *SWEET11-1* and *SWEET12-1* Genes are Expressed in the Phloem and the Xylem Tissues and Are Required for Proper Vascular Development and Cell Wall Composition.**

**(A and B)** *pSWEET11:GUS* and *pSWEET12:GUS* expression in the floral stem section of 5-week-old plants. Arrows point to cells showing blue GUS staining. ph, phloem; xy, xylem.

**(C and D)** *In situ* hybridization of *SWEET11* and *SWEET12* mRNA in transverse sections of floral stems from 6- or 7-week-old plants. Arrows point to cells expressing *SWEET11* or *SWEET12*. ph, phloem; xy, xylem.

**(E)** Uptake of [$^{14}$C]sucrose, [$^{14}$C]glucose, or [$^{14}$C] fructose catalyzed by SWEET11 and SWEET12 or water (control, black bars) after heterologous expression in *Xenopus laevis* oocytes. Values represent means ± SE (n = 9).

**(F and G)** Sucrose, glucose, and fructose levels in leaves of the different genotypes grown at 22°C under long-day (F) or short-day (G) conditions for 4 weeks and then transferred at 4°C. Values are means ±SD (n ≥ 4 for long days and n = 5 for short days).

**(H)** Electrolyte leakage from cold-acclimated wild-type, *sweet11-1*, *sweet12-1*, and *sweet11-1sweet12-1* plants. Three-week-old plants grown under short-day conditions at 22°C were cold adapted at 4°C for 4 days after which their electrolyte leakage was estimated by determining the electrical conductivity of a bathing solution of detached leaves frozen at −6°C. Values are means ± SE (n = 10).

**(I–N)** Plants were grown under long-day conditions and floral stem cross sections were obtained from stem segments taken 1 cm above the rosette. A minimum of five independent plants were analyzed. **(I)** The floral stem diameter. Values represent means ± SE (n ≥ 15 sections). **(J)** Phloem pole area. Values represent means ± SE (n ≥ 13 phloem poles). **(K)** Xylem pole area. Values represent means ± SE (n ≥ 15 xylem poles). **(L)** Average number of cells per phloem pole. Values represent means ± SE (n ≥ 13 phloem poles). **(M)** Average number of cells per xylem pole. Values represent means ± SE (n ≥ 15 xylem poles). **(N)** Average circle diameter of xylem cells. Values are means ± SE (Col-0, n = 44; *sweet11-1sweet12-1*, n = 81). **(E-N)** A one-way analysis of variance combined with Tukey's comparison post test indicated that the values marked with the same letter were not significantly different from each other, whereas different letters indicate significant differences ($P < 0.05$).

**(O)** Comparison of Fourier-transformed infrared spectra obtained from xylem cells in basal floral stem sections of wild-type plants as well as the *sweet11-1, sweet12-1*, and *sweet11-1sweet12-1* lines. Student's *t*-test was performed to compare the absorbances for the wild-type and the mutants, and the results were plotted against the corresponding wave numbers. *t* values (plotted on the vertical axis) between −2 and +2 correspond to non-significant differences ($P < 0.05$) between the tested genotypes (n ≥ 4). *t* values above +2 or below −2 correspond to significantly weaker and stronger absorbances, respectively, in the spectra of mutants relative to the wild-type.

pole size (Figure 1N). The defects in vascular development are supported by the expression of both AtSWEET11 and AtSWEET12 in the phloem and xylem cells. In addition, the decreased number of xylem cells as well as the smaller diameter of xylem vessels observed in the double mutant likely contribute to increase the resistance to ambient low temperatures. Indeed, it is known that xylem in the floral stem of *Arabidopsis* shares strong structure–function relationships with the xylem of woody plants for which it has been established that xylem vessels of small diameter are less

sensitive to cavitation caused by freezing (Pittermann and Sperry, 2003).

Both the developmental phenotype and increased freezing tolerance of the double mutant were reminiscent of the *eskimo1* mutant phenotype (Lefebvre et al., 2011). Although the xylem phenotypes we observed in the *sweet11-1* and *sweet11-1sweet12-1* mutants were less dramatic than the *esk1* xylem phenotype, this observation prompted us to analyze the chemical composition of the cell walls. Using Fourier-transformed infrared spectroscopy, we analyzed the cell walls of the xylem cell in flower stem cross sections (Figure 1O and Supplemental Figures 4A and 5). We showed that the spectra for the *sweet12-1* mutant exhibited significantly lower absorbance at wavenumbers between 1800 and 1700 $cm^{-1}$ than observed for the wild-type (Figure 1O and Supplemental Figure 4C). Absorbances in this region of the spectrum typically correspond to ester linkages, esterified pectins or C=O stretching on acetyl groups (1730 $cm^{-1}$). The absorbances for the single *sweet11-1*, *sweet12-1*, and double *sweet11-1sweet12-1* mutants also differed significantly from those for the wild-type in two other regions of the spectrum: the first between 1693 and 1530 $cm^{-1}$, and the second between 1130 and 1330 $cm^{-1}$. Based on previous IR analyses of plant cell walls, the first of these two regions corresponds to the COOH groups of pectic polysaccharides, which are also known as acidic pectins, and the second has been assigned to C=O and C–O vibrations in pectic rhamnogalacturonan I and rhamnogalacturonan II or *O*-acetyl moieties found in plant cell wall polymers (1230, 1240, 1245 $cm^{-1}$) (Figure 1O and Supplemental Figure 4B, 4C, and 4D). Although pectins are not abundant in secondary cell walls, pectin methylesterification appears to be a prerequisite for lignin modification during secondary cell wall deposition in xylem cells. In addition, some *Arabidopsis* mutants have been shown to exhibit defects in secondary cell wall formation associated with a deficiency in pectin content (Pelloux et al., 2007). A final set of differences was observed at around 890 $cm^{-1}$ and 1369 $cm^{-1}$; these regions are associated with crystalline polysaccharide components such as cellulose (β-linked glucan polymers at 898 $cm^{-1}$) or deformation of C–H linkages in the methyl group of *O*-acetyl moieties (1369 $cm^{-1}$). All three mutants exhibited significantly weaker absorbances than the wild-type in this region, suggesting that they have lower levels of crystalline cellulose and a modified xylan acetylation. When compared with each other, single and double mutants showed very similar average absorbance profiles (Supplemental Figure 5A, 5E–5G). Few significant differences could be observed. *sweet11-1* exhibited significantly higher absorbances at wave numbers between 1700 and 1800 $cm^{-1}$ and lower absorbances at 1450 and 1680 $cm^{-1}$ compared with *sweet12-1* (Supplemental Figure 5A and 5E). Compared with the double mutant, *sweet11-1* showed significantly lower absorbances between wave numbers 1735 and 1600 $cm^{-1}$, and *sweet12-1* showed significantly lower absorbances at wave numbers between 1740 and 1800 $cm^{-1}$ (Supplemental Figure 5A, 5F, and 5G). In conclusion, the xylem cell walls of both *sweet* single mutants and the double mutant exhibited severe chemical modifications. The overall phenotypical characteristics of single and double mutants as well as the fact that the double mutant phenotype differs from that of each single mutant suggest that SWEET 11 and SWEET 12 proteins play a synergistic role in the regulation of sugar transport.

Two important issues that currently remain unclear are how the pools of carbohydrate skeletons required for the synthesis of these secondary cell wall components are transported to this non-photosynthetic tissue, and how the depletion of sugar precursors in the xylem cells would affect the development and structure of the secondary cell wall. The results presented herein support the hypothesis that, in addition to their contribution to phloem loading in source leaves, the AtSWEET11 and AtSWEET12 proteins act as sugar exporters that deliver carbon-containing skeletons to developing xylem cells in order to support secondary cell wall formation. In addition, due to their expression in both the phloem and xylem of the flower stem, we cannot exclude the possibility that they may contribute, together with additional SWEET proteins or sugar transporters, to the transport of sugar from the phloem to nourish adjacent stem tissues.

## SUPPLEMENTAL INFORMATION

Supplemental Information is available at *Molecular Plant Online*.


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## AUTHOR CONTRIBUTIONS

C.B., R.L.H., and L.S. conceived and designed the experiments. D.C., S.C., F.D.M., C.G., P.A.W.K., R.L.H., B.P., L.S., F.V., and N.W. performed the experiments. C.B., S.D., P.A.W.K., R.L., R.L.H., G.M., H.E.N., and E.T. analyzed and discussed the data. C.B. and R.L.H. wrote the article. All authors contributed to the corrections.


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