Expression of Sucrose Transporter Complementary DNAs Specifically in Companion Cells Enhances Phloem Loading and Long-Distance Transport of Sucrose But Leads to an Inhibition of Growth and the Perception of a Phosphate Limitation[1][W][OPEN]

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Sucrose (Suc) is the predominant form of carbon transported through the phloem from source to sink organs and is also a prominent sugar for short-distance transport. In all streptophytes analyzed, Suc transporter genes (*SUT*s or *SUC*s) form small families, with different subgroups evolving distinct functions. To gain insight into their capacity for moving Suc in planta, representative members of each clade were first expressed specifically in companion cells of Arabidopsis (*Arabidopsis thaliana*) and tested for their ability to rescue the phloem-loading defect caused by the Suc transporter mutation, *Atsuc2-4*. Sequence similarity was a poor indicator of ability: Several genes with high homology to *AtSUC2*, some of which have phloem-loading functions in other eudicot species, did not rescue the *Atsuc2-4* mutation, whereas a more distantly related gene, *ZmSUT1* from the monocot *Zea mays*, did restore phloem loading. Transporter complementary DNAs were also expressed in the companion cells of wild-type Arabidopsis, with the aim of increasing productivity by enhancing Suc transport to growing sink organs and reducing Suc-mediated feedback inhibition on photosynthesis. Although enhanced Suc loading and long-distance transport was achieved, growth was diminished. This growth inhibition was accompanied by increased expression of phosphate (P) starvation-induced genes and was reversed by providing a higher supply of external P. These experiments suggest that efforts to increase productivity by enhancing sugar transport may disrupt the carbon-to-P homeostasis. A model for how the plant perceives and responds to changes in the carbon-to-P balance is presented.

Suc, whether from photosynthesis or storage reserves, is the principal form of assimilated carbon transported throughout plants. Suc distribution follows a diversity of routes and includes movement through the symplast and transport across plasma membranes for intercellular transport via the apoplast and across endomembranes for intracellular compartmentation (Ayre, 2011). As a relatively large and polar molecule, Suc movement across membranes requires facilitators, which may be passive or energized. SWEETs are a recently described family of transporters participating in passive movement (Chen et al., 2012), while Suc/H^+ symporters, alternatively called sucrose transporters (SUTs) or sucrose carriers (SUCs), couple the movement of Suc to the proton motive force to allow energized Suc accumulation. SUTs are best characterized for their role in apoplastic phloem loading in which Suc, after release into the apoplast from photosynthetic cells, is actively accumulated to high concentrations in phloem companion cells in preparation for long-distance transport to heterotrophic tissues. Reducing the activities of SUTs involved in phloem loading by mutation or transcript reduction strategies results in dramatically stunted plants and carbohydrate accumulation in source leaves (Kühn et al., 1996; Gottwald et al., 2000; Slewinski et al., 2009). However, SUTs are also found throughout the plant, and their regulation and role in carbohydrate distribution remain active research areas. The physiological and biochemical functions of SUTs have been regularly reviewed (Sauer, 2007; Braun and Slewinski, 2009; Kühn and Grof, 2010; Ayre, 2011).

In all species studied, SUTs form small families whose members have distinct gene expression patterns, and the encoded proteins have distinct kinetic properties and

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subcellular localizations. Different research groups divide the family into three to five clades and use different nomenclature for those subdivisions, but the basic structures of the trees are similar (Aoki et al., 2003; Sauer, 2007; Braun and Slewinski, 2009; Kühn and Grof, 2010). Reinders et al. (2012) recently analyzed the SUT clades in the context of land-plant evolution, following the type designation of Aoki et al. (2003; types I, IIA, IIB, and III; see Supplemental Table S1 for cross-referencing designations used by different laboratories). They propose that the earliest land plants had type II transporters localizing to the plasma membrane and type III transporters localizing to the tonoplast. Among angiosperms, eudicots adapted type III SUTs for phloem loading and other high-affinity uptake processes across plasma membranes, probably by losing the tonoplast localization signals, to form the type I clade, while monocots recruited type II SUTs for similar high-affinity uptake processes to form the type IIB branch (Reinders et al., 2012). The type IIA SUTs are more enigmatic: they are found in all land plants, have lower affinity for Suc, and have been postulated to be Suc sensors rather than functional SUTs (Barker et al., 2000; Barth et al., 2003).

Despite the fact that SUTs predate vascular plants, their current roles in Suc distribution and plant productivity made them mainstays of plant physiology well before their molecular characterization (Giaquinta, 1983; Bush, 1999). The physiological function of specific SUTs has been inferred mostly from gene repression and mutations, expression patterns, and protein localization; biochemical activity has been characterized predominantly by electrophysiology in *Xenopus laevis* oocytes and by Suc uptake assays into yeast (*Saccharomyces cerevisiae*). There are relatively few reports of overexpression (OE) in planta to characterize their contribution to growth and development or for possible gains in productivity. Both *AtSUC1* from Arabidopsis (*Arabidopsis thaliana*; type I; Wippel and Sauer, 2012) and *HvSUT1* from barley (*Hordeum vulgare*; type IIB; Reinders et al., 2012) restored phloem loading in an Arabidopsis *Atsuc2*$^{-/-}$ mutant when expressed from the companion cell-specific *AtSUC2* promoter (*AtSUC2p*), showing that their biochemical activity in planta can replace that of *AtSUC2*. *AtSUC2* (type I) encodes the exclusive, or at least the predominant, SUT catalyzing phloem loading in Arabidopsis (Gottwald et al., 2000; Srivastava et al., 2009a). With respect to ectopic expression of *SUT* genes to study the impact on carbon partitioning, constitutive expression of *SoSUT1* (type I) of spinach (*Spinacia oleracea*) from the *Cauliflower mosaic virus* 35S promoter in potato (*Solanum tuberosum*) resulted in shifts in metabolite levels with little effect on tuber morphology (Leggewie et al., 2003), while OE of *OsSUT5Z* (type IIB) and *OsSUT*2M (type III) of rice (*Oryza sativa*) from a tuber-specific promoter was reported to increase tuber yield (Sun et al., 2011). In other species, ectopic expression of potato *StSUT1* in the storage parenchyma of pea (*Pisum sativum*) cotyledons during seed development enhanced Suc influx and enhanced cotyledon growth rates (Rosche et al., 2002), and ectopic *SUT* expression in wheat (*Triticum aestivum*) grains increased levels of storage protein, indicating that enhanced Suc transport has benefits beyond carbohydrate accumulation (Weichert et al., 2010).

Altering phloem transport of Suc by manipulating the SUTs involved in phloem loading has been put forward as a means to improve plant productivity. As one example, Suc loading is modified in response to the physiological and environmental needs of the plant to maintain phloem hydrostatic pressure and coordinate source output with sink demand (Ayre, 2011), and it was proposed that heterologous promoters that are uncoupled from this natural regulation may be useful to keep loading rates constantly high (Srivastava et al., 2009b). Also, increased Suc transport from source leaves to sink organs was proposed to be an effective method to enhance crop productivity (Ainsworth and Bush, 2011), especially in conditions where carbohydrate is in excess (Stitt, 2013). This stems from two predictions: more carbohydrate would be sent to sink organs for growth and/or storage, and there would be more primary productivity because enhanced Suc transport would help remove Suc-mediated product inhibition on photosynthesis (Paul and Foyer, 2001; Stitt et al., 2010; Ainsworth and Bush, 2011).

The objectives of the research reported in this manuscript were (1) to identify a promoter that allows consistently high-level and specific expression in companion cells, (2) to systematically test the capacity of representative members from each SUT family subdivision to transport Suc into the phloem and rescue an *Atsuc2*$^{-/-}$ mutation when the genes are expressed specifically in companion cells, and (3) to test the ability of those *SUT* genes that rescue the *Atsuc2-4*$^{-/-}$ phenotype to increase phloem loading and enhance productivity when expressed in companion cells, in addition to the endogenous *SUT* expression of wild-type plants. For the first objective, we show that the companion cell-specific promoter from *Commelina yellow mottle virus* (*CoYMVp*) has regulation distinct from the endogenous *AtSUC2p*, because it is not subject to feedback inhibition by Suc. For the second objective, each representative *SUT* was expressed from *CoYMVp* in the homozygous *Atsuc2-4*$^{-/-}$ background, which is extremely stunted due to an inability to phloem load Suc and transport it long distance (Srivastava et al., 2008). The ability of each SUT to restore phloem loading and rescue the *Atsuc2-4*$^{-/-}$ phenotype was used to gauge the capacity of that SUT for moving Suc across the plasma membrane from the apoplast to symplast of the companion cells in planta. For the third objective, siblings of the plants used in the second objective that were segregating wild type (*AtSUC2*$^{+/+}$) instead of *Atsuc2-4*$^{-/-}$ were analyzed for the impact of added *SUT* expression in companion cells on Suc transport and plant growth.

The study shows that despite sequence conservation, comparable biochemical characteristics, and similar physiological functions in dicots (Supplemental Table S1; Reinders et al., 2012), only two of the tested type I *SUT* genes, *AtSUC2* and *AtSUC1*, rescued the *Atsuc2-4*$^{-/-}$ phenotype, whereas a divergent, monocot-specific type IIB *SUT*, *ZmSUT1* from *Zea mays*, effectively restored

growth. No or very poor rescue was achieved with representative type IIA (*AtSUC3* and tomato [*Solanum lycopersicum*] *LeSUT2*) and type III (*LeSUT4*) *SUT* genes. *SUT* expression from *CoYMVp* in wild-type siblings enhanced Suc loading and transport, but against expectations, the plants nevertheless accumulated Suc and other carbohydrate in rosette leaves and were stunted. This inhibition of growth was accompanied by increased expression of phosphate starvation-induced (*PSI*) genes, and the growth inhibition was relieved by including more P to the growth medium, implying that plants with enhanced Suc loading were perceiving a P deficiency. The implications of *SUT* OE to enhance phloem transport are discussed, and a model for how enhanced Suc transport to sink organs may induce perception of a P limitation is presented.

RESULTS

The *CoYMV* Promoter Is Activated by Suc while the *AtSUC2* Promoter Is Repressed

CoYMVp is known to confer strong companion cell-specific expression (Medberry et al., 1992; Matsuda et al., 2002). A construct in which *CoYMVp* was fused to *AtSUC2* complementary DNA (cDNA) was shown to rescue the *Atsuc2-4*$^{-/-}$ knockout mutation (Srivastava et al., 2009b). Both *BvSUT1* from sugar beet (*Beta vulgaris*; Vaughn et al., 2002) and *AtSUC2* (Osuna et al., 2007) are suppressed by high Suc levels. We previously suggested that the modified fine-tuning of exotic promoters, such as *CoYMVp*, compared to *AtSUC2p*, could benefit plant growth by maintaining high *AtSUC2* expression under conditions where it is otherwise repressed (Srivastava et al., 2009b).

To establish if *CoYMVp* and *AtSUC2p* sequences are differentially regulated, quantitative GUS assays were performed with plants grown on increasing levels of Suc and harboring *AtSUC2* cDNA (*cSUC2*) fused to the *uidA* reporter gene (encoding the GUS enzyme) downstream of either *AtSUC2p* or *CoYMVp*. As expected, GUS activity in the *AtSUC2p*::*cSUC2*::*uidA* line decreased with increasing levels of Suc but was enhanced in the *CoYMVp*::*cSUC2*::*uidA* line (Fig. 1). This demonstrates that *CoYMVp* does show different fine-tuning in response to carbohydrate (Suc, specifically) and may maintain high expression and transport levels when the natural gene is repressed. Quantitative GUS assays were also performed with plants grown on increasing mannitol levels for osmotic stress and sodium chloride to induce salt stress. In both lines, GUS activity increased with similar patterns (Supplemental Fig. S1, A and B). Therefore, under osmotic or salt stress, *CoYMVp* probably will not enhance phloem transport beyond the capacity of the natural *AtSUC2* promoter.

Sequence Similarity Is Not a Strong Indicator of *SUT* Capacity to Rescue *Atsuc2*$^{-/-}$ Mutants

Having established that *CoYMVp* can drive *cSUC2* expression in a manner sufficient to rescue *Atsuc2-4* $^{-/-}$

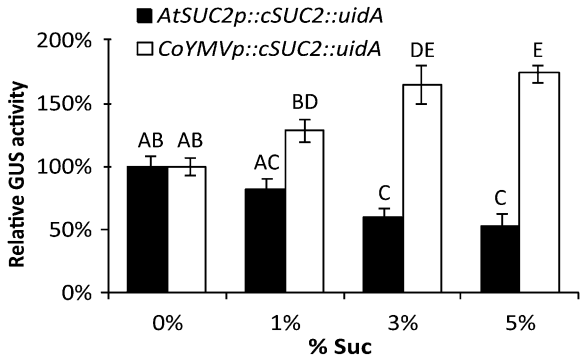

Figure 1. Comparison of *CoYMVp and AtSUC2p* phloem-specific promoter activities in the presence of increasing levels of exogenous Suc. Quantitative GUS analysis in *CoYMVp*::*cSUC2*::*uidA* and *AtSUC2p*::*cSUC2*::*uidA* plants grown on MS medium in the presence of 0%, 1%, 3%, and 5% (w/v) Suc, expressed relative to activity with 0% Suc, to test for potential Suc signaling. Variation is expressed as SE ($n = 6$). Different letters represent significant differences based on univariate ANOVA with Scheffe post hoc analysis at the 0.05 level. See also Supplemental Figure S1 for comparison of promoter activities in the presence of mannitol to mimic osmotic/drought stress and NaCl to mimic salt stress.

(Srivastava et al., 2009b) and that, unlike the endogenous *AtSUC2p*, activity of *CoYMVp* is not repressed by high Suc levels, it was used to test the capacity of other SUTs to restore or improve phloem loading and long-distance transport in *Atsuc2-4*$^{-/-}$. Representative *SUT* genes from each of the four major branches of the gene family were subcloned as cDNA downstream of *CoYMVp* by Gateway recombination (generically referred to as *CoYMVp*::*cSUTX*; Fig. 2). The kinetic properties and physiological role of each have been previously reported (see Supplemental Table S1 for references). The *CoYMVp*::*cSUTX* cassettes were transformed into heterozygous *Atsuc2-4*$^{+/-}$ plants, and transgenic progeny (T1) were genotyped as *AtSUC2*$^{+/+}$ or as *Atsuc2-4*$^{+/-}$ or *Atsuc2-4*$^{-/-}$ by PCR. For each construct, 25 to 30 independent heterozygous *Atsuc2-4*$^{+/-}$ T1 plants were identified that contained transfer DNA (T-DNA) at a single locus, based on a 3:1 segregation for glufosinate ammonium resistance in the T2 generation. From these, T3 or T4 plants that were homozygous *Atsuc2-4* knockouts and also homozygous for the rescuing *CoYMVp*::*cSUTX* cassette were identified, and growth parameters were compared in plants that had been grown for 21 d. Independent lines showed a range of phenotype rescue, as expected, and the most robust lines were selected for further analysis. The level of rescue expressed in terms of average rosette area of the two most robust lines for each cassette is shown in Figure 3.

Based on the visual phenotype, the *SUT* cDNA cassettes could be readily placed into one of two groups: those that promoted rescue (type I *AtSUC2* and *AtSUC1* and type IIB *ZmSUT1*) and those that did not (type I *AtSUC9*, *LeSUT1*, and tobacco [*Nicotiana tabacum*] *NtSUT3*; type IIA *AtSUC3* [also called *AtSUT2*] and *LeSUT2*; and type III *LeSUT4*). Transgene expression in the most robust lines of each cassette was determined by reverse transcription-quantitative

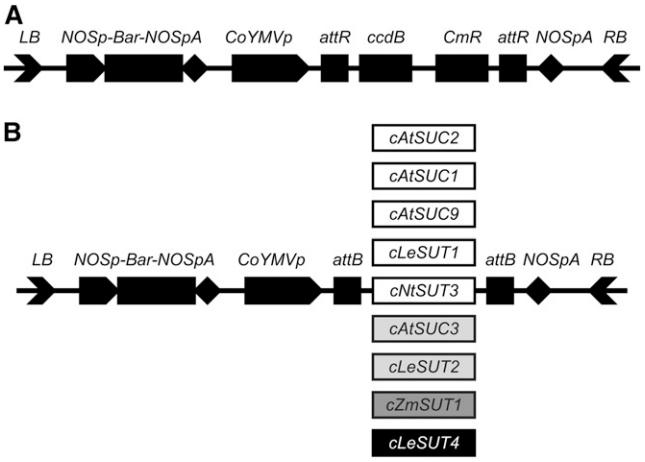

Figure 2. T-DNA cassettes used in this study. A, T-DNA cassette of the Gateway recombination compatible parent vector pGPTV-CoYMVp::ccdB::CmR. B, T-DNA cassettes of the expression vectors used for floral dip transformation and harboring representative *SUT* cDNA from each of the four branches of the *SUT* gene family. White indicates type I, light gray indicates type IIA, dark gray indicates type IIB, and black indicates type III. LB, T-DNA left border; RB, T-DNA right border; *NOSp-Bar-NOSpA*, nopaline synthase promoter-Bialaphos (glufosinate ammonium) resistance cDNA-nopaline synthase polyadenylation signal.

PCR (RT-qPCR). In all cases, transcript abundance was 50% to 80% that of *AtSUC2* in wild-type plants (Fig. 3C). We previously documented that a full gradient of rescue, from none to near-full rescue, could be observed among independent *Atsuc2-4* lines expressing *AtSUC2* cDNA (Srivastava et al., 2009b). Based on this, the lack of growth among those that did not rescue is striking and argues that the degree of rescue (i.e. restoration of vegetative growth) is not the direct result of transcript abundance (compare Fig. 3A and Fig. 3C). Possible reasons for the lack of rescue are addressed further in "Discussion" and further analysis focused on the *AtSUC2*, *AtSUC1*, and *ZmSUT1* lines that were rescued by their respective transgenes.

Each of the rescued lines was smaller than the wild type at 21 d postgermination (Fig. 3), suggesting that although growth was largely restored, there still may be some impairment to Suc transport. When Suc transport is impaired, transient carbohydrates accumulate in photosynthetic tissues. Suc, Glc, Fru, and starch in rosettes were therefore quantified to gauge rescue at the level of Suc export from leaves (Fig. 4). Carbohydrate accumulation showed the expected inverse relationship with rosette growth for impaired export: smaller rosettes (less rescued; e.g. *AtSUC1* lines) had more accumulated carbohydrate than larger rosettes (more rescued; e.g. *AtSUC2* lines). Collectively, these results suggest partial rescue based on relative expression and imply that the capacities of AtSUC2, AtSUC1, and ZmSUT1 for moving Suc across membranes in planta are roughly equal, consistent with kinetic parameters measured in heterologous systems (Chandran et al., 2003; Carpaneto et al., 2005; Sivitz et al., 2008).

Additional Companion Cell-Specific *SUT* Expression Enhances Phloem Loading But Not Productivity

The analysis above shows that *AtSUC2*, *AtSUC1*, and *ZmSUT1* cDNA fused to *CoYMVp* can restore phloem loading to appreciable levels in the *Atsuc2-4* $^{-/-}$ background. We hypothesized that an additive capacity to phloem load would be achieved if these cassettes were introduced into the wild type (*AtSUC2*$^{+/+}$). Because the *CoYMVp*::*cSUTX* cassettes were transformed into heterozygous *AtSUC2*$^{+/-}$ plants, *AtSUC2*$^{+/+}$ siblings of rescued lines (i.e. the same transformation event) were used to test if additional *SUT* expression can hyperload the phloem. These *AtSUC2*$^{+/+}$ lines with *CoYMVp*::*cSUTX* cassettes thus have *SUT* expression from both the endogenous *AtSUC2* promoter and the differentially regulated *CoYMVp*, and *CoYMVp*::*cAtSUC1* and *CoYMVp*::*cZmSUT1* plants, additionally, have two different symporters (AtSUC2 plus the transgene-encoded SUT), which may have different posttranslational regulation, participating in phloem loading. These lines are referred to as *SUT* OE lines because they have additional companion cell-specific *SUT* expression over the natural expression from the endogenous *AtSUC2* gene.

Additive *SUT* expression from the endogenous gene and the cDNAs was confirmed by RT-qPCR. Expression of the cDNA from the companion cell-specific *CoYMVp* roughly equaled or slightly exceeded that of the endogenous gene from the natural *AtSUC2* promoter (Fig. 5A; Supplemental Fig. S2). With additive *SUT* expression, phloem loading, Suc transport, and growth were expected to exceed or at least equal that of the wild type. Unexpectedly, the rosette areas of wild-type lines expressing *SUT* cDNA were not only smaller than wild-type plants but were also smaller than those of the rescued *Atsuc2-4*$^{-/-}$ siblings (Fig. 5, B and C).

To assess if the observed phenotype is linked to carbon partitioning, the major transient carbohydrates were quantified at the end-of-day (ED) and end-of-night (EN) time periods. At 21 d postgermination, *SUT* OE lines had more Glc (Fig. 6A), Fru (Fig. 6B), and Suc (Fig. 6C) at ED compared with the wild type. The *SUT* OE lines also contained more soluble sugar than the wild type at EN, but the differences were subtle and, in many cases, not statistically significant. Starch did not show any significant differences between the wild type and the *SUT* OE lines at ED or EN (Fig. 6D). Apart from the elevated levels of soluble sugar in the *SUT* OE lines, the diurnal patterns of carbohydrate patterns are consistent with published results: Diurnal starch turnover tends to keep the levels of soluble sugars somewhat constant through the 24-h period (Geiger and Servaites, 1994), with slightly higher levels during the day (Bläsing et al., 2005; Gibon et al., 2009; Sulpice et al., 2014). Photosynthesis was estimated by pulsed-amplitude-modulated fluorescence and revealed reductions consistent with the accumulation of sugar in the rosettes (Supplemental Fig. S3).

To test if carbohydrate accumulation in rosettes was affecting sugars in heterotrophic organs, the wild type

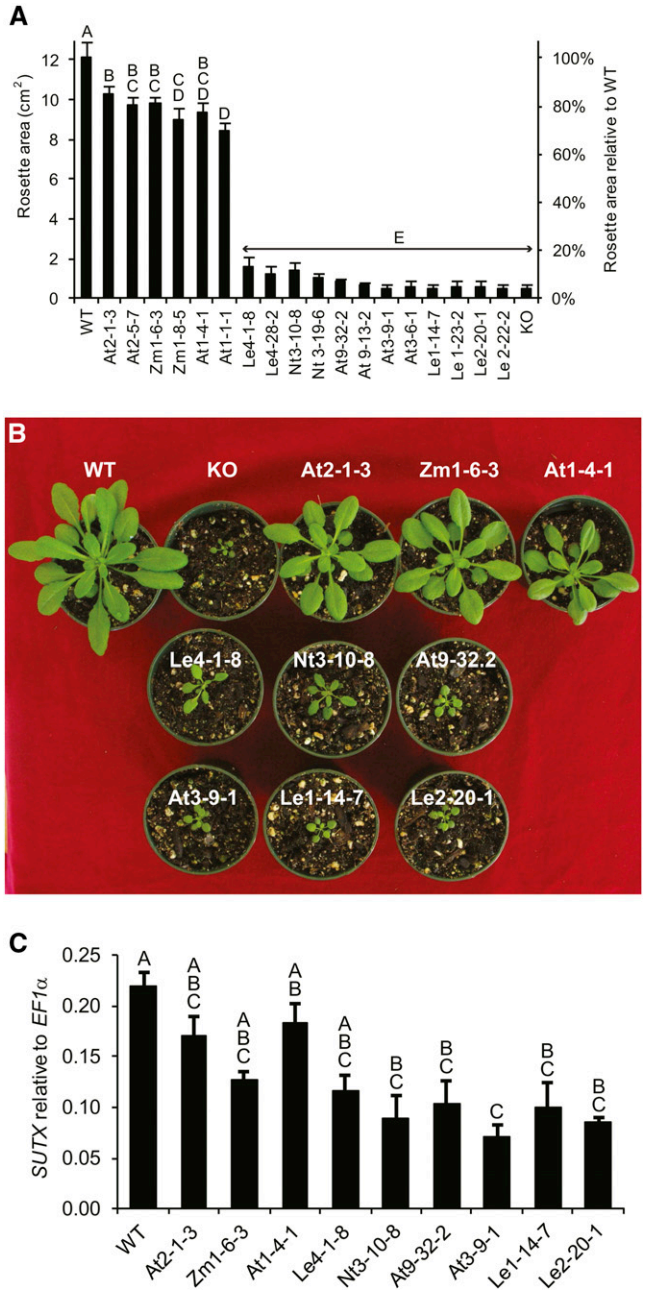

Figure 3. Growth characteristics of wild-type (WT), *Atsuc2-4*$^{-/-}$ knockout (KO), and *Atsuc2-4*$^{-/-}$ lines harboring the *CoYMVp*::*cSUTX* cassettes. A, Rosette area (cm^2) of 21-d-old wild-type and *Atsuc2-4*$^{-/-}$ knockout lines and two independent representative lines of *Atsuc2-4*$^{-/-}$ harboring each of the homozygous *CoYMVp*::*cSUTX* cassettes, arranged by the extent of rescue. The line designations indicate the *SUT* cDNA, the independent transformant number, and a sibling designation and are in the following order: *AtSUC2* (At2-1-3 and At2-5-7), *ZmSUT1* (Zm1-6-3 and Zm1-8-5), *AtSUC1* (At1-4-1 and At1-1-1), *LeSUT4* (Le4-1-8 and Le4-28-2), *NtSUT3* (Nt3-10-8 and Nt3-19-6), *AtSUC9* (At9-32-2 and At9-13-2), *AtSUC3* (At3-9-1 and At3-6-1), *LeSUT1* (Le1-14-7 and Le1-23-2), and *LeSUT2* (Le2-20-1 and Le2-22-2). Variation is expressed as SE (n = 6 sibling plants). Different letters represent significant differences based on univariate ANOVA with Scheffe post hoc analysis at the 0.05 level. B, Representative 21-d-old wild-type and *Atsuc2-4*$^{-/-}$ knockout lines and the indicated rescued *Atsuc2-4*$^{-/-}$ line. For scale, the round pots are 5 cm. C, *SUTX* transcript levels determined by RT-qPCR, relative to the *ELONGATION FACTOR1α* (*EF1α*) transcript (n = 3 individual plants as biological replicates, each with two technical replicates). Variation is expressed as SE. Different letters represent significant differences based on univariate ANOVA with Scheffe post hoc analysis at the 0.05 level. Note that all transgenic plants are in subgroups B or C with respect to *SUTX* expression.

and two *SUT* OE lines (At2-1-6 and Zm1-6-8) were grown on one-half-strength Murashige and Skoog (MS) medium without Suc, and shoots and root tips were analyzed separately. One centimeter of root tip was used for analysis because the tip is the strongest sink. Suc levels 4 h into the light period were higher in both the shoots and root tips of *SUT* OE lines compared with the wild type (Fig. 6, E and F). More Suc in the root provides evidence that transport to sink organs was improved rather than compromised and suggests that sink organ growth patterns may be altered (Kehr et al., 1998). To test for this, a high-resolution time lapse camera was used, and changes in the pattern of diurnal root growth were observed. In standard growth cabinet conditions, wild-type roots have a distinctive elongation pattern: there is a peak when the lights first come on, followed by a rapid drop and leveling off in the remainder of the light period; shortly after the transition to darkness, the root elongation rate drops, followed by recovery as the night period progresses (Yazdanbakhsh et al., 2011). This pattern is observed in the wild type (Fig. 6E) but is altered in the *SUT* OE lines: instead of showing a transient peak and subsequent decline, growth increases after illumination, but less markedly, and remains fairly constant for the remainder of the light period. All genotypes showed a similar response during the night, with a decrease in the rate of root elongation at the start of the night followed by a partial recovery in the middle of the night and a decrease in the last hours of the night (Fig. 6E). These alterations are consistent with the regulation of the *CoYMVp*::*cSUTX* transgenes relative to the endogenous *AtSUC2* gene: Suc accumulation during the day may increase *SUT* cDNA expression from *CoYMVp* and enhance transport to roots, resulting in more consistent elongation throughout the day compared with the wild type.

To further investigate if *SUT* OE increases phloem transport from source to sink organs, rosettes were photosynthetically labeled with [^{14}C]CO_2 for 20 min in the middle of the 14-h light period, and roots were harvested to measure transport of label by scintillation counting. As a percentage of total label in the plant, all three *SUT* OE lines transported more label to the root, indicating that more photoassimilate produced during the 20-min labeling period was loaded into the phloem and allocated to roots (Fig. 7A). When these values were standardized against the wild type, the *SUT* OE lines transported 25% to 30% more label to the root. There was no significant difference among the *SUT* OE

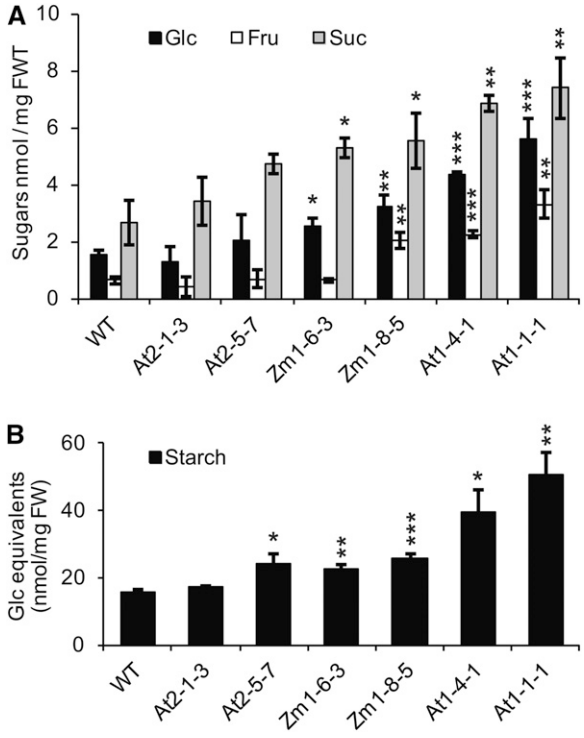

Figure 4. Principal soluble sugars and starch in 21-d-old rosettes of the wild type (WT) and the indicated rescued line. A, Glc, Fru, and Suc levels expressed as nanomoles per milligram of fresh weight (FW). B, Starch levels in the same samples represented in A, expressed as nanomoles Glc equivalents per milligram of fresh weight. Variation is SE ($n = 6$ rosettes of individual plants). Significant differences from the wild type for each carbohydrate are based on Student's t test (*$P \leq 0.05$, **$P \leq 0.01$, and ***$P \leq 0.001$).

lines, indicating that each of the rescuing constructs enhanced phloem transport equally well.

In another method to address the question of whether the *SUT* OE lines have enhanced long-distance transport, the rate of ^{14}C exuded from cut stems into EDTA solution was measured. In these experiments, the first 20 min of exudation, which would contain the contents of cut cells, was discarded, and ^{14}C exudation over the next two 1-h intervals was measured and expressed as counts per minute exuded per hour per milligram of fresh weight. The *SUT* OE plants had phloem exudation rates 4- to 5-fold higher than the wild type (Fig. 7B), indicating more efficient loading and transport of carbon in the phloem.

^{14}C allocation to roots and phloem exudation rates both measure long-distance transport, which is dependent on loading efficiency, but neither measure directly addresses phloem loading (Turgeon and Wolf, 2009). To compare loading among the wild type and *SUT* OE lines, uptake of ^{14}C into leaf veins was measured directly: leaf disks were infiltrated with a [^{14}C]Suc solution for 20 min, washed thoroughly, and freeze dried, and phloem loading was assessed by autoradiography and scintillation counting. The results show that there is more loading in the *SUT* OE lines compared with the wild type (Fig. 7C). Taken together, the experiments above show enhanced loading and long-distance transport, as well as a higher availability of carbon in both source and root sink tissues.

Enhanced Suc Loading and Transport Triggers Perception of a Phosphorous Limitation

The results above argue that phloem loading and transport are enhanced (Figs. 6 and 7), yet the *SUT* OE lines are smaller than the wild type and the rescued *Atsuc2-4*$^{-/-}$ lines (Fig. 5). This is counterintuitive because enhanced transport is predicted to increase carbon supply to growing sink organs while also reducing feedback inhibition on photosynthesis in source leaves (Ainsworth and Bush, 2011). In addition to being important for metabolic activities, sugars provide the majority of biomass for new growth and storage, are important signaling molecules for plant growth and development (Rolland et al., 2006), and interact with other essential nutrients. For example, more carbon from source organs is required for a response to a phosphorous limitation because P deficiencies induce many changes that have high demand for photoassimilate, including enhanced growth of lateral roots and root hairs, changes in gene expression, remodeling of metabolism, secretion of organic acids, and energization of membranes (Misson et al., 2005; Morcuende et al., 2007; Hammond and White, 2008, 2011; Plaxton and Tran, 2011; Veneklaas et al., 2012). Conversely, utilization of sugars and storage carbohydrates requires P for metabolism, which occurs mostly by ATP-dependent formation of phosphoesters. Recently, the mutation *hypersensitive to phosphate starvation1* (*hps1*) was isolated in an activation-tagging screen as having enhanced sensitivity to P deficiency (Lei et al., 2011). Interestingly, *hps1* results in ectopic and ubiquitous activation of *AtSUC2*, and growing *hps1* mutants in the presence of excess P reversed the pleiotropic effects. The authors argued that Suc is a global regulator of P starvation (Lei et al., 2011).

hps1 resulted in ectopic *SUT* OE in all tissues, which is likely to lead to major changes in the distribution of Suc and many pleiotropic responses. Our observation that phloem-specific *SUT* OE resulted in diminished growth prompted us to test if this also resulted in an increased requirement for P. Wild-type and *SUT* OE lines were grown on vertical plates with one-half-strength MS, which includes 0.6 mM P, and 0% Suc, with and without additional P. In the absence of additional P, rosettes and roots of *SUT* OE plants were stunted relative to the wild type. *SUT* OE roots were approximately two-thirds the length of wild-type roots and had fewer lateral roots (Fig. 8, A and C). On medium supplemented with additional PO_4 (ranging from 1.2 to 3 mM total PO_4), root length and rosette size of the *SUT* OE lines recovered and equaled the wild type (Fig. 8, B and C).

It was previously observed that expression levels of *PSI* genes in Arabidopsis seedlings positively correlate with Suc concentration in the culture medium (Karthikeyan

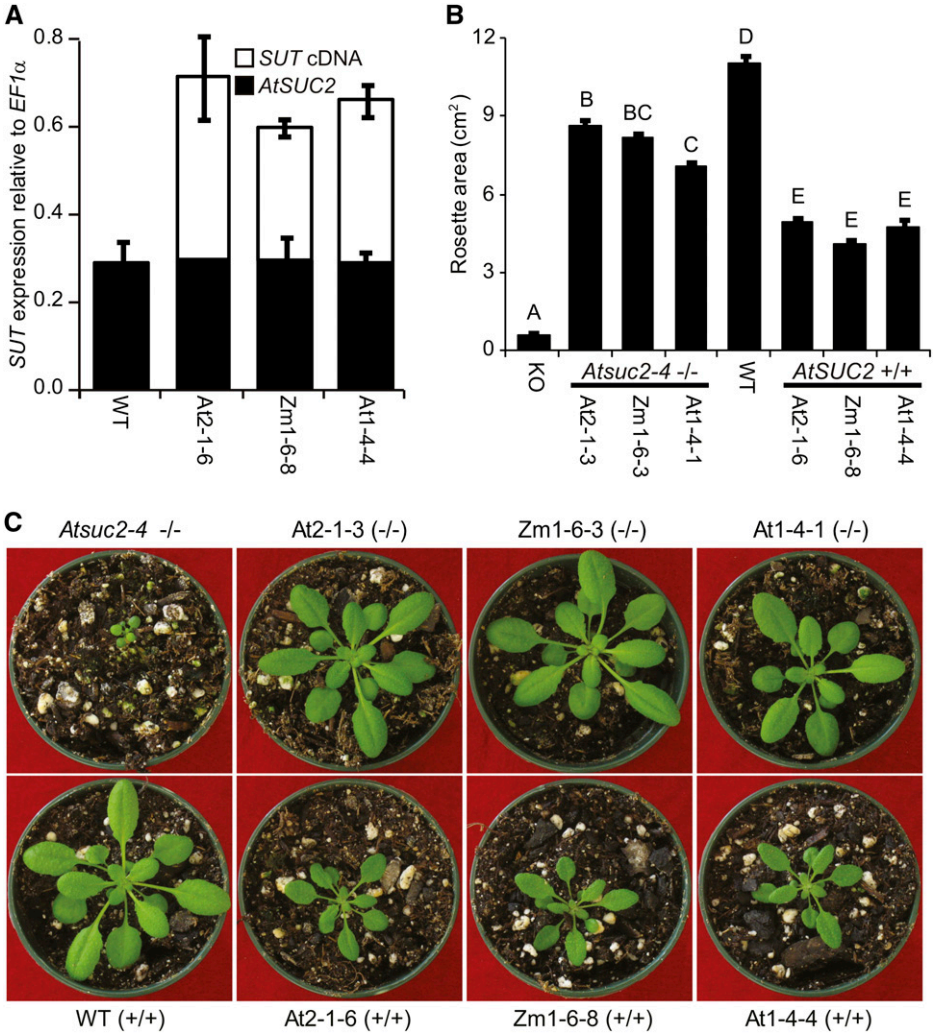

Figure 5. Phloem-specific *SUT* transcript abundance in the wild-type (WT; *AtSUC2*$^{+/+}$) background and growth characterization. A, Phloem-specific *SUT* transcript levels relative to *EF1α* determined by RT-qPCR in the wild-type (*AtSUC2*$^{+/+}$) background. cDNA expressed from *CoYMVp* (white) is superimposed on the endogenous *AtSUC2* expressed from the natural *AtSUC2* promoter (black). Variation is expressed as SE (n = 3 individual plants as biological replicates, each with two technical replicates). See also Supplemental Figure S2. B, Quantitative comparison of rosette area in 21-d-old *Atsuc2-4*$^{-/-}$ knockout (KO), *SUT* transgene-rescued *Atsuc2-4*$^{-/-}$ knockout lines (At2-1-3, Zm1-6-3, and At1-4-1), the wild type, and wild-type lines with additional companion cell-specific *SUT* transgene expression (At2-1-6, Zm1-6-8, and At1-4-4). Note that rescued knockout lines and the wild type with *SUT* expression are segregants of the same transformation event and are thus genetically identical with respect to the *SUT* transgene. Variation is SE (n = 8 plants). Different letters represent significant differences based on univariate ANOVA with Scheffe post hoc analysis at the 0.05 level. C, Representative 21-d-old knockout (*Atsuc2-4*$^{-/-}$), *SUT* transgene-rescued *Atsuc2-4*$^{-/-}$ knockout plants (At2-1-3, Zm1-6-3, and At1-4-1), the wild type, and wild-type plants overexpressing the *SUT* transgenes (At2-1-6, Zm1-6-8, and At1-4-4), as labeled. The genetic background of each line is indicated in parentheses behind the line designations: ($^{-/-}$) for the rescued knockout background and ($^{+/+}$) for the wild-type background with additional companion cell-specific *SUT* expression. For scale, the round pots have a 5-cm diameter.

et al., 2007). To test if companion cell-specific OE of *SUT* genes enhances *PSI* gene expression in the absence of exogenous Suc, representative *PSI* genes were analyzed by RT-qPCR. *AtPT2* (*PHT1;4*, *At2G38940*) and *PHT2;1* (*At3G26570*) are inorganic P transporters induced by P starvation (Karthikeyan et al., 2007; Lei et al., 2011). Both had higher expression levels in the *SUT* OE lines than in the wild type (Fig. 8, D and E). *PURPLE ACID PHOSPHATASE* (*PAP*) gene expression increases during P limitation, and in lines At1-4-4 and Zm1-6-8, *PAP14* (*At2G46880*) increased 400-fold and *PAP24* (*At4G24890*) increased 220-fold relative to the wild type (Fig. 8, F and G). These increases are consistent with those observed among seedlings grown in P-limited conditions relative to those grown in P-replete conditions (Morcuende et al., 2007) and support our premise that increased Suc loading and long-distance transport induces perception of a P deficiency. In addition, during P starvation, plants

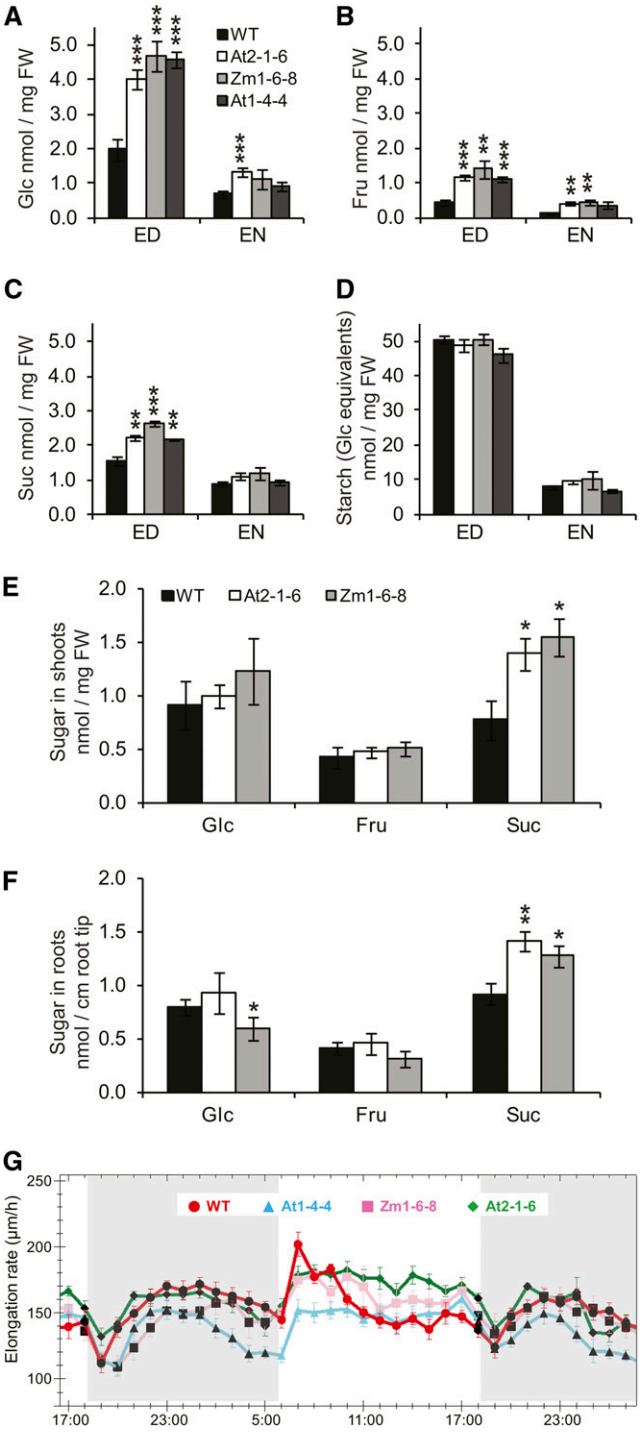

Figure 6. Sugar and starch levels in rosettes of *SUT* overexpressing lines at the end of the day and end of the night, sugar levels in roots, and the impact of additional companion cell-specific *SUT* expression on root growth. A to D, Glc (A), Fru (B), Suc (C), and starch (D; Glc equivalents) levels expressed as nanomoles per milligram of fresh weight (FW) in 21-d-old rosettes harvested within 30 min before the end of the 14-h light period (ED) and within 30 min before the end of the 10-h dark period (EN). Plants were grown on potting mix. E and F, Wild-type (WT) and two *SUT* lines (At2-1-6 and Zm1-6-8) grown on one-half-strength MS medium with Gamborg's vitamins in vertically oriented plates for access to roots. E, Sugar levels in rosettes expressed as nanomoles per milligram of fresh weight. F, Sugar levels in 1 cm of the primary root tip of the plants in E to emphasize the phloem-unloading zone and strongest heterotrophic sinks, expressed as nanomoles per centimeter of root tip. For A to F, black bars represent the wild type, white represents At2-1-6, light gray represents Zm1-6-8, and dark gray represents At1-4-4 (A–D only); variation is SE ($n = 6$ individual plants). Significant differences from the wild type for each carbohydrate are based on Student's *t* test (*$P \leq 0.05$, **$P \leq 0.01$, and ***$P \leq 0.001$). G, Root elongation expressed as micrometers per hour of the wild-type and *SUT* overexpressing lines, as indicated, grown on synthetic medium in vertically oriented plates ($n = 6$ individual plants).

secrete acids into the rhizosphere to help solubilize available P (Hammond and White, 2008). The wild type, At2-1-6, and Zm1-6-8 were grown on one-half-strength MS with 0% Suc and supplemented with bromocresol blue indicator dye. Only in the vicinity of the *SUT* OE lines did the media change from blue to yellow, indicating a drop from the starting pH of 6.8 to less than pH 5.0 and suggesting that these roots were secreting acid in response to perception of a P limitation (Supplemental Fig. S4).

One representative companion cell-specific *SUT* OE line, At2-1-6, was selected for further study. Plants were grown on a range of P concentrations and photosynthetically labeled with [^{14}C]CO_2 for 20 min. Relative to the wild type, At2-1-6 consistently accumulated a larger percentage of total assimilated carbon in the roots, implying enhanced transport to root sinks (Fig. 9A). This increase in ^{14}C export to the roots was seen with all PO_4 supplementations, although it was less pronounced with 3 mM PO_4. Because P deficiency can activate phospho*enol*pyruvate carboxylase to incorporate ^{14}C directly into roots independent of photoassimilate transport (Plaxton and Tran, 2011), roots that were freshly severed from their rosettes were also labeled with [^{14}C]CO_2. In all experiments, ^{14}C incorporation into severed roots was essentially indistinguishable from background, while roots attached to shoots showed substantial accumulation of ^{14}C (Fig. 9B). This indicates that the higher percentage of label in At2-1-6 roots relative to the wild type was the result of enhanced Suc import.

SUT OE from the Natural *AtSUC2* Promoter Causes the Same Phenotype as Expression from the *CoYMV* Promoter

It was previously shown that constitutive OE of *AtSUC2* from the *Cauliflower mosaic virus* 35S promoter could trigger a P limitation response (Lei et al., 2011). However, this might be due to inappropriate expression of Suc transport activity in cells that normally do not accumulate Suc from the apoplast. It is striking from our results that phloem-specific expression has a similar effect. While *CoYMVp* has the same spatial expression as *AtSUC2p*, it responds differently to various stimuli, particularly increased Suc levels (Fig. 1). It is possible that differences in the regulation of *SUT* expression, and not enhanced Suc transport per se, were responsible for

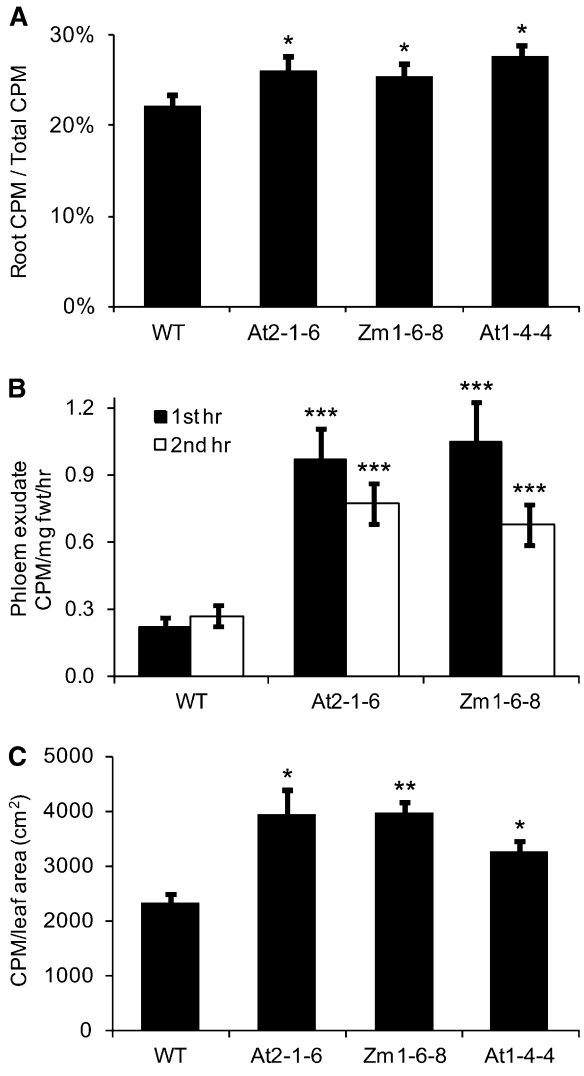

Figure 7. Additional companion cell-specific *SUT* expression enhances Suc loading and long-distance transport as determined by ^{14}C transport to roots, exudation from cut rosettes, and direct [^{14}C]Suc uptake. A, Plants grown on vertical plates were photosynthetically labeled with [^{14}C]CO_2, shoots and roots were analyzed separately by scintillation counting, and ^{14}C transported to the roots was expressed as a percentage of total ^{14}C incorporated. Variation is expressed as SE ($n = 6$). B, Phloem exudation from severed rosettes of soil-grown plants into EDTA-containing solution after photosynthetic labeling with [^{14}C]CO_2 shows enhanced phloem transport of [^{14}C] relative to the wild type (WT), expressed as a rate of counts per minute per hour per milligram of rosette fresh weight (FW). Variation is expressed as SE ($n = 12$ exudations for each line; three of each line in four labeling chambers). C, Uptake of [^{14}C]Suc into leaves of wild-type and *SUT* OE lines expressed as counts per minute per square centimeter of leaf area. The results are the same when expressed as counts per minute per gram of leaf tissue (not shown). CPM, Counts per minute; Variation is SE ($n = 16$ mature leaves from four plants). For A, B, and C, significant differences from the wild type are based on Student's *t* test (*$P \leq 0.05$, **$P \leq 0.01$, and ***$P \leq 0.001$).

the increased requirement for external P. To test this, *AtSUC2* cDNA was overexpressed from 2 kb of the natural *AtSUC2p*. Line KD1039 was previously described as an *Atsuc2-4*$^{-/-}$ mutant rescued with *AtSUC2* cDNA expressed from 2 kb of *AtSUC2p* (Srivastava et al., 2009b). Line KD476 is a sibling of KD1039 segregating *AtSUC2*$^{+/+}$. As shown in Figure 10, KD476 plants overexpressing *AtSUC2* cDNA from *AtSUC2p* have a similar growth phenotype to that of plants expressing *AtSUC2* from *CoYMVp*, implying that they are also perceiving a P limitation. This excludes the possibility that the inhibition of growth in lines in which *SUT* cDNA is expressed under the control of *CoYMVp* is due to a subtle difference in the expression pattern between this heterologous promoter and the endogenous *AtSUC2p*.

DISCUSSION

Solute accumulation in the phloem is a dynamic process that helps coordinate the needs of sink tissues with the output capacity of source tissues and, through osmotic adjustment, maintains phloem hydrostatic pressure during changes in plant water balance. Suc uptake and the activity of the predominant phloem Suc/H^+ symporters is regulated by numerous physiological and environmental stimuli, including leaf development, light, diurnal cycles, sugar signaling, and turgor (Vaughn et al., 2002; Ainsworth and Bush, 2011; Ayre, 2011). Dissecting these specific regulations will contribute to our understanding of how plants control transport processes and response to various stimuli and, from an applied perspective, may provide knowledge that can be used to maintain high rates of transport to target biomass partitioning to desired tissue and organs. Enhanced phloem transport has been proposed as a potential means to increase plant productivity by simultaneously enhancing transport to sink organs and reducing sugar-mediated feedback inhibition on photosynthesis (Ainsworth and Bush, 2011).

Plants contain multiple Suc transporters with differing expression patterns, properties, and subcellular locations. It was shown previously that *AtSUC2* cDNA expressed from various phloem-specific promoters can rescue *Atsuc2-4*$^{-/-}$ knockout plants (Srivastava et al., 2008, 2009b). It was predicted that although these promoters had similar spatial expression as the natural *AtSUC2p*, differences in responses to stimuli could be used to maintain high levels of *SUT* expression and, consequently, high levels of phloem transport in conditions where transport may normally be down-regulated (Srivastava et al., 2009b). *CoYMVp* is from *Commelina yellow mottle virus*, but despite its viral origin, it is specific to phloem companion cells (Medberry et al., 1992; Matsuda et al., 2002). Research presented here demonstrates that while *AtSUC2p* is repressed, *CoYMVp* is up-regulated in the presence of elevated Suc levels and thus can be used to bypass possible endogenous regulation of *AtSUC2* expression at the transcriptional level. In addition to transcriptional regulation, there is evidence for posttranslational regulation of SUT activity through protein-protein interactions (Schulze et al., 2003; Krügel et al., 2008, 2009; Fan et al., 2009). To potentially bypass these levels of regulation, *SUT* genes from each of the four subfamily types (Reinders et al., 2012) were tested for the capacity to

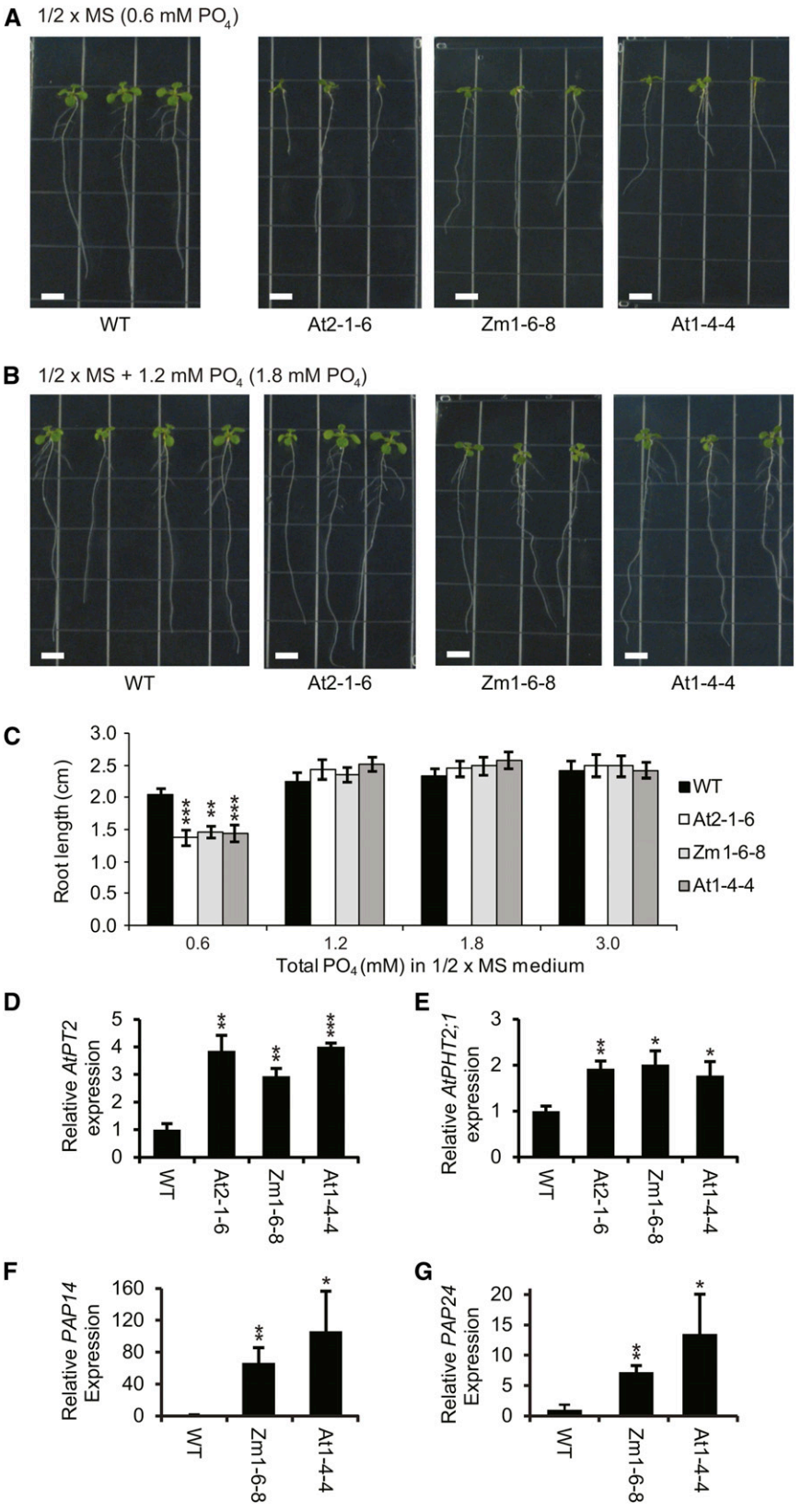

Figure 8. *SUT* OE lines perceive a P deficiency as indicated by rescue of wild-type (WT) levels of growth with P supplements and increased expression of *PSI* genes. A, Wild-type and *SUT* OE plants, as indicated, grown on one-half-strength MS medium with Gamborg's vitamins (0.6 mM total P). *SUT* OE lines have stunted rosettes and shorter roots. B, As in A, with 1.2 mM additional P (1.8 mM total). Rosette and root growth is rescued to wild-type levels. Bars in A and B = 0.5 cm. C, Quantitative assessment of root elongation (cm) with the indicated P concentrations (mM). Variation is SE ($n = 8$ plants per line). Significant differences from the wild type at each P concentration are based on Student's t test ($^{**}P \leq 0.01$ and $^{***}P \leq 0.001$). D to G, Increased expression of *PSI* genes *AtPT2* (D), *AtPHT2;1* (E), *AtPAP14* (F), and *AtPAP24* (G) among *SUT* OE lines relative to the wild type indicate that *SUT* OE lines are perceiving a P deficiency. All plants were grown on potting mix for 21 d as described in "Materials and Methods" ($n = 2$ biological replicates with 2 technical replicates). Significant differences in expression from the wild type are based on Student's t test ($^{*}P \leq 0.05$, $^{**}P \leq 0.01$, and $^{***}P \leq 0.001$).

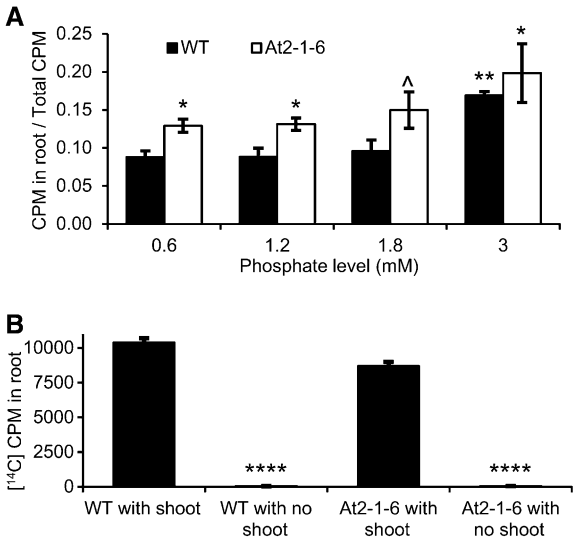

Figure 9. *SUT* OE lines, represented by At2-1-6, allocated more ^{14}C to roots at all tested P concentrations, and direct ^{14}C incorporation into roots was negligible. A, The ratio of ^{14}C counts per minute in roots relative to total counts per minute of plants photosynthetically labeled with $^{14}CO_2$ for 20 min indicates enhanced phloem transport in the *SUT* OE lines relative to the wild type (WT). Variation is SE ($n = 6$). Significant differences in the ratio of counts per minute transported to roots, relative to the wild type at 0.6 mM P, are based on Student's t test ($^{\wedge}P = 0.07$, $^{*}P \leq 0.05$, and $^{**}P \leq 0.01$). B, ^{14}C incorporation into roots without photosynthetic shoots is negligible; thus, the increased percentage of counts per minute in roots of *SUT* OE plants is the result of transport and not direct incorporation by, for example, phospho*enol*pyruvate carboxylase. Variation is SE ($n = 4$). ****, Significant differences in roots between the respective lines (wild type or At2-1-6) with and without shoots, based on Student's t test ($P \leq 1 \times 10^{-7}$). CPM, Counts per minute.

rescue the *Atsuc2-4*$^{-/-}$ mutation and restore efficient phloem transport.

A restoration-of-growth bioassay in *Atsuc2-4*$^{-/-}$ knockout plants was used as a proxy metric for the capacity of each SUT to load Suc into the phloem. The SUTs tested fell into two groups: those that did restore phloem transport and those that did not. As expected, expression of *AtSUC2* using *CoYMVp* complemented the *Atsuc2-4*$^{-/-}$ mutant. However, among the other eudicot-specific type I SUTs tested, only AtSUC1 restored phloem transport. It was previously shown that *AtSUC1* cDNA fused to *AtSUC2p* restores phloem loading in an *Atsuc2* mutant (Wippel and Sauer, 2012), and here, we restore phloem transport with *AtSUC1* expression driven from a viral promoter. Type IIB SUTs are used for phloem loading and other high-affinity uptake processes in monocots. Type IIB *ZmSUT1* cDNA efficiently rescued homozygous *Atsuc2-4*$^{-/-}$ mutant plants, even though the monocot type IIB and eudicot type I *SUT* genes separated in one of the earliest branches of the streptophyte *SUT* gene family (Reinders et al., 2012). It is also documented that *HvSUT1* from barley can restore phloem transport to *Atsuc2*$^{-/-}$ knockout mutants (Reinders et al., 2012). In the study reported here, rescued plants were slightly stunted and accumulated carbohydrate in rosette leaves, implying that Suc transport was not fully restored to wild-type levels but was sufficient for near-normal growth. Because RT-qPCR revealed that *SUT* expression was less than that in the wild type, compromised transport is not surprising.

Type IIA SUTs are characterized as low-affinity/high-capacity plasma membrane SUTs that predate the evolution of vascular plants (Reinders et al., 2012). The kinetic parameters of this group are sufficiently different from those that catalyze phloem loading that it is not surprising that the representative members tested did not rescue the *Atsuc2-4*$^{-/-}$ mutant. Type III SUTs from Arabidopsis, barley, and rice localize to the tonoplast and are proposed to be an ancient tonoplast-localized family, but tomato LeSUT4 and potato StSUT4 are reported to localize to the plasma membrane (Weise et al., 2000; Chincinska et al., 2008). Because of this discordance, we tested the full-length *LeSUT4* sequence in Arabidopsis with the rationale that restoration of phloem transport would support plasma membrane localization. However, transport was not restored, and this negative result does not illuminate the question of LeSUT4 localization.

Perhaps the more surprising outcome here is that many other type I SUTs did not restore phloem transport. AtSUC9 has the highest affinity for Suc among the plant SUTs, and the encoding gene is expressed broadly in sink organs, whereas NtSUT3 and LeSUT1 are the major transporters for phloem loading in the Solanaceae species tobacco and tomato (Lemoine et al., 1999; Hackel et al., 2006; Weise et al., 2008). One possible explanation for the lack of complementation is that these constructs are not expressed at a high enough level. However, we previously demonstrated a broad gradient of rescued phenotypes among *Atsuc2-4* lines that were independently transformed with constructs for companion cell-specific expression of *AtSUC2* cDNA (Srivastava et al., 2009b). This argues that the lack of rescue was not the result of a failure to achieve a required threshold of expression, although this possibility is not formally excluded. Posttranscriptional or posttranslational regulation that limited production, accumulation, or localization of the proteins are also possible explanations for the lack of rescue. For example, StSUT1 and BvSUT1 proteins in potato and sugar beet, respectively, were shown to have half lives in the range of several hours (Kühn et al., 1997; Vaughn et al., 2002), and differential rates of degradation among the proteins encoded by the transgenes tested here may have contributed to the lack of rescue. Notwithstanding, these negative results are worthy of mention because each of these SUTs localizes to the plasma membrane and catalyze membrane transport of Suc in heterologous organisms (yeast and/or *X. laevis* oocytes), showing that they do not have an obligate need of plant-specific cofactors or protein-protein interactions for activity. An intriguing possibility for why these type I SUTs did not rescue is that a plant-specific cofactor or protein, which is present in the phloem but missing in yeast and *X. laevis* oocytes, may act as an inhibitor that is removed to activate

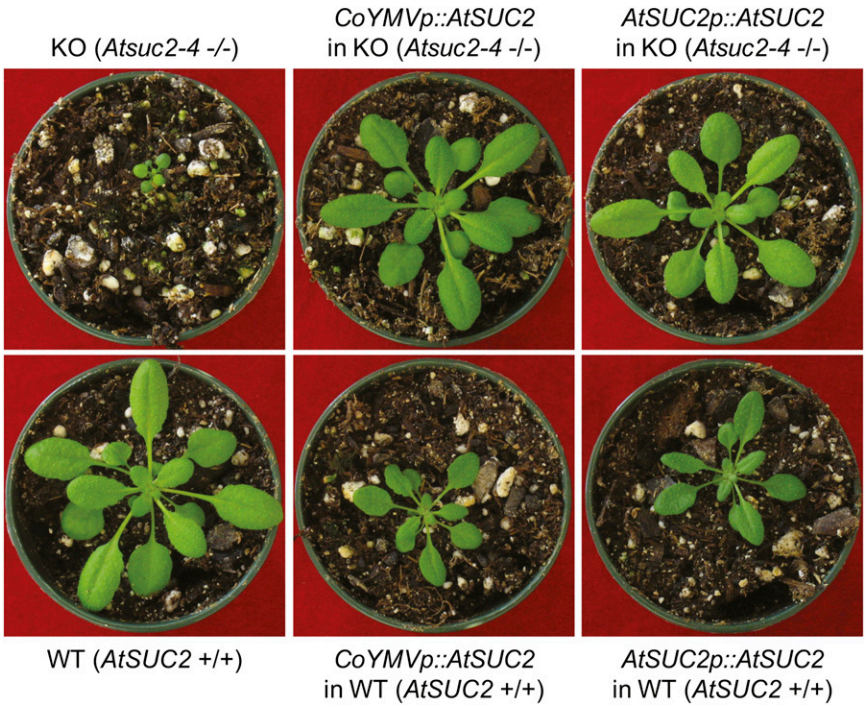

Figure 10. Representative 21-d-old knockout (KO; $^{-/-}$), wild-type (WT; $^{+/+}$), and wild-type and knockout backgrounds with *CoYMVp*::*AtSUC2* and *AtSUC2p*::*AtSUC2* (as labeled) show that *SUT* OE from 2 kb of natural *AtSUC2p* results in the same phenotype observed with the foreign *CoYMVp*.

these SUTs in specific organs or with specific stimuli.

We next tested if the three Suc transporters that could rescue *Atsuc2-4*$^{-/-}$ knockout plants (i.e. *AtSUC1*, *AtSUC2*, and *ZmSUT1*) increased phloem loading and plant growth when overexpressed in companion cells of wild-type *AtSUC2-4*$^{+/+}$ segregants. Unexpectedly, segregating *AtSUC2*$^{+/+}$ sibling lines with companion cell-specific expression of *SUT* cDNA, in addition to the wild-type expression from the endogenous *AtSUC2* gene, were more stunted and contained higher sugar levels in rosettes than their rescued *Atsuc2-4*$^{-/-}$ counterparts. Starch accumulation did not differ significantly between wild-type and *SUT* OE lines. Altered patterns of root elongation were also observed: wild-type plants showed an expected burst of elongation shortly after the onset of day, followed by a gradual decline toward night, but this pattern was distinctly muted in *SUT* OE lines. A possible explanation for these results is that the use of C for growth, as well as the storage and remobilization of transient C reserves, in both source and sink organs is subject to regulation by the circadian clock (Dodd et al., 2005; Graf et al., 2010; Stitt and Zeeman, 2012; Scialdone et al., 2013), but added *SUT* expression from the transgenes disrupts the coordinated distribution of C between source and sinks.

Stunting and carbohydrate accumulation in the *SUT* OE lines was unexpected because our premise was that up-regulating *SUT* expression should enhance phloem transport and growth. Transcript analysis indicated that introduction of *AtSUC1*, *AtSUC2*, and *ZmSUT1* cDNA did not lead to silencing of the endogenous *AtSUC2* gene (Fig. 5). Prior reports argue that SUT proteins can form homodimers and heterodimers when expressed in the same cells, and the ratio of monomers to dimers is influenced by protein abundance (Reinders et al., 2002; Krügel et al., 2008). These putative interactions could impart posttranslational control on SUT activity and lead to an inhibition of phloem loading. For this reason, multiple lines of experimentation were carried out to establish that OE of *AtSUC1*, *AtSUC2*, and *ZmSUT1* cDNA in companion cells leads to enhanced loading and long-distance transport compared with the wild type. First, after labeling intact plants with $^{14}CO_2$, a greater percentage of ^{14}C was transferred to roots in the *SUT* OE lines than the wild type. Second, in EDTA exudation experiments, in which transport is not limited by sink capacity to receive the contents of the translocation stream, exudation rates of ^{14}C were 2- to 4-fold higher in the *SUT* OE lines than the wild type. Third, ^{14}C-Suc uptake into the veins of leaf disks, which is a direct measure of phloem loading, was enhanced in the *SUT* OE lines compared with wild-type plants (Fig. 7).

But if phloem loading and transport are more efficient in *SUT* OE lines, why were the plants debilitated? There is growing evidence that long-distance Suc transport plays an important role in phosphorous responses (Liu et al., 2005; Karthikeyan et al., 2007; Hammond and White, 2008, 2011). The *pho3* mutation, which was identified in a screen for reduced acid phosphatase activity during P limitations, turned out to be a mutant allele of *AtSUC2* (Lloyd and Zakhleniuk, 2004). Both a reduction in photosynthesis and stem girdling, which blocks phloem transport, attenuate P deficiency responses in roots, including a decrease in the expression of many *PSI* genes (Liu et al., 2005; Karthikeyan et al., 2007). In addition, it was recently shown that ubiquitous *SUT*

OE can induce a P deficiency response (Lei et al., 2011), potentially by disrupting normal Suc distribution patterns. It is understandable that normal and efficient Suc transport is required for an effective response to P limitation, because P limitation induces processes that have a high demand for photoassimilate, such as growth of lateral roots and root hairs, changes in gene expression, remodeling of metabolism, secretion of organic acids, and energization of membranes (Misson et al., 2005; Morcuende et al., 2007; Plaxton and Tran, 2011; Veneklaas et al., 2012). However, what is significant and different about the result presented here compared to previous studies is that enhanced Suc transport along the normal distribution path triggers a P-limited phenotype. Our results imply not only that Suc is required to mount a response to a P deficiency, but also that too much Suc in sink organs is apparently interpreted as a P limitation. The same phenotype was obtained whether *AtSUC2*, *AtSUC1*, or *ZmSUT1* cDNA was overexpressed and, for *AtSUC2*, whether this was done using *CoYMVp* or *AtSUC2p*. Achieving the same phenotype with an *AtSUC2p*::*AtSUC2* cDNA construct is also noteworthy because it argues that *AtSUC2* expression, phloem loading, and Suc transport are under prominent dosage level control (i.e. the higher the gene copy number, the more severe the phenotype), in addition to transcription and post-transcriptional control.

We envision several possibilities, which are not mutually exclusive, to explain this link between the supply of Suc and the requirement for P (Fig. 11). One possibility is that the association between long-distance Suc transport and P deficiency is predominantly due to metabolic constraints. Although Suc is the major transport carbohydrate, it is not a prominent storage molecule in most species under physiological conditions. Rather, it is metabolized through a plethora of pathways, and many of the intermediates during its metabolism contain P. Thus, enhanced Suc transport to sink organs and its subsequent metabolism may sequester too much P into P-containing molecules. Any resulting restriction on Suc utilization would lead to an accumulation of Suc in sink organs, which might in turn decrease Suc import via the phloem.

Another possibility is that enhanced Suc transport to sinks may increase synthesis of other P-requiring cellular components related to general growth. In a classic study of barley growing on limiting P supply, about 26% was in free P, 17% was in phospholipids, 26% was in P-containing metabolites, including sugar Ps and nucleotide Ps, and 30% was in nucleic acids (Chapin and Bieleski, 1982). The vast majority of nucleic acids are in ribosomes, which are especially abundant in growing tissues (Detchon and Possingham, 1972; Dean and Leech, 1982; Baerenfaller et al., 2012), where they may represent an even greater proportion of the total P. Suc is known to increase gene expression for nucleotide synthesis and coordinately induce genes for ribosomal proteins and ribosome assembly, especially those for cytosolic ribosomes (Contento et al., 2004; Price et al., 2004; Bläsing et al., 2005; Zrenner et al., 2006; Kojima et al., 2007; Osuna et al., 2007; Usadel et al., 2008; Pal et al., 2013). It is thus plausible that an increased supply of Suc may induce pathways for nucleotide synthesis and ribosome biogenesis, which, in turn, creates an increased demand for P in growing sink tissues. Phospholipids are also important metabolites in growing tissues. Interestingly, among Proteaceae species that are adapted to grow on the extremely P-deficient soils in southwestern Australia, phospholipids are almost completely replaced by galactolipids in mature leaves but still represent the majority of the membrane lipids in young leaves. This implies that phospholipids are required in growing plant organs and cannot be substituted during P deficiencies (Lambers et al., 2012).

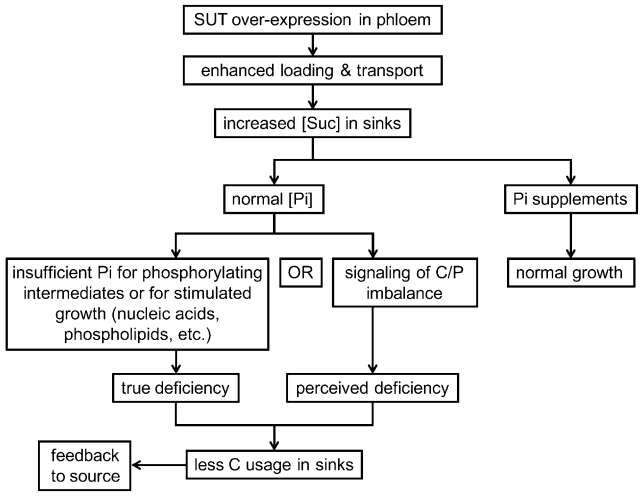

Figure 11. A model for possible relationships between C and P availability. OE of *SUT* genes in the companion cells of source leaves causes additional Suc mobilization to the sink organs. With regular P levels (left), there is insufficient P to activate, via phosphorylation, the incoming carbon, and/or there is insufficient P to support stimulated growth, resulting in a true P deficiency. Alternatively, the sink organs perceive a disruption in C-to-P homeostasis and, through signaling, activate a P starvation response. In either case, less carbon is used in the sinks, and a backlog is created throughout the plant. With P supplements (right), normal growth proceeds. [Pi], Inorganic phosphate concentration; [Suc], sucrose concentration.

Although additional P in the media restored growth among the overexpressing lines to wild-type levels, it is noteworthy that growth did not exceed the wild type. It is also noteworthy that growth did not change as P increased from 1.2 to 3.0 mM (Fig. 8C), suggesting that when P is provided in excess, another limitation prevents growth enhancements, despite additional phloem transport of Suc. While our study focused on Suc transport and the observed association with P, Kellermeier et al. (2014) recently reported on how plants integrate combinatorial nutritional stimuli of N, P, K, S, and light on root architecture. Their results confirm that P limitations are associated with reductions in main root growth. They also report that higher levels of light, which was used as a proxy for more photoassimilate, exasperated the effects of N starvation.

A more speculative possibility is that the apparent P deficiency is not linked directly to metabolism but is

the result of a signaling pathway that monitors the C-to-P balance (Fig. 11). In this scenario, the higher Suc levels in the transport stream may be perceived as a disruption in C-to-P homeostasis, provoking the plant to prepare for a limitation. The addition of more available P to the medium would alleviate this by putting the C-to-P ratio back into balance. In this regard, Suc may act as a global regulator of P starvation as proposed by Lei et al. (2011). Further experiments will be needed to test an interesting implication, which is that the rate of Suc export from source leaves and the allocation of the Suc to recipient tissues may be subject to feedback control to maintain an appropriate balance between the Suc import and P availability in growing sink tissues.

CONCLUSION

Increasing photosynthesis to enhance plant productivity is a prominent goal of plant biology (Ainsworth and Bush, 2011; Evans and von Caemmerer, 2011), as is increasing P use efficiency to minimize the need for this nonrenewable resource in agricultural production and to reduce the deleterious impacts of P used in fertilizers (Veneklaas et al., 2012). The implications of the study presented here are that efforts to enhance photosynthesis and growth by increasing Suc export may be compromised by the plant's perception that it requires more P, unless the links between carbohydrate and P homeostasis are better understood and uncoupled.

MATERIALS AND METHODS

Plasmid Construction

Gateway-compatible (Life Technologies) destination binary vectors with phloem-specific promoters were created by standard techniques (Sambrook and Russel, 2001). The AttR1-CmR-ccdB-AttR2 cassette of pMDC100 (Curtis and Grossniklaus, 2003) was isolated as a *Kpn*I-*Sac*I fragment and, using the same sites, cloned downstream of the *Cucumis melo GALACTINOL SYNTHASE1* promoter (*CmGAS1p*) and *AtSUC2p* of pGEM-CmGAS1p::cSUC2 and pGEM-SUC2p::cSUC2 (Srivastava et al., 2008) to create pGEM-CmGAS1p::CmR-ccdB and pGEM-SUC2p::CmR-ccdB. *CoYMVp* was isolated from pGEM-CoYMVp::cSUC2 (Srivastava et al., 2009b) with *Sbf*I and *Kpn*I and cloned into the same sites of pGEM-SUC2p::CmR-ccdB to create pGEM-CoYMVp::CmR-ccdB. The CmGAS1p::CmR-ccdB and SUC2p::CmR-ccdB cassettes were isolated with *Sbf*I and *Sac*I and cloned into the same sites of pGPTV-bar (Becker et al., 1992) to create the destination vectors pGPTV-CmGAS1p::CmR-ccdB and pGPTV-SUC2p::CmR-ccdB, and the CoYMVp::CmR-ccdB cassette was isolated with *Sbf*I and *Asc*I and cloned into the same sites of pGPTV-CmGAS1p::CmR-ccdB to create destination vector pGPTV-CoYMVp::CmR-ccdB.

pCR8 donor vectors carrying cDNA for *AtSUC1* (The National Center for Biotechnology Information nucleotide accession no. NM_105846), *AtSUT2* (*AtSUC3*; AJ289165), and *AtSUC9* (NM_120699) were gifts from Dr. John Ward (University of Minnesota). *NtSUT3* (AF149981; p195xE::NtSUT3), *LeSUT2* (AF166498; pDR195::LeSUT2), *LeSUT4* (AF176950; pDR195::LeSUT4), and *LeSUT1* (XM_004250320; p112A1::LeSUT1) cDNA were obtained from Dr. John Ward in conventional vectors. *ZmSUT1* (AB008464) was obtained in a pENTR donor vector from Dr. David Braun (University of Missouri). *AtSUC2* cDNA was previously described (Srivastava et al., 2008). cDNA in pCR8 donor vectors were recombined into pGPTV-CoYMVp::CmR-ccdB by LR recombination using Gateway LR Clonase II enzyme mix (Life Technologies). For those in conventional cloning vectors, the cDNA was PCR amplified with the oligonucleotides indicated in Supplemental Table S2, directionally cloned into pENTR/D using a pENTR/D-TOPO Cloning Kit (Life Technologies), and sequenced to confirm accuracy. For compatible antibiotic selection, the cDNA in pENTR donor vectors was first recombined into pMDC7 (Curtis and Grossniklaus, 2003) with Gateway LR Clonase II enzyme mix, then into pDonor-Zeo (Life Technologies) with BP Clonase II enzyme mix, and then into pGPTV-CoYMVp::CmR-ccdB with LR Clonase II enzyme mix. Electroporation into *Agrobacterium tumefaciens* GV3101 mp90, floral dip transformation of Arabidopsis (*Arabidopsis thaliana*) *Atsuc2-4*$^{+/-}$ heterozygous plants, and selection and genotyping of transgenic progeny were as previously described (Srivastava et al., 2008).

Plant Material and Growth Conditions

All plant material was Arabidopsis ecotype Columbia derived from the Arabidopsis Biological Research Center CS60000 or its progeny, CS70000. Unless otherwise noted, seeds were stratified for 72 h at 4°C, and plants were grown on Fafard 3B potting mix in Percival AR95L growth chambers with 14 h of light at 150 μmol photons m^{-2} s^{-1} and 24°C and 10 h of dark at 20°C. Twenty-five or more glufosinate ammonium-resistant lines segregating heterozygous *Atsuc2-4*$^{+/-}$ at the genomic locus were obtained from the T1 generation for each CoYMVp::cDNA construct. These were grown to seed, and among this T2 generation, glufosinate ammonium-resistant siblings were PCR genotyped to identify individual homozygous wild-type *AtSUC2*$^{+/+}$ and knockout (*Atsuc2-4*$^{-/-}$) at the endogenous locus. These were then taken through subsequent generations to identify lines that were homozygous for the companion cell-specific cDNA based on segregation of glufosinate ammonium resistance. All experiments were conducted with T3 or T4 generation seeds. From the 25 or more independent lines identified for each CoYMVp::cDNA construct, two representative lines were selected for further study based on rescue of the *Atsuc2-4*$^{-/-}$ phenotype that best approximated the growth habit of the wild-type control. Plants were digitally photographed, and rosette surface area was measured with ImageJ version 1.38x (Rasband, 2007).

Photosynthesis was measured by imaging pulsed amplitude modulated fluorescence on dark-adapted plants (variable fluorescence/maximal fluorescence; F_v/F_m) based on the equipment manufacturer's instructions (model IMAG-C; Walz).

For plants grown on sterile synthetic media, surface-sterilized seeds were distributed on one-half-strength MS media with Gamborg's vitamins (Phytotechnology Laboratories) and 1% (w/v) Suc (pH 5.8) and solidified with 2.8 g L^{-1} gellan gum (Sigma-Aldrich). Seeds were stratified for 72 h and germinated in the horizontal orientation for 7 d. The plants were then transferred to fresh one-half-strength media with appropriate supplements but without added Suc: for all experiments involving growth on synthetic media, plants were grown in the absence of Suc for at least 7 d before analysis. Media used during GUS analysis are described below. For growth on vertically oriented plates, 5.0 g L^{-1} gellan gum was used for stiffer media and to prevent the roots from growing into the media. Seedlings were transferred such that the rosettes were in a line at the top of the plate and the roots trailed behind on the media surface. The plates were sealed with Parafilm and oriented in the growth chamber 15° from vertical for an additional 7 d. Tissues were harvested between 4 and 6 h into the light period; rosettes and roots were collected and analyzed separately. KH_2PO_4 was used for P-supplemented media to achieve final PO_4 concentrations of 0.6 mM (no supplement), 1.2 mM, 1.8 mM, and 3.0 mM. Potassium levels were kept constant by balancing KH_2PO_4 supplements with K_2SO_4. To test for rhizosphere acidification by transgenic lines relative to wild-type lines, pH indicator dye bromocresol blue was added to one-half-strength MS medium (without Suc or P supplementations) as described (Yang et al., 2007). High-resolution analysis of root elongation on vertically oriented plates was as previously described (Yazdanbakhsh et al., 2011)

Quantitative GUS Analysis

The transgenic lines harboring *CoYMVp::cSUC2::uidA* and *AtSUC2p::cSUC2::uidA* that were used to analyze promoter strength under different environmental conditions were previously described (Srivastava et al., 2008, 2009b). Sterile seeds were stratified and germinated as above, except that full-strength media was used for 10 d. Seedlings were transferred to fresh media without Suc (unless otherwise noted) with different concentrations of Suc (0%, 1%, 3%, and 5% [w/v]), NaCl (0, 50, 100, and 200 mM), or mannitol (0, 100, 200, and 300 mM). After 3 d, seedlings were pooled and used in quantitative GUS assays with 4-methylumbelliferyl-β-D-glucuronide hydrate (Gold Biotechnology) and a VersaFluor fluorometer (Bio-Rad Life Science Research) as described (Weigel and Glazebrook, 2002).

Transcript Analysis

Total RNA was isolated from rosettes of 21-d-old plants using Trizol (Life Technologies) according to the manufacturer's instructions and treated with

RNase-Free DNase I (Ambion TURBO DNase). Six hundred nanograms of RNA from each plant was reverse transcribed with 50 μM oligo(dT) and SuperScript III reverse transcriptase (Life Technologies) according to the manufacturer's instructions. Gene-specific primers used for real-time quantitative PCR are listed in Supplemental Table S3. RT-qPCR was performed with SybrGreen PCR Master Mix (Life Technologies) on an Applied Biosystems ViiA 7 instrument (Life Technologies) using the following amplification protocol: 10-min polymerase activation and denaturation at 95°C followed by 40 cycles of 95°C for 10 s, 58°C for 30 s, and 72°C for 30 s. This was followed by a product melt to confirm a single PCR product. The level of *SUT* gene expression was normalized to that of *EF1α* by subtracting the cycle threshold value of *EF1α* from the cycle threshold value of the *SUT* genes. Three biological and two technical replicates were used for *SUT* measurements. For the *PSI* genes, transcript abundance of *AtPT2* and *AtPHT2;1* were determined relative to *EF1α*, and *AtPAP14* and *AtPAP24* were determined relative to *UBIQUITIN10* (*UBQ10*), using two biological replicates each with two technical replicates, and the transcript abundance in the transgenic lines was expressed relative to the wild type.

Transient Carbohydrate Analysis

Transgenic lines and controls were grown for 21 d on potting mix under the conditions described above or on synthetic medium as described above. All tissues were collected between 4 and 6 h into the light period, unless sampling was done at the end of the day or the end of the night, with plants removed from the growth chamber immediately before sampling. Rosettes (and roots for plants grown on synthetic media) were excised at the hypocotyls, fresh weight was determined, and rosettes and roots were immersed in a minimum of five volumes of ice-cold methanol:chloroform:water extraction solution (12:5:3) containing 10 μM lactose as a standard. Samples were kept on ice until all were collected. Further processing and analysis of the major neutral sugars by high-performance anion exchange chromatography with pulsed amperometric detection was as previously described (Srivastava et al., 2008), except that the Dionex CarboPac PA20 column was held at 30°C. The insoluble fraction of the harvested tissue was analyzed for starch as previously described (Srivastava et al., 2008). Similar experiments were conducted with tissues that were harvested into liquid nitrogen, ground with a pestle, and then extracted and processed as described. Among the carbohydrates analyzed, there was no difference when fresh or frozen tissues were used (not shown).

Radiolabeling and EDTA Exudate Analysis

To investigate transport efficiency among the transgenic lines, an EDTA exudation method (Srivastava et al., 2008) was used to collect phloem sap from severed rosettes of the wild type and representative *SUT* OE lines after photosynthetic labeling with $[^{14}C]CO_2$. Transparent polyethylene terephthalate containers with hinged lids (16.5 × 12.7 × 5.1 cm), commonly used for catering (Douglas Stevens Plastics), were adapted for plant growth by creating drainage holes and filling one-half full with potting mix. The tight seal of the lid made these ideal chambers for photosynthetic labeling with $[^{14}C]CO_2$. Nine plants, three of each wild type, At2-1-6, and Zm1-6-8, in a randomized design, were grown in each chamber with the hinged lids open. Four chambers were prepared. For labeling, the lid seal was supplemented with a film of vacuum grease, and the closed chambers were placed 60 cm below a 400-W metal halide lamp: chambers were processed individually so that each was directly under the light source during labeling, and all labeling was conducted between 6 and 8 h into the standard 14-h light period. $[^{14}C]CO_2$ was generated by mixing 5 μL of $[^{14}C]NaHCO_3$ (1 μCi μL^{-1}, 50 mCi $mmol^{-1}$, MP Biomedicals) with 15 μL of 80% lactic acid in a syringe barrel with a 20-gauge needle extending into the labeling chamber. The needle was removed, and the hole was sealed with modeling clay. Photosynthesis in the presence of $[^{14}C]CO_2$ was for 20 min, and then the chamber was pumped out through a column containing soda lime for 5 min to capture residual $[^{14}C]CO_2$. The chamber was then opened, and photosynthesis continued for 15 min in room air. The rosettes were cut at the hypocotyl, and fresh weight was determined. The hypocotyls were then cut again under EDTA, and collection of exudates from individual plants was as described (Cao et al., 2013). Twenty-five microliters of exudate solution was mixed with 5 mL of ScintiSafe Plus 50% scintillation cocktail (Fisher Scientific), and counts per minute were determined.

To analyze ^{14}C transport to roots in *SUT* OE and wild-type plants, plants were grown on vertical plates with P supplements as described above and labeled 6 to 8 h into the 14-h light cycle as previously described (Cao et al., 2013). The distribution of ^{14}C in rosettes and roots of individual plants was determined by scintillation counting.

For $[^{14}C]$Suc uptake studies, individual leaves of 21-d-old plants were harvested by cutting the petioles near the base of the lamina, fresh weight was established, and plants were submersed in MES buffer (20 mM, pH 5.5, with KOH) plus 2 mM $CaCl_2$ supplemented with $[^{14}C]$Suc (1 mM, 30 kBq mL^{-1}; MP Biomedicals), and weighted down with 4-mm glass beads in wells of 24-well microtiter plates. Each replicate contained four pooled leaves from the same plant. The leaves were vacuum infiltrated for 5 min and incubated at room temperature for 20 min, followed by three washes in fresh buffer without sugar. The leaves were gently blotted dry after washing, placed between sheets of filter paper, and frozen in powdered dry ice. Frozen rosettes were lyophilized in a –30°C chamber for 48 h, pressed flat between steel plates in a vice, and exposed to x-ray film (Kodak BioMax MR Film) for 24 h. The leaves were then cleared with 1 mL of 95% ethanol for 30 min and bleached with 1 mL of commercial bleach for 15 min. Five milliliters of scintillation fluid was added and $[^{14}C]$Suc uptake calculated as counts per minute per milligram of fresh weight (not shown) or counts per minute per leaf surface area (Fig. 7C). The leaf surface area was measured with ImageJ version 1.43u (Rasband, 2010).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Comparison of *CoYMVp* and *AtSUC2p* companion cell-specific promoter activities in the presence of different stimuli.

Supplemental Figure S2. RT-qPCR analysis of *AtSUC1*, *AtSUC2*, and *ZmSUT1* transcript levels in wild-type plants and wild-type lines with additional companion cell-specific *SUT* expression.

Supplemental Figure S3. Photosynthesis is reduced in *SUT* overexpressing lines.

Supplemental Figure S4. Increased rhizosphere acidification by plants with additional *SUT* expression in companion cells.

Supplemental Table S1. Cross reference for the most prevalent type, group, and clade designations used by different authors, and the biochemical characteristics and apparent functions of representative members.

Supplemental Table S2. Oligonucleotides used to create the indicated SUT cDNA by PCR for TOPO directional cloning.

Supplemental Table S3. Oligonucleotides used for RT-qPCR analysis of expression for the indicated genes.

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LITERATURE CITED

Ainsworth EA, Bush DR (2011) Carbohydrate export from the leaf: a highly regulated process and target to enhance photosynthesis and productivity. Plant Physiol **155:** 64–69

Aoki N, Hirose T, Scofield GN, Whitfeld PR, Furbank RT (2003) The sucrose transporter gene family in rice. Plant Cell Physiol **44:** 223–232

Ayre BG (2011) Membrane-transport systems for sucrose in relation to whole-plant carbon partitioning. Mol Plant **4:** 377–394

Baerenfaller K, Massonnet C, Walsh S, Baginsky S, Bühlmann P, Hennig L, Hirsch-Hoffmann M, Howell KA, Kahlau S, Radziejwoski A, et al (2012) Systems-based analysis of Arabidopsis leaf growth reveals adaptation to water deficit. Mol Syst Biol **8:** 606

Barker L, Kühn C, Weise A, Schulz A, Gebhardt C, Hirner B, Hellmann H, Schulze W, Ward JM, Frommer WB (2000) SUT2, a putative sucrose sensor in sieve elements. Plant Cell **12:** 1153–1164

Barth I, Meyer S, Sauer N (2003) PmSUC3: characterization of a SUT2/SUC3-type sucrose transporter from *Plantago major*. Plant Cell **15:** 1375–1385

Becker D, Kemper E, Schell J, Masterson R (1992) New plant binary vectors with selectable markers located proximal to the left T-DNA border. Plant Mol Biol **20:** 1195–1197

Bläsing OE, Gibon Y, Günther M, Höhne M, Morcuende R, Osuna D, Thimm O, Usadel B, Scheible WR, Stitt M (2005) Sugars and circadian regulation make major contributions to the global regulation of diurnal gene expression in *Arabidopsis*. Plant Cell **17:** 3257–3281

Braun DM, Slewinski TL (2009) Genetic control of carbon partitioning in grasses: roles of sucrose transporters and *tie-dyed* loci in phloem loading. Plant Physiol **149:** 71–81

Bush DR (1999) Sugar transporters in plant biology. Curr Opin Plant Biol **2:** 187–191

Cao T, Lahiri I, Singh V, Louis J, Shah J, Ayre BG (2013) Metabolic engineering of raffinose-family oligosaccharides in the phloem reveals alterations in carbon partitioning and enhances resistance to green peach aphid. Front Plant Sci **4:** 263

Carpaneto A, Geiger D, Bamberg E, Sauer N, Fromm J, Hedrich R (2005) Phloem-localized, proton-coupled sucrose carrier ZmSUT1 mediates sucrose efflux under the control of the sucrose gradient and the proton motive force. J Biol Chem **280:** 21437–21443

Chandran D, Reinders A, Ward JM (2003) Substrate specificity of the *Arabidopsis thaliana* sucrose transporter AtSUC2. J Biol Chem **278:** 44320–44325

Chapin FS, Bieleski RL (1982) Mild phosphorus stress in barley and a related low-phosphorus-adapted barleygrass: phosphorus fractions and phosphate absorption in relation to growth. Physiol Plant **54:** 309–317

Chen LQ, Qu XQ, Hou BH, Sosso D, Osorio S, Fernie AR, Frommer WB (2012) Sucrose efflux mediated by SWEET proteins as a key step for phloem transport. Science **335:** 207–211

Chincinska IA, Liesche J, Krügel U, Michalska J, Geigenberger P, Grimm B, Kühn C (2008) Sucrose transporter StSUT4 from potato affects flowering, tuberization, and shade avoidance response. Plant Physiol **146:** 515–528

Contento AL, Kim SJ, Bassham DC (2004) Transcriptome profiling of the response of Arabidopsis suspension culture cells to Suc starvation. Plant Physiol **135:** 2330–2347

Curtis MD, Grossniklaus U (2003) A gateway cloning vector set for high-throughput functional analysis of genes in planta. Plant Physiol **133:** 462–469

Dean C, Leech RM (1982) Genome expression during normal leaf development. I. Cellular and chloroplast numbers and DNA, RNA, and protein levels in tissues of different ages within a seven-day-old wheat leaf. Plant Physiol **69:** 904–910

Detchon P, Possingham JV (1972) Ribosomal-RNA distribution during leaf development in spinach. Phytochemistry **11:** 943–947

Dodd AN, Salathia N, Hall A, Kévei E, Tóth R, Nagy F, Hibberd JM, Millar AJ, Webb AA (2005) Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage. Science **309:** 630–633

Evans JR, von Caemmerer S (2011) Enhancing photosynthesis. Plant Physiol **155:** 19

Fan RC, Peng CC, Xu YH, Wang XF, Li Y, Shang Y, Du SY, Zhao R, Zhang XY, Zhang LY, et al (2009) Apple sucrose transporter SUT1 and sorbitol transporter SOT6 interact with cytochrome *b5* to regulate their affinity for substrate sugars. Plant Physiol **150:** 1880–1901

Geiger DR, Servaites JC (1994) Diurnal regulation of photosynthetic carbon metabolism in C_3 plants. Annu Rev Plant Physiol Plant Mol Biol **45:** 235–256

Giaquinta RT (1983) Phloem loading of sucrose. Annu Rev Plant Physiol Plant Mol Biol **34:** 347–387

Gibon Y, Pyl ET, Sulpice R, Lunn JE, Höhne M, Günther M, Stitt M (2009) Adjustment of growth, starch turnover, protein content and central metabolism to a decrease of the carbon supply when Arabidopsis is grown in very short photoperiods. Plant Cell Environ **32:** 859–874

Gottwald JR, Krysan PJ, Young JC, Evert RF, Sussman MR (2000) Genetic evidence for the in planta role of phloem-specific plasma membrane sucrose transporters. Proc Natl Acad Sci USA **97:** 13979–13984

Graf A, Schlereth A, Stitt M, Smith AM (2010) Circadian control of carbohydrate availability for growth in Arabidopsis plants at night. Proc Natl Acad Sci USA **107:** 9458–9463

Hackel A, Schauer N, Carrari F, Fernie AR, Grimm B, Kühn C (2006) Sucrose transporter LeSUT1 and LeSUT2 inhibition affects tomato fruit development in different ways. Plant J **45:** 180–192

Hammond JP, White PJ (2008) Sucrose transport in the phloem: integrating root responses to phosphorus starvation. J Exp Bot **59:** 93–109

Hammond JP, White PJ (2011) Sugar signaling in root responses to low phosphorus availability. Plant Physiol **156:** 1033–1040

Karthikeyan AS, Varadarajan DK, Jain A, Held MA, Carpita NC, Raghothama KG (2007) Phosphate starvation responses are mediated by sugar signaling in Arabidopsis. Planta **225:** 907–918

Kehr J, Hustiak F, Walz C, Willmitzer L, Fisahn J (1998) Transgenic plants changed in carbon allocation pattern display a shift in diurnal growth pattern. Plant J **16:** 497–503

Kellermeier F, Armengaud P, Seditas TJ, Danku J, Salt DE, Amtmann A (April 1, 2014) Analysis of the root system architecture of *Arabidopsis* provides a quantitative readout of crosstalk between nutritional signals. Plant Cell http://dx.doi.org/10.1105/tpc.113.122101

Kojima H, Suzuki T, Kato T, Enomoto K, Sato S, Kato T, Tabata S, Sáez-Vasquez J, Echeverría M, Nakagawa T, et al (2007) Sugar-inducible expression of the *nucleolin-1* gene of *Arabidopsis thaliana* and its role in ribosome synthesis, growth and development. Plant J **49:** 1053–1063

Krügel U, Veenhoff LM, Langbein J, Wiederhold E, Liesche J, Friedrich T, Grimm B, Martinoia E, Poolman B, Kühn C (2008) Transport and sorting of the *Solanum tuberosum* sucrose transporter SUT1 is affected by posttranslational modification. Plant Cell **20:** 2497–2513

Krügel U, Veenhoff LM, Langbein J, Wiederhold E, Liesche J, Friedrich T, Grimm B, Martinoia E, Poolman B, Kühn C (2009) Transport and sorting of the *Solanum tuberosum* sucrose transporter SUT1 Is affected by posttranslational modification. Correction. Plant Cell **21:** 4059–4060

Kühn C, Franceschi VR, Schulz A, Lemoine R, Frommer WB (1997) Macromolecular trafficking indicated by localization and turnover of sucrose transporters in enucleate sieve elements. Science **275:** 1298–1300

Kühn C, Grof CPL (2010) Sucrose transporters of higher plants. Curr Opin Plant Biol **13:** 288–298

Kühn C, Quick WP, Schulz A, Riesmeier JW, Sonnewald U, Frommer WB (1996) Companion cell-specific inhibition of the potato sucrose transporter SUT1. Plant Cell Environ **19:** 1115–1123

Lambers H, Cawthray GR, Giavalisco P, Kuo J, Laliberté E, Pearse SJ, Scheible WR, Stitt M, Teste F, Turner BL (2012) Proteaceae from severely phosphorus-impoverished soils extensively replace phospholipids with galactolipids and sulfolipids during leaf development to achieve a high photosynthetic phosphorus-use-efficiency. New Phytol **196:** 1098–1108

Leggewie G, Kolbe A, Lemoine R, Roessner U, Lytovchenko A, Zuther E, Kehr J, Frommer WB, Riesmeier JW, Willmitzer L, et al (2003) Overexpression of the sucrose transporter SoSUT1 in potato results in alterations in leaf carbon partitioning and in tuber metabolism but has little impact on tuber morphology. Planta **217:** 158–167

Lei MG, Liu YD, Zhang BC, Zhao YT, Wang XJ, Zhou YH, Raghothama KG, Liu D (2011) Genetic and genomic evidence that sucrose is a global regulator of plant responses to phosphate starvation in Arabidopsis. Plant Physiol **156:** 1116–1130

Lemoine R, Bürkle L, Barker L, Sakr S, Kühn C, Regnacq M, Gaillard C, Delrot S, Frommer WB (1999) Identification of a pollen-specific sucrose transporter-like protein NtSUT3 from tobacco. FEBS Lett **454:** 325–330

Liu JQ, Samac DA, Bucciarelli B, Allan DL, Vance CP (2005) Signaling of phosphorus deficiency-induced gene expression in white lupin requires sugar and phloem transport. Plant J **41:** 257–268

Lloyd JC, Zakhleniuk OV (2004) Responses of primary and secondary metabolism to sugar accumulation revealed by microarray expression analysis of the Arabidopsis mutant, *pho3*. J Exp Bot **55:** 1221–1230

Matsuda Y, Liang GQ, Zhu YL, Ma FS, Nelson RS, Ding B (2002) The Commelina yellow mottle virus promoter drives companion-cell-specific gene expression in multiple organs of transgenic tobacco. Protoplasma **220:** 51–58

Medberry SL, Lockhart BEL, Olszewski NE (1992) The Commelina yellow mottle virus promoter is a strong promoter in vascular and reproductive tissues. Plant Cell **4:** 185–192

Misson J, Raghothama KG, Jain A, Jouhet J, Block MA, Bligny R, Ortet P, Creff A, Somerville S, Rolland N, et al (2005) A genome-wide transcriptional analysis using *Arabidopsis thaliana* Affymetrix gene chips determined plant responses to phosphate deprivation. Proc Natl Acad Sci USA **102:** 11934–11939

Morcuende R, Bari R, Gibon Y, Zheng W, Pant BD, Bläsing O, Usadel B, Czechowski T, Udvardi MK, Stitt M, et al (2007) Genome-wide reprogramming of metabolism and regulatory networks of Arabidopsis in response to phosphorus. Plant Cell Environ **30:** 85–112

Osuna D, Usadel B, Morcuende R, Gibon Y, Bläsing OE, Höhne M, Günter M, Kamlage B, Trethewey R, Scheible WR, et al (2007) Temporal responses of transcripts, enzyme activities and metabolites after adding sucrose to carbon-deprived Arabidopsis seedlings. Plant J **49:** 463–491

Pal SK, Liput M, Piques M, Ishihara H, Obata T, Martins MCM, Sulpice R, van Dongen JT, Fernie AR, Yadav UP, et al (2013) Diurnal changes of

polysome loading track sucrose content in the rosette of wild-type Arabidopsis and the starchless *pgm* mutant. Plant Physiol **162:** 1246–1265

Paul MJ, Foyer CH (2001) Sink regulation of photosynthesis. J Exp Bot **52:** 1383–1400

Plaxton WC, Tran HT (2011) Metabolic adaptations of phosphate-starved plants. Plant Physiol **156:** 1006–1015

Price J, Laxmi A, St Martin SK, Jang JC (2004) Global transcription profiling reveals multiple sugar signal transduction mechanisms in *Arabidopsis*. Plant Cell **16:** 2128–2150

Rasband WS (2010) ImageJ. http://rsb.info.nih.gov/ij

Reinders A, Schulze W, Kühn C, Barker L, Schulz A, Ward JM, Frommer WB (2002) Protein-protein interactions between sucrose transporters of different affinities colocalized in the same enucleate sieve element. Plant Cell **14:** 1567–1577

Reinders A, Sivitz AB, Ward JM (2012) Evolution of plant sucrose uptake transporters. Front Plant Sci **3:** 22

Rolland F, Baena-Gonzalez E, Sheen J (2006) Sugar sensing and signaling in plants: conserved and novel mechanisms. Annu Rev Plant Biol **57:** 675–709

Rosche E, Blackmore D, Tegeder M, Richardson T, Schroeder H, Higgins TJV, Frommer WB, Offler CE, Patrick JW (2002) Seed-specific overexpression of a potato sucrose transporter increases sucrose uptake and growth rates of developing pea cotyledons. Plant J **30:** 165–175

Sambrook J, Russel DW (2001) Molecular Cloning: A Laboratory Manual, Ed 3. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Sauer N (2007) Molecular physiology of higher plant sucrose transporters. FEBS Lett **581:** 2309–2317

Schulze WX, Reinders A, Ward J, Lalonde S, Frommer WB (2003) Interactions between co-expressed Arabidopsis sucrose transporters in the split-ubiquitin system. BMC Biochem **4:** 3

Scialdone A, Mugford ST, Feike D, Skeffington A, Borrill P, Graf A, Smith AM, Howard M (2013) Arabidopsis plants perform arithmetic division to prevent starvation at night. Elife **2:** e00669

Sivitz AB, Reinders A, Ward JM (2008) Arabidopsis sucrose transporter AtSUC1 is important for pollen germination and sucrose-induced anthocyanin accumulation. Plant Physiol **147:** 92–100

Slewinski TL, Meeley R, Braun DM (2009) Sucrose transporter1 functions in phloem loading in maize leaves. J Exp Bot **60:** 881–892

Srivastava AC, Dasgupta K, Ajieren E, Costilla G, McGarry RC, Ayre BG (2009a) *AtSUC2*, the predominant sucrose/proton symporter involved in phloem loading, is not an essential gene as plants harbouring a null allele are able to complete their life cycle and produce viable seed. Ann Bot (Lond) **104:** 1121–1128

Srivastava AC, Ganesan S, Ismail IO, Ayre BG (2008) Functional characterization of the Arabidopsis AtSUC2 Sucrose/H^+ symporter by tissue-specific complementation reveals an essential role in phloem loading but not in long-distance transport. Plant Physiol **148:** 200–211

Srivastava AC, Ganesan S, Ismail IO, Ayre BG (2009b) Effective carbon partitioning driven by exotic phloem-specific regulatory elements fused to the *Arabidopsis thaliana AtSUC2* sucrose-proton symporter gene. BMC Plant Biol **9:** 7

Stitt M (2013) Progress in understanding and engineering primary plant metabolism. Curr Opin Biotechnol **24:** 229–238

Stitt M, Sulpice R, Keurentjes J (2010) Metabolic networks: how to identify key components in the regulation of metabolism and growth. Plant Physiol **152:** 428–444

Stitt M, Zeeman SC (2012) Starch turnover: pathways, regulation and role in growth. Curr Opin Plant Biol **15:** 282–292

Sulpice R, Flis A, Ivakov AA, Apelt F, Krohn N, Encke B, Abel C, Feil R, Lunn JE, Stitt M (2014) Arabidopsis coordinates the diurnal regulation of carbon allocation and growth across a wide range of photoperiods. Mol Plant **7:** 137–155

Sun A, Dai Y, Zhang X, Li C, Meng K, Xu H, Wei X, Xiao G, Ouwerkerk PB, Wang M, et al (2011) A transgenic study on affecting potato tuber yield by expressing the rice sucrose transporter genes *OsSUT5Z* and *OsSUT2M*. J Integr Plant Biol **53:** 586–595

Turgeon R, Wolf S (2009) Phloem transport: cellular pathways and molecular trafficking. Annu Rev Plant Biol **60:** 207–221

Usadel B, Bläsing OE, Gibon Y, Retzlaff K, Höhne M, Günther M, Stitt M (2008) Global transcript levels respond to small changes of the carbon status during progressive exhaustion of carbohydrates in Arabidopsis rosettes. Plant Physiol **146:** 1834–1861

Vaughn MW, Harrington GN, Bush DR (2002) Sucrose-mediated transcriptional regulation of sucrose symporter activity in the phloem. Proc Natl Acad Sci USA **99:** 10876–10880

Veneklaas EJ, Lambers H, Bragg J, Finnegan PM, Lovelock CE, Plaxton WC, Price CA, Scheible WR, Shane MW, White PJ, et al (2012) Opportunities for improving phosphorus-use efficiency in crop plants. New Phytol **195:** 306–320

Weichert N, Saalbach I, Weichert H, Kohl S, Erban A, Kopka J, Hause B, Varshney A, Sreenivasulu N, Strickert M, et al (2010) Increasing sucrose uptake capacity of wheat grains stimulates storage protein synthesis. Plant Physiol **152:** 698–710

Weigel D, Glazebrook J (2002) Arabidopsis: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Weise A, Barker L, Kühn C, Lalonde S, Buschmann H, Frommer WB, Ward JM (2000) A new subfamily of sucrose transporters, SUT4, with low affinity/high capacity localized in enucleate sieve elements of plants. Plant Cell **12:** 1345–1355

Weise A, Lalonde S, Kühn C, Frommer WB, Ward JM (2008) Introns control expression of sucrose transporter LeSUT1 in trichomes, companion cells and in guard cells. Plant Mol Biol **68:** 251–262

Wippel K, Sauer N (2012) Arabidopsis SUC1 loads the phloem in suc2 mutants when expressed from the SUC2 promoter. J Exp Bot **63:** 669–679

Yang H, Knapp J, Koirala P, Rajagopal D, Peer WA, Silbart LK, Murphy A, Gaxiola RA (2007) Enhanced phosphorus nutrition in monocots and dicots over-expressing a phosphorus-responsive type I H^+-pyrophosphatase. Plant Biotechnol J **5:** 735–745

Yazdanbakhsh N, Sulpice R, Graf A, Stitt M, Fisahn J (2011) Circadian control of root elongation and C partitioning in *Arabidopsis thaliana*. Plant Cell Environ **34:** 877–894

Zrenner R, Stitt M, Sonnewald U, Boldt R (2006) Pyrimidine and purine biosynthesis and degradation in plants. Annu Rev Plant Biol **57:** 805–836