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Report

Leaf Fructose Content Is Controlled by the Vacuolar Transporter SWEET17 in *Arabidopsis*

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Summary

In higher plants, soluble sugars are mainly present as sucrose, glucose, and fructose [1]. Sugar allocation is based on both source-to-sink transport and intracellular transport between the different organelles [2, 3] and depends on actual plant requirements [4]. Under abiotic stress conditions, such as nitrogen limitation, carbohydrates accumulate in plant cells [5]. Despite an increasing number of genetic studies [6, 7], the genetic architecture determining carbohydrate composition is poorly known. Using a quantitative genetics approach, we determined that the carrier protein SWEET17 is a major factor controlling fructose content in *Arabidopsis* leaves. We observed that when *SWEET17* expression is reduced, either by induced or natural variation, fructose accumulates in leaves, suggesting an enhanced storage capacity. Subcellular localization of SWEET17-GFP to the tonoplast and functional expression in *Xenopus* oocytes showed that SWEET17 is the first vacuolar fructose transporter to be characterized in plants. Physiological studies in planta provide evidence that SWEET17 acts to export fructose out of the vacuole. Overall, our results suggest that natural variation in leaf fructose levels is controlled by the vacuolar fructose transporter *SWEET17*. *SWEET17* is highly conserved across the plant kingdom; thus, these findings offer future possibilities to modify carbohydrate partitioning in crops.

Results

Fine Mapping of a QTL for Fructose Content to the *SWEET17* Gene

A fine-mapping population was developed from the heterogeneous line HIF195, and a total of 960 individuals were genotyped with nine markers covering the candidate region (Figure 1A). Thirty-two recombinants between the two extreme markers CAPS15270 and CAPS16770 were self-fertilized to generate newly recombined heterogeneous inbred families (HIFs), hereafter referred to as rHIFs. Geno- and phenotyping of these families found four rHIFs that reduced the FR3.4 locus to the interval between CAPS897 and CAPS16060 (see Figure S1A available online). By sequencing the haplotypes of these rHIFs, we mapped the recombination points and delimited the quantitative-trait locus (QTL) FR3.4 to a 3.03 kb interval (Figure 1B). According to the TAIR10 genome release, the only gene located in this interval is *SWEET17* (At4g15920). The QTL candidate region covers the third exon to the 3′ UTR, excluding the promoter and the first 94 amino acids. The *SWEET* gene family has been recently described as a new class of plasma membrane-located glucose and sucrose transporters in several kingdoms, including plants [8, 9].

SWEET17 Is Associated with Fructose Levels in the Worldwide *Arabidopsis* Population

Comparison of the *SWEET17* sequences of the Bay-0 and Shahdara parents revealed several polymorphisms (Figure 1B; Table S1), but all except one in the last exon are silent. We analyzed 59 *Arabidopsis* natural accessions for relative fructose levels compared to the total sugar (fructose, glucose, and sucrose) content under nitrogen (N)-limiting conditions. Association mapping revealed that sequence variations in the fourth intron are highly and significantly correlated with relative fructose levels (Figure 1C). The amino acid change in the last exon is therefore unlikely to be primarily responsible for variation in fructose contents.

SWEET17 Controls Leaf Fructose Contents

To confirm the role of *SWEET17* in controlling leaf fructose levels, we analyzed two null mutants, *sweet17-1* and *sweet17-2*, available in the Col-0 genetic background (Figures S1B and S1C). Under N limitation, fructose accumulated 2.2 and 1.8 times more in the leaves of *sweet17-1* and *sweet17-2*, respectively, compared to the wild-type (WT) (Figure 1D), showing that *SWEET17* controls the fructose content in leaves. Thus, mutations in *SWEET17* and the Shahdara allele induce similar effects.

Validation of Cloning of the FR3.4 QTL by Quantitative Complementation

To further demonstrate the role of *SWEET17* in the FR3.4 QTL, we performed a quantitative complementation approach by comparing the four different allelic combinations in F1 plants obtained after crossing each FR3.4 allele to either the *sweet17-1* mutant or its wild-type. The expected result was that only the functional Bay-0 allele would complement the *sweet17-1* mutation. Accordingly, fructose levels were lowest in F1 HIF195-Bay × WT plants, which carry two functional alleles. The F1 HIF195-Bay × *sweet17-1* and HIF195-Sha × WT plants, both carrying only one functional allele, showed slightly higher fructose levels and fructose levels were the highest in the F1 HIF195-Sha × *sweet17-1* plants, which carry two defective alleles (Figure 1E). Altogether, these results confirm that variation at the *SWEET17* gene explains the FR3.4 QTL.

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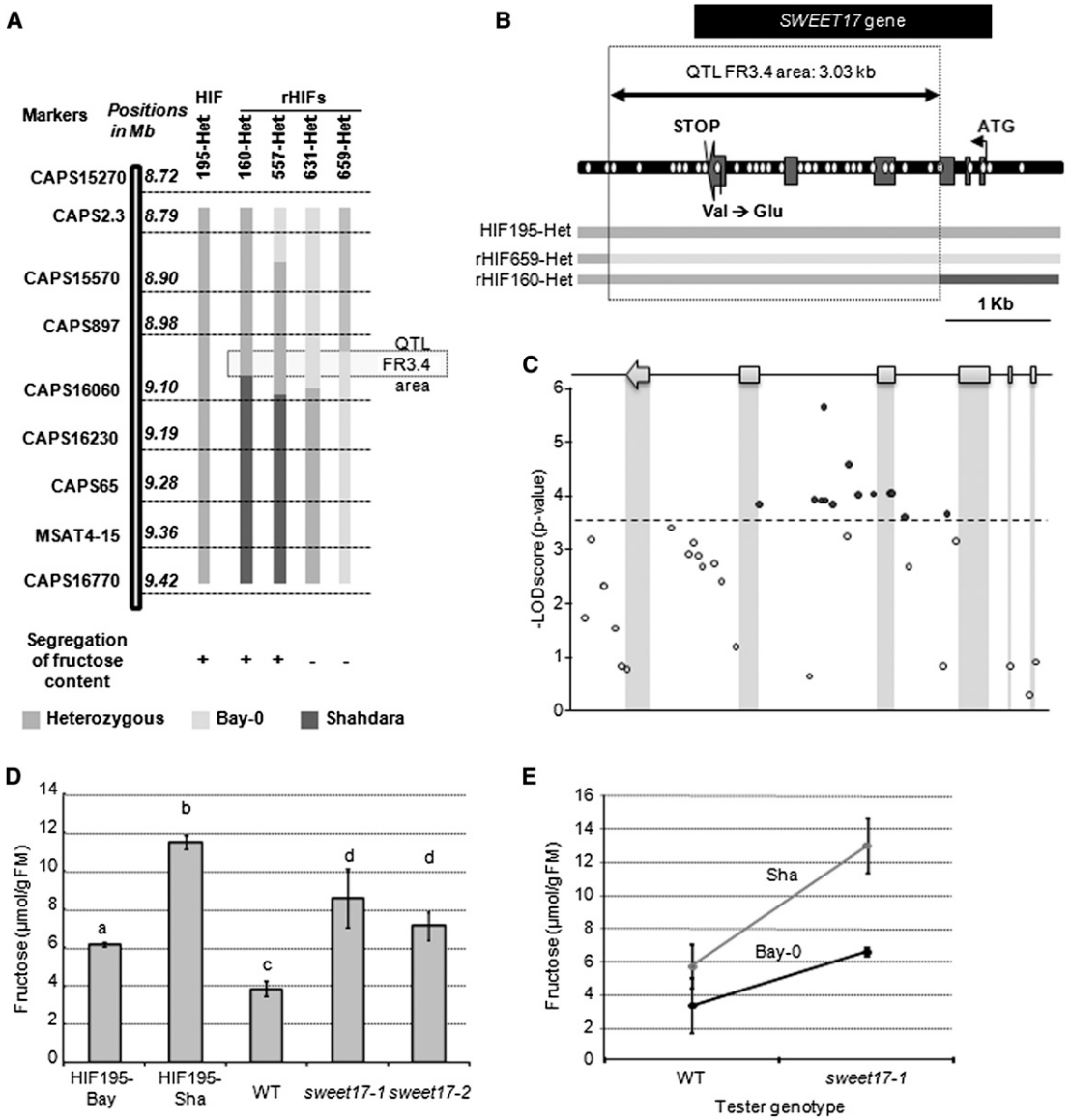

Figure 1. Fine Mapping of a QTL for Fructose Levels in Wild-Type and Mutant Plants

(A) Fine map of FR3.4 using recombined heterogeneous inbred families (rHIFs). The listed genetic markers were used to delimit the interval. Genetic structure is represented by gray bars.

(B) Genomic structure and polymorphic sites in QTL FR3.4. Exons are indicated by dark gray boxes, and polymorphic variations are indicated by white dots. Polymorphic sites were used to fine map FR3.4 to a 3.03 kb interval. Shahdara, Bay-0, and heterozygous genotypes are shown in dark, light, and medium gray, respectively.

(C) Association mapping between *SWEET17* polymorphic sites and the fructose-to-total sugar ratio. Vertical axis represents $-\log_{10}(p)$ values of the marker trait association. The dashed horizontal line indicates the threshold (Bonferroni-corrected $p < 0.01$). Dark gray circles indicate significant associations; white circles indicate nonassociations. Light gray rectangles show the positions of exons.

(D and E) Fructose content in shoots of plants grown under limiting nitrogen.

(D) Comparison of HIF195-Bay, HIF195-Sha, *sweet17-1*, *sweet17-2*, and their corresponding wild-type (WT). Letters indicate significant difference at $p < 0.01$.

(E) Quantitative complementation of FR3.4. Comparison of the F1 allelic combinations obtained by crosses of either HIF195-Bay or HIF195-Sha with *sweet17-1* or its WT background is shown.

Errors bars indicate SD (n = 5). The FR3.4 allele × SWEET genotype interaction term is significant at $p < 0.05$. See also Figure S1 and Table S1.

FR3.4 Overlaps with an eQTL of the *SWEET17* Gene

Interestingly, the expression of *SWEET17* was lower in plants carrying a fixed Shahdara allele (HIF195-Sha) compared to plants with a fixed Bay-0 allele (HIF195-Bay), indicating that the QTL FR3.4 overlaps with an expression QTL (eQTL) of *SWEET17* (Figure 2A). This difference was not due to a shift in the circadian-regulated expression pattern of *SWEET17* alleles because transcript accumulation in both HIF195-Bay and HIF195-Sha followed a diurnal rhythm with a peak in the middle of the day (Figure 2B). This expression pattern is consistent with substrate induction by sugars that accumulate in leaves during the light phase [10]. The eQTL still segregated

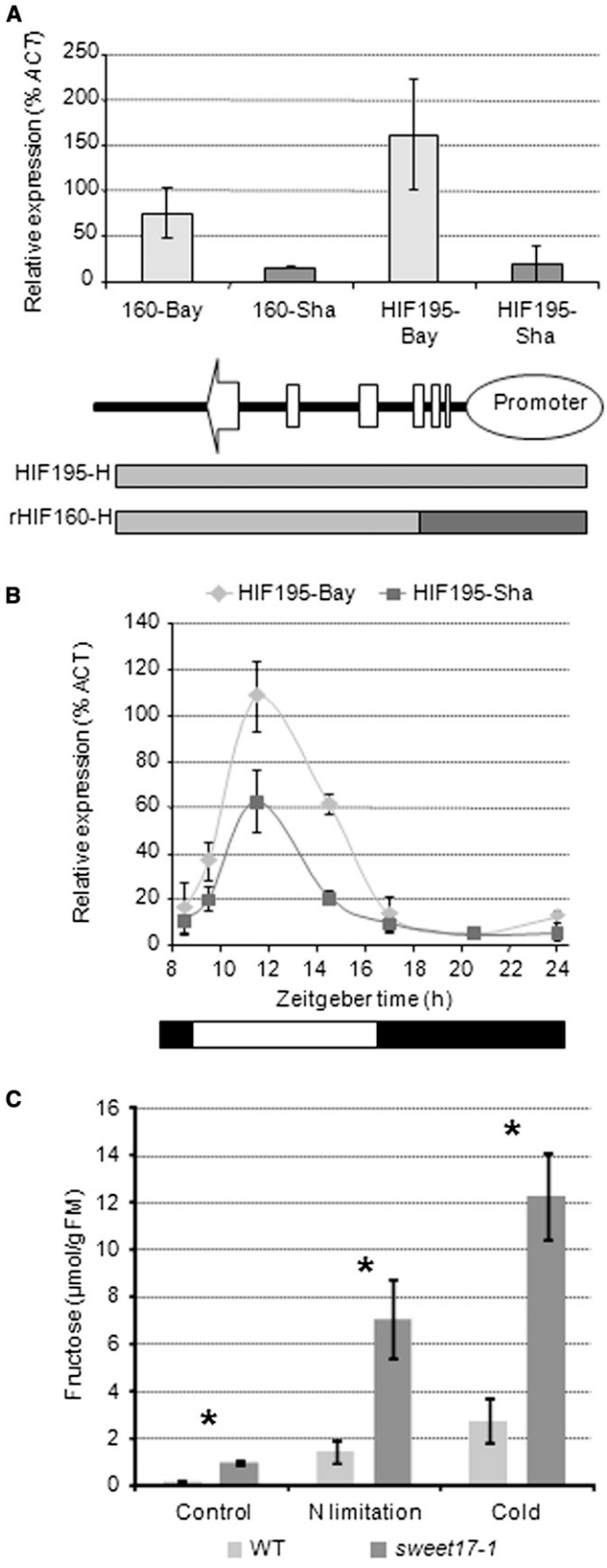

Figure 2. Expression Pattern of *SWEET17* and Effect of *sweet17-1* on Fructose Levels in Response to Abiotic Stress

(A) Differential *SWEET17* expression in rHIFs defines an expression QTL (eQTL). Comparative transcript accumulation of *SWEET17*, normalized to the levels of the *ACT* reference gene for plants grown under N limitation. The fixed Shahdara promoter region (in dark gray) in the rHIF160-H excludes the eQTL from the promoter.

(B) The expression profile of *SWEET17* follows the circadian cycle in the both the HIF195-Bay and HIF195-Sha backgrounds.

(C) Fructose content in shoots of plants grown under control, N limitation, or cold stress (4°C) conditions.

Error bars indicate SD between biological repeats (n = 5). *p < 0.05 between WT and mutants.

in rHIF160 (Figure 2A), whereas in this family the *SWEET17* promoter is fixed with the Shahdara allele, indicating that the eQTL is not only due to variation in the promoter region. The region involved in the variation of the steady-state amount of *SWEET17* mRNA includes intron 4, in which SNPs are also potentially causal for fructose content (Figure 1C). Further studies are needed to determine the regulatory mechanisms that control *SWEET17* expression.

Response to Environmental Stress

Abiotic stresses other than N deficiency, such as cold stress, also lead to the accumulation of carbohydrates [11, 12]. To distinguish a specific N limitation effect from responses to other stresses, we compared the impact of the loss of function of *SWEET17* on plants under N limitation, cold stress, and control conditions. Figure 2C shows that more fructose accumulated in the mutants for both stress conditions compared to the controls, revealing the broad effect of *SWEET17* on fructose storage.

Impact of SWEET17 on Carbohydrate Metabolism

To investigate the impact of SWEET17 on carbohydrate metabolism, we analyzed soluble sugar and starch contents under N limitation. Glucose and starch contents were not affected by the defective alleles (Figures 3A and 3B). The sucrose content of both null mutants was reduced 2-fold compared to WT, although the QTL effect on sucrose content was weak (Figure 3C), suggesting either a difference in metabolic regulation in the two genetic backgrounds (HIF195 versus Col-0) or an as yet unidentified effect due to the single amino acid change at the C terminus of the Shahdara protein. Analysis of the global metabolite profiles of these genotypes showed that fructose was the only metabolite whose level was significantly modified in the different genetic backgrounds (Figure S2). This result suggested that variation in fructose content is uncoupled from further metabolic pathways, which could result from the sequestration of fructose into the vacuole, the main storage compartment for soluble sugars [2]. Thus, SWEET17 appeared to be involved in fructose efflux from the vacuole.

SWEET17 Acts as a Vacuolar Fructose Exporter

Subcellular localization of SWEET17 was examined using translational fusions of SWEET17 with GFP, transiently expressed under the control of the cauliflower mosaic virus 35S promoter in *Arabidopsis* and tobacco protoplasts. Both allelic versions of SWEET17-GFP clearly localized to the tonoplast (Figures 4A–4F and S3A–S3C). Thus, SWEET17 is the first member of the *SWEET* gene family to be localized to the tonoplast, supporting a role for SWEET17 in fructose transport across this membrane. The transport capacity of SWEET17 was then investigated in a heterologous expression system. Expression of the Col-0 allele of *SWEET17* in *Xenopus* oocytes allowed both time-dependent uptake and efflux of ^{13}C-fructose (Figures 4G and 4H) to be measured. Other members of

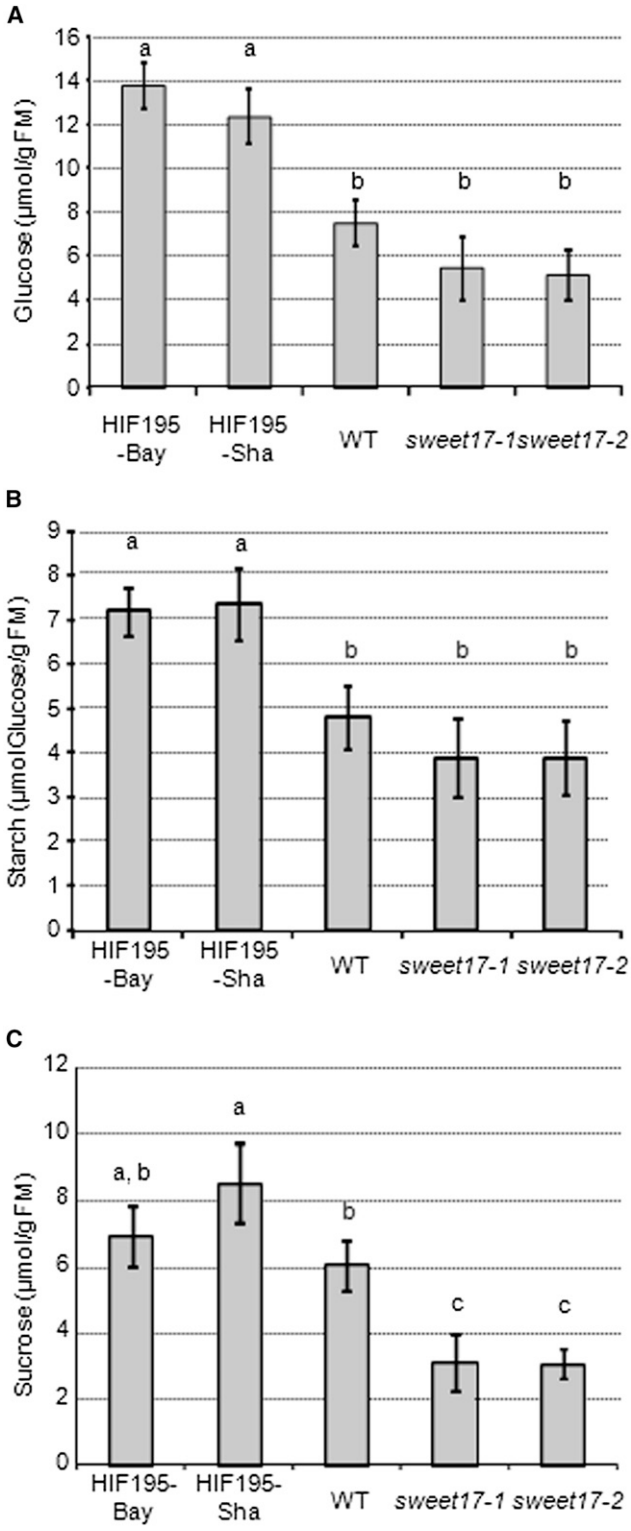

Figure 3. *SWEET17* Regulates Leaf Fructose Content

Carbohydrate levels in plants expressing the two fixed alleles HIF195-Bay and HIF195-Sha, the mutants *sweet17-1* and *sweet17-2*, and their corresponding WT grown under N limitation.

(A) Glucose content.

(B) Starch content (given as glucose equivalents).

(C) Sucrose content.

Error bars indicate SD between biological repeats (n = 5). Letters indicate significant difference at $p < 0.01$. See also Figure S2.

the *SWEET* gene family were previously described as bidirectional glucose and sucrose transporters [8, 9]; SWEET17 is the first member that mediates fructose transport. To provide functional evidence that SWEET17 is involved in fructose transport in planta, we investigated the effect of exogenously supplied fructose. Fructose feeding in liquid culture led to a substantial increase in fructose levels in *sweet17* lines compared to WT (Figure S3D). However in contrast to the strong stimulatory effect of glucose feeding on plant growth, fructose feeding did not increase the growth rates of the mutant (Figures S3E–S3G). Because most cellular fructose is in vacuoles [2] and *Arabidopsis* requires additional sugars during growth under liquid culture conditions [13], this suggests that vacuolar fructose export is impaired in *sweet17* mutants even though their cells are highly loaded with this sugar. However, another hypothesis would be that the mutant cells accumulate toxic fructose levels in the cytosol because they exhibit already high vacuolar fructose levels (Figure 4J). In the latter case, energy supply and fructose toxicity may counteract.

Next, we fed liquid-culture-grown WT and *sweet17* mutant lines with labeled ^{14}C-fructose (Figure 4I). WT seedlings released $^{14}CO_2$ at a rate of 0.32 nmol/6 hr, whereas both mutant lines exhibited markedly reduced rates of $^{14}CO_2$ release at 0.19 and 0.24 µmol/6 hr, respectively. In plant cells, the rate of respiratory CO_2 release is determined by the concentration of cytosolic sugars [14, 15]. Thus, this result shows that the excess fructose is not due to an increased fructose content in the cytosol. Given that fructose enters the vacuole via the monosaccharide carrier TMT [11], we can conclude that both *sweet17* lines sequester exogenously supplied ^{14}C-fructose into the vacuole more efficiently than WT plants.

Finally, isolation of vacuoles from cold-stressed plants accumulating fructose demonstrated directly that the stored fructose in *sweet17* mutants was located in the vacuoles, whereas the vacuolar glucose content was not significantly altered (Figure 4J). In summary, this functional evidence establishes that SWEET17 is involved in vacuolar fructose transport and that vacuolar fructose export is impaired in *sweet17* mutants.

SWEET17 Is Expressed in Parenchyma and the Vascular System

SWEET17 is expressed in whole leaves, but its expression is enriched in the vascular tissues (Figure S4A). Histochemical staining to detect GUS activity in plants carrying a *pSWEET17::uidA* construct (Figures S4B–S4G) showed that *SWEET17* is mainly expressed in the xylem. Paired with the observation that *SWEET17* mutations cause stunted growth and affect seed yield (Figures S4H–S4J), we suggest that this transporter plays a role in interorgan fructose allocation.

Discussion

In the present study, we used a quantitative genetics-based approach to identify the main genetic element involved in the variation of fructose levels between two wild *Arabidopsis* accessions, Bay-0 and Shahdara, previously described by Calenge et al. [16]. Fine mapping of the FR3.4 QTL restricted the interval to a region within the *SWEET17* gene, a member of the *SWEET* gene family (Figure 1). The *SWEET* gene family was recently described, and all characterized members appear to be glucose and sucrose transporters located at the plasma membrane [8, 9]. However, our functional analysis of SWEET17 revealed that SWEET17 is located at the vacuolar

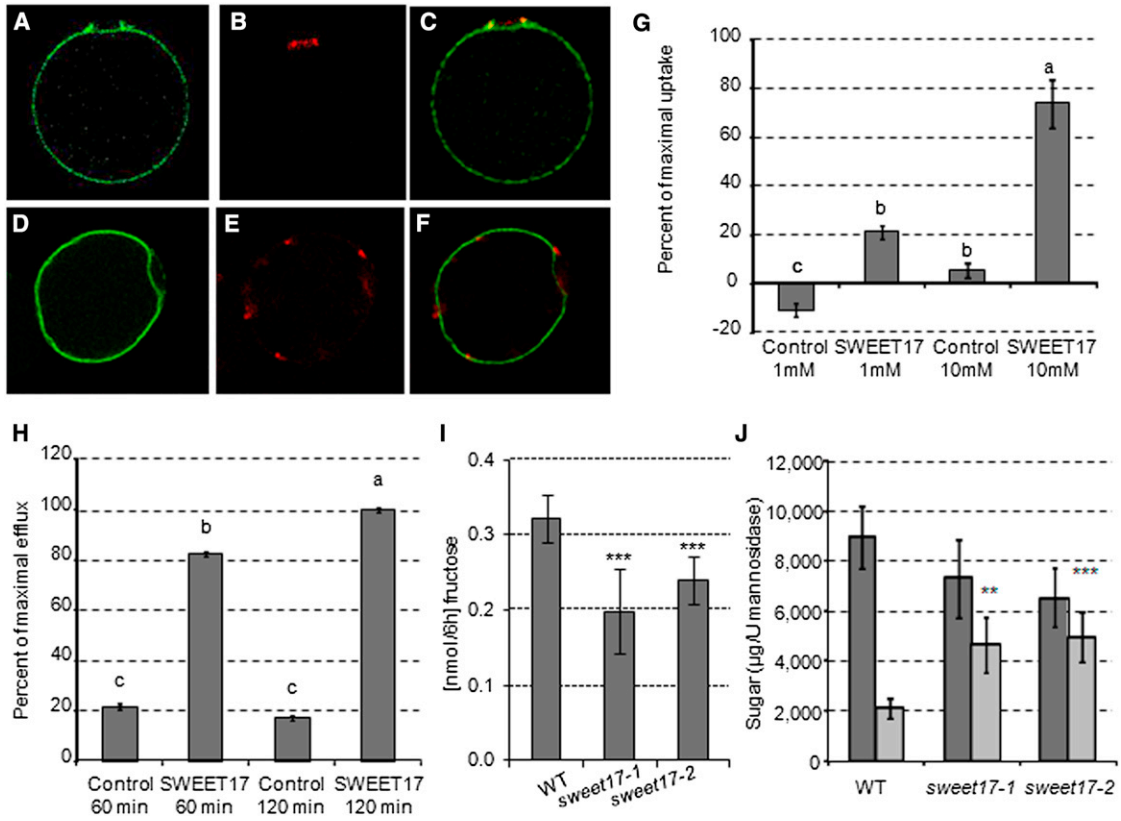

Figure 4. SWEET17 Acts as a Vacuolar Fructose Exporter

(A–F) Confocal image of the SWEET17-GFP fusion protein after transient expression in *Arabidopsis* protoplasts. Protein alleles of Bay-0 and Sha are shown in (A)–(C) and (D)–(F), respectively.

(A, B, D, and E) GFP fluorescence is shown in green (A and D); chlorophyll autofluorescence is shown in red (B and E).

(C and F) Overlay of GFP fluorescence and chlorophyll autofluorescence.

(G) Oocyte uptake assay: ^{13}C-fructose uptake in control and SWEET17-expressing oocytes supplied with 1 mM or 10 mM ^{13}C-fructose. The natural abundance of ^{13}C in oocytes was subtracted to plot the isotopic excess. Data are the means ± SE in percent of maximal uptake (n = 4–5 batches of two oocytes). Letters indicate significant difference at $p < 0.01$.

(H) Oocyte efflux assay: ^{13}C-fructose efflux activity resulting from the expression of SWEET17 in *Xenopus* oocytes. Results are the percent of maximal efflux at 120 min. The natural abundance of ^{13}C in ND96 medium was subtracted. Data are the means ± SE (n = 3). Letters indicate significant variation at $p < 0.01$.

(I) Respiration of exogenously applied fructose in WT and *sweet17* plants. Seedlings were grown in liquid culture medium for 10 days before transfer into fresh medium supplemented with 100 µmol ^{14}C-fructose. $^{14}CO_2$ released within 6 hr was trapped with 1 N KOH, and the radioactivity was quantified. Data represent the means ± SE (n = 6). In (I) and (J), ** and *** indicate significant difference between WT and mutants at $p < 0.05$ and $p < 0.01$, respectively.

(J) Fructose accumulation in isolated WT and *sweet17* vacuoles. Vacuoles were isolated from plants grown in soil for 4 weeks under cold conditions (3 days at 4°C, 10 hr light). The glucose and fructose contents are shown in dark and light gray, respectively. Data are the means ± SE (n = 3).

See also Figures S3 and S4.

membrane and that in a heterologous system it could mediate both the influx and efflux of fructose (Figures 4A–4H). Further physiological approaches demonstrated that SWEET17 acts as a vacuolar fructose exporter in planta (Figures 4I and 4J). This in vivo function is consistent with the relative fructose concentrations in the cytosol and the vacuole [17] and thus the resulting concentration gradient. Plants have several small gene families encoding mono- or disaccharide transporters (e.g., MST, TMT, VGT, or SUT gene families) at the plasma membrane or tonoplast [11, 18–20], but no fructose-specific vacuolar carrier has been identified so far. Thus, here we describe SWEET17 as the first vacuolar fructose exporter in plants.

In higher plants, the accumulation of soluble sugars rapidly leads to metabolic changes and starch production [21]. Interestingly, metabolite profiles of *sweet17* knockout mutants and HIFs developed for FR3.4 showed that *SWEET17* dysfunction leads to fructose accumulation without further significant effects on overall metabolism (Figures 3 and S2). Thus, in cells with reduced *SWEET17* activity, fructose appears to be trapped in the vacuole without any other significant changes to metabolism. During N assimilation, nitrate is reductively converted to ammonium, leading to the de novo synthesis of amino acids. The carbon backbones required for amino acid biosynthesis derive mainly from sugars. Thus, under conditions of limiting N, a substantial accumulation of sugars is typical for plant tissues, and excess monosaccharides (glucose and fructose) accumulate in the vacuole by the action of the vacuolar monosaccharide transporter TMT [14]. Under these conditions, SWEET17 obviously exports a fraction of the vacuolar fructose back to the cytosol. In *sweet17* loss-of-function mutants, we find that this re-export into the cytosol is impaired, leading to a specific accumulation of fructose in the tissue. This observation is moreover clear evidence for the substrate specificity of SWEET17 under in vivo conditions (intact cell).

SWEET17 is highly conserved in a number of agriculturally important crop species; thus, the clear evidence presented in this study that it functions as a vacuolar fructose exporter opens the way for new studies aimed at modifying carbohydrate partitioning in crops.

Supplemental Information

Supplemental Information includes four figures, four tables, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.03.021.

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