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Supplemental Information

Leaf Fructose Content Is Controlled

by the Vacuolar Transporter

SWEET17 in *Arabidopsis*

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Author Contributions

F. Chardon designed, conducted, and analyzed experiments and wrote the manuscript; M.B isolated homozygote mutants, cloned the GFP constructs, and conducted and analyzed expression analyses and sugar measurement; F. Calenge conducted part of the initial fine mapping; P.A.W.K. conducted fructose feeding, vacuole isolation, and seed yield experiments; L.S. constructed and analyzed Pro:GUS lines; G. Clement conducted and analyzed metabolite profiling; S.L. conducted the heterologous expression experiment; B.L. supervised S.L. and contributed to the discussion; C.B. and S.D. supervised L.S. and contributed to the discussion; H.E.N. supervised P.A.W.K., designed experiments, and contributed to the discussion; G.Chietera conducted association mapping experiments; M.F. sequenced the *Arabidopsis* accessions for the association mapping experiment; O.L. contributed to discussion and supervised M.F.; F.D.-V. contributed to the discussion and the design of the experiments; and A.K. helped with the design, performed and analyzed experiments, and cowrote the manuscript. All authors read and approved the final version of the manuscript.

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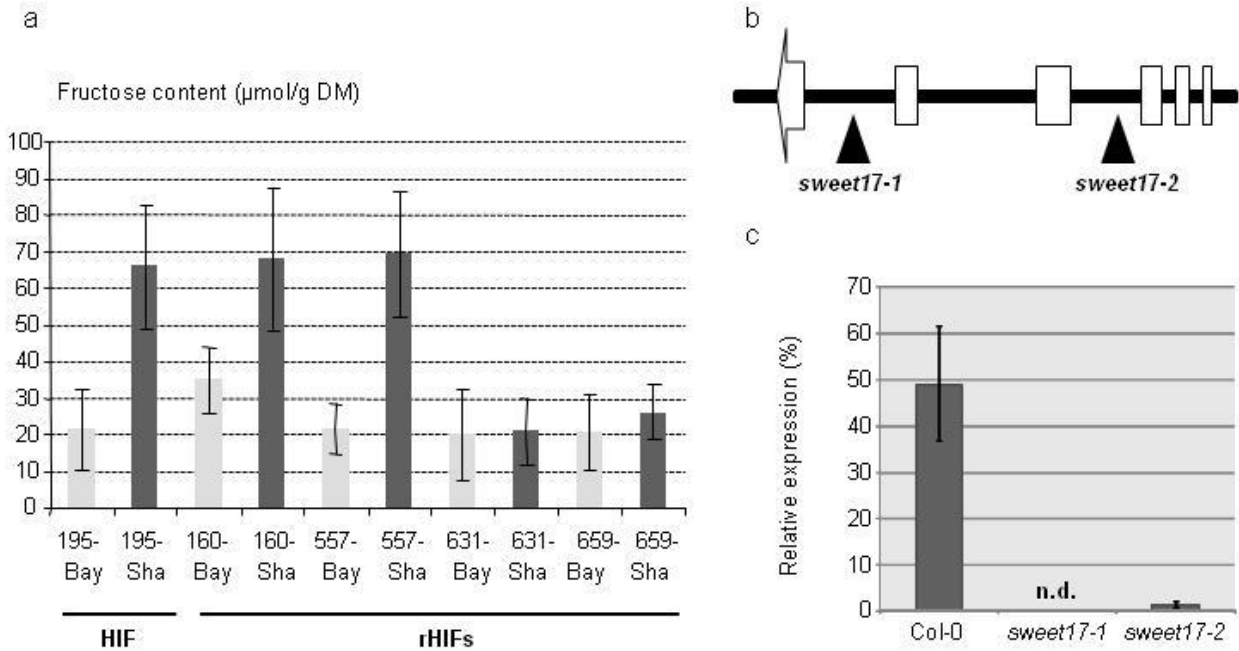


Figure S1.

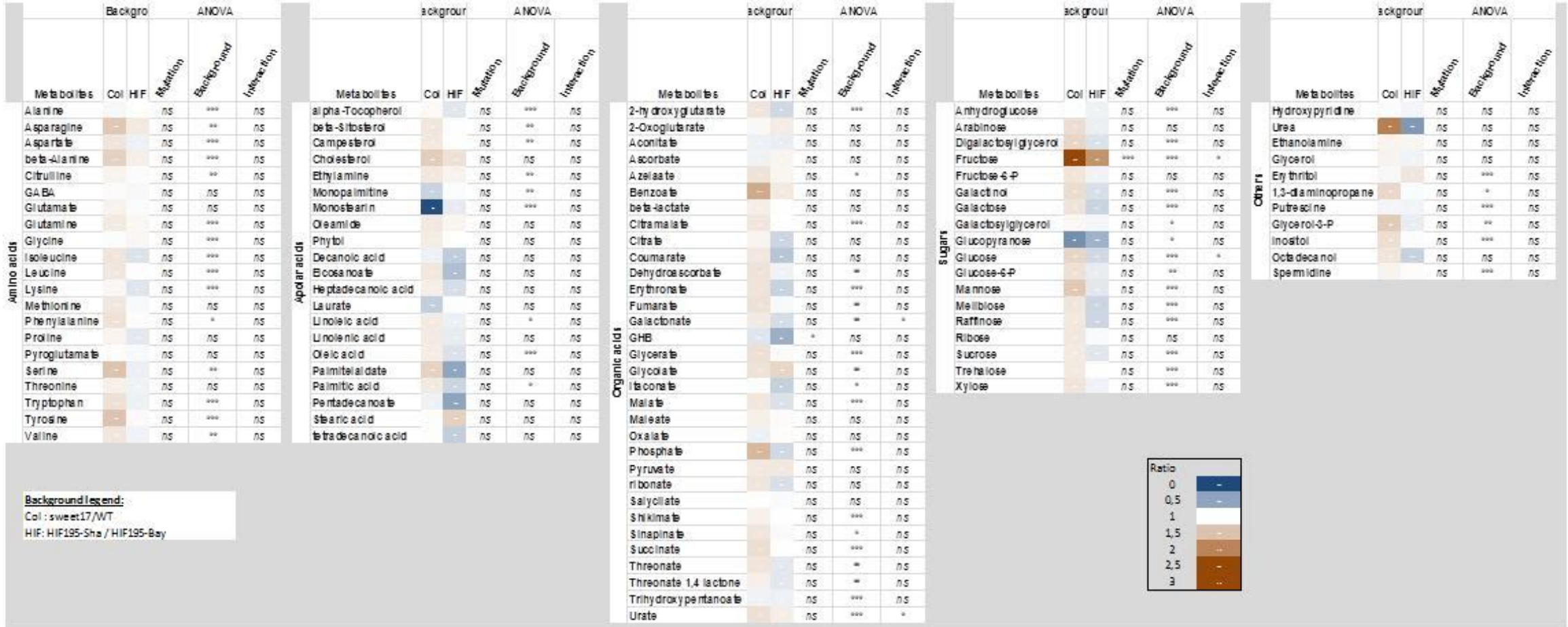
(A) Fine mapping of QTL FR3.4. QTL segregation into the HIF195 and rHIF 160, 557, 631 and 659. Fructose content in leaves of plants grown under limiting nitrogen supply. Errors bars indicate standard deviation between 5 biological repeats.

(B) Schematic map of the insertion sites in *sweet17-1* and *sweet17-2* mutants. Mutant *sweet17-1* carried a T-DNA insertion in the 5th intron, mutant *sweet17-2* carried T-DNA insertion in the 3rd exon (black boxes and black lines represent exons and introns, respectively).

(C) *SWEET17* expression in mutant lines. Expression of *SWEET17* measured by qRT-PCR using the primers listed in Table S2. Relative expression levels are presented as a percentage of *ACTIN* gene. Errors bars indicate standard deviation between 5 biological repeats, n.d., not detectable.

Figure S2. Metabolite Ratio of HIF195-Bay to HIF195-Sha and *sweet17-1* to WT

Metabolites were measured by GC-MS. Ratios of relative values are given for each comparison using a color code. ANOVAs for metabolic traits were performed keeping *SWEET17* mutation (*sweet17-1* or Shahdara alleles) and Background (Col-0 or HIF195) as the main effects with the potential interaction MutationXBackground. They were carried out using the general linear models (GLM) procedure of SAS software (<http://www.sas.com>). *, **, and *** indicate significant variation at 0.05, 0.01 and 0.01, respectively, *ns*, non significant.



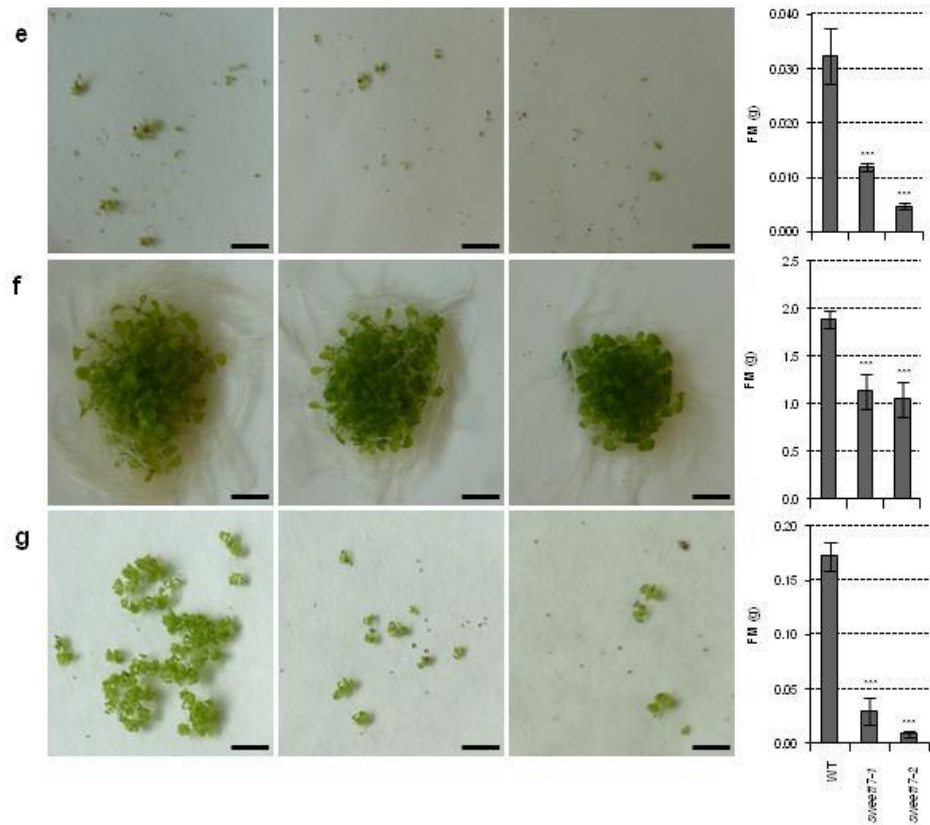
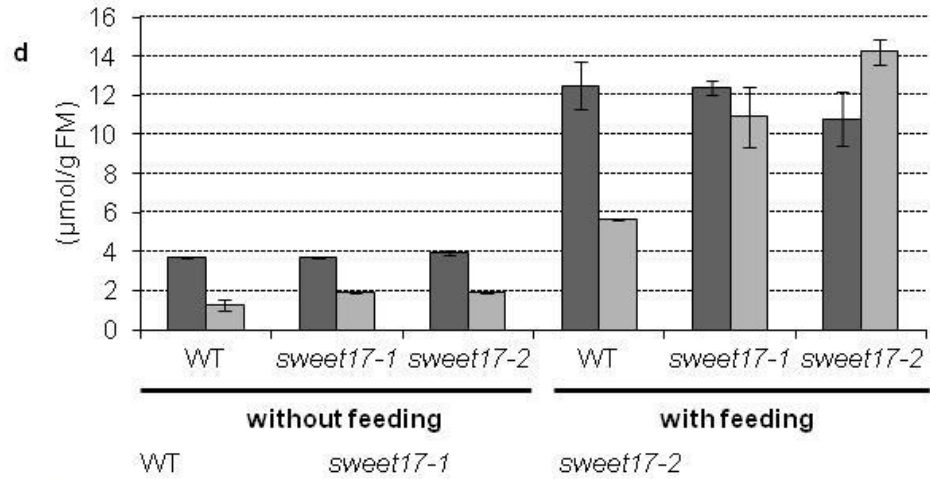
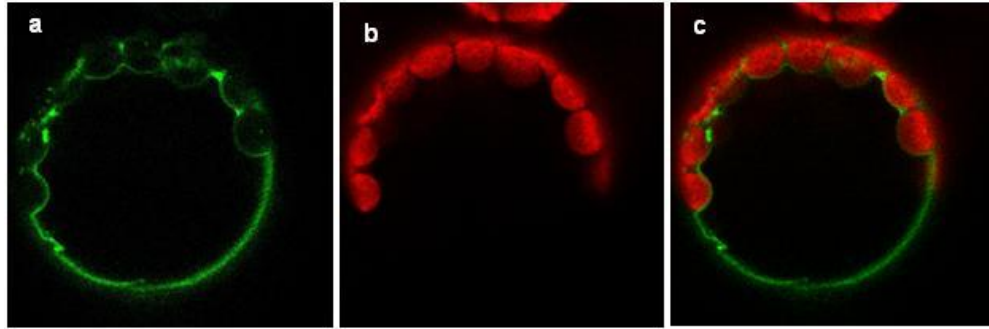


Figure S3.

Figure S3. Confocal Image of SWEET17-GFP Fusion Protein (Sha-Protein Allele) after Transient Expression in Tobacco Protoplasts

(A and B) GFP fluorescence is shown in green (A) and chlorophyll autofluorescence in red (B).

(C) Overlay of GFP fluorescence and Chlorophyll autofluorescence.

(D) Sugar accumulation after fructose feeding into WT and *sweet17* plants. Seedlings grown for 7 days in liquid-culture medium (Scheible *et al.*, 2004) were fed for 7 days with 1% fructose. Sugars were measured in whole plant extracts. Dark and light grey boxes correspond to glucose and fructose, respectively. Data are means \pm SE (n=3).

(E-G) Effect of sugars on WT and *sweet17* growth under liquid culture conditions. Plants were grown in liquid-culture medium (Scheible *et al.*, 2004) for 14 days without sugar (E), or supplemented with 1% glucose (F), or 1% fructose (G) and the fresh masses (FM) were quantified. Data represent means \pm SE (n=3).

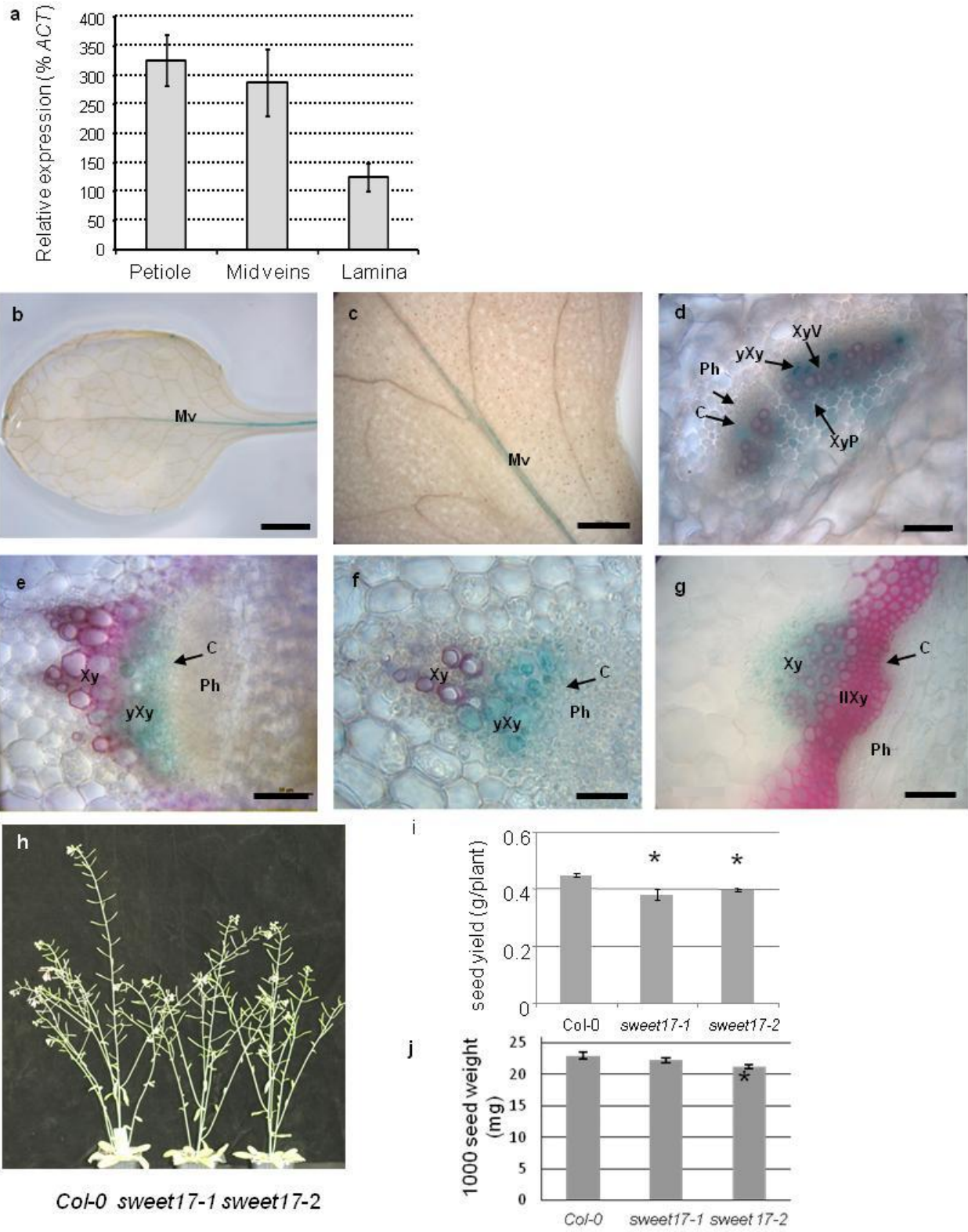


Figure S4.

Figure S4.

(A) *SWEET17* is mainly expressed in vascular tissues. Expression in three parts of the leaves (petiole, mid-veins and lamina) of plants grown under N-limitation. Error bars indicate standard deviation between biological repeats (n= 5). Expression of *SWEET17* in the xylem. Pro*SWEET17*::GUS expression in plants grown under N limitation.

(B-D) Rosette leaf (B), mid-vein of leaf of a 14 day-old plant (C), transversal section of petiole (D). GUS staining is indicated in blue, lignin staining with Phloroglucinol is shown in pink.

(E-G) transversal section of floral stem from a 7 week-old plant (E and F) and 10 week-old plant (G).

C Cambium, Xy Xylem, Ph Phloem, yXy young differentiating xylem, XyV xylem vessels, XyP xylem parenchyma, lIXy secondary xylem, Mv midvein. Scale bar: 1.20 mm (B and C), 50 μm (D), 120 μm (E), 50 μm (F and G).

(H-J) The *sweet17* mutants have shorter flower stalks (H), produce less seeds (I) than wild type, and 1000 seed weight is slightly reduced (J). Plants grown in long day (16h light, $150 \mu\text{mol.m}^{-2} \text{s}^{-1}$, 8h dark) on soil (N limiting condition), plants flowered at the same time. Errors bars indicate standard error between biological repeats (n= 8). Asterisks indicate significant differences between wild type and mutants at $P < 0.05$.

Table S1. Polymorphism Sites between the Bay-0 and Sha Alleles of the *SWEET17* Gene

Polymorphism (Bay-0/Shahdara)	Distance from ATG position (bp)	Gene structure	Genomic position (Tair 10)
SNP(T/C)	-349	Promoter	9033692
SNP(C/T)	-110	Promoter	9033453
INDEL(GAGAGACA/-)	-53	Promoter	9033396
SNP(A/C)	-2	Promoter	9033345
SNP(A/G)	39	Intron 1	9033304
SNP(G/A)	146	Exon 2	9033197
SNP(T/A)	500	Intron 3	9032843
SNP(T/C)	557	Intron 3	9032786
SNP(T/G)	583	Intron 3	9032760
SNP(G/C)	807	Intron 3	9032536
SNP(A/G)	832	Intron 3	9032511
SNP(C/A)	910	Exon 4	9032433
SNP(T/C)	914	Exon 4	9032429
SNP(C/G)	926	Exon 4	9032417
SNP(T/A)	1033	Intron 4	9032310
SNP(A/T)	1129	Intron 4	9032214
SNP(A/T)	1186	Intron 4	9032157
SNP(T/A)	1200	Intron 4	9032143
SNP(G/T)	1215	Intron 4	9032128
SNP(T/G)	1222	Intron 4	9032121
SNP(G/A)	1288	Intron 4	9032055
SNP(A/G)	1304	Intron 4	9032039
SNP(C/T)	1335	Intron 4	9032008
INDEL(AAA/-)	1337	Intron 4	9032006
SNP(A/G)	1365	Intron 4	9031978
INDEL(-/T)	1406	Intron 4	9031937
SNP(C/G)	1438	Intron 4	9031905
SNP(A/T)	1767	Intron 4	9031576
Indel(-/A)	1910	Intron 5	9031433
SNP(C/T)	1998	Intron 5	9031345
SNP(T-ATC/AGAT)	2044	Intron 5	9031299
SNP(T/G)	2081	Intron 5	9031262
Indel(TCTAATTGGGG/-)	2096	Intron 5	9031247
SNP(A/C)	2197	Intron 5	9031146
SNP(T/G)	2157	Intron 5	9031186
SNP(TT/AA)	2189	Intron 5	9031154
Indel(-/A)	2302	Intron 5	9031041
SNP(A/T)	2586	Exon 6	9030757
SNP(GCAT/ATAA)	2618	3' UTR	9030725
SNP(T/C)	2659	3' UTR	9030684
SNP(T/A)	2732	3' UTR	9030611
INDEL(T/C)	2737	3' UTR	9030606
INDEL(G/A)	2816	3' UTR	9030527
INDEL(A/-)	2852	3' UTR	9030491
SNP(TT/C-)	2896	3' UTR	9030447
SNP(T/C)	3223	3' UTR	9030120
SNP(T/G)	3248	3' UTR	9030095
SNP(C/A)	3513	3' UTR	9029830
INDEL(TGT/-)	3736	3' UTR	9029607

Supplemental Experimental Procedures

Plant Material

Heterogeneous Inbred Families: HIF195 was described by Calenge *et al.* [1] This F6 line from the Bay-0 x Shahdara RIL population [2] (<http://dbsgap.versailles.inra.fr/vnat/>) which is heterozygous for the QTL FR3.4 region was self-fertilised. We obtained in the descendants (F7) the segregating lines rHIF 160, 557, 631 and 659. The lines HIF195 and rHIF160 were fixed for either Bay-0 or Shahdara alleles in the region of interest (HIF195-Bay, rHIF160-Bay, HIF195-Sha, rHIF160-Sha).

Mutants: *sweet17-1* (SALK_012485.27.15.x) and *sweet17-2* (SAIL_535_H02) were obtained from T-DNA-mutagenized populations of the Col-0 accession [3] available at Nottingham Arabidopsis Stock Centre. Homozygous mutant plants were identified by PCR with the primers listed in Table S2. *SWEET17* transcript levels in the mutants were determined by quantitative RT-PCR with the primers listed in Table S2.

Table S2. Oligonucleotides Used for Quantitative RT-PCR Mutant Screening and Cloning. Sequences are given from the 5' end to the 3' end.

	Forward primer	Reverse primer
sweet17-1 mutant screening	TGATGTGAGGCTTC CTCTT	CCGTTTTGGTTGTCGTTTTT
sweet17-2-2 mutant screening	TTTTATTGC AAAACGTGTGTGA	TGTGTTTGACGCAGGAAATG
SWEET17 – qRT-PCR for sweet17-1	ACGTCGTGTTGGAGTAAAGCA	GATGTGGATGCGATGTTGAA
SWEET17 – qRT-PCR for sweet17-2	TGA TGT GAG GCC TTC CTC TT	CCG TTT TGG TTG TCG TTT TT
SWEET17 – qRT-PCR (AT4G15920)	TGA TGT GAG GCC TTC CTC TT	CCG TTT TGG TTG TCG TTT TT
ACTIN –qRT-PCR (AT3G18780)	GCCATCCAAGCTGTTCTCTC	CCCTCGTAGATTGGCACAGT
ProSWEET17:G US cloning	CACCGAAAGAGATAAATTAATGA	TATTGGAGAAAGAGTTTCTGAGAG
SWEET17-GFP cloning	GGAGATAGAACCATGGCAGAGGCAA GTTTC	TCCACCTCCGGATCMAGAGAGGAGA GGTTC

Arabidopsis thaliana Accessions: 59 representative natural *Arabidopsis thaliana* accessions were selected from the 48 core-collection of McKhann *et al.* [4] and from the study of Simon *et al.* [5] to cover the worldwide genetic diversity (Table S3). Accessions were obtained from the Versailles seed stock centre (<http://dbsgap.versailles.inra.fr/vnat/>).

Table S3. List of Accessions Analyzed in the Association Mapping Experiment (Data from Simon et al. [5])

Natural variant name	Code name (in cluster analysis)	Versailles Arabidopsis Stock Center line	Locality of origin	Country of origin	Core collection membership (McKahn et al. 2009)
Pyl-1	Pyl.1	008AV	Le Pyla	France	16
Ran	Ran	021AV	Cale de Mordeuc (estuaire de la Rance)	France	48
Jea	Jea	025AV	St Jean Cap Ferrat	France	8
Pi-0	Pi.0	040AV	Pitztal (Tirol)	Austria	48
Bay-0	Bay.0	041AV	Bayreuth	Germany	
Bl-1	Bl.1	042AV	Bologna	Italy	24
Pa-1	Pa.1	050AV	Palermo	Italy	48
Sp-0	Sp.0	053AV	Berlin (Spandau)	Germany	40
Ta-0	Ta.0	056AV	Tabor	Czechoslovakia	32
St-0	St.0	062AV	Stockholm	Sweden	16
Lip-0	Lip.0	063AV	Lipowiec (Chrzanow)	Poland	48
Te-0	Te.0	068AV	Tenela	Finland	40
Kn-0	Kn.0	070AV	Kaunas	Lithuania	24
Bla-1	Bla.1	076AV	Blanes (Gerona)	Spain	32
Edi-0	Edi.0	083AV	Edinburgh	United Kingdom	24
Lz-0	Lz.0	087AV	Lezoux (Puy-de-Dome)	France	
Ra-0	Ra.0	088AV	Randan (Puy-de-Dome)	France	
Tsu-0	Tsu.0	091AV	Tsu	Japan	24
Stw-0	Stw.0	092AV	Stobowa (Orel)	Russia	24
Ms-0	Ms.0	093AV	Moscow	Russia	32
Mt-0	Mt.0	094AV	Martuba (Cyrenaika)	Libya	16
Nok-1	Nok.1	095AV	Noordwijk	Netherlands	48
Ge-0	Ge.0	101AV	Geneva	Switzerland	16
Ga-0	Ga.0	117AV	Gabelstein	Germany	
Gu-0	Gu.0	118AV	Guckingen	Germany	
Da-0	Da.0	126AV	Darmstadt	Germany	
Mz-0	Mz.0	138AV	Merzhausen (Ts)	Germany	
Rd-0	Rd.0	142AV	Rodenbach (Dill)	Germany	
Hn-0	Hn.0	145AV	Hennetalsperre	Germany	
Mr-0	Mr.0	148AV	Monterosso	Italy	
Ita-0	Ita.0	157AV	Ibel Tazekka (Jebel Tazekka)	Morocco	8
Ri-0	Ri.0	160AV	Richmond (B.C.)	Canada	40
Ct-1	Ct.1	162AV	Catania	Italy	8
Can-0	Can.0	163AV	Canary islands	Spain	16
Cvi-0	Cvi.0	166AV	Cape Verde Islands	Cape Verde Islands	8
Bur-0	Bur.0	172AV	Burren	Ireland	8
Alc-0	Alc.0	178AV	Alcalá de Henares (Madrid)	Spain	16
Blh-1	Blh.1	180AV	Bulhary	Czechoslovakia	8
Col-0	Col.0	186AV	Gorzow Wielkopolski (Landsberg/Warthe)	Poland	
Kondara	Konda	190AV	Kondara	Tajikistan	40
Enkheim-T	Enk.T	197AV	Enkheim (Frankfurt)	Germany	48
Gre-0	Gre.0	200AV	Greenville (MI)	USA	24
Jm-0	Jm.0	206AV	Jamolice	Czechoslovakia	40
Mh-1	Mh.1	215AV	Mülhen (OstPr)	Poland	16
Oy-0	Oy.0	224AV	Oystese	Norway	8
Rld-2	Rld.2	229AV	Rschew	Russia	32
Rubezhnoe-1	Rubez.1	231AV	Rubezhnoe	Ukraine	32
Sah-0	Sah.0	233AV	Sierra Alhambra	Spain	32
Sap-0	Sap.0	234AV	Slapy	Czechoslovakia	40
Sav-0	Sav.0	235AV	Slavice	Czechoslovakia	40
Shahdara	Shah	236AV	Shakdara River (Pamir)	Tajikistan	8
Yo-0	Yo.0	250AV	Yosemite Nat. Park (CA)	USA	40
Akita	Akita	252AV	Akita Pref	Japan	24
Ishikawa	Ishika	253AV	Uchinada (Ishikawa Pref)	Japan	32
Sakata	Sakata	257AV	Sakata (Yamagata Pref)	Japan	24
N6	N6	262AV	Karelian region	Russia	48
N7	N7	263AV	Pinguba	Russia	48
N13	N13	266AV	Konchezero	Russia	16
N14	N14	267AV	Sampo Mountain	Russia	32

Growth Conditions

Seeds were stratified for 48 h in 0.1% (w/v) agar solution (in water) at 4 °C in the dark. They were sown in small pots (L 60 mm, W 65 mm, H 60 mm) filled with medium particle size sand (0.6-1.6 mm). One week after sowing, six seedlings were retained in each pot, and the surface was covered with black sand. Plants were grown for 35 days under short days (8 h light, 16 h dark) with 21 °C day and 17 °C night temperatures and 65% humidity. The photon flux density was 160 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Plants were watered with a modified Hoagland solution containing 1 mM nitrate (except for the cold stress experiment and the control condition where 5 mM nitrate was included in the nutrient solution). Pots were watered three times per week for 2 h by immersing the base of the pots. For cold stress, 37 days old plants were transferred to 4°C for 4 days under the same environmental conditions.

For all measurements samples were taken 3 hours after the beginning of the light period and snap-frozen in liquid nitrogen. All experiments were repeated at least twice and representative experiments are presented.

Growth in liquid culture was carried out according to Kirchberger *et al.* [6].

Sugars and Starch Quantification

Sugars were extracted in a two-step ethanol-water extraction using 50 mg of frozen leaf material as in Ikram *et al.* [7]. The glucose, fructose and sucrose contents were determined using an enzymatic assay based on a commercial kit (RBIOPHARM kit Sucrose/D-Glucose/D-Fructose). The starch content was analyzed in the dry pellet of the ethanol-water extraction after digestion with amyloglucosidase and α -amylase as in Ikram *et al.* [21]. The resulting glucose was measured as described. The starch content was expressed in equivalents of glucose molecules. For the association mapping experiment, the ratio of fructose to total sugars was calculated to homogenize variation in total sugar levels among the different accessions.

Quantitative RT-PCR

RNA was extracted using the Trizol method (Invitrogen) and first strand cDNAs were synthesized according to Daniel-Vedele and Caboche [8] using M-MLV reverse transcriptase and oligo(dT)₁₅ primers (Promega). PCR was performed on a Realplex Mastercycler (Eppendorf) with the Eurogentec Sybergreen mix (MESA FAST qPCR™ Mastermix Plus) according to the manufacturer's protocol. Each reaction was performed on a 1:20 dilution of the first cDNA strands, synthesized as described above, in a total reaction of 20 μL . With this dilution, the SYBR green signal was linear. Specific primer sets for *SWEET17* and *ACTIN* as the reference gene are shown in Table S2.

Isolation of Protoplasts and Vacuoles

Protoplasts and vacuoles were enriched exactly as described previously in Schulze *et al.* [9].

***SWEET17* Sequencing**

SWEET17 alleles of each accession were sequenced using primers shown in Table S4.

Table S4. List of Primers Used for Haplotype Sequencing for the Association Mapping Experiment

Primer Name	Sequence	Melting Temperature	Primer Size
fr3.4_F1	ATCACGATCCGAACGCTAAA	60.61	20
fr3.4_F2	ACACAAACCCCACTCCATTT	59.16	20
fr3.4_F3	CCAAAACGGCATGTATTTCA	59.42	20
fr3.4_F4	GCCATGTGGTTGTTGGTTTA	59.3	20
fr3.4_F5	TTCAACATCGCTTCCACATC	59.65	20
fr3.4_F6	GAGATGTGCGAGGGGCATAAA	60.04	20
fr3.4_F7	TGGTGAGAGGAAAAGTAGTACGG	59.7	23
fr3.4_R1	AAATGGAGTGGGGTTTGTGT	59.16	20
fr3.4_R2	TACATGCCGTTTTGGTTGTC	59.44	20
fr3.4_R3	TGTTTCATATCCACTTGGTCCTTT	59.75	23
fr3.4_R4	GATGTGGAAGCGATGTTGAA	59.65	20
fr3.4_R5	CGGAGATCAACGGAGGAGTA	60.21	20
fr3.4_R6	ATGGCAGAGGCAAGTTTCTA	57.58	20
fr3.4_R7	TCACGTTTCCCCCAATTAAG	59.79	20

Metabolite Profiling by GC-MS

Metabolome analyses were performed at the IJPB-Plant chemistry platform (LC2V, Versailles, France) using gas chromatography coupled to mass spectrometer (GC-El-quad-MS) exactly as described in Fontaine *et al.* [10].

Construction of SWEET17-GFP Translational Fusions

cDNAs for both *SWEET17* alleles (Bay-0 and Shahdara) were obtained by reverse transcription (see above) on total leaf RNA from 35 days old plants grown on 3mM nitrate solution using gene specific primers (Table S2). PCR products were cloned into pDONR207 (Invitrogen) and inserted using Invitrogen Gateway® technology with Clonase™ II into the binary vector pMDC83[11] to create 2xPro35S::SWEET17-GFP. Sequences of the final constructs were confirmed by sequencing.

Transient Expression

Protoplasts were isolated from Arabidopsis cell suspensions, and GFP fusions were transiently expressed in the protoplasts by polyethylene glycol (PEG)-mediated transformation, as described in Thomine *et al.* [12], and incubated in the dark for 48h. Confocal microscopy analysis was performed on a TCSNT confocal microscope (Leica) equipped with an argon/krypton laser (Omnichrome). GFP fluorescence was detected using the laser line 488 nm (Alexa 488). The images were coded green (fluorescent GFP) and red (chlorophyll autofluorescence). Each image represents one single focal plane. Transient expression in tobacco protoplasts was performed as in Palmieri *et al.*[13].

Construction of ProSWEET17:GUS Fusions

The *AtSWEET17* promoter was amplified from genomic DNA of 7 day-old seedlings using Finnzyme's PHUSION high fidelity DNA polymerase and gene specific primers (Table S2). The 2004 bp PCR product was cloned into pENTR/D-TOPO (Invitrogen) and recombined by Gateway technology into pKGWFS7 [14] to create ProSWEET17:GUS. Recombination was performed using Gateway LR Clonase enzyme Mix (Invitrogen). The resulting construct was confirmed by sequencing. The binary vector was transferred into *Agrobacterium tumefaciens* strain GV310::pMP90 (C58C1) via electroporation [14]. Transformants were selected on LB plates containing rifampicin (50 µg L⁻¹), gentamycin (20 µg L⁻¹) and spectinomycin (50 µg L⁻¹). *Arabidopsis* (Col-0) was transformed using the floral dip method[15]. Transgenic seedlings were recovered on Estelle and Somerville media [16] containing 1% sucrose and 50 µg L⁻¹ kanamycin.

GUS Staining

Three independent homozygous T3 lines were grown for 35 days on sand under 65% humidity, 8 hours of light (150 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, 21°C) and 16 hours of night (17°C). Histochemical GUS staining was performed on plants from each line according to Sorin et al. [17] and then observed using a Nikon SMZ1111 stereomicroscope equipped with a ProgResC3 camera and ProgResCapturePro 2.8 software (JENOPTIK).

For thin sections, samples were embedded in 8% agarose and sectioned with a Leica VT100S vibratome (LEICA). Phloroglucinol staining was applied for 2 min on thin sections. Pictures were taken using a Nikon microphot-FXA microscope equipped with a ProgResC10plus camera and ProgResCapturePro 2.7 software (JENOPTIK).

Expression in *Xenopus* Oocytes and Transport Measurements

Xenopus oocytes were obtained and injected as previously described in Krouk *et al.*[18]. Briefly, for uptake studies oocytes (injected or controls) were incubated for 2 hours in 2 mL of ND96 medium (pH 6.5) containing 1mM or 10 mM of ^{13}C -Fructose (atom % ^{13}C abundance: 99%, Sigma-Aldrich). Oocytes were then washed five times in 15 ml of ND96 medium at 4°C. Batches of two oocytes were then analyzed for total C content and atom% ^{13}C abundance by Continuous-Flow Mass Spectrometry, using an Euro-EA Eurovector elemental analyzer coupled with an IsoPrime mass spectrometer (GV instruments, Crewe, UK). For efflux studies, three days after injection, control and SWEET17-expressing oocytes were placed in ND96 solution supplemented with 10 mM fructose. Each batch of oocytes was injected with 20 nL of 250 mM ^{13}C -Fructose (Sigma). After 5 min of recovery, oocytes were placed in 2 mL of ND96 for efflux measurement. After 60 min and 120 min, 100 μL of efflux buffer was sampled in replicates and the ^{13}C abundance (atom %) was analyzed by continuous-flow mass spectrometry.

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