Supporting Information for

Dissection of the role of sucrose and gibberellin transport activities of SWEET13 in male fertility of Arabidopsis

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Figure S1. Distribution and time evolution of the RMSDs for apo and bound forms of SWEET13

(A) Clustering results for ligand docking. The final docking poses are marked in red. The binding poses are grouped in three clusters for sucrose and one cluster for GA3. (B) The Root Mean Square Distance (RMSD) distribution of sucrose (black) and GA3 (red) in the binding pockets during the MD simulations. RMSDs were calculated on ligand atoms after least square fitting of the binding pocket atoms of each snapshot of MD trajectory on the same atoms of the first equilibrated structure of the MD run. The RMSD values for sucrose and GA3 range from 0.3 Å to 3.9 Å and from 0.3 Å to 2.5 Å, respectively. (C) Time dependence of the ligand center of mass (COM) position projected
on the channel axis for sucrose (black) and GA$_3$ (red). (D) Time evolution of the overall RMSDs compared to the initial coordinates ($t = 0$ ns) for apo SWEET13 (black), and the models of SWEET13 bound to sucrose (SWEET13*Suc; red) and gibberellic acid (SWEET13*GA$_3$; green). RMSDs were calculated on the backbone atoms of the transporter after least square fitting the backbone atoms of each snapshot on the backbone atoms of the first structure of the MD run. (E) 3D structures of the SWEET13*Suc averaged for the first (0-900 ns, black cartoon) and second (900-2100 ns, red cartoon) part of the simulation.
Figure S2. Comparison of predicted binding modes of sucrose and GA₃ with dCMP

Representative conformation of (A) sucrose (magenta sticks) - cluster 1 binding mode - with its binding residues (magenta lines) in side and (B) bottom view, (C) GA₃ (cyan sticks) - cluster 1 binding mode - with its binding residues (cyan lines) in side view and (D) bottom view, E) sucrose (magenta sticks) / GA₃ (cyan sticks) - cluster 1 binding mode - with their binding residues (magenta/cyan lines, correspondingly) in side view and in F) bottom view, superimposed onto the carbon alpha atoms of the 5XPD crystal structure (gray ribbon) with dCMP bound in the central cavity (green sticks). The backbone is shown only for the 5XPD crystal structure for the sake of clarity. The dCMP binding residues are shown as yellow sticks.
Fig. S3. Sucrose uptake by SWEET13 variants carrying mutations in the proposed substrate-binding pocket

(A) Sucrose uptake activity was determined in HEK293T cells coexpressing the sucrose sensor FLIPsuc-90μΔ1 with SWEET13 or one of seven variants carrying mutations in the proposed binding site. Addition of 10 mM sucrose triggered a time-dependent negative ratio change, consistent with the accumulation of sucrose in the cells. In the absence of SWEET13 (Empty vector), no significant change in ratio was observed. (mean – s.e.m., n ≥ 14 cells). The experiment was repeated 3 times with comparable results. (B) Mean of intensity ratio from 125 to 150 s after addition of sucrose from the data in (A). Transport activity was determined by subtracting the value of the control (Empty), and the value of SWEET13 was set to 100%. Data indicated with gray were used in Fig. 2F.
Fig. S4. GA\textsubscript{1} uptake by SWEET13 variants carrying mutations in the proposed substrate binding pocket

GA\textsubscript{1} uptake assay by Y3H. Ten-fold serial dilution assay using yeast carrying both pDEST22-GID\textsubscript{1}a and pDEST32-GAI and either pDRf1-SWEET13, pDRf1-SWEET13 with mutations (S20N, N76Q, S142L, S142N, V145L, N196Q), or empty vector (negative control). Yeast cells were grown on SD (-Leu, -Trp, -Ura) or selective SD (-Leu, -Trp, -Ura, -His) medium, containing 3 mM 3-AT and 0.001% (v/v) DMSO as mock or 0.1 µM GA\textsubscript{1}, for 3 days at 30 ºC. Comparable results were obtained in three independent replicates.
**Fig. S5.** GA$_3$ uptake by SWEET13 variants carrying mutations in the proposed substrate binding pocket

(A) Boxplot of GA$_3$ uptake assays in HEK293T cells coexpressing the GA sensor GPS1 and SWEET13 (n = 32 cells) or SWEET13 with mutations such as S142N (n = 29), V145L (n = 30), S20Q (n = 32), N196A (n = 21), S20N (n = 20), S142A (n = 31), S142L (n = 12), V145A (n = 32), N196Q (n = 30), V23A (n = 24), N76Q (n = 16), S54A (n = 23), S20A (n = 13). Empty vector served as negative control (n = 29). The YFP/CFP fluorescence ratio of GPS1 was measured after incubation with 1 µM GA$_3$ for 3 h. Asterisk indicates significant difference compared to ‘Empty vector’ control (**$P < 0.0001$, *$P < 0.01$ by one-way ANOVA with Dunnett’s post hoc test). n.s.: no significant difference. In box plots, box represents the range from 25$^{th}$ to 75$^{th}$ percentile, the horizontal line marks the median value, and the whiskers from the 2.5 to 97.5 percentile. Each data point shown was generated by determining the mean emission ratio during 3-minute recordings. The experiment was repeated 3 times with comparable results. (B) Mean of intensity ratio from the data in (A). Transport activity was determined by subtracting the value of the control (Empty); the value of SWEET13 was set to 100%. Data indicated with gray were used in Fig. 2F.
Fig. S6. SWEET13 protein accumulation in anthers at different stages of flower development

Phenotypes and GFP fluorescence at stages from 12 to 14 for sweet13; sweet14 mutants expressing translational SWEET13-GFP fusions driven from the SWEET13 promoter in (A) buds/flowers, (B) pistil and stamen (C) anther, and (D) Confocal fluorescence images of sum slices projection of confocal images of anthers (yellow). Cyan indicates autofluorescence. Arrowhead indicates dehiscence. Scale bars: 200 µm in (A) and (B), and 100 µm in (C) and (D). Comparable results were obtained in 3 independent analyses.
**Fig. S7.** Tissue specificity of SWEET13 and SWEET14 mRNA levels

SWEET13 (AT5G50800), SWEET14 (AT4g25010) expression data from database (Arabidopsis eFP Browser 2.0).
Fig. S8. Accumulation of the mutated versions of SWEET13 protein in Arabidopsis anthers

Confocal images of stage 13 anther expressing (A, B, C, D) translational SWEET13<sup>N76Q</sup>-GFP and (E, F, G, H) SWEET13<sup>S142N</sup>-GFP fusions driven from the SWEET13 promoter in sweet13; sweet14 mutants. (A, D) surface of anther, (B, F) epidermal cell surface, (C, G) middle section of anther, and (D, H) enlarged view of boxed area in (B, E). Cyan indicates autofluorescence. Ep, epidermis; En, endothecium. (I) A framework for quantification of GFP fluorescence intensity of GFP-tagged SWEET13 expressed in anthers. To quantify the fluorescence intensity, the mean intensity of the area inside the red line was measured. (J) Normalized fluorescence intensity of anther from sweet13: sweet14 complemented with either SWEET13-GFP (WT), SWEET13<sup>N76Q</sup>-GFP (N76Q), or SWEET13<sup>S142N</sup>-GFP (S142N). Scatter plots with mean ± s.e.m (n=8). The same letters above the plots indicate no significant differences among means (one-way ANOVA with Tukey’s post hoc test, \( P = 0.1804 \) (WT - N76Q); \( P = 0.9415 \) (WT - S142N); and \( P = 0.3059 \) (N76Q - S142N)). Scale bars: 100 µm in (A, C, E, G), 5 µm in (B, F), and 20 µm in (D, H). Comparable results were obtained in 3 independent analyses.
Fig. S9. Sequence alignment of SWEET proteins from Arabidopsis and rice. Amino acids in the substrate binding pocket are highlighted in orange. SWEET homologs labeled in pink are able to transport GA3 confirmed based on Y3H assays (1-3).
Fig. S10. Glucose uptake by SWEET13, SWEET13\textsuperscript{N76Q} and SWEET13\textsuperscript{S142N}

A) Glucose uptake assay in yeast strain EBY.VW4000. Ten-fold serial dilution assay using yeast carrying either pDRf1-SWEET13, pDRf1-SWEET13 with mutations (N76Q, S142N), pDRf1-SWEET1 (positive control), or empty vector (negative control). Yeast cells were grown on SC (-Ura) containing either 2% maltose or 2% glucose for 3 days at 30 °C. Comparable results were obtained in three independent replicates. (B) Glucose uptake activity was determined in HEK293T cells coexpressing the glucose sensor FLII\textsuperscript{Pglu700μδ6} with SWEET13, SWEET13\textsuperscript{N76Q} or SWEET13\textsuperscript{S142N}. SWEET1 was used as a positive control. Addition of 10 mM glucose triggered a time-dependent ratio change, consistent with the accumulation of glucose in the cells. In the absence of SWEET13 (Empty), no significant change in ratio was observed. Each plot indicates mean value of 5 independent experiments: n≥14 cells were analyzed per experiment. (mean – s.e.m.). (C) Scatter plots with mean ± s.e.m (n = 5) of average emission ratios from 125 to 150 s after addition of glucose from the data in (B). Different letters on each plot represent significant differences determined by one-way ANOVA with Tukey’s post hoc test (P < 0.05). (D) Mean of intensity ratio from the data in (C). Glucose transport activity was determined by subtracting the value of the control (Empty), and the value of SWEET13 was set to 100%.
Table S1. Primers used in this study

| Primer   | Sequence (5’ > 3’)                                      |
|----------|--------------------------------------------------------|
| Ser20Ala-F | TTGGGTAACATCATAGCTTTTCGCTGTTTTCTTGCCCCAGTGCC         |
| Ser20Asn-F | TTGGGTAACATCAAATACTTCGCTGTTCTTGCCCCAGTGCC         |
| Ser20Gln-F | TTGGGTAACATCAAATCTTCGCTGTTCTTGCCCCAGTGCC         |
| Ser20-R   | TATGATGTTACCCAAGATTTCCAACAAACAAAATGCC              |
| Asn76Gln-F | CTTCTCATCACCATAACAAGCTTTTGATGCGTCATCGGAAA         |
| Asn76Gln-R | TATGGTGAAGAAAAGGCTGTCATCTTTTTTG                  |
| Ser142Ala-F | ATTTGCGTTGGATTTGCTGAGTGTGTTTTCGACGTCCTTTTG   |
| Ser142Leu-F | ATTTGCGTTGGATTTGCTGAGTGTGTTTTCGACGTCCTTTTG   |
| Ser142Asn-F | ATTTGCGTTGGATTTAATTGAGTGTGTTTTCGACGTCCTTTTG   |
| Ser142-R   | AATCCAACGAAATCCCTCCGAGAAATTTCTACGTTTGAACC        |
| Val145Ala-F | GGATTCCCCGTCAGTGTGTTTTCGACGTCCTTTTGAGATC  |
| Val145Leu-F | GGATTCCCCGTCAGTGTGTTTTCGACGTCCTTTTGAGATC  |
| Val145-R   | ACTGACGGAAAAATCCCAAACGGAATCCCTCCGAGAC          |
| Asn196Ala-F | TACGTTGCCCCATTCCAGCTGTATTTAGGCGCTTTTAGGAGCTGTT   |
| Asn196Gln-F | TACGTTGCCCCATTCCACAGATTTAGGCGCTTTTAGGAGCTGTT   |
| Asn196-R   | TGGAAAGGCAACGTAAAGCTTTTAAATACGCGAGACCGTAGAAAGAGCC |
| SWEET13a-1-LP | TACGCTATGCAAAAAAGATGCC          |
| SWEET13a-1-RP | CGACAAAAAGAGTGGCAAGAG          |
| SWEET14aLP  | AAGCATCATCGATCTCAAACG           |
| SWEET14aRP  | CGCACCCAAATATATTTTGAAG           |
Supporting Information Text

SI Materials and Methods

**Missing side chain and loop modeling**

The 3D model of SWEET13 from *Arabidopsis* was derived from the X-ray crystallographic structure of the SWEET13 sugar transporter of *Arabidopsis thaliana* in the inward open state, (PDB ID 5XPD) (4) (resolution 2.79 Å). The structure was generated with a SWEET13 carrying thermostabilizing mutations (V23L, S54N, V145M, and S176N) as a fusion with rubredoxin and bound to 2'-deoxycytidine 5'-monophosphate (dCMP). Rubredoxin and dCMP were removed computationally from 5XPD to create an apo SWEET13 model. The side chains of V23, S54, V145, and S176 were built using SCWRL4 (5). The missing first 5 residues of the N-terminal loop were modeled using MODELLER 9.14 (6). The model was optimized using the VTFM method applying conjugate gradients with a maximum iteration number of 300. The degree of the VTFM was set by the autosched module applying the long, thorough optimization schedule of autosched.slow. Subsequently, the model was refined by short molecular dynamics (MD) simulation (of 2 ns) and simulated annealing using the predefined function, refine.slow of refining module of MODELLER. Optimization was repeated two times with an objective function cutoff of 10^6. Protonation states of titratable residues were determined by visual inspection and calculations with PropKa 3.1 (7).

**Molecular docking**

To construct the models of SWEET13 bound to sucrose (SWEET13*Suc) and gibberellic acid (SWEET13*GA3), molecular docking of sucrose and GA3 were performed using AutoDock 4.2, respectively (8). The docking sites of sucrose and GA3 were assumed to be similar to that of dCMP based on the dCMP-bound SWEET13 structure (4). Docking sites were obtained by superimposition of the apo-SWEET13 model generated here onto the 5XPD structure for sucrose and GA. The centers of the grid boxes were derived by superposition of the glucosyl ring of sucrose onto the cytosine base of dCMP. The coordinates of the ether bond oxygen atom of sucrose served as the position of the grid center for both sucrose and GA3. The number of grid points was chosen as 34, 34, 34 in X, Y, Z directions with a grid spacing of 0.375 Å. 150 independent docking runs were performed using the Lamarckian genetic algorithm (LGA) with a maximum number of 27000000 energy evaluations, the maximum number of 27000 generations and population size of 300 for each ligand. The ligands and side chains of assumed binding residues were set to be flexible. The torsional degrees of freedom were 13 and 3 for sucrose and GA3, respectively. The docking poses of each ligand were ranked by binding free energy estimations and population numbers of resulting clusters. Docking poses with the lowest binding free energy in the most populated clusters were selected as final conformations. All experimentally suggested sucrose binding residues are in contact with sucrose in its final docking pose. The protonation states of titratable residues were determined by PropKa 3.1 and visual inspection.

**Preparation for MD simulation**

A pre-equilibrated 1-palmitoyl-2-oleoyl-d-glycero-3-phosphatidylcholine (POPC) bilayer consisting of 161 lipid molecules was packed around the transporter of each system using the Membrane Builder facility of CHARMM-GUI (9, 10). The transporter was oriented with respect to the membrane in a way that charged side chains of the transmembrane domain were surrounded either by lipid head groups or water molecules. The membrane position relative to the transporter was determined based on the database of Orientations of Proteins in Membranes (OPM) (11). Each transporter-membrane system was solvated with 17433 TIP3P (12) water molecules resulting in 80.27x80.27x129.65 Å³ simulation box cells with a distance of at least 15 Å between protein surface and box face. Each box was replicated by periodic boundary conditions. Sodium and
chloride ions corresponding to a physiological ion strength of 150 mM were added. Additional chloride counterions were added to achieve a neutral net charge of all systems.

The minimization procedure and set-up of MD simulations were performed with GROMACS 5.0.6 program package (13, 14) using the CHARMM-36 all-atom additive force field containing carbohydrate parameters (15). The parameters of GA3 were generated by the CHARMM-GUI using the CHARMM General Force Field (CGenFF) (16). The real space summation of electrostatic interactions was truncated at 12 Å, and the Particle Mesh Ewald (PME) method was used to calculate the electrostatic interactions beyond 12 Å with a grid spacing of 1.2 Å and an interpolation order of 4. Van der Waals interactions were calculated using a cut-off of 12 Å. The solvated systems were energy minimized to eliminate unfavorable positions. Harmonic positional restraints were applied on protein, ligands, lipids, and dihedral restraints on lipid heavy atoms to achieve smooth minimization. 5000 steps steepest descent algorithm was used adopting harmonic force constants for protein backbone/side-chain atoms -4000/2000 kJmol⁻¹nm⁻², for the ligand atoms - 4000 kJmol⁻¹nm⁻², for the lipid phosphor (P) atom in Z direction (orthogonal to the membrane) - 1000 kJmol⁻¹nm⁻², for the improper dihedral angle formed by the glycerol carbon and the oleoyl ester oxygen atoms of POPC (restricted to 120°) and for the dihedral angle around the double bond of the oleoyl chain of POPC (restricted to 0°) - 1000 and 1000 kJmol⁻¹rad⁻², respectively.

The minimized systems were equilibrated over 6 successive runs: 2 x 25 ps (NVT, 1 fs timestep), 25 ps (NPT, 1 fs timestep) and 3 x 100 ps (NPT, 2 fs timestep). Gradually decreasing harmonic restraints were applied to the protein, ligands, and the lipid heavy atoms. The force constant values were decreased every run according to the following procedure: the protein backbone/side-chain atoms − 4,000/2,000, 2,000/1,000, 1,000/500, 500/200, 200/50, 50/0 kJmol⁻¹nm⁻²; the ligand atoms − 4000, 2000, 1000, 500, 200, 50 kJmol⁻¹nm⁻²; the lipid phosphor (P) atom in Z direction − 1,000, 1,000, 400, 200, 40, 0 kJmol⁻¹nm⁻²; improper dihedral angle formed by the glycerol carbon and the oleoyl ester oxygen atoms of POPC (restricted to 120°) and dihedral angle around the double bond of the oleoyl chain of POPC (restricted to 0°) − 1000, 400, 200, 100, 0 kJmol⁻¹rad⁻².

Production of MD trajectories

All-atom MD simulations were performed on 96 nodes of a local computer cluster with CHARMM-36 force field using GROMACS 5.0.6 package. 2100 ns trajectory was performed for each system (SI Appendix, Fig. S1). The first 100 ns of each simulation was used as non-restrained equilibration and was not included in binding pocket clustering. The following MD protocols were used: the integration time step was 2 fs; the isobaric–isothermal (NPT) ensemble was employed; the pressure was set to 1 bar using semi-isotropic coupling (uniform scaling of X-Y box vectors, independent Z) to the Parrinello-Rahman barostat with a time constant of 5 ps and an isothermal compressibility of 4.5*10⁻⁵ bar⁻¹; the temperature was kept constant at 300 K using the Nosé-Hoover thermostat with a time constant of 1 ps. Bonds with hydrogen atoms were constrained using the Linear Constraint Solver (LINCS). Atomic coordinates were recorded every 10 ps.

The MD trajectories were analyzed (root mean square deviations (RMSDs), root mean square fluctuations (RMSFs), principal component analysis (PCA), clustering, and geometric measurements) with tools included in the GROMACS 5.0.6 package. The RMSDs were calculated on the backbone atoms of the transporter after the least square fitting of each snapshot on the first structure of the MD run. According to the RMSD curves, the initial 100 ns of each trajectory was omitted as non-equilibrated. It was assumed that GA3 makes similar contacts with the transporter as sucrose, reasoning the same choice of possible binding residues. The conformational clustering of the ensemble of the ligand and its binding site was performed after superimposition of each snapshot onto the atoms of binding residues and ligands of the first equilibrated structure. The clustering was based on RMSD comparison. The Gromos algorithm of Gromacs 5.0.6 was applied for clustering analysis using a cut-off of 2 Å for both ligand-bound states. The central structure of
each cluster was considered as *representative conformation*. The center of a cluster is the structure with the smallest average RMSD from all other structures of the cluster.

**Sucrose transport assays in mammalian cells**

The ORF of *Arabidopsis* SWEET13 was cloned into pcDNA3.2/V5-DEST. Mutations (S20A, S20Q, V23A, S54A, N76Q, S142A, S142L, S142N, V145A, V145L, N196A, N196Q) were introduced by PCR mutagenesis using PrimeSTAR® GXL DNA Polymerase (Takara) and NEBuilder® HiFi DNA Assembly (NEB) (*SI Appendix*, Table S1). For sucrose transport assays, HEK293T cells were co-transfected with constructs carrying the sucrose sensor FLIPSuc-90μA1 (17) and SWEET13, or SWEET13 variants carrying mutations, by Lipofectamine LTX (Invitrogen) in 8-well glass-bottom chambers (Iwaki) and incubated for 48 h. Fluorescence images were acquired on a Nikon Ti2-E microscope equipped with PRIME BSI sCMOS camera (Photometrics) and SPECTRA X LED Light (Lumencor), a 40x dry objective lens CFI PlanFluor (Nikon) under excitation at 440 nm and emission channels for CFP (ET480/40m, Chroma Technology) and YFP (ET535/30m, Chroma Technology). Culture media were replaced with 150 μL Hanks Balanced Saline Salt (HBSS) buffer (pH7.4) followed by the addition of 150 μL HBSS buffer containing 20 mM sucrose. Images were taken at 5-sec intervals. Image quantification was performed with Fiji/ImageJ software (NIH). Data were analyzed and the box plots were generated with Prism8 (GraphPad Software).

**Glucose transport assays in mammalian cells**

For glucose transport assays, HEK293T cells were co-transfected with constructs carrying the glucose sensor FLII12Pglu700µδ6 (18) and SWEET1, SWEET13, or SWEET13 variants carrying mutations. Fluorescence images were acquired by the same method in sucrose transport assays. Culture media were replaced with 150 μL HBSS buffer containing 20 mM glucose. Images were acquired as for sucrose transport assays and the scatter plots were generated with Prism8.

**Immunofluorescence imaging of mammalian cells**

HEK293T cells were co-transfected with constructs FLIPSuc-90μA1 and SWEET13 or SWEET13 variants carrying mutations using the same method for sucrose transport assay. Cells were washed with phosphate-buffered saline (PBS) and subsequently fixed with ice-cold 4% paraformaldehyde in D-MEM followed by permeabilization using Triton X-100 (19). Mouse monoclonal Anti-V5 antibodies (Invitrogen) were diluted at 1:5000 in PBS with 4% fetal bovine serum (FBA) and incubated at 4°C overnight. After washing with PBS, secondary antibodies, goat anti-mouse Alexa488 (Abcam), diluted at 1:1000 in PBS with 4% FBA were incubated for 1 hour at room temperature. The cells were counter-stained with the nuclear probe DAPI 0.6 µM for 4 min. After washing with PBS, cells were mounted with glycerol with 10xPBS for observation.

**Glucose transport assays in yeast**

Glucose transport assays were performed using hexose transporter mutant EBY4000 strain transformed with pDRF1-GW (empty vector control), pDRf1-SWEET1 (positive control), pDRF1-SWEET13, or pDRf1-SWEET13 with mutations using conventional lithium acetate/PEG transformation. Three independent colonies were used for each assay. Colonies were incubated in synthetic complete (SC) (-Ura) liquid media containing 2% (v/v) maltose at 30°C overnight. Cultures were diluted sequentially to 10, 102,103, and 104 cells/µL. 10 µL cell suspension was spotted on SC (-Ura) containing either 2% maltose or 2% glucose and incubated for 3 days at 30°C. Plates were photographed.

**GA transport assays in mammalian cells**
For GA transport assays, HEK293T cells were co-transfected with constructs carrying the GA sensor GPS1 (20) and SWEET13, or SWEET13 variants carrying mutations, by Lipofectamine LTX (Invitrogen) in 8-well glass-bottom chambers (Iwaki) and incubated for 48h. Culture medium was replaced with 300 µL Dulbecco’s Modified Eagle Medium (D-MEM) without phenol red containing either 0.001% (v/v) DMSO or 1.0 µM GA3 dissolved in 0.001 % DMSO. Cells were incubated for 3h. D-MEM containing either DMSO or GA3 were replaced with HBSS buffer (pH 7.4) containing either DMSO or GA3 for observation. Fluorescence images were acquired as for sucrose transport assays.

**GA transport assays in a Yeast three-hybrid system**

GA transport assays were performed using a previously described Y3H system (1, 3). The yeast strain PJ69-4a [MATa trp1-901 leu2-3,112 ura3-52 his3-200 A gal4 A gal80 LYS2::GAL1-HIS3 GAL2-ADE2 met2::GAL7-lacZ] (BY5625) was obtained from the National Bio-Resource Project (NBRP, Japan). Yeast cells were cotransformed with the GA receptor components in pDEST22-GAI and pDEST32-GID1a and either pDRf1-GW (empty vector control), pDRf1-SWEET13, or pDRf1-SWEET13 with mutations by conventional lithium acetate/PEG transformation. In the pDRf1-vectors, ORF expression is driven from the strong PMA1 promoter fragment {Loque:2007ia}. Three independent colonies were used as technical replicates for each assay. Colonies were incubated in Synthetic Defined (SD -Leu, -Trp, -Ura) liquid media and incubated overnight at 30°C. Cultures were diluted sequentially to 10, 10², 10³, and 10⁴ cells/µL. 10 µL cell suspension was spotted on SD (-Leu, -Trp, -Ura) or selective (-Leu, -Trp, -Ura, -His) media containing 3 mM 3-amino-1,2,4-triazole (3-AT) in 0.001% (v/v) DMSO, and 0.1 µM GA3 or 0.1 µM GA1 in 0.001% (v/v) DMSO, and incubated for 3 days at 30°C. Plates were photographed.

To observe the localization of SWEET13 and SWEET13 with mutations in yeast, yeast cells were cotransformed with monomeric YFP (mYFP) -fusion SWEET13, pDEST22-GAI and pDEST32-GID1. For the yeast expression vector, pDRf1-GW-mYFP, was amplified from a pGWB441m kindly provided by Dr. Shoji Segami and introduced into the pDRf1-GW backbone amplified by an inverse PCR. Colonies were cultured in SD (-Leu, -Trp, -Ura) at 30°C overnight. Fluorescence images were acquired on a Nikon Ti2-E microscope equipped with PRIME BSI sCMOS camera (Photometrics) and SPECTRA X LED Light (Lumencor), a 100x oil objective lens CFI PlanFluor (Nikon) under excitation at 470 nm and emission channels for YFP (FF01-512/25-25, Semrock).

**Plant materials and growth conditions**

Single sweet13 and sweet14 mutants were obtained from the Arabidopsis Biological Resource Center (ABRC) and homozygous mutants were selected by PCR using primers (Table 1). The sweet13;14 double mutant was generated by crossing sweet13 (SALK_087791: a T-DNA is inserted before the 39th base T of the fifth exon.) with sweet14 (SALK_010224: a T-DNA is inserted before 16th base A of the fifth). For complementation and other purposes, a translational fusion SWEET13-turboID-GFP (SWEET13-GFP) construct was generated from a genomic clone of SWEET13 (At5g50800), including 4059-bp upstream from translational start site and a 2178-bp region downstream from the stop codon. The turboID-GFP (21) sequence was fused to SWEET13 just before the stop codon. Using SWEET13-turboID-GFP as a template, SWEET13N76Q and SWEET13S142N mutations were introduced by inverse PCR using PrimeSTAR® GXL DNA Polymerase (Takara) and NEBuilder® HiFi DNA Assembly (NEB) (SI Appendix, Table S1). The turboID-GFP fused constructs were transferred to pBIN40 (22). Agrobacterium-mediated transformation by flower dipping was used to generate transgenic plants (23). Seeds were surface-sterilized with 70% (v/v) ethanol and washed with sterile water four times. After stratification at 4°C in the dark for 3 days, seeds were sown on mineral wool blocks. Plants were grown in a growth chamber at 22°C in a 16 h light / 8 h dark cycle (40 µmol m⁻² s⁻¹ of white light) with 60% humidity for 2 weeks. Mineral wool blocks with two-week-old seedlings were transferred to soil containing
vermiculite and Metro-mix at a 2:1 ratio and grown in a growth chamber at 23°C under continuous light (60 μmol m$^{-2}$ s$^{-1}$ of white light). Plants were supplied with nutrients (1/1000 dilution of Hyponex) once every two weeks.

**FDA-PI staining of pollen**

To evaluate the plasma membrane integrity of pollen, pollen was stained with 1 μg/mL propidium iodide (PI) and 2 μg/ml fluorescein diacetate (FDA) in MilliQ water. After staining, pollen was observed under an LSM800 confocal microscope using a 20x dry objective Plan-APOCHROMAT (Zeiss). FDA was excited at 488 nm and detected with a window of 500-550 nm. PI was excited at 561 nm and detected with a window of 570-640 nm. Bar graphs were generated and statistical analyses were performed with Prism8 (GraphPad Software).

**In vitro pollen germination assays**

To evaluate the pollen germination efficacy, pollen grains were incubated on pollen germination medium (0.01% boric acid, 5 mM CaCl$_2$, 5 mM KCl, 1 mM MgSO$_4$, 10% sucrose, adjusted to pH7.5 with KOH, 1.5% low-melting agarose supplemented with 10 μM epibrassinolide) at 23°C in a humid chamber (24). Images were obtained using an Axio Imager A2 upright microscope (Zeiss) equipped with a CCD camera (Axiocam 512 color; Zeiss).

**Confocal imaging of anthers**

Fluorescence images of anthers from plants stably expressing SWEET13-turboID-GFP were captured with an LSM800 confocal laser scanning microscope (Zeiss) equipped with a 20x dry objective. GFP was excited at 488 nm and detected in a window of 500-550 nm; autofluorescence was excited at 561 nm and detected in a window of 570-640 nm. For sum slices projection, 35 slices of Z-stacks in 1.5 μm intervals were taken. Fixation, clearing, and staining of anther were conducted following ClearSee protocol (25). Images were taken with a Z-stack in 1.5 μm intervals using a 40x water-immersion objective lens. GFP proteins were excited at 488 nm and detected in a window of 410-546 nm; autofluorescence was excited at 488 nm and detected in a window of 656-700 nm; CalcofluorWhite for cell wall staining was excited at 405 nm and detected in a window of 410-546 nm. For the quantification of fluorescence in anthers, the fluorescence of SWEET13-GFP in anthers was obtained by taking z-stack in 1.5 μm intervals with 50 slices with an LSM800 (Zeiss) using a 20x dry objective lens. The fluorescence of SWEET13- GFP in the sum image of anthers was quantified by Fili/ImageJ software. In brief, the mean intensity of the selected area was measured (shown in Fig S8). Each data point was normalized by the mean value of wild type SWEET13 and the scattered dot plots were generated with PRISM8.
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