AtSWEET13 and AtSWEET14 regulate gibberellin-mediated physiological processes

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Transmembrane transport of plant hormones is required for plant growth and development. Despite reports of a number of proteins that can transport the plant hormone gibberellin (GA), the mechanistic basis for GA transport and the identities of the transporters involved remain incomplete. Here, we provide evidence that Arabidopsis SWEET proteins, AtSWEET13 and AtSWEET14, which are members of a family that had previously been linked to sugar transport, are able to mediate cellular GA uptake when expressed in yeast and oocytes. A double sweet13 sweet14 mutant has a defect in anther dehiscence and this phenotype can be reversed by exogenous GA treatment. In addition, sweet13 sweet14 exhibits altered long distant transport of exogenously applied GA and altered responses to GA during germination and seedling stages. These results suggest that AtSWEET13 and AtSWEET14 may be involved in modulating GA response in Arabidopsis.
Transport of solutes across non-permeable biological membranes consisting of a lipid bilayer is required for many aspects of biological processes, including generation of electrochemical potentials, energy production, metabolism, signal transduction and so on, and membrane transporters are the proteins that mediate these processes. Plants produce a variety of compounds and metabolites, which accumulate in specific tissues, cell types and subcellular compartments via transport, indicating the presence of highly sophisticated transport mechanisms in plants.

Plant hormones are a group of small molecules that induce a wide range of physiological responses at low concentrations (less than μM if applied exogenously). The transport of auxin (indole-3-acetic acid; IAA) is well known to be required for many physiological processes during plant growth and development. Plant-specific PIN-FORMED (PIN) transporters mediate IAA efflux from cells, whereas the amino acid permease-like AUX/LUX family proteins import IAA into cells. In addition, some members in subgroup B of the ATP-binding cassette (ABC)-type transporter family (ABCB) function as IAA transporters. In contrast, transporters of ABC transporters in the transport of other plant hormones has also been reported. In Arabidopsis, AtABC25 functions as an abscisic acid (ABA) transporter that mediates ABA export from ABA biosynthesizing cells around vascular tissues, whereas AtABC40 is an ABA importer involved in the uptake of ABA into guard cells to regulate stomatal aperture. Furthermore, in Arabidopsis, AtABC14 regulates root-to-shoot transport of the cytokinin trans-zeatin, to modulate shoot development. In petunia, the ABCG protein PDR1 regulates symbiotic interactions with arbuscular mycorrhiza and shoot branching by regulating cellular strigolactone export.

Recently, some of the Arabidopsis NRT1/PTR FAMILY (NPF) proteins, initially characterized as nitrate or di/tri-peptide transporters, were also identified as plant hormone transporters. AtNPF6.3 was originally identified as a dual affinity nitrate transporter CHL1/NRT1.1 that is regulated by protein phosphorylation. However, AtNPF6.3/CHL1/NRT1.1 reportedly transports IAA with competition between nitrate and IAA playing an important role in lateral root development. In our previous study, we identified the low affinity nitrate transporter NPF4.6/NRT1.2 (ref. 15) as a protein capable of transporting ABA by a modified two-hybrid (Y2H) screening system using the ABA receptor complex as a sensor to detect ABA concentrations in yeast cells. Mutants defective in AtNPF4.6/NRT1.2 had more open stomata compared with the wild type, indicating that the protein functions as an ABA transporter in vivo. We subsequently identified additional Arabidopsis NPF proteins capable of transporting gibberellin (GA) and jasmonoyl-isoleucine (JA-Ile) as well as ABA, although the functions of these proteins in vivo are unknown. Independently, AtNPF2.10/GTR1 was identified as a gene that is co-expressed with jasmonate biosynthesis genes. AtNPF2.10/GTR1 had been characterized as a glucosinolate transporter; however, AtNPF2.10/GTR transports not only glucosinolate but also GA and JA-Ile when expressed in X. oocytes.

Furthermore, the gtr1 mutant is less sensitive to exogenously applied methyl jasmonate in seedlings, and the reduced fertility observed in gtr1 was restored by GA application, suggesting that this protein can function as a GA/JA-Ile transporter. AtNPF3.1 was reported recently to function as a GA transporter in vivo. In addition to the above-mentioned transporters, the multidrug and toxin extrusion (MATE) family of proteins has been implicated in salicylic acid (SA) and ABA transport. The existence of multiple plant hormone transporters and transporter families for a particular plant hormone suggests that plants have highly redundant hormone transport systems.

GA regulates diverse aspects of plant growth and development, including seed germination, stem elongation, leaf expansion and flower and seed development. As mentioned above, Arabidopsis AtNPF2.10/GTR1 and AtNPF3.1 have been identified as GA transporters; however, since the GA-related phenotypes of the mutants defective in AtNPF2.10/GTR1 or AtNPF3.1 are limited compared with those observed in severe GA-deficient or signalling mutants, there might be other GA transporters that have not yet been identified. In this work, we conducted a functional screen for GA transporters using a Y2H system with the GA receptor GID1a and the DELLA protein GAI, and found that some Arabidopsis SWEET proteins transport GA when expressed in yeast and X. oocytes. SWEET proteins were originally identified as a novel class of sugar transporters that facilitate transmembrane transport of sugars like glucose, fructose and/or sucrose bi-directionally depending on their concentrations. In Arabidopsis, AtSWEET11 and AtSWEET12 are localized to the plasma membrane of phloem parenchyma cells and mediate phloem loading of sucrose. AtSWEET11 and AtSWEET12 are also expressed in developing seeds together with AtSWEET15, and they regulate sucrose transport from the seed coat to the embryo through the endosperm. In contrast, AtSWEET2, AtSWEET16 and AtSWEET17 mediate vacuolar sugar transport. AtSWEET17 regulates the fructose content in leaf vacuoles, whereas AtSWEET2 modulates sugar secretion from roots and is involved in resistance to the pathogen Pythium.

These diverse functions suggest that SWEET proteins play important roles in various physiological processes. In the present study we show that AtSWEET13 and AtSWEET14 are required for proper development of anthers, seeds and seedlings. Our data suggest that the functions of AtSWEET13 and AtSWEET14 are associated with GA-mediated physiological responses.

**Results**

**Screen for potential GA transporters.** To identify novel GA transporters, we screened for cDNAs that induced interactions between the Arabidopsis GA receptor GID1a and the DELLA protein GAI at a low GA concentration (0.1 μM) using a Y2H system. GAs are the major bioactive GA produced in the fungus Gibberella fujikuroi, was used as a substrate because it is commercially available in relatively large quantities. Furthermore, GA3 is resistant to hydroxylation at the C-2 position due to the presence of a double bond between C-2 and C-3, and is therefore predicted to have stable biological activity. We transformed ~ 1.9 × 10⁶ cDNAs cloned in a yeast expression vector, which originated from ~ 0.5 × 10⁶ initial independent cDNAs that were synthesized from RNA extracted from 2-week-old Arabidopsis seeds, into yeast cells containing the BD-GID1a and AD-GAI constructs. In the first screen, we obtained 131 colonies that grew on selection media containing 0.1 μM GA3 using HIS3 as a positive selection marker. In the second screen, 11 clones that grew on selection media in the presence, but not in the absence, of 0.1 μM GA3 were selected. Among the 11 clones, three contained cDNAs corresponding to AtNPF1.2 (At1g52190), and another three clones contained cDNAs corresponding to AtSWEET13 (At5g50800). AtNPF1.2 has been reported already to transport GA, which indicated that the screening system performed as expected. We hypothesized that AtSWEET13, which belongs to the recently identified sugar transporter family, could transport multiple substrates as has been shown for Arabidopsis NPF proteins.

Next, we examined whether some other proteins related to AtSWEET13 would be able to induce BD-GID1a/AD-GAI interactions in yeast (Fig. 1). We cloned five additional cDNAs encoding AtSWEET9, 10, 11, 12 and 14 (Fig. 1a), and expressed...
expression of the interaction between GID1a and GAI were determined by Y2H assays based on the b-galactosidase (b-gal) assay (Fig. 1c). As predicted from yeast growth on selection media, AtSWEET14 promoted BD-GID1a/AD-GAI interactions more efficiently than did the other AtSWEETs, and 10 nM GA3 was sufficient to induce detectable b-gal activity. In addition, AtSWEET10 and AtSWEET12 enhanced the BD-GID1a/AD-GAI interactions to levels similar to AtSWEET14 but to a lesser extent than AtSWEET14.

GA transport activities of AtSWEET proteins. The close phylogenetic relationship as well as similar gene expression patterns predicted by a public database (Arabidopsis eFP Browser; http://bar.utoronto.ca/eFP/cgi-bin/eFPWeb.cgi; Supplementary Fig. 1)34–36 suggested that AtSWEET13 and AtSWEET14 have redundant physiological roles. AtSWEET13 and AtSWEET14 have been shown to transport sucrose when expressed in human embryonic kidney (HEK) 293T cells27. AtSWEET13 has also been reported to regulate pollen wall pattern formation in association with AtSWEET8 (ref. 37); however, the in vivo functions of AtSWEET13 and AtSWEET14 remain largely unknown.

To assess GA transport activity more directly, we quantified GA taken into yeast cells or Xenopus oocytes by mass spectrometry (Fig. 2). In the buffer conditions used previously for AtNPFs (50 mM potassium phosphate buffer, pH 5.8–7.0)16,17,38, we were unable to detect significant GA transport activities by AtSWEET13 and AtSWEET14 in yeast (Supplementary Fig. 2a). As shown in Fig. 1, AtSWEET13 and AtSWEET14 induced BD-GID1a/AD-GAI interactions in yeast when the cells were incubated in growth media. Thus, we suspected that elements present in the media were required for these activities. We found that a significant amount of GA3 was taken into yeast expressing AtSWEET13 or AtSWEET14 when 100 mM glucose was added to the reaction buffer at acidic pH conditions (Fig. 2a; Supplementary Fig. 2a). This phenomenon appeared not to be direct regulation of AtSWEET13 and AtSWEET14 by glucose because similar induction of GA transport activity was observed for AtNPF2.5 that was previously shown to induce BD-GID1a/AD-GAI interactions in yeast17 (Supplementary Fig. 2b). In the same conditions, yeast cells expressing AtSWEET13 or AtSWEET14 accumulated ABA, jasmonic acid (JA) and JA-Ile at levels similar to control cells that did not possess the transporters (Fig. 2b), suggesting that the proteins mediated specific GA transport. We further confirmed the GA transport activities of AtSWEET13 and AtSWEET14 by glucose because similar induction of GA transport activity was observed for AtNPF2.5 that was previously shown to induce BD-GID1a/AD-GAI interactions in yeast17 (Supplementary Fig. 2b). In the same conditions, yeast cells expressing AtSWEET13 or AtSWEET14 accumulated ABA, IAA, jasmonic acid (JA) and JA-Ile at levels similar to control cells that did not possess the transporters (Fig. 2b), suggesting that the proteins mediated specific GA transport. We further confirmed the GA transport activities of AtSWEET13 and AtSWEET14 by glucose because similar induction of GA transport activity was observed for AtNPF2.5 that was previously shown to induce BD-GID1a/AD-GAI interactions in yeast17 (Supplementary Fig. 2b). In the same conditions, yeast cells expressing AtSWEET13 or AtSWEET14 accumulated ABA, IAA, jasmonic acid (JA) and JA-Ile at levels similar to control cells that did not possess the transporters (Fig. 2b), suggesting that the proteins mediated specific GA transport. We further confirmed the GA transport activities of AtSWEET13 and AtSWEET14 by glucose because similar induction of GA transport activity was observed for AtNPF2.5 that was previously shown to induce BD-GID1a/AD-GAI interactions in yeast17 (Supplementary Fig. 2b). In the same conditions, yeast cells expressing AtSWEET13 or AtSWEET14 accumulated ABA, IAA, jasmonic acid (JA) and JA-Ile at levels similar to control cells that did not possess the transporters (Fig. 2b), suggesting that the proteins mediated specific GA transport. We further confirmed the GA transport activities of AtSWEET13 and AtSWEET14 by glucose because similar induction of GA transport activity was observed for AtNPF2.5 that was previously shown to induce BD-GID1a/AD-GAI interactions in yeast17 (Supplementary Fig. 2b). In the same conditions, yeast cells expressing AtSWEET13 or AtSWEET14 accumulated ABA, IAA, jasmonic acid (JA) and JA-Ile at levels similar to control cells that did not possess the transporters (Fig. 2b), suggesting that the proteins mediated specific GA transport. We further confirmed the GA transport activities of AtSWEET13 and AtSWEET14 by glucose because similar induction of GA transport activity was observed for AtNPF2.5 that was previously shown to induce BD-GID1a/AD-GAI interactions in yeast17 (Supplementary Fig. 2b). In the same conditions, yeast cells expressing AtSWEET13 or AtSWEET14 accumulated ABA, IAA, jasmonic acid (JA) and JA-Ile at levels similar to control cells that did not possess the transporters (Fig. 2b), suggesting that the proteins mediated specific GA transport. We further confirmed the GA transport activities of AtSWEET13 and AtSWEET14 by glucose because similar induction of GA transport activity was observed for AtNPF2.5 that was previously shown to induce BD-GID1a/AD-GAI interactions in yeast17 (Supplementary Fig. 2b). In the same conditions, yeast cells expressing AtSWEET13 or AtSWEET14 accumulated ABA, IAA, jasmonic acid (JA) and JA-Ile at levels similar to control cells that did not possess the transporters (Fig. 2b), suggesting that the proteins mediated specific GA transport.
was technically difficult to determine the uptake kinetics in this system. In contrast, GA$_3$ uptake into *Xenopus* oocytes mediated by AtSWEET13 and AtSWEET14 was more gradual (Supplementary Fig. 2e). In this system, the Km values of both AtSWEET13 and SWEET14 for GA$_3$ were estimated to be several hundred μM (Supplementary Fig. 2f,g).

Next, we tested whether AtSWEET13 and AtSWEET14 could transport different types of GAs using the yeast assay system (Fig. 2d). We used a mixture of 11 GAs (5 μM each), including two major bioactive GAs in *Arabidopsis*, GA$_1$ and GA$_4$, as well as their precursors and metabolites, for which isotope-labelled internal standards were available, as substrates. Most of the GAs accumulated at higher levels in yeast expressing AtSWEET13 or AtSWEET14, compared with yeast that were not expressing the transporters, indicating that these proteins transport various GAs; however, each of the 11 GAs appeared to have a different membrane permeability. These differences in membrane permeability are possibly due to their different hydrophobicities; GA$_{12}$ uptake was high in yeast cells even in the absence of transporters, and the permeability was gradually lower after successive oxidations of GA$_{12}$ by GA$_{13}$ox, GA$_{20}$ox, GA$_{3}$ox and GA$_{2}$ox (Fig. 2d; Supplementary Fig. 2h). This result suggests that specific transporters are required for GAs that have a lower membrane permeability, such as GA$_1$.

Spatial expression patterns of AtSWEET13 and AtSWEET14.

To investigate the spatial expression patterns of AtSWEET13 and AtSWEET14, we generated transgenic plants expressing the GUS gene under the control of the AtSWEET13 and AtSWEET14 promoters (pAtSWEET13:GUS and pAtSWEET14:GUS, respectively) (Fig. 3). As predicted by a public database (Supplementary Fig. 1), the promoter activities of AtSWEET13 and AtSWEET14 were detected in stamens during the later stages of flower development (Fig. 3a,g). Closer investigation revealed that this expression was localized to the anthers (Fig. 3b,h). Although the database indicated that AtSWEET13 and AtSWEET14 were expressed abundantly in stamens, promoter activity was also detected at other developmental stages with similar expression patterns (Fig. 3c–i,l). In seedlings of pAtSWEET13:GUS and pAtSWEET14:GUS transgenic plants, GUS activity was detected around the vascular tissues in leaves and roots (Fig. 3c,d,i,j). Dot-like GUS staining at the junctions of stems and petioles suggested that AtSWEET13 and AtSWEET14 were also expressed in axillary buds (Fig. 3e,k). During seed development, the promoter activity of AtSWEET13 and AtSWEET14 was detected in embryonic cotyledons (Fig. 3f,j). When AtSWEET13 and AtSWEET14 were expressed as GFP fusion proteins under the control of the 35S promoter, GFP fluorescence was associated with the plasma membrane (Fig. 4).
AtSWEET13 and AtSWEET14 regulate anther dehiscence. To delineate the physiological roles of AtSWEET13 and AtSWEET14, we obtained mutants defective in the respective proteins (sweet13 and sweet14). As the expression patterns of AtSWEET13 and AtSWEET14 were similar, we generated a double mutant of sweet13 and sweet14 (sweet13 sweet14). We noted that the double mutant had reduced fertility and produced fewer seeds per silique compared with the wild type, whereas this phenotype was not observed in the sweet13 and sweet14 single mutants (Fig. 5a,b). The phenotype was complemented by the introduction of either AtSWEET13 or AtSWEET14 genomic DNA (Supplementary Fig. 3), indicating that this phenotype resulted from the double mutation. Expression of AtSWEET13 and AtSWEET14 in anthers (Fig. 3) suggested that this phenotype might be related to anther development as was observed for some GA-deficient and signalling mutants.23 Closer investigation revealed that anther dehiscence was delayed in sweet13 sweet14 compared with the wild type, whereas filament elongation was similar to the wild type (Fig. 5c). Treatment of flower buds with an excess amount (100 μM) of GA3 restored the delayed anther dehiscence of the double mutant (Fig. 5d). Pollen from sweet13 sweet14 is likely to have developed normally because siliques of the double mutant set at later stages sometimes developed similarly to those of the wild type (Fig. 5a). GA synthesized in stamens has been hypothesized to regulate flower development25,39–41; however, the phenotype of sweet13 sweet14 was restricted to anthers. GA content in whole anthers and developing seeds and seedlings (Fig. 3). Careful investigation revealed that mature seeds of sweet13 sweet14 were larger than those of the wild type and were approximately 1.5-fold heavier, whereas this phenotype was not observed in the sweet13 and sweet14 single mutants (Fig. 6a,b). Seed and seedling phenotypes of sweet13 sweet14. Promoter–reporter analyses indicated that AtSWEET13 and AtSWEET14 were expressed not only in anthers but also in developing seeds and seedlings (Fig. 3). Careful investigation revealed that mature seeds of sweet13 sweet14 were larger than those of the wild type and were approximately 1.5-fold heavier, whereas this phenotype was not observed in the sweet13 and sweet14 single mutants (Fig. 6a,b). Interestingly, in contrast to sweet13 sweet14, the ga3ox1 mutant, which is defective in the GA biosynthesis gene AtGA3ox1, produced smaller seeds than the wild type (Fig. 6b). Introduction of the ga3ox1 mutation reduced the size of the sweet13 sweet14 double mutant seeds (Fig. 6b). Although the seed size of another GA-deficient mutant ga20ox1 was not significantly different from the wild type, introduction of the ga20ox1 mutation clearly reduced the seed size of sweet13 sweet14 (Fig. 6b). These results indicate that GA is required for the increased seed size of the sweet13 sweet14 double mutant.
for the seed size phenotype observed in sweet13 sweet14. In Arabidopsis, the early 13-hydroxylatation pathway to produce GA1 as a bioactive GA is dominant during the middle stages of seed development42. Although the GA1 content in developing seeds at 10 days after flowering (DAF) was comparable between wild type and sweet13 sweet14, the levels of deactivated forms of GAs, GA8 and GA29, were significantly reduced in sweet13 sweet14 compared with wild type (Table 1). Endogenous levels of other hormones (IAA, ABA, JA, JA-Ile, SA, isopentenyladenine and trans-zeatin) in the developing seeds were not extremely different (<1.5-fold) in sweet13 sweet14 compared with wild type, although there are some statistically significant differences (Supplementary Table 1).

We subsequently found that sweet13 sweet14 seedlings grew larger than wild-type seedlings and were characterized by longer roots and heavier shoot dry weights (Fig. 6b,c). The phenotype was observed even when 1% sucrose was present in the media (Supplementary Fig. 4). As observed for the seeds, the phenotype of ga3ox1 seedlings contrasted to that of sweet13 sweet14 seedlings; ga3ox1 seedlings had shorter root lengths compared with wild-type seedlings. Introduction of the ga3ox1 mutation reduced the size of the sweet13 sweet14 double mutant seedlings. Although the seedling size of ga20ox1 was comparable to that of wild type, the ga20ox1 mutation also significantly reduced seedling size in the sweet13 sweet14 double mutant background. Again, these data suggest that the phenotype observed in sweet13 sweet14 is dependent on GA. Endogenous levels of GAs and other hormones were quantified in the shoots and roots of the seedlings; however, these hormones accumulated at similar levels in wild type and sweet13 sweet14 with only a few statistically significant differences (Table 1; Supplementary Table 1).

The phenotypes observed in sweet13 sweet14 seeds and seedlings were complemented by transformation with either AtSWEET13 or AtSWEET14 genomic DNA (Supplementary Fig. 5a,b), indicating that either gene is sufficient for normal seed and seedling development. Vegetative growth in the later developmental stages was comparable between sweet13 sweet14 and wild type (Supplementary Fig. 6).

As the seed and seedling size phenotype of sweet13 sweet14 contrasted to that of the GA-deficient mutants, we hypothesized that GA-mediated physiological processes were promoted in the double mutant. GA and ABA are known to have antagonistic effects on seed germination; GA promotes, whereas ABA inhibits seed germination43,44. Thus, we determined the effects of ABA and the GA biosynthesis inhibitor paclobutrazol on seed germination (Fig. 6d). Both wild type and sweet13 sweet14 germinated similarly under normal conditions after stratification; however, in the presence of ABA, sweet13 sweet14 germinated better than wild type. Similarly, sweet13 sweet14 was more resistant to paclobutrazol in terms of inhibition of seed germination compared with wild type. These results are consistent with GA responses being overactivated in sweet13 sweet14.

Transport of exogenously applied GA. We then investigated the movement of exogenously applied GA from the roots to the shoots in wild type and sweet13 sweet14 (Fig. 7). GA3 was used as a tracer for this experiment because it is hardly detected in Arabidopsis and can be discriminated from endogenous GAs. Droplets of GA3-containing water were applied to the main root tip of wild type and sweet13 sweet14 seedlings, and GA3 transported to the shoots was detected by LC-MS/MS. A product ion corresponding to GA3 was detected at background levels both in wild type and sweet13 sweet14 seedlings; GA3 transported to the shoots was detected by LC–MS/MS. A product ion corresponding to GA3 was detected at background levels both in wild type and sweet13 sweet14 seedlings; GA3 transported to the shoots was detected by LC–MS/MS. A product ion corresponding to GA3 was detected at background levels both in wild type and sweet13 sweet14 seedlings; GA3 transported to the shoots was detected by LC–MS/MS. A product ion corresponding to GA3 was detected at background levels both in wild type and sweet13 sweet14 seedlings; GA3 transported to the shoots was detected by LC–MS/MS. A product ion corresponding to GA3 was detected at background levels both in wild type and sweet13 sweet14 seedlings; GA3 transported to the shoots was detected by LC–MS/MS. A product ion corresponding to GA3 was detected at background levels both in wild type and sweet13 sweet14 seedlings; GA3 transported to the shoots was detected by LC–MS/MS. A product ion corresponding to GA3 was detected at background levels both in wild type and sweet13 sweet14 seedlings; GA3 transported to the shoots was detected by LC–MS/MS. A product ion corresponding to GA3 was detected at background levels both in wild type and sweet13 sweet14 seedlings; GA3 transported to the shoots was detected by LC–MS/MS. A product ion corresponding to GA3 was detected at background levels both in wild type and sweet13 sweet14 seedlings; GA3 transported to the shoots was detected by LC–MS/MS. A product ion corresponding to GA3 was detected at background levels both in wild type and sweet13 sweet14 seedlings; GA3 transported to the shoots was detected by LC–MS/MS. A product ion corresponding to GA3 was detected at background levels both in wild type and sweet13 sweet14 seedlings; GA3 transported to the shoots was detected by LC–MS/MS. A product ion corresponding to GA3 was detected at background levels both in wild type and sweet13 sweet14 seedlings; GA3 transported to the shoots was detected by LC–MS/MS. A product ion corresponding to GA3 was detected at background levels both in wild type and sweet13 sweet14 seedlings; GA3 transported to the shoots was detected by LC–MS/MS. A product ion corresponding to GA3 was detected at background levels both in wild type and sweet13 sweet14 seedlings; GA3 transported to the shoots was detected by LC–MS/MS.

Discussion

Using a Y2H system with the GA receptor GID1a and the DELLA protein GAI, we identified Arabidopsis SWEET proteins as novel
candidate GA transporters. Among the six closely related AtSWEET proteins tested, AtSWEET10, AtSWEET12, AtSWEET13 and AtSWEET14 were found to enhance GA-dependent interactions between GID1a and GAI in yeast (Fig. 1), suggesting that these proteins mediate GA uptake into yeast cells. Among these, AtSWEET13 and AtSWEET14 are close homologues and their spatial gene expression patterns were predicted to be similar. Thus, we hypothesized that AtSWEET13 and AtSWEET14 play similar physiological roles in plants and examined their functions in more detail.

Although the sucrose transport activities of AtSWEET13 and AtSWEET14 were reported previously, we confirmed that AtSWEET13 and AtSWEET14 could mediate cellular GA uptake when expressed in yeast or Xenopus oocytes using mass spectrometry (Fig. 2). The sugar transport activities of AtSWEET13 and AtSWEET14 are expressed in stamens (Fig. 3), and the fertility of the sweet13 sweet14 double mutant was reduced and anther dehiscence was delayed (Fig. 5). This phenotype was reversed by exogenous application of an excess amount of GA (Fig. 5). The stamen is thought to be an active site for GA biosynthesis and severe mutants impaired in GA biosynthesis or signalling have defective stamen development and reduced fertility. Despite the fact that GA regulates multiple steps during stamen development including pollen maturation, filament elongation and anther dehiscence, the sweet13 sweet14 phenotype was restricted to anther dehiscence. This is in contrast to mutants lacking AtNPF2.10/GTR1 (ref. 18) and reduced fertility. GA synthesized in stamens regulates flower development; however, we do not believe that AtSWEET13 and AtSWEET14 are involved in this process because flower tissues, other than the anthers, appeared normal in wild-type Arabidopsis. AtSWEET13 and AtSWEET14 function possibly results in reduced cellular GA levels without affecting the bulk GA content.

Promoter–reporter analyses showed that AtSWEET13 and AtSWEET14 are expressed in stamens (Fig. 3), and the fertility of the sweet13 sweet14 double mutant was reduced and anther dehiscence was delayed (Fig. 5). This phenotype was reversed by exogenous application of an excess amount of GA (Fig. 5). The stamen is thought to be an active site for GA biosynthesis and severe mutants impaired in GA biosynthesis or signalling have defective stamen development and reduced fertility. Despite the fact that GA regulates multiple steps during stamen development including pollen maturation, filament elongation and anther dehiscence, the sweet13 sweet14 phenotype was restricted to anther dehiscence. This is in contrast to mutants lacking AtNPF2.10/GTR1 (ref. 18) and reduced fertility. GA synthesized in stamens regulates flower development; however, we do not believe that AtSWEET13 and AtSWEET14 are involved in this process because flower tissues, other than the anthers, appeared normal in wild-type Arabidopsis. AtSWEET13 and AtSWEET14 function possibly results in reduced cellular GA levels without affecting the bulk GA content.

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Table 1 | GA levels in wild type and sweet13 sweet14.

| GA | WT | anther | filament | flower | root | leaf | root1 | leaf1 |
|----|-----|-------|----------|--------|------|------|-------|-------|
| GA1 | 61.68 ± 22.16 | ND | ND | ND | ND | ND | ND | ND |
| GA2 | 13.0 ± 6.8 | ND | ND | ND | ND | ND | ND | ND |
| GA3 | 2.57 ± 1.15 | ND | ND | ND | ND | ND | ND | ND |
| GA4 | 0.8 ± 0.4 | ND | ND | ND | ND | ND | ND | ND |
| GA6 | 1.57 ± 0.8 | ND | ND | ND | ND | ND | ND | ND |
| GA13ox | 1.58 ± 0.84 | ND | ND | ND | ND | ND | ND | ND |
| GA2ox | 1.22 ± 0.61 | ND | ND | ND | ND | ND | ND | ND |
| GA3ox | 0.77 ± 0.38 | ND | ND | ND | ND | ND | ND | ND |

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| GA13ox | 1.58 ± 0.84 | ND | ND | ND | ND | ND | ND | ND |
| GA2ox | 1.22 ± 0.61 | ND | ND | ND | ND | ND | ND | ND |
| GA3ox | 0.77 ± 0.38 | ND | ND | ND | ND | ND | ND | ND |

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| GA2ox | 1.22 ± 0.61 | ND | ND | ND | ND | ND | ND | ND |
| GA3ox | 0.77 ± 0.38 | ND | ND | ND | ND | ND | ND | ND |

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| GA3 | 2.57 ± 1.15 | ND | ND | ND | ND | ND | ND | ND |
| GA4 | 0.8 ± 0.4 | ND | ND | ND | ND | ND | ND | ND |
| GA6 | 1.57 ± 0.8 | ND | ND | ND | ND | ND | ND | ND |
| GA13ox | 1.58 ± 0.84 | ND | ND | ND | ND | ND | ND | ND |
| GA2ox | 1.22 ± 0.61 | ND | ND | ND | ND | ND | ND | ND |
| GA3ox | 0.77 ± 0.38 | ND | ND | ND | ND | ND | ND | ND |

| GA | WT | anther | filament | flower | root | leaf | root1 | leaf1 |
|----|-----|-------|----------|--------|------|------|-------|-------|
| GA1 | 61.68 ± 22.16 | ND | ND | ND | ND | ND | ND | ND |
| GA2 | 13.0 ± 6.8 | ND | ND | ND | ND | ND | ND | ND |
| GA3 | 2.57 ± 1.15 | ND | ND | ND | ND | ND | ND | ND |
| GA4 | 0.8 ± 0.4 | ND | ND | ND | ND | ND | ND | ND |
| GA6 | 1.57 ± 0.8 | ND | ND | ND | ND | ND | ND | ND |
| GA13ox | 1.58 ± 0.84 | ND | ND | ND | ND | ND | ND | ND |
| GA2ox | 1.22 ± 0.61 | ND | ND | ND | ND | ND | ND | ND |
| GA3ox | 0.77 ± 0.38 | ND | ND | ND | ND | ND | ND | ND |
of GA on seed size have not been discussed in previous studies, but our study demonstrated that ga3ox1 produced smaller seeds than did the wild type (Fig. 6b). In contrast, the seed size phenotype was not obvious in ga20ox1 mutants (Fig. 6b), possibly due to different degrees of redundancies between ga3ox1 and ga20ox1. Nevertheless, mutations in either GA3ox1 or GA20ox1 reduced seed size in the sweet13 sweet14 mutant background, indicating that GA is required for the phenotype observed in sweet13 sweet14. Therefore, the larger size of sweet13 sweet14 seeds might be attributed to enhanced GA responses. Again, we cannot exclude the possibility that the larger seed size of sweet13 sweet14 is unrelated to differences in GA transport. For example,
In conclusion, we demonstrated that SWEET proteins are multifunctional transporters. Among the 17 homologues present in the Arabidopsis SWEET family, some members might transport compounds other than GA and sugars. Interestingly, multifunctionality has recently been reported for NPF11,17. Further elucidation of novel transporter functions will explain how plants are able to transport a variety of compounds with only a limited number of transporters.

**Methods**

**Yeast screening.** The BD-GIId1a and AD-GA Y2H system17 was used to screen for a DNA library constructed from RNA extracted from 2-week-old Arabidopsis seedlings16. Colonies that grew on selection medium (synthetic dextrose (SD): -Leu, -Try, -Ura, -His) in the presence of 0.1 μM GA3 were selected.

**Cloning AtSWEET cDNA.** Coding sequences of six AtSWEET cDNAs were amplified by PCR using the following primer combinations: 5′-CACCATGTTCTCCTCAAGGTCTCATGAAATGTTG-3′ (forward) and 5′-TCACTCTATTGGGCTTAC-3′ (reverse) for AtSWEET7 (AT2G39060), 5′-CACCATGTTCTCCTCAAGGTCTCATGAAATGTTTG-3′ (forward) and 5′-TCACTCTATTGGGCTTAC-3′ (reverse) for AtSWEET10 (AT5G07090), 5′-CACCATGTTCTCCTCAAGGTCTCATGAAATGTTTG-3′ (forward) and 5′-TCACTCTATTGGGCTTAC-3′ (reverse) for AtSWEET14 (AT5G08800), and 5′-CACCATGTTCTCCTCAAGGTCTCATGAAATGTTTG-3′ (forward) and 5′-TCACTCTATTGGGCTTAC-3′ (reverse) for AtSWEET14 (AT4G25010). Underlined sequences denote overhangs that were used to clone the sequence into pENTR/D-TOPO (Invitrogen).

**Transport assays in yeast.** For the Y2H-based indirect assays, AtSWEET cDNAs were cloned into a yeast expression vector pYES-DEST52 (Invitrogen), in which the GAL1 promoter had been replaced with the ADH1 promoter by LR reactions16. BD-GIId1a/AD-GA interactions were detected based on the expression of HIS3 or LacZ markers.

For direct assays in yeast, AtSWEET13, AtSWEET14 or AnNPF2.5 cDNA cloned in pYES-DEST52 was transformed into the yeast strain INVSc1 (Invitrogen). As a negative control, the empty pYES-DEST52 vector was transformed. AnNPF2.5 cDNA was amplified using primers 5′-CACCATGTTCTCCTCAAGGTCTCATGAAATGTTTG-3′ (forward) and 5′-TCACTCTATTGGGCTTAC-3′ (reverse) and cloned into pENTR/D-TOPO and then into pYES-DEST52. Underlined sequence denotes overhangs that were used to clone the sequence into pENTR/D-TOPO. Precultured yeast cells were cultured overnight in medium (SD: -Ura), and cells were collected when the OD600 reached 0.7 to 1.0. Media containing 2% (w/v) galactose and 1% (w/v) raffinose were used for the culture. Cells were washed with 50 mM potassium phosphate buffer (KPB; pH 5.8) and the OD600 was adjusted to 10 with 10 mM MES, pH 5.0) containing substrates. Substrate concentrations and incubation time are indicated in each figure legend. Oocytes were washed twice with 100 mM KCl, and then washed three times with 1 ml of 50 mM KPB (pH 5.8).

**Transport assays in Xenopus oocytes.** AtSWEET13 and AtSWEET14 cDNAs were amplified with following primers: 5′-TGGGATCCATTTGCTGATCTAGGTCTCATGAAATGTTTG-3′ (forward)/5′-TCTAGGATCCATTTGCTGATCTAGGTCTCATGAAATGTTTG-3′ (reverse) for AtSWEET13, and 5′-TCTAGGATCCATTTGCTGATCTAGGTCTCATGAAATGTTTG-3′ (forward)/5′-TCTAGGATCCATTTGCTGATCTAGGTCTCATGAAATGTTTG-3′ (reverse) for AtSWEET14. The amplification products were subcloned into the EcoRI and BamHI sites of the plasmid to synthesize cRNAs49. Capped cRNA of each gene was prepared using the mMESSAGE mMACHINE kit (Life Technologies). Each oocyte was injected with 500 ng cRNA. After incubation for 24 h, the buffer was replaced with 100 μl Kuliory-based solution (90 mM sodium gluconate, 1 mM potassium glutonate, 1 mM calcium chloride, 1 mM magnesium glutonate, 1 mM L-CaCl, and 10 mM MES, pH 5.0) containing substrates. Substrate concentrations and incubation time are indicated in each figure legend. Oocytes were washed twice with 200 mM sorbitol and homogenized in 40 μl extraction buffer (28% methanol, 0.05% acetic acid). After incubation at 4°C for 24 h, samples were centrifuged at 20,000g at room temperature for 20 min and supernatants were collected. Samples (10 μl) were subjected to ultra-performance liquid chromatography (UPLC) coupled to time-of-flight mass spectrometry (TOFMS)45. An Agilent 1290 Infinity (Agilent Technologies) equipped with a ZORBAX Eclipse Plus C18 column (1.8 mm, 2.1 x 50 mm; Agilent Technologies) and a micrOTOF II (Bruker Daltonics) were used for the analysis. The mobile phases used for UPLC were as.

**Figure 7** | Transport of exogenously applied GA from roots to shoots. The primary root tips of 8-day-old wild-type (WT) and sweet13 sweet14 (13 14) seedlings were spotted with 2μl of 10 μM GA3. After incubation for 0, 1, 3 and 6 h, the fragment ion with a m/z of 221.2 derived from the molecular ion with a m/z of 345.2 was detected from shoots by LC-MS/MS. Peak areas relative to those of wild type before the treatment (0 h) are shown as means of three biological replicates with standard deviations. *P < 0.05; NS, not significant (P > 0.05); Student’s t-test.
follows: A, 20% (v/v) aqueous methanol with 0.05% (v/v) acetic acid, and B, methanol with 0.05% (v/v) acetic acid. The gradient program was as follows: 0 to 3.5 min, isocratic 90% A, 3.5 min to 6 min, linear gradient 90 to 0% A; 6.1 min to 9 min, isocratic 90% A, with a flow rate of 0.15 ml min⁻¹. The TOFMS analysis was performed in the negative mode with scan range of 100–700 m/z. The capillary voltage was set at 4,200 V, the nebulizer gas pressure was set at 1.6 bar, the desolvation gas flow was set at 80 ml min⁻¹, and the temperature was set at 180 °C. GA levels were quantified based on extracted ion chromatograms and the corresponding peak position of the standard compound.

**Chemicals.** ABA, IAA and D₂-I AA were purchased from Sigma-Aldrich. GA₃ was purchased from Wako. JA, JA-Ile and 13C JA-Ile (ref. 50) were gifted from Dr. Yusuke Jikumaru (RIKEN). D₂-JA was purchased from Tokyo Kasei. D₆-ABA and D₆-iP were purchased from Icon Isotope. D₂-GAs, D₅-tZ, D₆-iP and D₃-dihydrozeatin were obtained from the Resource Center (ABRC). The mutant (SALK_010224) was selected by PCR using primers designed according to the NimBioS (Salk Institute Genomic Analysis Laboratory) website (http://signal.salk.edu/tdnaprimers.2.html). A homozygous ga20ox1 mutant (ga2ox1-3; SALK_016701C) was obtained from the Arabidopsis Biological Resource Center (ABRC). The ga2ox1 mutant (ga2ox1-3) was a gift from Dr. Shinjiro Yamaguchi (Tohoku University).

To generate pAsgTET13GUS and pAsgSWEET14·GUS lines, approximately 2 kb upstream regions from the start codon were amplified by PCR and cloned into pENTR/D-TOPO (Invitrogen). After the DNA sequences were confirmed, the promoter regions were cloned into pGWB3 (ref. 53) by LR reactions. Primer combinations used to amplify AtSWEET13 and AtSWEET14 promoter regions were as follows; 5'-ACACCACCTTTCTTTAACAAACAGGTTTGG-3' (forward)/5'-TTCTTTCTGCACAGTTTCC-3' (reverse), and 5'-CACCAAGTTTGGGTTATTTTGTGAAT-3' (reverse), respectively. Underlined sequences in the primers identify overhangs that were used for cloning into pENTR/D-TOPO. Each construct was amplified from genomic DNA of wild-type Col-0 using the following primers: 5'-ACCCCTTTCTTTACAAACAGGTTTGG-3' (forward)/5'-TTCTTTCTGCACAGTTTCC-3' (reverse), respectively, and were cloned into pENTR/D-TOPO. Underlined sequences in the primers identify overhangs that were used for cloning into pENTR/D-TOPO. After the DNA sequences were confirmed, the coding sequences were cloned into pGWB5 (ref. 53) by LR reactions. Each construct was introduced into Agrobacterium strain GV3101 and transformed into the Arabidopsis Col-0 accession by the floral-dip method. GFP fluorescence was observed by confocal laser scanning microscopy (LSM700, Carl Zeiss).

For complementation of sweet13 sweet14, the AtSWEET13 genomic sequence containing the coding region was amplified from wild-type Col-0 DNA using the following primers: 5'-ACCCCTTTCTTTACAAACAGGTTTGG-3' (forward)/5'-TTCTTTCTGCACAGTTTCC-3' (reverse), and 5'-CACCAAGTTTGGGTTATTTTGTGAAT-3' (reverse), respectively, and were cloned into pENTR/D-TOPO. Underlined sequences in the primers identify overhangs that were used for cloning into pENTR/D-TOPO. After the DNA sequences were confirmed, the coding sequences were cloned into pGWB5 (ref. 53) by LR reactions. Each construct was introduced into Agrobacterium strain GV3101 and transformed into Arabidopsis Col-0 accession by the floral-dip method.

**Plant growth conditions.** Arabidopsis seeds were surface-sterilized in a solution containing 5% (v/v) NaClO and 0.05% (v/v) Tween 20, rinsed with water, and sown on Murashige and Skog media (one-half strength Murashige and Skog salts, MES (0.5 g l⁻¹), pH 6.5) containing 0.8% (w/v) agar. After stratification at 4 °C in the dark for 3 days, plates were transferred to continuous light conditions at 22 °C. One-week-old seedlings were transferred to soil containing vermiculite and Metro-mix 350 (Sun Gro Horticulture) at a 3:1 ratio, supplied with nutrients (0.1,000 dilution of Hyperon; Hyperon Japan) and grown in growth chambers at 22 °C under continuous white light (approximately 10 W m⁻²).

To observe seedlings (Fig. 6), surface-sterilized seeds were germinated on 1.5% (w/v) agar plates containing Murashige and Skog medium. After stratification at 4 °C in the dark for 3 days, plates were incubated vertically under continuous light conditions at 22 °C.

For germination assays, surface-sterilized seeds were sown on Murashige and Skog media containing 0.8% (w/v) agar plates. ABA and paclobutrazol stock solutions (1,000 × concentration) prepared in DMSO were added to the cooled media after autoclaving. Plates containing 0.1% (v/v) DMSO were used for control experiments. After incubation for 1 day, plates were incubated under continuous light conditions at 22 °C. Germination was defined as cotyledon greening.

To observe the different anther dehiscence of sweet13 sweet14 by GA treatment, main inflorescence stems containing flower buds were excised (approximately 3–4 cm) and the bottom ends were placed in water containing 100 μM GA₃. After incubation for 1 day, anthers were observed with a stereomicroscope (Leica MZ FLIII).

To observe the movement of exogenously applied GA₃, roots to shoots, surface-sterilized seeds were germinated on 1.5% (w/v) agar plates containing Murashige and Skog medium. After stratification at 4 °C in the dark for 3 days, plates were incubated vertically under continuous light conditions at 22 °C for 8 days. Two μl of 10 μM GA₃ were spotted onto the main root tip. After incubation for 0, 1, 3 and 6 h, fragment ions corresponding to GA₃ were detected by LC-MS/MS as detailed below.

**Hormone analysis by LC-MS/MS.** To determine levels of GA in anthers and filaments, flowers at stage 13 (ref. 54) were dissected using a stereomicroscope. The GA levels in whole flowers at approximately stage 13 were also analysed. To measure the level of hormones in seedlings, 8-day-old plants grown on vertical plates were separated into shoots and roots. To collect developing seeds, siliques at 10 DAF were dissected using a stereomicroscope. All samples were frozen in liquid nitrogen and weighed after lyophilization.

The samples were ground and homogenized in extract solution (Supplementary Table 2) with defined amounts of deuterium-labelled internal standards. The mixtures were incubated for 12 h at 4 °C and then centrifuged at 3,000g for 20 min at 4 °C. The supernatants were dried in a vacuum and dissolved in 1 mL of water containing 1% (v/v) acetic acid. After several steps of purification on solid phase columns, extracts were dried in a vacuum and dissolved in 20 μL of water containing 1% (v/v) acetic acid. The purification steps are summarized in Supplementary Tables 2 and 3. The LC-MS/MS system consisting of a quadrupole/time-of-flight tandem mass spectrometer (Triple TOF 5600, AB SCIEX), and a Nexera HPLC system (SHIMADZU) were used in these analyses. LC separations were performed at a flow rate of 400 μl min⁻¹ using the conditions presented in Supplementary Table 4. MS/MS conditions are presented in Supplementary Table 5. We used a software tool (MultiQuant 2.0, AB SCIEX) to calculate plant hormone concentrations from the LC-MS/MS data.

**Data availability.** The authors declare that all other data supporting the findings of this study are available within the article and its Supplementary Information files or are available from the corresponding author upon request.

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Author contributions
Y. Kanno screened GA transporters. Y. Kanno and Y.C. cloned the AtSWEET genes and
constructed the vectors. Y. Kanno and T.S. conducted the yeast transport assays.
T.O. and Y.I. conducted the transport assays using oocytes. Y. Kanno, T.S. and N.S.
measured plant hormones. Y. Kanno, N.S. and M.S. characterized the mutant
phenotypes and observed the GUS and GFP expression patterns. Y. Kanno, T.O., Y.C.,
Y.I., T.S., T.K., Kamiya, M.U. and M.S. designed the experiment and discussed the
results. Y. Kanno, T.O., Y.C., Y.I., T.S., M. Kamiya, M.U. and M.S. wrote the manuscript.
M.S. planned the project. All authors discussed the results and commented on the
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