New Phytologist

SWEET11 and 15 as key players in seed filling in rice

Jungil Yang[1,2,3]*, Dangping Luo[4]*, Bing Yang[4], Wolf B. Frommer[1,2,3,5] and Joon-Seob Eom[1,2,3]

[1]Institute for Molecular Physiology, Heinrich-Heine University Duesseldorf, 40225 Duesseldorf, Germany; [2]Max Planck Institute for Plant Breeding Research, 50829 Cologne, Germany; [3]Department of Plant Biology, Carnegie Institution for Science, Stanford, CA 94305, USA; [4]Department of Genetics, Development, and Cell Biology, Iowa State University, Ames, IA 50011, USA; [5]Institute of Transformative Bio-Molecules (ITbM), Nagoya University, Furo-cho, Chikusa-ku, Nagoya, Aichi 464-8602, Japan

Authors for correspondence:
Joon-Seob Eom
Tel: +49 2215062385
Email: eom@mpipz.mpg.de

Wolf B. Frommer
Tel: +49 2118114826
Email: frommew@hhu.de

Received: *1 October 2017*
Accepted: *15 December 2017*

New Phytologist (2018) **218:** 604–615
doi: 10.1111/nph.15004

Key words: apoplasmic pathway, caryopsis, endosperm, rice, sucrose transporter, SWEET.

Summary

- Despite the relevance of seed-filling mechanisms for crop yield, we still have only a rudimentary understanding of the transport processes that supply the caryopsis with sugars. We hypothesized that SWEET sucrose transporters may play important roles in nutrient import pathways in the rice caryopsis.
- We used a combination of mRNA quantification, histochemical analyses, translational promoter–reporter fusions and analysis of knockout mutants created by genomic editing to evaluate the contribution of SWEET transporters to seed filling.
- In rice caryopses, *SWEET11* and *15* had the highest mRNA levels and proteins localized to four key sites: all regions of the nucellus at early stages; the nucellar projection close to the dorsal vein; the nucellar epidermis that surrounds the endosperm; and the aleurone. *ossweet11;15* double knockout lines accumulated starch in the pericarp, whereas caryopses did not contain a functional endosperm.
- Jointly, SWEET11 and 15 show all the hallmarks of being necessary for seed filling with sucrose efflux functions at the nucellar projection and a role in transfer across the nucellar epidermis/aleurone interface, delineating two major steps for apoplasmic seed filling, observations that are discussed in relation to observations made in rice and barley regarding the relative prevalence of these two potential import routes.

Introduction

Population growth is expected to lead to an increasing need for rice production, especially in Africa (Sharma, 2014). A key question is thus how to optimize yield potential. Rice grains are composed mainly of starch (over 90% in many cases; www.knowledgebank.irri.org/ricebreedingcourse/Grain_quality.htm), which derives from imported soluble carbohydrates. These carbohydrates are produced and delivered from leaves (source tissues) to caryopses (sink tissues) via the phloem (Patrick, 1997; Patrick & Offler, 2001). With a concentration of *c.* 600 mM, sucrose is the major sugar translocated in rice phloem sap (Fukumorita & Chino, 1982). To fill the seeds, massive amounts of sucrose have to be imported into developing caryopses. Sucrose is unloaded from the phloem strands that enter the seed coat, from where sugars are transferred into the developing caryopsis to supply cells with nutrients, in particular sugars as sources of energy and as carbon skeletons for cell wall and starch biosynthesis (Oparka & Gates, 1984; Patrick & Offler, 2001; Zhang *et al.*, 2007).

In plants, cell-to-cell transport of sugars is thought to be mediated by apoplasmic (export from one cell by a plasma membrane transporter and subsequent import into the adjacent cell by another transport protein) or by symplasmic transfer via plasmodesmata. Other routes are conceivable, but evidence for vesicular transport processes is sparse (van den Broek *et al.*, 1997). Two of the key processes for long-distance translocation are phloem loading and seed filling. In maize, SUT1 and SWEET13a,b,c are essential for efficient phloem loading (Slewinski *et al.*, 2009; Bezrutczyk *et al.*, 2018). The transport processes that ultimately lead to cell wall synthesis and storage product accumulation in seeds, in particular in cereal caryopses are not fully understood. To delineate sym- and apoplasmic pathways in rice caryopses, Oparka carried out a combination of ultrastructural and dye tracer studies (Oparka & Gates, 1981a,b, 1982, 1984). The rice caryopsis is supplied by three vascular bundles that pass through the pericarp. The dorsal vascular bundle is the major route for sugar delivery to the developing caryopsis (Oparka & Gates, 1981a; Krishnan & Dayanandan, 2003). Symplasmic connections were found from the parenchyma of the dorsal vascular bundle throughout the nucellar projection and the nucellar epidermis. By contrast, the apoplasmic connection between the nucellar epidermis and pericarp (inner integument) is blocked by a lipid barrier. Furthermore, nucellar epidermis and aleurone are symplasmically isolated, thus requiring transporters at the maternal/filial boundary for release from maternal tissues and subsequent uptake into filial tissues. Comprehensive analyses that included radiotracer

*These authors contributed equally to this work.

analyses led Oparka to propose that sucrose, after unloading from the phloem, diffuses along the nucellar epidermis from where a yet-unknown set of sugar transporters transfers the sugars into the developing endosperm (Oparka & Gates, 1981b). Candidates for such transporters include two classes of plasma membrane sucrose transporters (SUTs and clade 3 SWEETs) (Aoki *et al.*, 2003; Chen *et al.*, 2012) and three classes of plasma membrane hexose transporters (MSTs (STPs), ERDs, and clade 1 and 2 SWEETs) (Toyofuku *et al.*, 2000; Johnson & Thomas, 2007; Chen *et al.*, 2015a).

In legume seeds, during early developmental stages, cwINVs produce hexoses from the incoming sucrose. These hexoses are thought to stimulate mitotic activity to increase cell number, whereas subsequently at later stages the cwINVs are switched off and sucrose transporters are induced. Sucrose is thought to then act as a differentiation signal that triggers storage product accumulation (Weber *et al.*, 2005). Specialized sucrose facilitators (SUFs, members of the SUT family), PsSUF1, PsSUF4 and PvSUF1 contribute to sucrose efflux from seed coats of pea and common bean (Zhou *et al.*, 2007). In monocots, specifically in maize and rice, cwINVs also play important roles in seed filling (Cheng & Chourey, 1999; Wang *et al.*, 2008a). Because sucrose, upon arrival in the caryopsis through the phloem of the dorsal vascular bundle, is partially hydrolyzed by cell wall invertases (cwINVs; in particular OsGIF1/OsCIN2) into glucose and fructose, one would predict the adjacent expression of hexose transporters (Wang *et al.*, 2008a). Several hexose transporters possibly involved in import of cwINV-derived hexoses into the caryopsis or endosperm have been identified. Rice *MST4* and *MST6* are expressed in maternal tissues including the dorsal vascular bundle, nucellus including nucellar projection and nucellar epidermis, and the aleurone layer of the filial endosperm (Wang *et al.*, 2007, 2008b). However, sucrose transporters of the SUT family also have been identified as important for seed filling in rice (Hirose *et al.*, 1997, 2002, 2010; Furbank *et al.*, 2001; Aoki *et al.*, 2003; Scofield *et al.*, 2007a,b; Eom *et al.*, 2012; Reinders *et al.*, 2012). *OsSUT1* was found to be expressed in the aleurone of developing caryopses (Furbank *et al.*, 2001; Hirose *et al.*, 2002). OsNF-YB1, an aleurone-specific transcription factor, directly regulates *OsSUT1*, *OsSUT3* and *OsSUT4* (Bai *et al.*, 2016). Notably, antisense inhibition of *OsSUT1* caused seed-filling defects (Scofield *et al.*, 2002). SWEETs are a class of seven transmembrane hexose and sucrose uniporters that function as oligomers (Chen *et al.*, 2010; Xuan *et al.*, 2013). It has been proposed that AtSWEET13 functions as a 'revolving door' mechanism to accelerate the transport efficacy (Feng & Frommer, 2015; Han *et al.*, 2017; Latorraca *et al.*, 2017). Roles of SWEETs include phloem loading and nectar secretion, and they have been shown to act as susceptibility factors for pathogen infections (Chen *et al.*, 2010, 2012; Lin *et al.*, 2014). *ZmSWEET4c* in maize is expressed in the basal endosperm transfer layer (BETL) and necessary for seed filling and BETL differentiation. The apparent orthologue *OsSWEET4* also appears to have a role in grain filling, although its detailed cell specificity has yet to be determined (Sosso *et al.*, 2015). Because we also found that several sucrose transporting SWEETs contribute to seed filling in Arabidopsis (Chen *et al.*, 2015b), we speculated that one or several rice orthologues may play analogous roles in supplying sucrose to either the rice SUTs or cwINVs.

Here, we show that similar to the situation in legumes, the hexose transporter *OsSWEET4* is predominantly expressed during early stages of caryopsis development, whereas OsSWEET11 and 15 mRNA levels gradually increased from early stages and accumulated at higher levels during later developmental stages. Expression was found in the ovular vascular trace, nuclear epidermis and endosperm. We show that *ossweet11* single and *ossweet11;15* double mutants show defects in endosperm development and filling. Our findings are supported by work performed in parallel, which also identified OsSWEET11 as critical for seed filling (Ma *et al.*, 2017). We found that the phenotype of *ossweet11;15* double mutants was more severe in having an empty seed phenotype, which was accompanied by accumulation of more starch in the pericarp. Together, these results indicate that OsSWEET11 and OsSWEET15 are necessary for sugar efflux from the maternal nuclear epidermis as well as efflux from the ovular vascular trace to the apoplasm and also may contribute to sucrose influx into the aleurone.

Materials and Methods

Plant materials and growth conditions

The *Oryza sativa* ssp. *japonica* cultivar Kitaake was used for CRISPR-Cas9 and TALEN-mediated mutagenesis of *OsSWEET11* and *OsSWEET15* genes. Methods for CRISPR-Cas9 induced mutant line (*ossweet11-1*) were described previously (Zhou *et al.*, 2014). Briefly, guide RNA genes targeting the start codon (5′-TCACCAGTAGCAATGGCAGG-3′) of *OsSWEET11* were coexpressed with Cas9 (see Supporting Information Fig. S1). The methods for TALEN-induced genome editing (*ossweet11-2*, *ossweet15-1* and *ossweet15-2*) have been described also (Li *et al.*, 2014). Pairs of TALENs for *OsSWEET11* and *OsSWEET15* were used for transformation of *O. sativa* japonica cv Kitaake (Fig. S1) (Li *et al.*, 2012). Double mutants (*ossweet11-1; 15-1 and ossweet11-2; 15-2*) were created by crossing. Wild-type (WT) plants and mutants were grown either in field conditions (paddy field summer 2016, Carnegie, Stanford, CA, USA) or in glasshouses (Carnegie and ISU) under long-day conditions (14 h : 10 h, day : night, 28–30°C).

Genotyping of rice plants

Rice genomic DNA was extracted using CTAB (http://gsl.irri.org/services/dna-extraction-king-fisher/met). PCR was performed using ExTaq DNA polymerase (Clontech, Mountain View, CA, USA) with a melting temperature of 56°C and 53°C for *OsSWEET11* and *OsSWEET15,* respectively (for primers, see Table S1). The PCR-amplicons from the mutant alleles were validated by DNA sequencing. Chromatograms were analysed and aligned using BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html).

RNA isolation and transcript analyses

Total RNA was isolated using Spectrum™ Plant total RNA kits (Sigma) or the Trizol method (Invitrogen), and first strand cDNA was synthesized using the Quantitect reverse transcription kit (Qiagen). qRT-PCR was performed using a LightCycler 480 (Roche), with the $2^{-\Delta Ct}$ method for relative quantification (Livak & Schmittgen, 2001). Primers for *OsSWEET11, OsSWEET15* and *OsUBI1* (Takahashi *et al.*, 2005) are listed in Table S1.

Generation of *OsSWEET11* and *OsSWEET15* reporter constructs

The 2334-bp GUSplus (GUS, β-glucuronidase) coding sequence fused to the nopaline synthase terminator was amplified by PCR from pC1305.1 (Cambia, Canberra and Brisbane, Australia). The amplified fragment was subcloned into the pJET2.1/blunt vector (Thermo Fisher, Waltham, MA, USA) and sequencing was performed for validation. After *Sac*I-*EcoR*I double restriction the resulting fragment was transferred into the plant transformation vector pC1300intC to generate a promoterless GUSplus vector. For tissue specificity analysis, a 4354-bp genomic fragment containing 2106 bp of the 5′ upstream region and 2248 bp comprising the entire coding region of *OsSWEET11,* and a 4193-bp genomic fragment containing 2069 bp of the 5′ upstream region and 2124 bp comprising the entire coding region of *OsSWEET15* (without stop codon) was amplified by PCR using Kitaake genomic DNA as a template, respectively (primers: Table S1). The amplified product was subcloned into a pJET2.1/blunt vector and confirmed by sequencing. The cloned fragment digested with *Hind*III and *BamH*I for *OsSWEET11,* or *Xba*I and *Kpn*I for *OsSWEET15,* was inserted in front of the GUSplus coding sequence (previously restricted with *Hind*III/*BamH*I and *Xba*I/*Kpn*I, respectively). The resulting *pOsSWEET11*:gOsSWEET11-GUSplus and *pOsSWEET15*:gOsSWEET15-GUSplus constructs were used to transform *O. sativa* japonica cv Kitaake. Eighteen and 13 independent lines were obtained for *pOsSWEET11*:gOsSWEET11-GUSplus and *pOsSWEET15*:gOsSWEET15-GUSplus, respectively, with similar expression patterns.

Histochemical GUS analyses

Immature seeds at 5 or 9 d after pollination (DAP) from *pOsSWEET11*:gOsSWEET11-GUS-8 and *pOsSWEET15*:gOsSWEET15-GUS-14 transformants were collected and analysed for cell-type specific expression patterns in developing caryopses. Samples were collected in 90% cold acetone for fixation, vacuum infiltrated for 10 min and incubated for 30 min at room temperature. Seeds were vacuum infiltrated in GUS staining buffer (staining solution w/o 5-bromo-4-chloro-3-indole-beta-glucuronide (X-Gluc)) on ice for 10 min. The solution was changed with GUS staining solution (50 mM sodium phosphate pH 7.0, 10 mM EDTA, 20% (v/v) methanol, 0.1% (v/v) Triton X-100, 1 mM potassium ferrocyanide, 1 mM potassium ferricyanide, 2 mM 5-bromo-4-chloro-3-indole-beta-glucuronide (X-Glc) dissolved in dimethyl sulfoxide). Samples were incubated at 37°C. After 20 min staining, samples were cleared in an ethanol series (20%, 35%, 50%) at room temperature for 30 min each. For paraffin sections, seeds were fixed using FAA for 30 min (50-% (v/v) ethanol, 3.7% (v/v) formaldehyde, 5% (v/v) acetic acid). Dehydration was performed with an ethanol series (70%, 80%, 90%, 100%, 30 min each) and 100% tert-butanol. Samples were transferred and embedded in Histosec pastilles (Millipore). Sections (8 µm) were obtained with a rotary microtome (Jung RM 2025, Wetzlar, Germany). Specimens were observed with a Nikon eclipse e600 microscope. Images for GUS histochemistry in Fig. 1 were enhanced uniformly in PHOTOSHOP by adjusting brightness (+6), contrast (+12), and blue colour balance (+4).

Plastic embedding and sectioning

Wild-type, *ossweet11-1* and *ossweet11-1;15-1* seeds were fixed using 4% PFA (1× PBS buffer (37 mM NaCl, 10 mM Na_2HPO_4, 2.7 mM KCl, 1.8 mM KH_2PO_4, pH 7.4) with 4% paraformaldehyde), vacuum infiltrated for 15 min and incubated overnight. Dehydration of samples were performed in a concentration series of ethanol (10%, 30%, 50%, 75%, 95%). Plastic embedding was performed according to the LR White embedding kit protocol (Electron Microscopy Science). Cross-sections (1 µm) were performed using an Ultracut (Reichert-Jung, Wetzlar, Germany), counterstained with 0.1% Safranin O for 30 s and washed twice with distilled water, followed by 3 min staining with Lugol's staining solution to visualize starch. Specimens were observed under a Nikon eclipse e600 microscope.

FRET sucrose sensor analysis in HEK293T cells

For functional analyses in HEK293T cells, *OsSWEET11* and *OsSWEET15* coding sequences were cloned into the Gateway

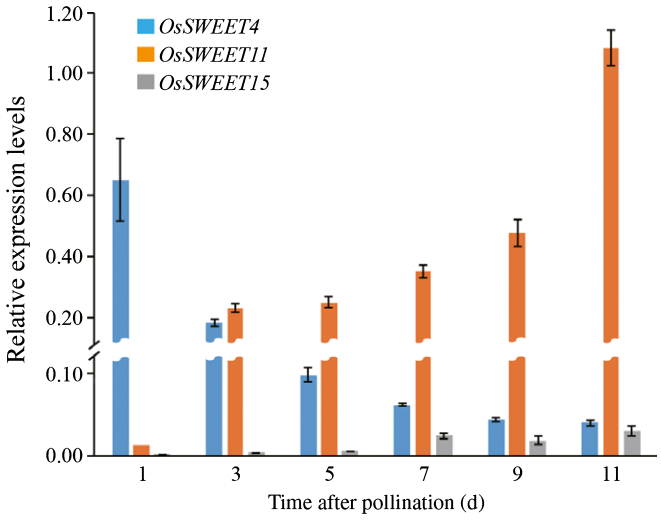

Fig. 1 Relative expression of *OsSWEET4*, *OsSWEET11* and *OsSWEET15* during rice seed development after pollination. Expression was measured by quantitative reverse transcription polymerase chain reaction (qRT-PCR) in wild-type glasshouse-grown seeds. Data shown as mean ± SEM, $n = 3$; expression levels were normalized to rice *Ubiquitin1* levels.

entry vector pDONR221f1, then transferred into pcDNA3.2V5 by LR recombination reactions. HEK293T cells were co-transfected with a plasmid carrying the *OsSWEET11* or *OsSWEET15* and the sucrose sensor FLIPsuc90μ-sCsA, using Lipofectamine 2000 (Invitrogen). For FRET imaging, HBSS medium with and without sucrose was used to perfuse FLIPsuc90μ-sCsA-expressing HEK293T cells. Image acquisition and analysis were performed as described previously (Chen *et al.*, 2012).

Results

Identification of OsSWEET11 and OsSWEET15 in rice caryopses

The elongation of caryopses occurs in the first 6 DAP, whereas width and thickness expand mainly between 4 and 12 DAP (Xu *et al.*, 2008; Wu *et al.*, 2016b). To identify SWEETs expressed specifically in rice caryopses, we analysed public microarray data from RiceXPro (ricexpro.dna.affrc.go.jp). Among the five clade 3 SWEETs analysed, *OsSWEET11* and *15* had the highest mRNA levels in the endosperm between 7 and 14 DAP (Fig S2). To validate the microarray data, we harvested immature seeds at different developmental stages from glasshouse-grown plants and re-analysed mRNA levels of *OsSWEET11* and *15* by quantitative reverse transcription polymerase chain reaction (qRT-PCR). For comparison, we used *OsSWEET4*, which had been shown to play a role as a hexose transporter in seed development (Sosso *et al.*, 2015; Fig. 1). At 1 DAP, *OsSWEET4* mRNA levels were at a maximum, and levels declined *c.* three-fold at 3 DAP. In comparison, *OsSWEET11* was low at 1 DAP, but equal to *OsSWEET4* at 3 DAP. *OsSWEET11* gradually increased throughout development. Although the microarray showed that *OsSWEET15* was only 2–3× lower compared to *OsSWEET11*, our analysis indicated a much lower relative level. Nevertheless, the developmental pattern of *OsSWEET15* mRNA levels was similar to that of *OsSWEET11*. Although the *c.* two-fold increase in *OsSWEET11* mRNA levels between 9 and 11 DAP coincides with the onset of rapid biomass accumulation, substantial mRNA levels were also detected at both earlier and later stages, possibly implicating OsSWEET11 and 15 in both rapid size expansion of the caryopses and in processes before and after.

OsSWEET11 plays a key role in seed filling

OsSWEET11 had previously been shown to function as a plasma membrane sucrose transporter (Chen *et al.*, 2012). Because OsSWEET11 was by far the most highly expressed clade 3 SWEET gene in the caryopsis, we hypothesized that knockout mutants might be affected in seed filling. Two independent *ossweet11* mutants, one carrying a single base pair deletion that led to a frameshift was created by CRISPR-Cas9, together with a second TALEN-derived mutant carrying a 489-bp deletion were characterized phenotypically (Fig. S1). Under glasshouse conditions, both mutants had incompletely filled seeds at the time the WT had reached maturity (Fig. 2). At 40 DAP, WT panicles were fully mature with brown spikelets, whereas *ossweet11* mutants had immature panicles that still contained chlorophyll (Fig. 2c,d). Full maturity of the *ossweet11* mutants, when there was complete loss of chlorophyll, was reached only much later (> 60 DAP). Even after the extended maturation period, mutants showed significantly reduced yield (both percentage of mature seeds after harvest and 1000-grain weight; Fig. S3a,b). Depending on the growth conditions, the phenotype was more or less severe (Figs 2, S3); defects became more severe in paddy field conditions (single field experiment in 2016; similar also as in the parallel study with multiple field trials; Ma *et al.*, 2017). Of note, however, plant height, spikelet number and panicle length appeared unaffected in both glasshouse and paddy field conditions (Figs 2c, S3c,d).

OsSWEET11 in the nucellar projection, nucellar epidermis and aleurone

Oparka had predicted symplasmic diffusion of sugar in the pericarp and apoplasmic transport at the nucellar epidermis–aleurone interface all around the endosperm. Notably, this pattern is different from that found in developing barley seeds, where the main import route was through the nucellar projection (Oparka & Gates, 1984; Melkus *et al.*, 2011). To determine whether OsSWEET11 exports sucrose at the nucellar projection or the nucellar epidermis/aleurone interface, we analysed transgenic rice plants expressing translational GUS fusions containing a 2-kb promoter fragment and the whole coding region including all introns. Crude histochemical GUS analysis of caryopses showed comparable GUS staining in seeds in eight of 18 independent transformants. Two independent lines were used for a more detailed analysis. In early stages (up to 3 DAP), we observde GUS activity in maternal tissues including the ovular vascular trace and the nucellus, possibly indicating a role in remobilization of carbohydrates during nucellar degradation (Fig. S4). At 5 DAP, GUS activity was detected in the ovular vascular trace, the nucellar projection, the nucellar epidermis surrounding the developing endosperm, the remaining nucellar layers and also in the aleurone layer of the endosperm (Fig. 3a–c). To our surprise, we found OsSWEET11 expression in nucellar projection as well as the nucellar epidermis, and in addition also in the outermost endosperm cell layer, the aleurone, providing a potential path for sucrose export out of the nucellar projection into the endosperm, and a parallel pathway for export from the circumferential nucellar epidermis and then subsequently a potential import via OsSWEET11 into the aleurone. A parallel study obtained similar expression patterns using a transcriptional GUS fusion (Ma *et al.*, 2017).

Potential compensation for *ossweet11* deficiency by other SWEETs

The relatively weak phenotype of the *ossweet11* mutants may either indicate the existence of alternative genes and pathways, or indicate compensation. Analysis of the expression levels of clade 3 SWEET genes in the *ossweet11* mutant showed that

Fig. 2 Phenotypes of wild-type (WT), *ossweet11* and *ossweet15* rice mutants. (a) Mature caryopses of WT and *ossweet11* mutants (Stanford (CA, USA) glasshouse). (b) Mature caryopses of WT and *ossweet15* mutants (Stanford glasshouse). (c) Mature *ossweet11-2* and WT plant grown in a paddy field and transferred to pots for photography (Stanford field, 40 d after pollination (DAP)). (d) Panicle and grains for WT and *ossweet11-2* mutants in paddy conditions (Carnegie field, 40 DAP). Scale bars: (a, b) 1 mm; (c) 10 cm; (d) 1 cm (upper panel), 1 mm (lower panel).

OsSWEET13 mRNA levels were slightly increased, but that the absolute levels were extremely low. The mRNA levels of *OsSWEET15* were *c.* two-fold higher in *ossweet11* seeds compared to the WT (Fig. 4). Because, depending on the experiment, *OsSWEET15* was expressed at only slightly lower levels compared to *OsSWEET11* and was furthermore candidate that may contribute to compensation in the mutant, we first tested whether it functions as a sucrose transporter, determined its expression pattern in developing caryopses and then analysed knockout mutants. As one may have predicted, OsSWEET15 also functioned as a sucrose transporter when co-expressed with a sucrose sensor in HEK293T cells (Fig. S5). Translational GUS fusions of the OsSWEET15 gene driven by their native promoter showed similar tissue specificity as compared to OsSWEET11 (13 independent lines): GUS activity was detected at early stages in all regions of the nucellus, and later in the ovular vascular trace, the nucellar projection and the aleurone (Fig. 3d,e). At 9 DAP, OsSWEET15 GUS activity was also detected in the nucellar epidermis (Fig. 3f). The two SWEET transporters exhibit similar expression patterns in developing seeds, especially the ovular vascular and the interface between the nucellar epidermis and the aleurone, intimating redundant roles during seed development. However, on their own, two independent *ossweet15* knockout mutants generated via CRISPR-Cas9 (frameshift mutations that prevent production of a functional OsSWEET15 protein; Fig. S1) did not show any detectable phenotypic differences compared to WT in four independent experiments (Fig. 2b).

OsSWEET11 and 15 are essential for seed filling

Because the seed filling of *ossweet11* mutants was only partially affected relative to *ossweet4* mutants (Sosso *et al.*, 2015), and OsSWEET15 appeared to be expressed in the same cell types to substantial levels, and even possibly compensates in part for

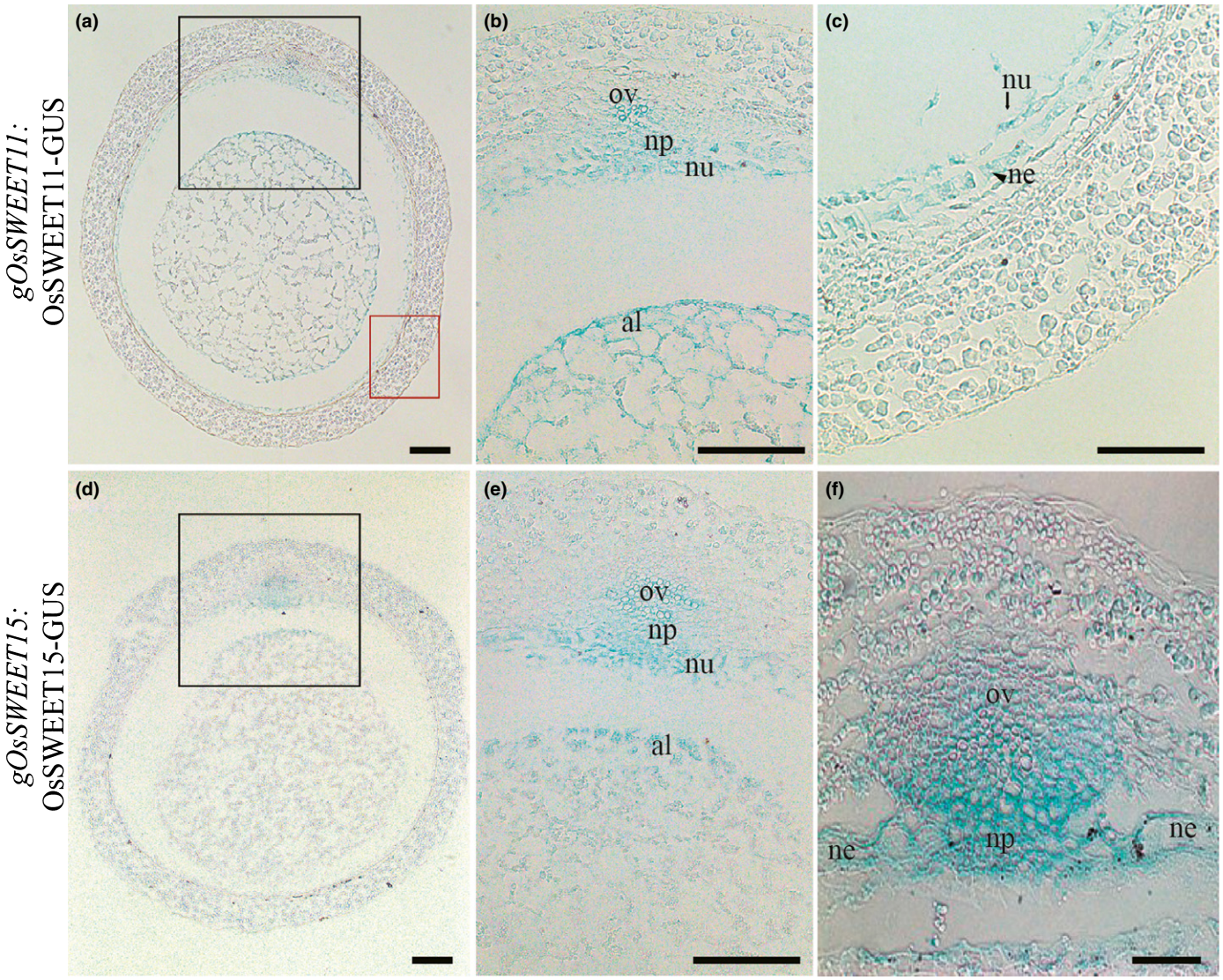

Fig. 3 Tissue-specific protein concentrations of OsSWEET11 and OsSWEET15 in rice grains. (a) Transverse section of *gOsSWEET11:*gOsSWEET11-GUS (GUS, β-glucuronidase) grain at 5 d after pollination (DAP). (b) Black boxed area in (a), showing GUS activity in the ovular vascular trace (ov), nucellus (nu), nucellar projection (np) and aleurone (al). (c) Red boxed area in (a), showing activity in the nu (black arrow) and ne, nucellar epidermis (black arrowhead). (d) OsSWEET15 GUS activity in grains at 5 DAP. (e) Black boxed area in (d), GUS activities detected in ov, np, nu and al. (f) OsSWEET15 GUS activity in developing grains was detected to the ov, np and ne at 9 DAP. Bars, 50 μm.

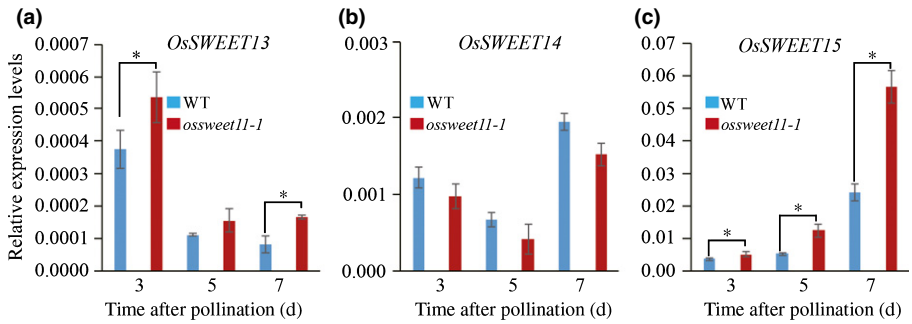

Fig. 4 Transcript levels of clade 3 SWEET genes in *ossweet11-1* rice mutants. Relative mRNA levels of (a) *OsSWEET13*, (b) *OsSWEET14* and (c) *OsSWEET15* determined by quantitative RT-PCR in wild-type (WT) and *ossweet11*. *OsSWEET13* and *OsSWEET15* levels were increased in the *ossweet11-1* mutant compared to WT (*, $P < 0.05$). Data shown as mean ± SEM, $n = 3$; expression levels were normalized to rice *Ubiquitin1* levels. *OsSWEET12* transcripts were not detected.

OsSWEET11 deficiency in the mutant, we generated *ossweet11;15* double mutants for both alleles of the two loci. In glasshouse conditions both at ISU and Stanford, the double mutant phenotype was very severe, much more than in the single *ossweet11* mutants (Fig. 5). The differences were even more severe in the ISU glasshouses, where *ossweet11* showed only a minor phenotype, whereas the caryopses of the double mutant were severely affected (Fig. S6). A detailed time series showed that phenotypic differences became apparent at *c.* 5 DAP (Fig. 5a). Differences became bigger at 7 DAP, a time point at which *ossweet11* mutants had already started to develop a wrinkled grain morphology, whereas *ossweet11;15* was characterized by grains that were flattened with a smaller diameter (Fig. 5a,b). Sections through the grain showed that the mutants were endosperm-deficient and either had only remnants of the endosperm or had lost the endosperm completely (Fig. 5c). Histological analyses of resin-embedded sections showed that the endosperm developed at *c.* 3 DAP in both WT and *ossweet11*, whereas a functional endosperm did not form in the *ossweet11;15* mutant (Fig. S7). The cellularization of the endosperm was completed and nucellar

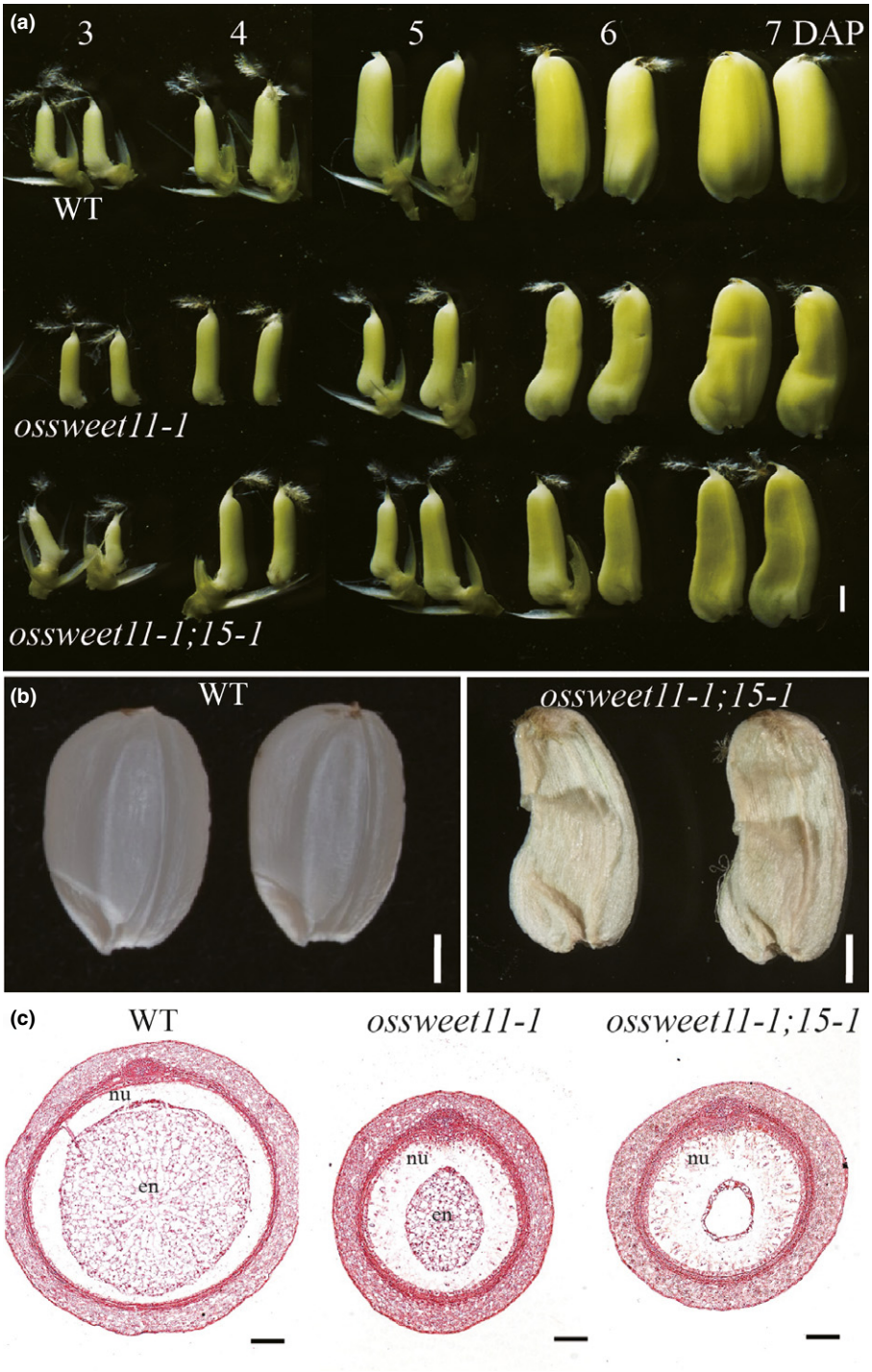

Fig. 5 Endosperm deficiency in the *ossweet11;15* double mutant. (a) Morphological changes wild-type (WT), *ossweet11-1* and *ossweet11-1;15-1* rice grains from 3 to 7 d after pollination (DAP) (Carnegie (Stanford, CA, USA) glasshouse). (b) WT and *ossweet11-1;15-1* double mutant seeds at maturity (Carnegie glasshouse). (c) Transverse sections of WT, *ossweet11-1* and *ossweet11-1;15-1* seeds at 5 DAP stained with Safranin O. nu, nucellus; en, endosperm. Bars: (a, b) 1 mm; (c) 50 μm.

cells (adjacent to the nucellar epidermis) had degenerated in the WT *c.* 5–7 DAP (Fig. S7a) (Wu *et al.*, 2016a). In the *ossweet11* mutant, the endosperm was defective and degeneration of the nucellar cells was delayed by *c.* 2–3 d (Fig. S7b). OsSWEET11 and 15 are both essential for seed filling as evidenced by a poorly developed endosperm and a defect in nucellar degeneration (Fig. S7c).

Starch accumulation in the pericarp of *ossweet11;15* double mutants

Based on the localization of OsSWEET11 and 15, we predicted that inhibition of the transporters would lead to starch accumulation in cells that export sucrose and cells peripheral to the endosperm. In the WT, starch is stored transiently in the pericarp until 7–9 DAP. Starch degradation in the pericarp correlated with starch accumulation in the endosperm starting *c.* 7 DAP (Wu *et al.*, 2016b). As expected, we also found starch in the endosperm, as well as residual amounts in the pericarp of WT caryopses (Fig. 6a,d). By contrast, starch accumulated to high levels in the pericarp of the *ossweet11* mutant (Fig. 6b,e). In *ossweet11;15*, starch accumulated to even higher concentrations in the pericarp, whereas the endosperm did not show substantial starch grains (Fig. 6c,f). The accumulation of starch in the pericarp of *ossweet11* and *ossweet11;15* double mutants supports the critical roles of OsSWEET11 and OsSWEET15 in sucrose translocation and mobilization towards the developing endosperm.

Discussion

We draw five key conclusions from the results of a combination of analyses comprising gene expression, translational reporters to map tissue-specific protein accumulation, and knockout mutants

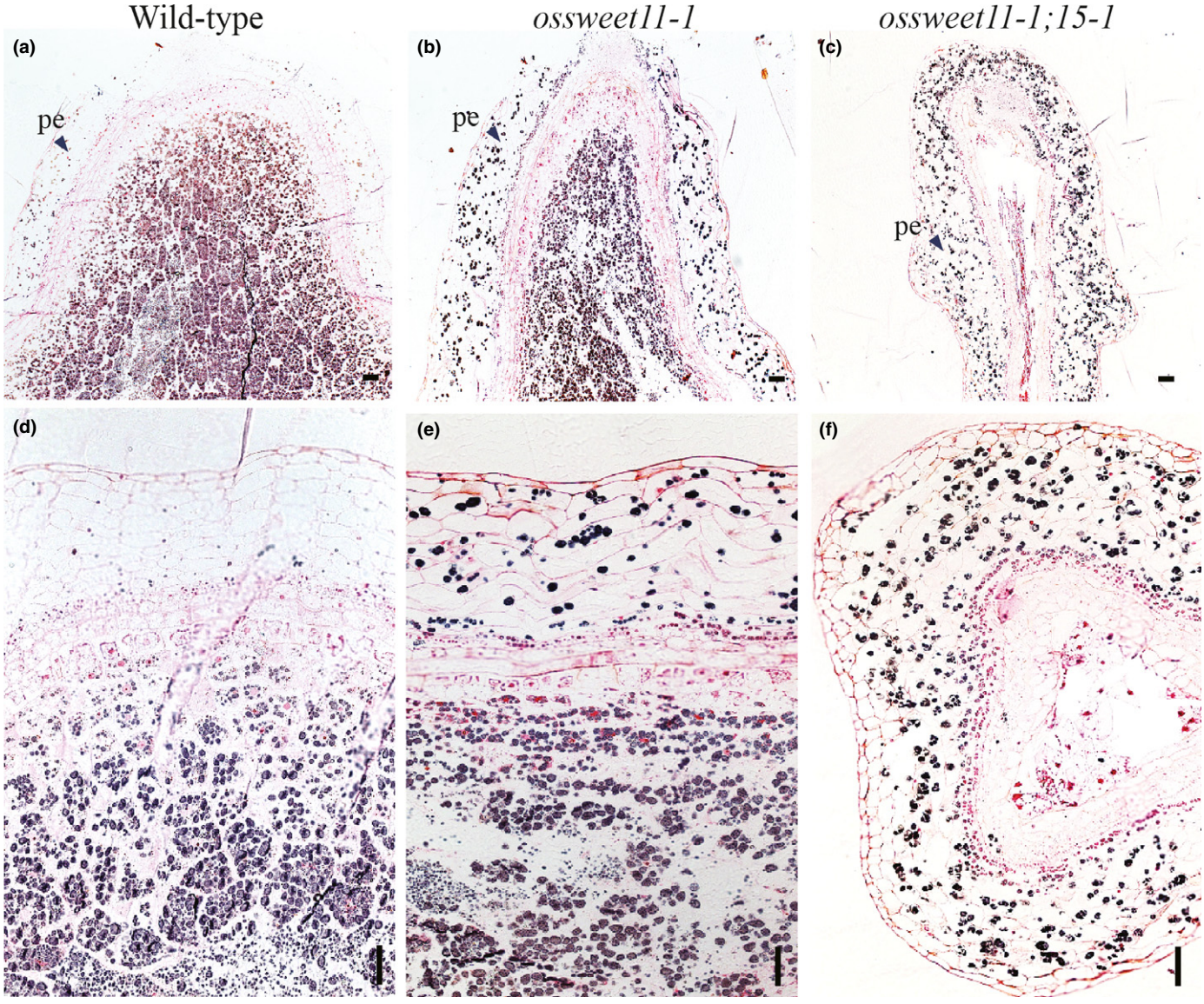

Fig. 6 Accumulation of starch in the pericarp of *ossweet11-1* and *ossweet11-1;15-1* rice mutants relative to the wild-type. (a–c) Cross sections of caryopses stained with Lugol's iodine solution at 9 d after pollination (DAP). pe, pericarp. (d–f) Magnified images of (a–c). Bars 50 μm.

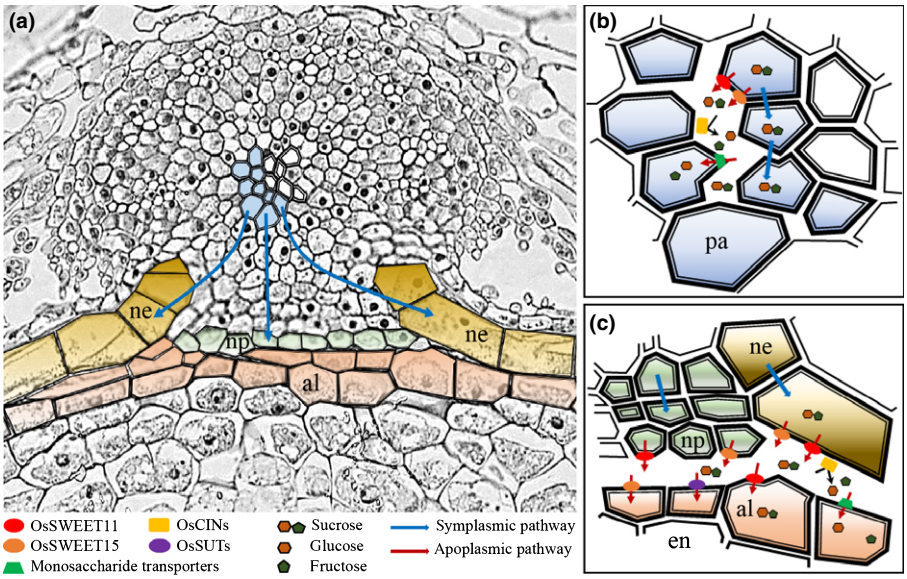

Fig. 7 Proposed model for sugar unloading in rice caryopses. Possible apoplasmic transport routes as indicated by SWEET sucrose transporter localization. The model is made based on observations from this and previous studies (Oparka & Gates, 1981a,b; Wu *et al.*, 2016a). Sucrose may move from the phloem to parenchyma cells in the ovular vascular bundle and then to the nucellar projection and nucellar epidermis through symplasmic pathways via plasmodesmata (a). We surmise that OsSWEET11 and OsSWEET15 mediate sucrose export from vascular parenchyma into the apoplasmic space (b). In addition, OsSWEET11 and OsSWEEET15 may be involved in sucrose export from cells belonging to the nucellar projection and the nucellar epidermis to the apoplasm, followed by import into the aleurone (endosperm) (c). SWEET-mediated transfer of sucrose across the nucellar epidermis/aleurone interface would require a sucrose gradient across both cell types. al, aleurone; en, endosperm; ne, nucellar epidermis; np, nucellar projection; pa, parenchyma.

that were generated by genome editing: (1) OsSWEET11 and 15 are the most highly expressed sucrose transporting SWEETs (both belong to clade 3) in the rice caryopsis; (2) if we assume that they mark apoplasmic import routes, sucrose can enter both directly below the vein via the nucellar projection as well as via the circumferential nucellar epidermis; (3) SWEET11 and 15 may not only play roles in cellular efflux at these two sites, but may also be responsible for importing sucrose into the aleurone; (4) OsSWEET11 and 15 both contribute to seed filling with seemingly redundant roles. Because the single *ossweet15* mutants had no apparent phenotypic differences to wild-type under the tested conditions, OsSWEET11 appears to function as the dominant transporter, consistent with having higher relative levels of mRNA. (5) Because *OsSWEET4* is predominantly expressed at early stages of development, it may cooperate with cell wall invertase cwINV2 (OsCIN2, GIF1) in hexose import into cells surrounding the dorsal vein, whereas OsSWEET11/15 may play more important roles at later stages. The findings made here for OsSWEET11 in *Oryza sativa* cv Kitaake are similar to those from a parallel study that used *O. sativa* cv Nipponbare (Ma *et al.*, 2017). Notably, the phenotype of the *ossweet11* mutant was more severe in field vs glasshouse conditions.

Pathways for seed filling

The tissue-specific expression of OsSWEET11 and 15 in parenchymatic cells of the vascular bundle, the nucellar projection, the nucellar epidermis and the aleurone indicates specific roles of OsSWEET11 and 15 in sucrose translocation into developing caryopses. Here we propose a possible model for sucrose translocation from the vascular bundle to the endosperm (Fig. 7). We propose four sites where sucrose transport occurs: (1) vascular parenchyma cells in the vascular bundle: SWEET effluxers may be required to supply OsCIN2 with sucrose (Wang *et al.*, 2008a). Of note, the *oscin2/gif1* cwINV mutant has a clearly distinct phenotype with markedly greater grain chalkiness and is thus not similar to *ossweet11* (Wang *et al.*, 2008a). (2) Nucellar epidermis: SWEET expression in the nucellar epidermis is compatible with Oparka's anlyses which indicated that there is no symplasmic pathway to the filial tissues, thus requiring sugar transporters at the nucellar epidermis (Oparka & Gates, 1981a,b, 1982, 1984). (3) Aleurone: an unexpected location for SWEET-mediated efflux because the aleurone likely requires sucrose influx. Because SWEETs appear to function as uniporters, a sucrose gradient across nucellar epidermis and aleurone, driven by a high rate of delivery from the maternal side and rapid conversion in the endosperm, would allow the use of the same uniporters on both cell types. This situation is remotely similar to the human intestine, where transcellular transport across the intestinal epithelia is mediated by GLUT2 on both the apical and basal membrane under conditions where the glucose concentrations in the lumen exceed those of the blood stream (Kellett *et al.*, 2008). In addition to the two SWEETs, *SUT* sucrose transporters, which are expressed in the aleurone, may contribute to secondary active sucrose import into the aleurone (Scofield *et al.*, 2002; Bai *et al.*, 2016). (4) Nucellar projection: the presence of OsSWEET11 and 15 in the nucellar projection may appear as the most surprising site, because plasma membrane sucrose transport is not in line with radiotracer import studies, which had indicated that in rice, the import of sugars occurs exclusively via

the nucellar epidermis–aleurone pathway (Oparka & Gates, 1981b). However, others had suggested that the nucellar projection may contribute to sugar import into the developing endosperm (Krishnan & Dayanandan, 2003). Notably, the nucellar projection pathway appears to be the main pathway for sugar import in barley caryopses as shown by magnetic resonance imaging(MRI) (Melkus *et al.*, 2011). We are aware that in contrast to Oparka's radiotracer studies, we did not measure the actual translocation of assimilates, but rather the presence of a transport protein, and we do not know whether the two SWEETs are active at the plasma membrane of these cells. Nevertheless, we suggest that it may be useful to reassess sugar entry pathways, for example by MRI at different stages and in different varieties. One possible cause for the discrepancy could be that Oparka used an *indica* rice variety (IR 2153-338-3), whereas the *japonica* variety Kitaake was used for all of the experiments described here; Nipponbare was used by the parallel study that localized a transcriptional GUS fusion of the OsSWEET11 promoter to the same cells as the translational fusions in our work (Ma *et al.*, 2017). Alternatively, the two pathways may be used at different stages of development. Notably, the three Arabidopsis transporters SWEET11, 12 and 15 which play critical roles for sucrose efflux from the seed coat showed very complex changes in cellular expression during seed development (Chen *et al.*, 2015b).

Starch in the pericarp as a buffer

During the development of the rice caryopsis, starch grains accumulated in pericarp at 6 days after pollination (DAP), which disappeared at *c.* 7–9 DAP (Wu *et al.*, 2016b). Transient starch accumulation also had been observed in the pericarp of barley and wheat (Radchuk *et al.*, 2009; Xiong *et al.*, 2013). Starch accumulation in the pericarp was also observed in *ossweet11* single and *ossweet11;15* double mutants at 9 DAP (Fig. 6), but it remains to be determined whether this accumulation was caused by defects in sucrose import at the nucellar projection or at the nucellar epidermis–aleurone interface.

Ma *et al.* (2017) also localized transcriptional reporter fusions of OsSWEET11 to the pigment strand close to the main vascular trace and found a severe seed-filling defect in field-grown plants. In our glasshouse experiments, the phenotypic effect of *ossweet11* mutations was a lot less severe, in some cases even marginal, intimating a strong effect of the growth conditions – possibly light and nutrition – on the phenotype. Our work indicates, based on the similarity in steady-state RNA levels, timing of mRNA accumulation, and tissue specificity and the combined effect observed in double knockout mutants, that OsSWEET15 partially compensates for OsSWEET11 deficiency.

Relevance for pathogen susceptibility

The finding that OsSWEET11 and 15 play important roles in seed filling also is relevant with respect to the role of OsSWEET11 in rice blight susceptibility (Yang *et al.*, 2006; Antony *et al.*, 2010; Chen *et al.*, 2010; Yuan *et al.*, 2010). Ectopic expression of *OsSWEET11* is activated by pathovar-specific effectors of the blight pathogen *Xanthomonas oryzae pv oryzae* (Xoo). Mutations in the effector binding site of the *OsSWEET11* promoter lead to resistance to Xoo (Yang *et al.*, 2006; Antony *et al.*, 2010; Yuan *et al.*, 2010). It will therefore be important to ensure that genome editing of the promoter of *OsSWEET11* – with the purpose of engineering resistance – does not impact proper OsSWEET11 expression in seeds, to ensure that resistant lines do not carry a yield penalty. This goal appears feasible because apparently mutants (*xa13*) that are used by breeders do not show yield deficiencies (Laha *et al.*, 2016).

Conclusions

The analysis of SWEET gene expression in rice caryopses together with the characterization of knockout mutants demonstrates that OsSWEET11 and 15 play central roles in seed filling. The cellular expression patterns of OsSWEET11 and 15 may indicate the presence of two parallel apoplasmic pathways for sugar entry into the endosperm. A careful analysis of the timing and localization of other sugar transporters of the SWEET, SUT and MST families. as well as the cell wall invertases, and an analysis of sucrose import by MRI in a variety of rice cultivars may help to further delineate the precise sugar import pathways and contribute to the knowledgebase for engineering improved yield potential in rice.

Acknowledgements

We thank Davide Sosso for comments and advice. This research was supported by grants from the National Science Foundation research (IOS-1258103) to W.B.F. and B.Y., and the U.S. Department of Energy (DE-FG02-04ER15542), the Bill and Melinda gates Foundation and the Alexander von Humboldt Foundation to W.B.F.

Author contributions

J.Y., J-S.E., B.Y. and W.B.F. conceived and designed the experiments; J.Y., J-S.E. and D.L. performed the experiments and collected the data, executed the data analyses and rendered the figures; and all authors contributed to the interpretation of the results, wrote and revised earlier drafts – they approved the final version of this manuscript and agree to be held accountable for the content. J.Y. and D.L. contributed equally to this work.

References

Antony G, Zhou J, Huang S, Li T, Liu B, White F, Yang B. 2010. Rice *xa13* recessive resistance to bacterial blight is defeated by induction of the disease susceptibility gene *Os-11N3*. *The Plant Cell* **22**: 3864–3876.

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

Bai A-N, Lu X-D, Li D-Q, Liu J-X, Liu C-M. 2016. NF-YB1-regulated expression of sucrose transporters in aleurone facilitates sugar loading to rice endosperm. *Cell Research* **26**: 384–388.

Bezrutczyk M, Hartwig T, Horschman M, Char SN, Yang J, Yang B, Frommer WB, Sosso D. 2018. Impaired phloem loading in *zmsweet13a,b,c* sucrose transporter triple knock-out mutants in *Zea mays*. *New Phytologist* **218**: 594–603.

van den Broek PJ, van Gompel AE, Luttik MA, Pronk JT, van Leeuwen CC. 1997. Mechanism of glucose and maltose transport in plasma-membrane vesicles from the yeast *Candida utilis*. *Biochemical Journal* **321**: 487–495.

Chen L-Q, Cheung LS, Feng L, Tanner W, Frommer WB. 2015a. Transport of sugars. *Annual Review of Biochemistry* **84**: 865–894.

Chen L-Q, Hou B-H, Lalonde S, Takanaga H, Hartung ML, Qu X-Q, Guo W-J, Kim J-G, Underwood W, Chaudhuri B *et al.* 2010. Sugar transporters for intercellular exchange and nutrition of pathogens. *Nature* **468**: 527–532.

Chen L-Q, Lin IW, Qu X-Q, Sosso D, McFarlane HE, Londoño A, Samuels AL, Frommer WB. 2015b. A cascade of sequentially expressed sucrose transporters in the seed coat and endosperm provides nutrition for the Arabidopsis embryo. *The Plant Cell* **27**: 607–619.

Chen L-Q, Qu X-Q, Hou B-H, Sosso D, Osorio S, Fernie AR, Frommer WB. 2012. Sucrose efflux mediated by SWEET proteins as a key step for phloem transport. *Science* **335**: 207–211.

Cheng W-H, Chourey PS. 1999. Genetic evidence that invertase-mediated release of hexoses is critical for appropriate carbon partitioning and normal seed development in maize. *Theoretical and Applied Genetics* **98**: 485–495.

Eom J-S, Choi S-B, Ward JM, Jeon J-S. 2012. The mechanism of phloem loading in rice (*Oryza sativa*). *Molecules and Cells* **33**: 431–438.

Feng L, Frommer WB. 2015. Structure and function of SemiSWEET and SWEET sugar transporters. *Trends in Biochemical Sciences* **40**: 480–486.

Fukumorita T, Chino M. 1982. Sugar, amino acid and inorganic content in rice phloem sap. *Plant and Cell Physiology* **23**: 273–283.

Furbank RT, Scofield GN, Hirose T, Wang X-D, Patrick JW, Offler CE. 2001. Cellular localisation and function of a sucrose transporter OsSUT1 in developing rice grains. *Functional Plant Biology* **28**: 1187–1196.

Han L, Zhu Y, Liu M, Zhou Y, Lu G, Lan L, Wang X, Zhao Y, Zhang XC. 2017. Molecular mechanism of substrate recognition and transport by the AtSWEET13 sugar transporter. *Proceedings of the National Academy of Sciences, USA* **114**: 1089–1094.

Hirose T, Imaizumi N, Scofield GN, Furbank RT, Ohsugi R. 1997. cDNA cloning and tissue specific expression of a gene for sucrose transporter from rice (*Oryza sativa* L.). *Plant and Cell Physiology* **38**: 1389–1396.

Hirose T, Takano M, Terao T. 2002. Cell wall invertase in developing rice caryopsis: molecular cloning of OsCIN1 and analysis of its expression in relation to its role in grain filling. *Plant and Cell Physiology* **43**: 452–459.

Hirose T, Zhang Z, Miyao A, Hirochika H, Ohsugi R, Terao T. 2010. Disruption of a gene for rice sucrose transporter, *OsSUT1*, impairs pollen function but pollen maturation is unaffected. *Journal of Experimental Botany* **61**: 3639–3646.

Johnson DA, Thomas MA. 2007. The monosaccharide transporter gene family in Arabidopsis and rice: a history of duplications, adaptive evolution, and functional divergence. *Molecular Biology and Evolution* **24**: 2412–2423.

Kellett GL, Brot-Laroche E, Mace OJ, Leturque A. 2008. Sugar absorption in the intestine: the role of GLUT2. *Annual Review of Nutrition* **28**: 35–54.

Krishnan S, Dayanandan P. 2003. Structural and histochemical studies on grain-filling in the caryopsis of rice (*Oryza sativa* L.). *Journal of Biosciences* **28**: 455–469.

Laha GS, Sailaja B, Srinivas Prasad M, Ladhalakshmi D, Krishnaveni D, Singh R, Prakasam V, Yugander A, Kannan C, Valarmathi P *et al.* 2016. *Changes in rice disease scenario in India: an analysis from production oriented survey.* Rajendranagar, Hyderabad, India: ICAR-Indian Institute of Rice Research.

Latorraca NR, Fastman NM, Venkatakrishnan AJ, Frommer WB, Dror RO, Feng L. 2017. Mechanism of substrate translocation in an alternating access transporter. *Cell* **169**: e12.

Li T, Liu B, Chen CY, Yang B. 2014. TALEN utilization in rice genome modifications. *Methods* **69**: 9–16.

Li T, Liu B, Spalding MH, Weeks DP, Yang B. 2012. High-efficiency TALEN-based gene editing produces disease-resistant rice. *Nature Biotechnology* **30**: 390–392.

Lin IW, Sosso D, Chen L-Q, Gase K, Kim S-G, Kessler D, Klinkenberg PM, Gorder MK, Hou B-H, Qu X-Q *et al.* 2014. Nectar secretion requires sucrose phosphate synthases and the sugar transporter SWEET9. *Nature* **508**: 546–549.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT Method. *Methods* **25**: 402–408.

Ma L, Zhang D, Miao Q, Yang J, Xuan Y, Hu Y. 2017. Essential role of sugar transporter OsSWEET11 during the early stage of rice grain filling. *Plant and Cell Physiology* **58**: 863–873.

Melkus G, Rolletschek H, Fuchs J, Radchuk V, Grafahrend-Belau E, Sreenivasulu N, Rutten T, Weier D, Heinzel N, Schreiber F *et al.* 2011. Dynamic $^{13}C/^{1}H$ NMR imaging uncovers sugar allocation in the living seed. *Plant Biotechnology Journal* **9**: 1022–1037.

Oparka KJ, Gates P. 1981a. Transport of assimilates in the developing caryopsis of rice (*Oryza sativa* L.): ultrastructure of the pericarp vascular bundle and its connections with the aleurone layer. *Planta* **151**: 561–573.

Oparka KJ, Gates P. 1981b. Transport of assimilates in the developing caryopsis of rice (*Oryza sativa* L.). The pathways of water and assimilated carbon. *Planta* **152**: 388–396.

Oparka KJ, Gates PJ. 1982. Ultrastructure of the developing pigment strand of rice (*Oryza sativa* L.) in relation to its role in solute transport. *Protoplasma* **113**: 33–43.

Oparka KJ, Gates P. 1984. Sink anatomy in relation to solute movement in rice (*Oryza sativa* L.): a summary of findings. *Plant Growth Regulation* **2**: 297–307.

Patrick JW. 1997. Phloem unloading: sieve element unloading and post-sieve element transport. *Annual Review of Plant Physiology and Plant Molecular Biology* **48**: 191–222.

Patrick JW, Offler CE. 2001. Compartmentation of transport and transfer events in developing seeds. *Journal of Experimental Botany* **52**: 551–564.

Radchuk VV, Borisjuk L, Sreenivasulu N, Merx K, Mock H-P, Rolletschek H, Wobus U, Weschke W. 2009. Spatiotemporal profiling of starch biosynthesis and degradation in the developing barley grain. *Plant Physiology* **150**: 190–204.

Reinders A, Sun Y, Karvonen KL, Ward JM. 2012. Identification of amino acids important for substrate specificity in sucrose transporters using gene shuffling. *The Journal of Biological Chemistry* **287**: 30296–30304.

Scofield GN, Aoki N, Hirose T, Takano M, Jenkins CLD, Furbank RT. 2007a. The role of the sucrose transporter, OsSUT1, in germination and early seedling growth and development of rice plants. *Journal of Experimental Botany* **58**: 483–495.

Scofield GN, Hirose T, Aoki N, Furbank RT. 2007b. Involvement of the sucrose transporter, OsSUT1, in the long-distance pathway for assimilate transport in rice. *Journal of Experimental Botany* **58**: 3155–3169.

Scofield GN, Hirose T, Gaudron JA, Furbank RT, Upadhyaya NM, Ohsugi R. 2002. Antisense suppression of the rice transporter gene, *OsSUT1*, leads to impaired grain filling and germination but does not affect photosynthesis. *Functional Plant Biology* **29**: 815–826.

Sharma R. 2014. Outlook for rice demand, supply and trade. In: Dawe D, Jaffee S, Santos N, eds. *Rice in the shadow of skyscrapers: policy choices in a dynamic East and Southeast Asian setting*. Rome, Italy: FAO, 8–31.

Slewinski TL, Meeley R, Braun DM. 2009. Sucrose transporter1 functions in phloem loading in maize leaves. *Journal of Experimental Botany* **60**: 881–892.

Sosso D, Luo D, Li Q-B, Sasse J, Yang J, Gendrot G, Suzuki M, Koch KE, McCarty DR, Chourey PS *et al.* 2015. Seed filling in domesticated maize and rice depends on SWEET-mediated hexose transport. *Nature Genetics* **47**: 1489–1493.

Takahashi S, Ishimaru K, Yazaki J, Fujii F, Shimbo K, Yamamoto K, Sakata K, Sasaki T, Kishimoto N, Kikuchi S. 2005. Microarray analysis of sink–source transition in rice leaf sheaths. *Breeding Science* **55**: 153–162.

Toyofuku K, Kasahara M, Yamaguchi J. 2000. Characterization and expression of monosaccharide transporters (*OsMSTs*) in rice. *Plant and Cell Physiology* **41**: 940–947.

Wang E, Wang J, Zhu X, Hao W, Wang L, Li Q, Zhang L, He W, Lu B, Lin H *et al.* 2008a. Control of rice grain-filling and yield by a gene with a potential signature of domestication. *Nature Genetics* **40**: 1370–1374.

Wang Y, Xiao Y, Zhang Y, Chai C, Wei G, Wei X, Xu H, Wang M, Ouwerkerk PBF, Zhu Z. 2008b. Molecular cloning, functional characterization and expression analysis of a novel monosaccharide transporter gene *OsMST6* from rice (*Oryza sativa* L.). *Planta* **228**: 525–535.

Wang Y, Xu H, Wei X, Chai C, Xiao Y, Zhang Y, Chen B, Xiao G, Ouwerkerk PBF, Wang M *et al.* 2007. Molecular cloning and expression analysis of a monosaccharide transporter gene *OsMST4* from rice (*Oryza sativa* L.). *Plant Molecular Biology* **65**: 439–451.

Weber H, Borisjuk L, Wobus U. 2005. Molecular physiology of legume seed development. *Annual Review of Plant Biology* **56**: 253–279.

Wu X, Liu J, Li D, Liu C-M. 2016a. Rice caryopsis development I: dynamic changes in different cell layers. *Journal of Integrative Plant Biology* **58**: 772–785.

Wu X, Liu J, Li D, Liu C-M. 2016b. Rice caryopsis development II: dynamic changes in the endosperm. *Journal of Integrative Plant Biology* **58**: 786–798.

Xiong F, Yu XR, Zhou L, Wang F, Xiong AS. 2013. Structural and physiological characterization during wheat pericarp development. *Plant Cell Reports* **32**: 1309–1320.

Xu SB, Li T, Deng ZY, Chong K, Xue Y, Wang T. 2008. Dynamic proteomic analysis reveals a switch between central carbon metabolism and alcoholic fermentation in rice filling grains. *Plant Physiology* **148**: 908–925.

Xuan YH, Hu YB, Chen LQ, Sosso D, Ducat DC, Hou BH, Frommer WB. 2013. Functional role of oligomerization for bacterial and plant SWEET sugar transporter family. *Proceedings of the National Academy of Sciences, USA* **110**: E3685–E3694.

Yang B, Sugio A, White FF. 2006. *Os8N3* is a host disease-susceptibility gene for bacterial blight of rice. *Proceedings of the National Academy of Sciences, USA* **103**: 10503–10508.

Yuan M, Chu Z, Li X, Xu C, Wang S. 2010. The bacterial pathogen *Xanthomonas oryzae* overcomes rice defenses by regulating host copper redistribution. *The Plant Cell* **22**: 3164–3176.

Zhang W-H, Zhou Y, Dibley KE, Tyerman SD, Furbank RT, Patrick JW. 2007. Nutrient loading of developing seeds. *Functional Plant Biology* **34**: 314–331.

Zhou H, Liu B, Weeks DP, Spalding MH, Yang B. 2014. Large chromosomal deletions and heritable small genetic changes induced by CRISPR/Cas9 in rice. *Nucleic Acids Research* **42**: 10903–10914.

Zhou Y, Qu H, Dibley KE, Offler CE, Patrick JW. 2007. A suite of sucrose transporters expressed in coats of developing legume seeds includes novel pH-independent facilitators. *Plant Journal* **49**: 750–764.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Fig. S1 Mutagenesis of *OsSWEET11* and *OsSWEET15* using CRISPR-Cas9 and TALEN genome editing.

Fig. S2 Heat map of the tissue-specific mRNA levels of *SWEET*, *SUT1* and *cwINV* genes from public microarray data.

Fig. S3 Phenotypical characterization of the *ossweet11-2* mutant in paddy conditions.

Fig. S4 Histochemical analysis of OsSWEET11 accumulation at early stages of caryopsis development.

Fig. S5 OsSWEET11 and OsSWEET15 function as sucrose transporters.

Fig. S6 Wild-type and mutant grains in ISU glasshouse.

Fig. S7 Endosperm development in wild-type, *ossweet11-1* and *ossweet11-1;15-1*.

Table S1 Primer sequences

Please note: Wiley Blackwell are not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.