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LETTER TO THE EDITOR

Sucrose transport involves in disease response to *Xanthomonas oryzae* pathovar *oryzae*

Yunfei Wu, Wangmenghan Peng, and Fei Xiong

Jiangsu Key Laboratory of Crop Genetics and Physiology, Co-Innovation Center for Modern Production Technology of Grain Crops, Joint International Research Laboratory of Agriculture & Agri-Product Safety, Yangzhou University, Yangzhou, China

ABSTRACT

Sugar acts as an important nutrient for plant development. Previously, we reported that *Oryza sativa DNA BINDING WITH ONE FINGER 11* (*OsDOF11*) plays a crucial role in sucrose transport by binding promoters of sucrose transporter genes, *OsSUT1*, *OsSWEET11*, and *OsSWEET14*. Meanwhile, sucrose transport activity abnormal also involved susceptibility to infection of *Xanthomonas oryzae* pathovar *oryzae* (*Xoo*) by *OsSWEET* genes. Here, we provid an addendum, that *OsDOF11* expression pattern in spikelet development stage, and transcript levels of *OsSWEET12, OsSWEET13, OsSWEET15*, and *OsSWEET16* in the mutants of *OsDOF11* at different developmental stages. This information further supplied a new insight that sucrose transport activity mediated susceptibility to *Xoo* infection.

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Sugar is the key element for plant development, and its transport activity is a crucial factor for crop biomass.This transport is initiated by different phloem loading processes in the source tissue and then move into sink tissues, especially in the reproductive organ.[1] Sucrose is the main material for starch synthesis. Thus, sucrose transporter genes, *SUT* (*Sucrose Transporter*; also called *SUC* for *Sucrose Carrier*) and *SWEET* (*Sugars Will Eventually be Exported Transporters)-type transporters*, directly or indirectly mediate sink organ development processes.[1-5] Previous reported data demonstrate that the *OsSUTs* genes, *OsSUT1,OsSUT2, OsSUT3, OsSUT4*, and *OsSUT5*, perform sucrose import by the membrane.[6,7] *OsSUT1* is preferentially expressed in leaf phloem cells along with the stamens of mature spikelet formation and *OsSUT1 Tos17* insertional mutant is unable to produce homozygous progeny due to impaired pollen.[3] *OsSUT3* and *OsSUT4* are highly expressed in developing pollen.[3,8] But *OsSUT2* expressed in the cell layers of seed coats.[9] Meanwhile, *OsSWEET* genes, *OsSWEET11* and *OsSWEET14*, function in sucrose export.[10,11] *OsSWEET11 is* expressed in vascular cells at the reproductive stage and loss-of-function mutation shows stamen defects, reduced pollen viability, and smaller seeds.[10] Mutation of *OsSWEET14* also showed semi-dwarf phenotype, with a smaller grain.[11] These reports suggest that the sucrose transporters function in reproductive organ development, possibly in consistent with vegetative stage in rice.

As the sucrose is transferred into the grain, it has been reported that sucrose reaches the caryopsis through phloem loading at the dorsal vascular bundle.[12] Bai et al. (2017) reported that *OsNF-YB1*(*Nuclear Factor Y B1*) expressed in caryopses from 4 to 21 DAP, and mutants of *OsNF-YB1* generated by CRSPER 9 and RNA interference (RNAi) showed chalky endosperm possibly due to transcript level reduction of *OsSUT1, OsSUT3, OsSUT4*, and *OsMST4* (monosaccharide transporter 4).[12] In our previous study, it has been reported that *OsDOF11* is expressed in the phloem cells of leaves, mature spikelet, developmental seed, and root. T-DNA insertion mutants and RNAi transgenic plants displayed semi-dwarf phenotypes with smaller panicles and grains.[13] We also reported that OsDOF11 involved in sucrose transport by *OsSUTs* and *OsSWEETs*, as shown in Figure 1a.[13] In the main manuscript, the researchers showed the expression level of almost all the *SUT* and 2 *SWEETs* in different developmental stages in *OsDOF11* T-DNA insertional mutant were reduced by binding were checked and validated in OsSUT1, OsSWEET11, and OsSWEET14, no all its promoter.[13] *osdof11* also showed smaller seeds as shown in Figure 1b, probably due to OsDOF11's expression pattern during spikelet development stages as well as in the filling stage.

We found that *OsDOF11* also affect susceptibility to infection of *Xoo* which might through OsSWEET11 and OsSWEET14 in leaf blade.[13] Sugar acts as an important nutritent in bacterial proliferation. Published data showed that *OsSWEET11, OsSWEET12, OsSWEET13, OsSWEET14, OsSWEET15* not only mediate sugar transport, but also involved in the response to the infection of *Xoo*.[10,11,13-15] Mutants of *OsSWEET11 and OsSWEET14* were less susceptible to infection. Also, the transcript levels of *OsSWEET12, OsSWEET13*, and *OsSWEET15* in leaf were

CONTACT Yunfei Wu 006949@yzu.edu.cn; Fei Xiong feixiong@yzu.edu.cn Jiangsu Key Laboratory of Crop Genetics and Physiology, Co-Innovation Center for Modern Production Technology of Grain Crops, Joint International Research Laboratory of Agriculture & Agri-Product Safety, Yangzhou University, Yangzhou 225009, China

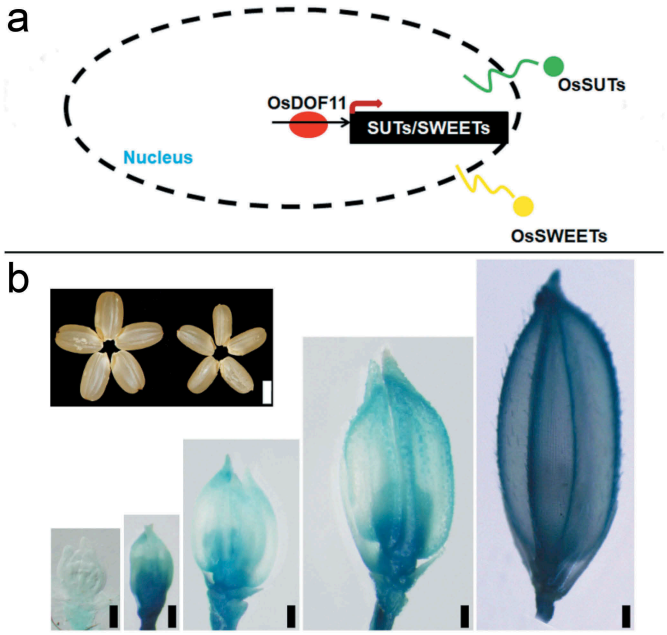

Figure 1. OsDOF11 functions in sucrose transport in rice. (a) OsDOF11 functions in sucrose transport by *OsSUTs* and *OsSWEETs*; (b) OsDOF11 functions during reproductive stage, the upper left picture showed seed phenotype, left: WT; right: osdof11-1. the bottom-right picture showed expression pattern. Bar: 2 mm, 200 um, 200 μm; 200 μm; 200 μm; 200 μm.

also increased to *Xoo (PXO99*-modified *or PXO339* lines) by different effectors.[15] Yuan et al. (2010) reported that *OsSWEET11* is induced by PXO99.[14] *Xa25/ OsWEET13* were induced by *PXO399* but not *PXO99* (Liu et al., 2011). And there is no native TAL effectors have been reported for finding *OsSWEET12* and *OsSWEET15*, although TAL protein AAW76267 and Tal7b/Tal8b may be two putative candidates, respectively.[15] Designed effectors of TALE-S1/TALE-S2 /artTAL12-2 and artTAL15-1 were used for finding *OsSWEET12* and *OsSWEET15* functions.[12,15]As the infection of *Xoo PXO99* in mutants of *OsDOF11*, relatively lower expression of all *OsSWEET11, OsSWEET12, OsSWEET13, OsSWEET14*, and *OsSWEET15* were observed in *osdof11-1*and *RNAi-9* lines than WT. However, other diseases affect rice panicle or grain development, such as *Gibberellazeae and Fusarium graminearum*.

Decreased expressions were observed for *OsSUT1, OsSUT3, OsSUT5, OsSWEET11*, and *OsSWEET14* in the embryo of germination stage, leaf of vegetative stage, young panicle, and spikelet of reproductive stage. Here, we also found that the transcript level of *OsSWEET13* was reduced (Figure 2). Also, *OsSWEET15* transcript level was decreased (Figure 2), although there was no native effector. Recently, Yang et al. (2017) reported that *ossweet11* single and *ossweet11/ossweet15* double mutants showed defects in endosperm development and filling, which indicated that *OsSWEET15* also contributes to the reproductive stage.[16] Compared to the kit genes, *OsSWEET12, OsSWEET13, OsSWEET15*, and *OsSWEET16*, expression of *OsSWEET15* was higher in 3 days after immersion (DAI), 1–2 cm inflorescence meristem (IF), and mature spikelet flower (MF)) than 35 days after germination (DAG) leaf, which means *OsSWEET15* functions in sugar loading.

The present results reveal that sucrose transporter–related gene *SWEET* paralog can function as a sugar transporter involved in plant development and also supported bacterial (*Xoo*) to amplify and increase disease susceptibility. This inference gives a new insight into susceptibility to bacterial infection.

RT-PCR analyses

Total RNA was isolated from leaf blades of greenhouse-grown plants at 35 DAG as well as from 3 DAG seedlings and 1–2-cm-long young panicles. The complementary DNA were synthesized and quantitative real-time Reverse Transcription-Polymerase Chain Reaction was performed as previously described. The internal control was rice *UBQ5* (*LOC_Os01g22490*). All experiments were conducted at least three times, with three or more samples taken at each point. To ensure primer specificity, we performed the experiments when the melting curve showed a single sharp peak. The PCR products were sequenced to verify the specificity of the reaction. All primers used for studying gene expression were listed in our previous report.[13]

Histochemical GUS Analysis and Subcellular Localization of OsDOF11

The β-glucuronidase assays were performed as previously described,[13] using GUS-stained samples that were

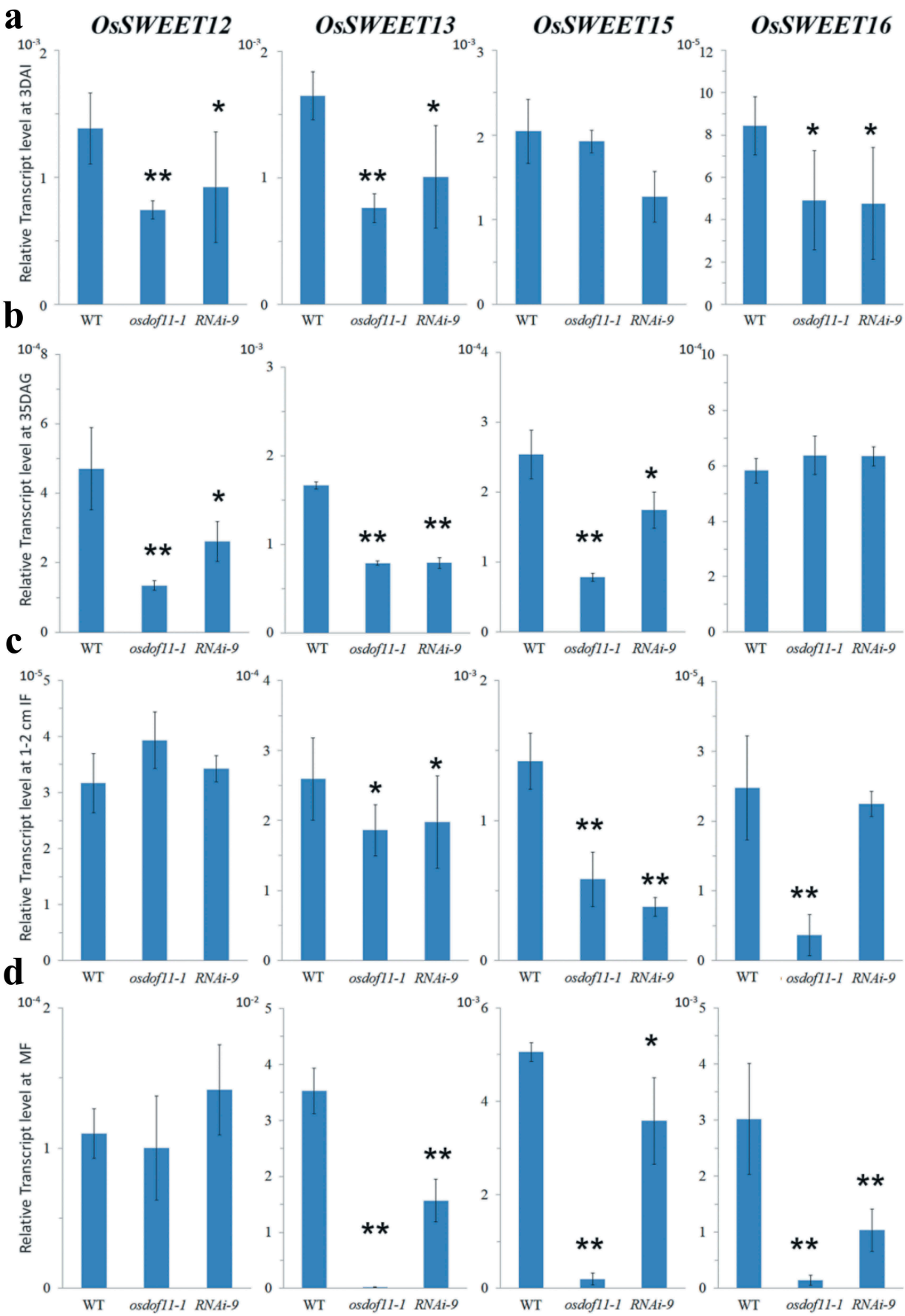

Figure 2. Relative transcript levels (RTL) of OsSWEETs in WT and OsDOF11 mutants (*osdof11-1* and *RNAi-9*). (a) RTL at 3 DAI stage; (b) RTL at 35 DAG stage; (c) RTL at 1–2 mm IF stage; (d) RTL at MF stage.Error bars represent SE of at least three samples. *$P < .05$; **$P < .01$.

fixed in 3% (w/v) paraformaldehyde, 5% (v/v) acetic acid, and 63% (v/v) ethanol. Following ethanol dehydration, samples were embedded in Technovit Embedding Kits 7100 (Germany).

Disclosure statement

The authors declare that they have no competing interest.

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