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Characterization of *OsSUT2* Expression and Regulation in Germinating Embryos of Rice Seeds

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Abstract *OsSUT2* encodes a putative sucrose transporter containing 12 transmembrane domains in rice plants. Subcellular localization of the OsSUT2::GFP fusion protein indicated that OsSUT2 is a cell membrane protein. In embryos of germinating seeds, the expression of *OsSUT2* gradually increased during the early germinating stage. The developmental regulations of *OsSUT2* in germinating embryos could be mediated by sugars transported from endosperms. *OsSUT2* expression was up-regulated by glucose through a hexokinase-independent pathway. Exogenous sucrose was sensed by a sensor localized on the plasma membrane and functioned as an enhancer to promote *OsSUT2* expression. Based on *OsSUT2* promoter::*GUS* expression in germinating seeds of transgenic rice, *OsSUT2* was significantly expressed in the embryos and aleurone layers. In embryos, strong GUS expression was detected in the scutellum and vascular bundle tissues. Developmental stage- and sugar-dependent *OsSUT2* expression was suggested to be controlled by transcriptional regulation of the promoter region.

Keywords Embryo · *Oryza sativa* · Seed germination · Sucrose transporter

Abbreviations

DAI	Days after imbibition
Glc	Glucose
GUS	β-Glucuronidase
Man	Mannitol
MU	4-Methylumbelliferone
3-OMG	3-*O*-Methylglucose
Pal	Palatinose
QRT-PCR	Quantitative real-time reverse transcriptase polymerase chain reaction
Sc	Scutellum cells
Suc	Sucrose
SUT	Sucrose transporter
Ubi	Ubiquitin
V	Vascular bundle

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Introduction

In plants, carbohydrates translocate from the source to sink tissues through symplastic and apoplastic pathways. Sugar transport between cells in symplasts is mediated by plasmodesmata. In apoplasts, sugar translocators are responsible for carbohydrate uptake into cells. Because sucrose is the major transported form of carbohydrate between plant tissues, sucrose transporter (SUT) plays an important role in long-distance disaccharide transport. SUT1 in sugarcane functions to partition sucrose between the vascular bundle and storage cells (Rae et al. 2005). Maize SUT, ZmSUT1, is responsible for sugar uptake into phloem in source tissues and sugar unloading from phloem into cells in sink tissues (Carpaneto et al. 2005). Changes in carbohydrate allocation and the inhibition of photosynthesis have been observed in transgenic plants with reduced SUT activity due to antisense *SUT* genes; thus, SUT has been indicated to play an important role in carbohydrate partitioning and physiological processing (Kühn et al. 1996; Bürkle et al. 1998).

To date, SUT genes have been identified from several plants, most belonging to gene families (Lemoine 2000; Lalonde et al. 2004). The SUT gene families of dicot and monocot species were classified into five sequence-based groups by Braun and Slewinski (2009). In rice, the SUT gene family consists of five genes, *OsSUT1* to *OsSUT5* (Aoki et al. 2003). *OsSUT1* and *3* were classified into group 1. The group 1 *SUT* family is composed of several Poaceae *SUT* members; so far, no dicot *SUTs* have been found in group 1. *OsSUT2* was classified with *Arabidopsis SUT4* (*AtSUT4*) in group 2. AtSUT4 has been demonstrated to be responsible for the low-affinity/high-capacity transport system (reviewed by Lalonde et al. 2004). *OsSUT4* and *OsSUT5* were classified into group 3 and 5, respectively.

During plant development from seed germination to seed establishment and seedling growth, starch reserved in the seed is the primary carbon and energy source for sprouting and early seedling establishment, and then the sugar sources for later stages of seedling growth come from the photosynthetic shoots. In rice plants, carbohydrate transport from germinating seeds to other developing sink tissues is a fundamental process for plant development and growth. Sugar derived from starch degradation exported to the cytosol from storage organelles is the first step for long-distance carbohydrate translocation. In cereal endosperms, starch granules are attacked by α-amylase (Murata et al. 1968), and the maltose and linear/short-branch-chain oligosaccharides are generated for further degradation by α-glucosidase (Stanley et al. 2011). The hexoses produced by starch degradation can be taken up by the scutellum cells of the embryo. Hexoses have been suggested to be re-synthesized into sucrose in the scutellum (Edelman et al. 1959; Nomura et al. 1969). Sucrose in the scutellum is loaded into the vascular bundle, transported, and then unloaded to growing tissues through apoplastic or symplastic pathways (Aoki et al. 2006). In addition, it was also suggested that sucrose transported from aleurone layers to endosperm at early germination stage could be further uptaked by scutellum (Aoki et al. 2006). Based on our previous work (Liu et al. 2010), soluble sugar can also be converted to starch in scutellum or the cells surrounding vascular bundles at the post-germination stage, and the amount of transitory starch in embryonic tissues was dependent on the demand of growing sink tissues. Phloem functions as an important pathway for carbon source transport among various plant tissues. In rice germinating seeds, *OsSUT1* was the first *OsSUT* family member identified to have a tissue-specific expression pattern (Hirose et al. 1997; Scofield et al. 2007). Developmental expression and regulation of *OsSUT1* has been observed in germinating seeds (Chen et al. 2010). However, the regulation of other members of the *OsSUT* gene family still needs to be established.

In the present study, we identified the subcellular localization of *OsSUT2*. The expression of *OsSUT2* in germinating embryos was detected by real-time quantitative RT-PCR. The spatial and temporal transcriptional activities of the *OsSUT2* promoter were detected in the germinating seeds of transgenic rice plants carrying the *OsSUT2* promoter*::GUS* fusion gene. Furthermore, the signal transduction of sugars for regulating *OsSUT2* expression was examined and discussed.

Materials and methods

Plant materials and treatment

Rice (*Oryza sativa* L. cv. Tainung 67) seeds were obtained from the Hualien District Agricultural Research and Extension Station in Taiwan. For germination, seeds were sterilized in 2.5% sodium hypochlorite with Tween 20 for 20 min and subsequently washed with distilled H_2O four times. Seeds were then germinated at 37°C in the dark for 3 days and then moved to the phytotron for growing at 30/25°C under natural daylight. To analyze the sugar content and *OsSUT2* expression in embryos, the growing shoots and roots were cut and the embryos isolated after 1- to 5-day seed imbibition.

To isolate embryos from dry seeds, the grain hulls were removed by machine and the embryos picked by razor blade. The isolated embryos were sterilized in 0.25% sodium hypochlorite for 10 min and subsequently washed with distilled H_2O four times (Matsukura et al. 2000). For isolated embryo culturing, the embryos were placed on MS medium and incubated at 28°C in dark or light. For sugar and sugar analog treatments, the chemicals were added in MS medium. The concentration of all sugars and sugar analogs used in this study was 100 mM.

Homology analysis of amino acid sequences and transmembrane domain prediction

Multiple sequence alignments of SUT amino acid sequences were carried out using SDSC Biology WorkBench 3.2 (http://workbench.sdsc.edu/). The transmembrane domains on the OsSUT2 amino acid sequence were predicted according to the Hidden Markov Models using TMMOD software (Kahsay et al. 2005).

RNA extraction

Embryos isolated from ten seeds or harvested from medium were ground in liquid nitrogen, homogenized in 1 mL Trizol reagent (Invitrogen, Carlsbad, CA, USA), and centrifuged at 8,000×*g*. The supernatant was treated with 0.2 mL chloro-

form, shaken for 15 s, and incubated at room temperature for 3 min. After centrifugation at 12,000×*g* for 15 min at 4°C, the upper layer was transferred to a new tube. RNA was precipitated with 0.5 mL isopropanol and incubated for 10 min at room temperature. After centrifugation, the pellet was dissolved in 0.2 mL H_2O. Before the gene expression analysis, the total RNA extracted from the embryos was treated with DNase to remove contaminating genomic DNA.

Quantitative real-time reverse transcriptase-PCR

Total RNA (200 ng) was used as the template for quantitative real-time RT-PCR analysis using the Brilliant SYBR Green QRT-PCR Master Mix (Stratagene, La Jolla, CA, USA), and PCR reactions were performed using a Multiplex 3000P Real-Time PCR System (Stratagene). The gene-specific RT-PCR primers are listed on Table 1. RT-PCR was carried out as follows: 50°C for 30 min and 95°C for 10 min, followed by 40 cycles of 95°C for 1 min, 58°C for 1 min, and 72°C for 1 min. To quantify relative gene expression levels accurately, the C_T values of *OsSUT1* to *5* were normalized to the C_T value of the ubiquitin (Ubi) gene. For all real-time RT-PCR analyses, three independent experiments were carried out, and the data are presented as mean±SD.

Sugar analysis

Ten embryos were ground to powder in liquid nitrogen and extracted with 1 mL of 80% (*v*/*v*) ethanol at 80°C for 5 min before centrifugation at 3,000×*g*. The supernatant was analyzed for glucose and sucrose following the method described by Spackman and Cobb (2002).

Subcellular location of OsSUT2

The coding region cDNA of *OsSUT2* was cloned from rice embryos using RT-PCR. Total RNA was extracted from embryos 5 days after imbibition (DAI) and RT-PCR was performed using specific primers. The forward primer (5′-TTCTAGAATGCCGCGGCGGCCTAGGCGG-3′) contained the *Xba*I cloning site sequence, the reverse primer (5′-AGGATCCATCGGTGACCTCTCCTCCTTG-3′) contained the *Bam*HI cloning site, and the stop codon sequence was not included. *OsSUT2* cDNA was cloned in-frame with the N-terminus of the green fluorescent protein (GFP) gene, driven by a CaMV35S promoter in a transient expression vector. The *OsSUT2-GFP* fusion construct was transiently expressed in barley aleurone layer cells mediated by a He Biolistic particle delivery system (model PDS-1000, Bio-Rad). Half-de-embryonated barley seeds were sterilized before soaking in a shooting buffer (20 mM Na–succinate and 20 mM $CaCl_2$, pH 5.0) for 48 h at 24°C in the dark (Mena et al. 2002). Next, the pericarp was removed from the seeds and the aleurone layer exposed for bombardment. After bombardment, the seeds were incubated with shooting buffer for 24 h at 24°C. Finally, fluorescence localization in the aleurone layer cells was observed using an AXIO Imager M1 fluorescence microscope (Carl Zeiss, Germany).

Promoter cloning

Rice genomic DNA was extracted from the leaf tissues with plant DNA_{ZOL} reagent (Invitrogen). The *OsSUT2* DNA fragment upstream of the translation start site, located from −830 to −1 bp, was amplified by PCR using specific primers (forward primer with *Sac*I site: 5′-GAGCTCT TAAGGAGCACCAA-3′; reverse primer with *Sma*I site: 5′-CCCGGGCTTCTTCTCGTGTT-3′). Putative *cis*-elements on the *OsSUT2* promoter sequence were characterized by searching for similar motifs in the Database of Plant *Cis*-acting Regulatory DNA Elements (PLACE) (Higo et al. 1999). To generate the plasmid for the promoter activity assay in transgenic rice plants, the *OsSUT2* promoter

Table 1 Primer pairs for real-time RT-PCR

Gene	Accession number	Primer pair (F: forward primer; R: reverse primer)	Amplicon size (bp)
OsSUT1	D87819	F: 5′-CTGTGATTTTCCTGTCCCTG-3′ R: 5′-AACACTGCTAGTGGACCAGT-3′	136
OsSUT2	AB091672	F: 5′-AGGAGGAGAGGTCACCGATAA-3′ R: 5′-CCAACATCCAATGTACAACAGCA-3′	240
OsSUT3	AB071809	F: 5′-GCCCAAGGTCTCCGTCC-3′ R: 5′-TGCTATAGTACCCGCTCTAA-3′	137
OsSUT4	AB091673	F: 5′-TTTGGCTGAGCAGAACACCA-3′ R: 5′-ATGTCATTCGGGCAGAGCTT-3′	249
OsSUT5	AB091674	F: 5′-CTAGTGCGAAACTCCATCAAA-3′ R: 5′-AAAATATTTGGGTTTCCTGAGAT-3′	249
Ubi	D12629	F: 5′-CGCAAGTACAACCAGGACAA-3′ R: 5′-TGGTTGCTGTGACCACACTT-3′	101

The nucleotide sequences used for primer designing were presented by their accession numbers in GenBank

(−830/−1) was inserted into the *Sac*I and *Sma*I sites of the pCHY10 vector (a gift from Dr. Chwan-Yang Hong), which contained the first intron of the Ubi gene fused to the β-glucuronidase (*GUS*) reporter gene. We used restriction enzymes to isolate the DNA cassettes containing the *OsSUT2* promoter fragment with the *Ubi* intron and GUS gene from the pCYH10 constructs. These cassettes were then ligated into the *Sac*I and *Hind*III sites of the pCAMBIA1302 plasmid.

Generation of transgenic rice plants

To produce *OsSUT2* promoter*::GUS* transgenic plants, the constructed pCAMBIA1302 plasmid was transformed into *Agrobacterium tumefaciens* EHA105. The cultured *A. tumefaciens* harboring the constructed plasmids were used to infect rice embryo-derived calli. The transformed calli were selected on medium containing 50 μg/mL hygromycin and 250 μg/mL cefotaxime. Finally, the transgenic rice plants were regenerated from the transformed calli (Hiei et al. 1994; Toki 1997).

Histochemical GUS assay

Histochemical GUS activity assays were performed as described previously (Jefferson 1987). Half-cut germinated seeds from transgenic plants were placed in a solution containing 10 mM EDTA, 0.5 mM potassium ferricyanide, 0.5 mM potassium ferrocyanide, 1.0 mM 5-bromo-4-chloro-3-indolyl-β-D-glucuronide, 0.1% Triton X-100, and 0.1 M sodium phosphate buffer (pH 7.0) and incubated at 37°C for 4 h. The staining reaction was stopped by adding 75% ethanol.

Quantitative GUS activity assay

Developmental regulation of the *OsSUT2* promoter was analyzed in germinating embryos of transgenic rice seeds. Seeds were germinated in water containing hygromycin, and embryos were isolated from 3 and 5 DAI. To assay the effect of glucose on *OsSUT2* promoter activity, the seeds were imbibed in hygromycin-containing water for 3 days and the germinated embryos were isolated from transgenic seeds. The isolated embryos were cultured in MS medium containing 100 mM glucose solution for 5 days at 28°C in the dark. The protruding shoots and roots were excised and discarded before the embryo proteins were extracted with the buffer containing 100 mM sodium phosphate (pH 7.0), 5 mM dithiothreitol, 20 μg/ml leupeptin, and 20% (*v*/*v*) glycerol. Next, 4-methylumbelliferyl β-D-glucuronide was added as a substrate and GUS activities were measured fluorometrically in the embryos as described previously (Jefferson 1987).

Results

Subcellular localization of OsSUT2 protein

To characterize the protein structure and identify the subcellular localization of OsSUT2 protein, the *OsSUT2* coding sequence was amplified by RT-PCR. The *OsSUT2* cDNA (accession no. HQ875341) encodes a protein of 501 amino acids in length. According to membrane protein topology prediction using Hidden Markov Models in TMMOD software (Kahsay et al. 2005), the OsSUT2 protein contains 12 transmembrane domains (Fig. 1a, b). The amino- and carboxyl-terminal sequence tails predicted a cellular localization (Fig. 1b). Based on a previous phylogenetic analysis of the *SUT* gene family by Braun and Slewinski (2009), *OsSUT2* was grouped with *Arabidopsis AtSUT4*. Furthermore, Kühn and Grof (2010) showed that AtSUT4 is also classified with StSUT4 and LeSUT4 in the same group. The amino acid identities between OsSUT2 and StSUT4, LeSUT4, and AtSUT4 are 66%, 66%, and 64%, respectively. The significant differences within the above SUT genes are at the amino terminus and central inside loop (Fig. 1a). In addition, the central inside loop of OsSUT2 (35 amino acids in length) is smaller than that of LeSUT2 (94 amino acid residues) and AtSUT2 (87 amino acid residues), which belong to the other SUT group (Fig. 1b). To determine the subcellular localization of OsSUT2 protein, the expression of OsSUT2-GFP fusion protein was observed in the aleurone layer cells of barley seeds. Fluorescence imaging showed that the fusion protein was localized on the plasma membrane (Fig. 2).

Developmental expression of OsSUT2 in embryos during germination

To examine the expression of five *OsSUT* family genes in the embryos of germinating seeds, rice seeds were germinated in the dark for 3 days and then grown in phytotron with natural sunlight. The expression levels of *OsSUT3* and *5* were lower than those of the other three OsSUT genes at 1 and 5 DAI (Fig. 3a). Quantitative RT-PCR showed that the *OsSUT2* transcript level was significantly higher than that of other *OsSUT* genes at 5 DAI (Fig. 3a). Among *OsSUT1*, *2*, and *4*, only the *OsSUT2* mRNA of embryos was increased at 5 DAI compared to 1 DAI (Fig. 3a). Furthermore, the expression of *OsSUT2* was observed to gradually increase during the early germination stage, and the transcript level at 5 DAI was 4.6-fold that of dry seed embryos (Fig. 3b). In addition, the expression of *OsSUT2* in embryonic tissues increased with growth stage, even seedlings continuously grew in the dark after germination (Fig. 4a). However, the phenomenon of

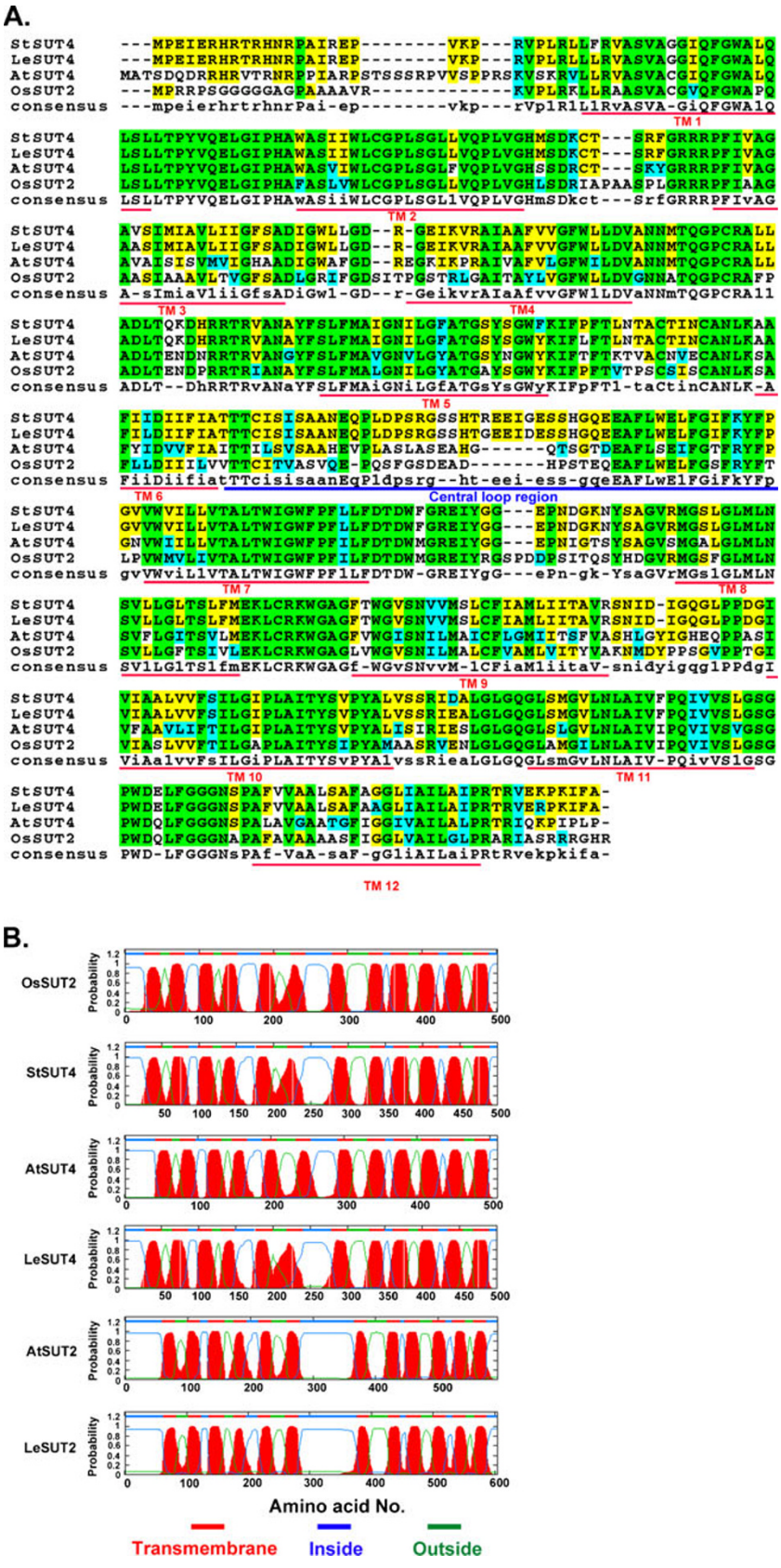

Fig. 1 Multiple amino acid sequence alignment and transmembrane domain analysis of SUT proteins. **a** Alignment of amino acid sequences from potato SUT4 (StSUT4; Genbank accession AF237780), tomato SUT4 (LeSUT4; AF176950), *Arabidopsis* SUT4 (AtSUT4; AY072092), and rice OsSUT2 (HQ875341). *TM* transmembrane domain. **b** Comparison of SUT protein structures. The inside and outside membrane regions and transmembrane domains of OsSUT2, StSUT4, AtSUT4, LeSUT4, AtSUT2 (AK226970), and LeSUT2 (AF166498) were predicted using TMMOD software.

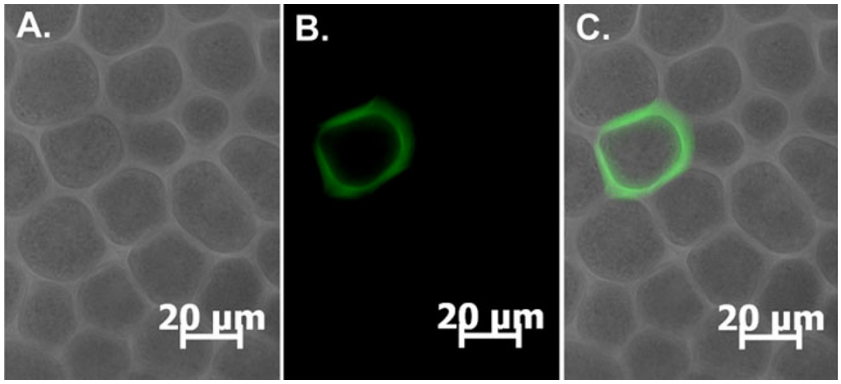

Fig. 2 Subcellular localization of OsSUT2 by transient expression of OsSUT2-GFP fusion proteins in barley aleurone layer cells. **a** Bright field image. **b** GFP fluorescence image. **c** Merged field and fluorescence image.

OsSUT2 up-regulation during germination was not obvious in dark-cultured isolated embryos (endosperm-free) (Fig. 4c).

Expression of *OsSUT2* regulated by exogenous sugars

OsSUT2 expression in germinating embryos was different from those with and without endosperm status. It was considered whether the sugar transported from endosperm during seed germination plays a role in the regulation of *OsSUT2* expression in embryos. To evaluate this possibility, the levels of glucose and sucrose were analyzed in the embryos of germinating seeds from 0 to 5 DAI. Glucose levels gradually increased with the days after imbibition, but sucrose content fluctuated (Fig. 5). The changes in glucose content corresponded to the expression pattern of *OsSUT2* transcripts in embryos during seed germination (Figs. 3b and 5). Even for seed germination in the dark, changes in glucose content and *OsSUT2* transcript levels were consistent (Fig. 4a, b). In order to determine whether sugars are the factors up-regulating *OsSUT2* expression in the embryos of germinating seeds, the embryos isolated from dry seeds were cultured on sugar-containing MS medium in the dark for 5 days. Although glucose (100 mM) and sucrose (100 mM) slightly enhanced *OsSUT2* mRNA levels in 1-day sugar-treated embryo samples, the effect was not significant (Fig. 6a). In 5-day cultured embryos, the *OsSUT2* expression was obviously up-regulated by both glucose and sucrose, but not by the same concentration of mannitol (Fig. 6b).

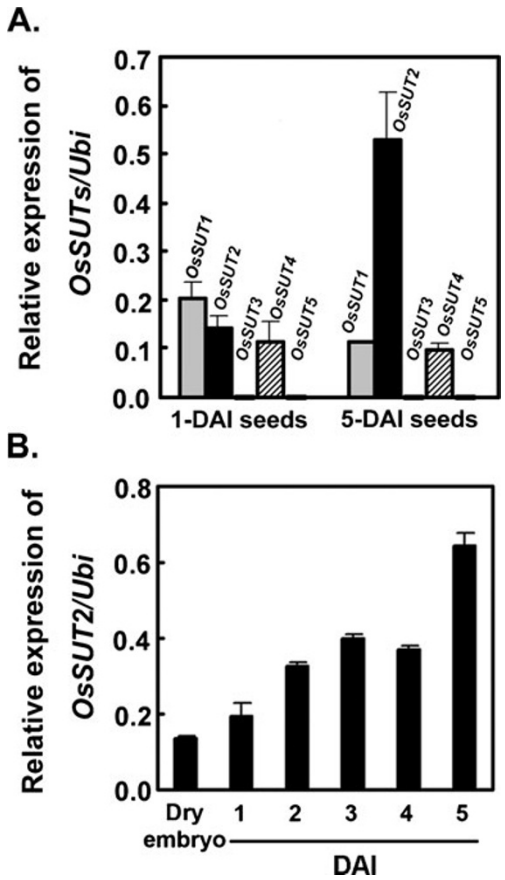

Fig. 3 Developmental expression of rice *OsSUT* in the embryos of germinating seeds. **a** Comparison of the expression of five OsSUT genes in the embryos of 1-DAI and 5-DAI seeds. **b** Changes in *OsSUT2* expression in embryos during seed germination. The data are presented as mean±SD.

Sugar analogs were applied to study the sugar-sensing pathway for regulating *OsSUT2* expression. 3-*O*-Methylglucose (3-OMG), a nonmetabolizable glucose analog, was taken up by cells but was not phosphorylated by hexokinase (Dixon and Webb 1979). The results showed that 3-OMG (100 mM) had the same effect as glucose (100 mM) to enhance *OsSUT2* expression (Fig. 7a). Palatinose, a nonmetabolizable sucrose analog, cannot be imported into plant cells (Bouteau et al. 1999). The effect of palatinose (100 mM) on OsSUT2 gene expression was similar to that of sucrose (100 mM) (Fig. 7b), suggesting that embryo cells sense sucrose signals to regulate *OsSUT2* expression through a membrane sensor.

OsSUT2 promoter::GUS expression in embryos

Transgenic rice plants harboring the *OsSUT2* promoter::*GUS* construct was used to investigate the spatial expres-

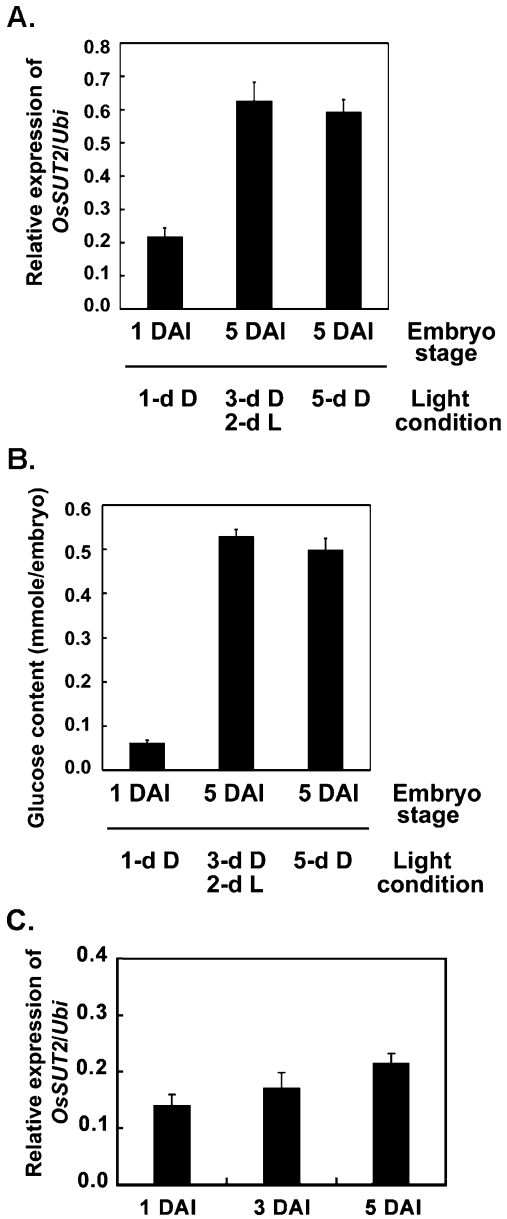

Fig. 4 *OsSUT2* expression and glucose content in embryonic cells from light- and dark-grown seedlings. Rice seeds were germinated in the dark for 3 days. Some germinated seeds were moved to a phytotron with natural daylight for 2 days (labeled 3-day D/2-day L), and some germinated seeds were kept in the dark (labeled 5-day D) for growth. The seedlings were collected at 1 and 5 DAI, and the embryos were isolated for *OsSUT2* expression analysis (**a**) and to measure glucose content (**b**). **c** The *OsSUT2* expression in germinating isolated embryos was analyzed. The embryos were dissected from dry seeds and dark-grown in MS medium. *OsSUT2* expression was detected after 1, 3, and 5 days of culture. The data are presented as mean±SD.

sion of *OsSUT2* in rice embryos. Rice seeds of three independent transgenic lines were germinated in water, and GUS expression in germinating seeds was observed. GUS activities were performed in aleurone layers and embryos. In embryos, significant GUS staining was detected in the

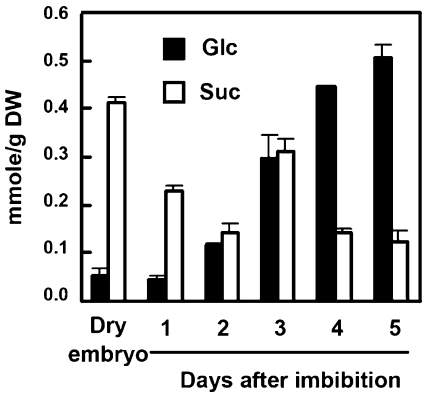

Fig. 5 Changes in the glucose and sucrose content of embryos during seed germination. The data are presented as mean±SD. *Glc* glucose, *Suc* sucrose.

vascular tissues and scutellum cells of embryos (Fig. 8). In addition, the quantitative GUS activity assay showed that the GUS activity in embryos from 5-DAI seeds was significantly higher than that of 3-DAI seeds (Fig. 9a). To identify the effect of glucose on *OsSUT2* promoter activity, the germinated embryos of transgenic rice seeds were isolated and incubated in 100 mM glucose solution for

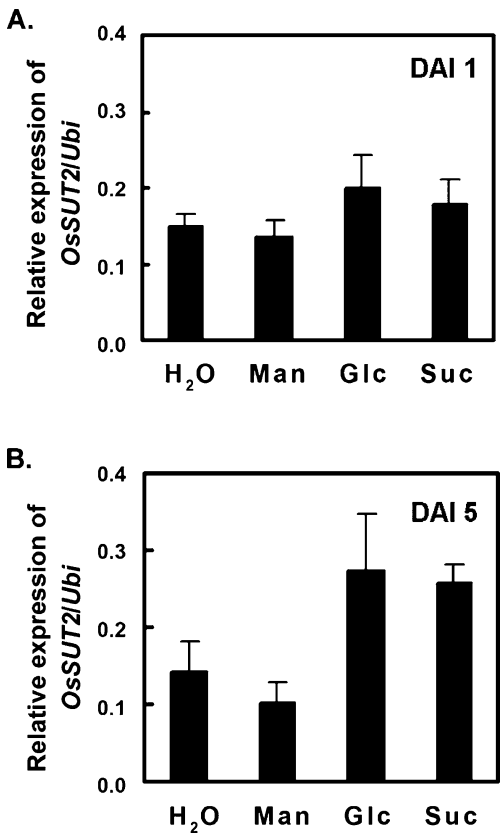

Fig. 6 Effects of sugars on *OsSUT2* expression. *OsSUT2* transcript levels were determined in isolated embryos cultured in medium containing mannitol (*Man*), glucose (*Glc*), or sucrose (*Suc*) for 1 day (**a**) and 5 days (**b**). The data are presented as mean±SD.

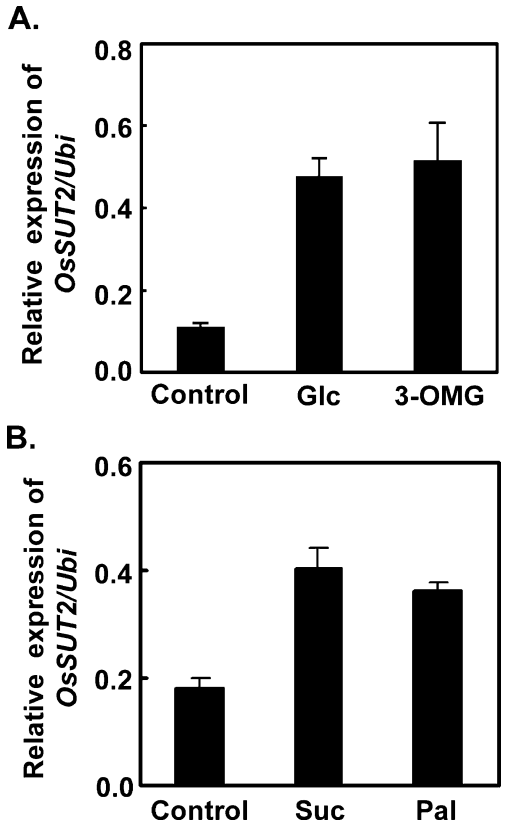

Fig. 7 Sugar-sensing pathways for OsSUT2 gene regulation in germinating embryos. **a** Effect of glucose analog 3-O-methylglucose (*3-OMG*) on *OsSUT2* expression after 5 days of treatment. **b** Effect of the sucrose analog palatinose (*Pal*) on *OsSUT2* expression after 5 days of treatment. The data are presented as mean±SD.

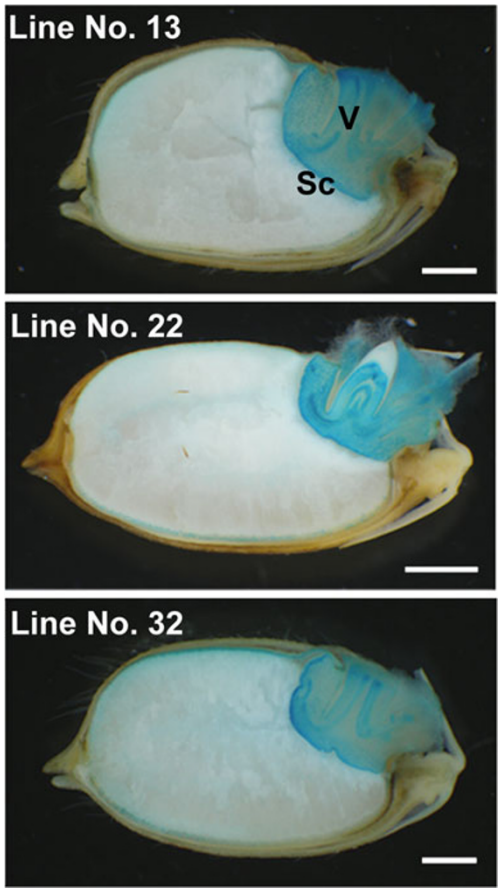

Fig. 8 Histochemical localization of β-glucuronidase activity in the embryos of transgenic rice plants harboring the *OsSUT2* promoter::*GUS* constructs. The seeds of transgenic rice lines 13, 22, and 32 were germinated in the dark for 3 days, and then the GUS activity was analyzed in half-cut germinated seeds. *Sc* scutellum cells, *V* vascular bundle. *Bar*=1 mm.

5 days. The *OsSUT2* promoter activities were obviously enhanced (Fig. 9b).

Discussion

SUT proteins are important carriers for transporting sucrose across the plasma membrane or vacuolar membranes (reviewed by Kühn and Grof 2010). *Arabidopsis* SUT protein has also been found on the chloroplast membrane (Rolland et al. 2003). Rice OsSUT2 is classified in the same group with *Arabidopsis* AtSUT4, tomato LeSUT4, potato StSUT4, *Lotus japonicus* LjSUT4, and barley HvSUT2 (Braun and Slewinski 2009; Kühn and Grof 2010). Some of the above-mentioned SUTs, including AtSUT4, StSUT4, and LjSUT4, have been identified as low-affinity/high-capacity transporters (Weise et al. 2000; Reinders et al. 2008). In addition, AtSUT4, LjSUT4, and HvSUT2 are vacuolar transporters according to a previous analysis of transient SUT–GFP fusion protein expression (Endler et al. 2006; Reinders et al. 2008). On the other hand, LeSUT4 and StSUT4 are located on the plasma membrane of sieve elements (Reinders et al. 2002). OsSUT2 contains 12 transmembrane domains and was localized on plasma membrane. Amino acid alignment showed that the number of amino acids in the central inside loops are similar among OsSUT2 and other SUTs in group 4 (according to the classification by Braun and Slewinski 2009) (Fig. 1); however, the length of the OsSUT2 central loop is shorter than that of SUTs belonging to group 2, i.e., AtSUT2 and LeSUT2. LeSUT2 is considered to function as a putative sucrose sensor (Barker et al. 2000). The conserved domains in the extended cytoplasmic loop of LeSUT2 and AtSUT2 play an important role in signal sensing and transduction (Barker et al. 2000). Because the lengths and amino acid sequences of the central loops are significantly different between OsSUT2 and LeSUT2, the regulatory mechanism and function of OsSUT2 might not be completely identical to that of LeSUT2.

Carbohydrate transport from endosperms and embryos to coleoptiles, shoots, and roots is an important process for supplying developing tissues with a carbon source during

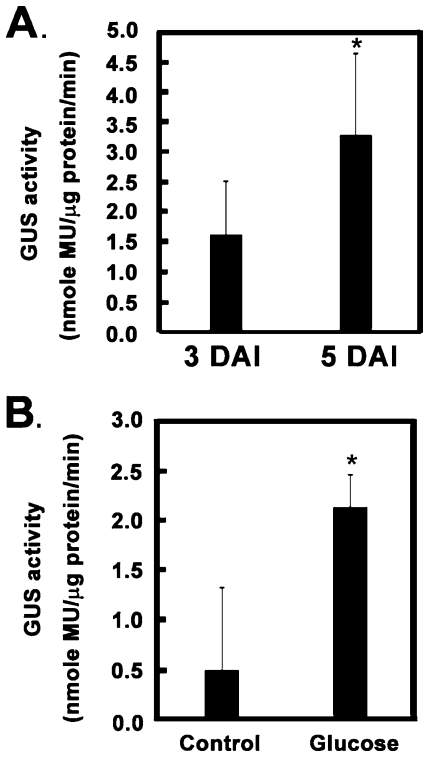

Fig. 9 Developmental regulation and effect of glucose on *OsSUT2* promoter activity. **a** GUS activity was quantitatively analyzed in the embryos from germinating seeds of transgenic rice carrying the *OsSUT2* promoter::*GUS* construct. **b** GUS activity was determined in isolated embryos cultured in medium with or without glucose. *MU* 4-methylumbelliferone. The data are presented as mean±SD, and statistical significance is set at $p<0.05$ (*asterisk*).

seed germination and seedling establishment. The levels of *OsSUT2* mRNA gradually increased from 1 to 5 DAI (Fig. 3b). However, our previous report showed that the *OsSUT1* transcript significantly increased after 1 to 2 DAI and then quickly decreased at 3 DAI (Chen et al. 2010). The expression level of *OsSUT1* was the highest among *OsSUT* members in embryos at early imbibition stages (i.e., DAI 1), but *OsSUT2* was the dominantly expressed *OsSUT* member 5 DAI (Fig. 3a). Thus, the functions and regulations of individual OsSUT genes are different in the embryos of germinating seeds. As shown by the data in Fig. 8, the expression levels of *OsSUT2* in scutellum and vascular bundles were higher than in other embryo ground cells, and the *OsSUT2* promoter activity was also significantly observed in aleurone layers. According to the *OsSUT2* expression patterns in germinating seeds, it was suggested that OsSUT2 play a role to release sucrose from aleurone layers, transport sucrose into scutellum, and also load sucrose into the phloem in germinating seeds. On the other hand, OsSUT1 gene was expressed in the scutellar vascular bundle of germinating embryos but not in the scutellar epithelial cell layer. Therefore, it was suggested that OsSUT1 functioned to load sucrose into phloem for transport to developing shoot and roots but not play a role to transport sucrose from endosperm to embryos (Scofield et al. 2007).

The increased *OsSUT2* expression in isolated embryos (without endosperms) during germination was not obvious in dark conditions; however, the significant up-regulation of *OsSUT2* was observed in the embryos of germinating seeds (with endosperms) in dark conditions. Thus, it was suggested that the sugar transported from endosperm was the key factor for promoting *OsSUT2* expression. Sugars not only act as nutrients and energy for supporting plant growth but also function as signals controlling plant development and the expression of various genes (reviewed by Gibson 2005; Rolland et al. 2006). The data in Fig. 6 showed that the transcript levels of rice *OsSUT2* could be slightly enhanced by glucose and sucrose after 1 day of treatment and significantly enhanced after 5 days of treatment. Moreover, since the *OsSUT2* expression was not affected by mannitol, the sugar-enhanced *OsSUT2* expression was not caused by osmotic effect. Sugar-enhanced expression has also been observed with *OsSUT1* (Matsukura et al. 2000; Chen et al. 2010). However, the positive effect of sugar on *OsSUT1* expression occurs after 5 days of treatment, and 1-day sugar treatment down-regulates *OsSUT1* expression (Chen et al. 2010). Thus, the mechanisms of sugar-mediated regulation are different for *OsSUT1* and *OsSUT2*. Sucrose-induced signal transduction for gene regulation could involve sucrose as a direct signal that is sensed by a sensor located on the cell membrane or an intracellular sensor. In addition, sucrose metabolites, such as glucose, could be signals to trigger the downstream transduction pathway (reviewed by Halford et al. 1999). The nonmetabolizable sucrose analog palatinose had a similar effect on *OsSUT2* expression in rice embryos as sucrose, suggesting that sucrose acts as a direct molecule for triggering the up-regulation of *OsSUT2* expression. In addition, the sensor for sucrose signal transduction is expected to be located on the cell membrane because of the lack of palatinose transport into plant cells (Sinha et al. 2002; Rolland et al. 2006). Moreover, since there was no effect of mannitol on *OsSUT2* expressions (Fig. 6), it was suggested that the up-regulation of *OsSUT2* expressions by palatinose was not caused by osmotic effect. Moreover, the data showing that 3-OMG can also conduct the same positive effect on *OsSUT2* expression in germinating embryos as glucose suggests that the glucose-induced *OsSUT2* expression was mediated by a hexokinase-independent pathway. In contrast, glucose-regulated *OsSUT1* expression in germinating embryos was via a hexokinase-dependent pathway (Chen et al. 2010).

Changes in the transcriptional activity of the *OsSUT2* promoter in embryos during germination correlated with the mRNA levels. *OsSUT2* promoter activity was also up-

regulated by glucose in embryos. Thus, promoter regulation was a key step for controlling *OsSUT2* expression. The predicted *cis*-acting elements on the *OsSUT2* promoter were searched in the PLACE database (Higo et al. 1999), identifying a SUSIBA2 transcription factor-binding site (WBOXHVISO1; W-box) 477 bp upstream of the ATG translation start codon. SUSIBA2 is a sugar-inducible WRKY protein that can bind the sugar-responsive element on the barley *isoamylase 1* promoter (Sun et al. 2003). Further study is needed regarding whether the interaction of SUSIBA2 and the W-box on the *OsSUT2* promoter is a key factor to controlling sugar-responsive *OsSUT2* expression.

In conclusion, we identified OsSUT2 as a plasma membrane transporter. *OsSUT2* expression in germinated embryos correlates with the developmental stage, with regulation depending on the sugar transported from endosperms. The developmental regulation and signaling pathways of sugar-responsive *OsSUT2* expression in germinating embryos are different from those of *OsSUT1*. Glucose-enhanced *OsSUT2* expression was mediated by a hexokinase-independent pathway, and sucrose is sensed by a sensor located on the plasma membrane to up-regulate *OsSUT2* expression. We also found that promoter activity is the major factor controlling the developmental stage- and sugar-dependent mRNA accumulation of *OsSUT2* in the embryos of germinating seeds. Future studies of the activity of the deleted promoter element and finding the factors that interact with the *cis*-acting element would be helpful for elucidating the molecular mechanism of *OsSUT2* expression in germinating rice embryos.

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