

RESEARCH PAPER

# Reduced expression of a gene encoding a Golgi localized monosaccharide transporter (*OsGMST1*) confers hypersensitivity to salt in rice (*Oryza sativa*)

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## Abstract

**Sugar transport is critical for normal plant development and stress responses. However, functional evidence for the roles of monosaccharide transporters in rice (*Oryza sativa*) has not previously been presented. In this study, reversed genetics was used to identify *OsGMST1* as a member of the monosaccharide transporter family in rice. The predicted 481 amino acid protein has the typical features of a sugar transporter in the plastid glucose transporter subfamily consistent with reduced monosaccharide accumulation in plants with reduced *OsGMST1* expression. *OsGMST1*-green fluorescent protein is localized to the Golgi apparatus. *OsGMST1* expression is induced by salt treatment and reduced expression confers hypersensitivity to salt stress in rice. *OsGMST1* may play a direct or an indirect role in tolerance to salt stress in rice.**

**Key words:** Golgi, monosaccharide transporter, NaCl stress, *Oryza sativa* L., rice, SGB1.

## Introduction

In higher plants, sugars function as an energy source, signal molecules, building blocks of polysaccharide structures, and osmotic regulators. Sugars are synthesized in the photosynthetic tissues (source) of the plant and then exported to heterotrophic tissues (sink), and this process requires several transport steps across membranes (Lalonde *et al.*, 1999; Williams *et al.*, 2000). Sugar transporters play key roles in sugar allocation at both the subcellular level and in long-distance transport via the phloem (Buttner, 2007). Long-distance transport is required for sinks to import photosynthetic assimilates from sources in the form of sucrose (Sauer, 2007). In sink tissues, released sucrose travels to sink cells via plasmodesmata and/or sucrose transporters (SUCs) or via monosaccharide transporters (MSTs) after hydrolysis

to glucose and fructose by cell wall-bound invertases (Williams *et al.*, 2000; Weschke *et al.*, 2003). As such, the process of long-distance transport involves both cell-to-cell transport as well as to different organelles in a single cell. The sugar transport mode is dependent on the plant developmental stage and the sugar location (Williams *et al.*, 2000). SUCs and MSTs are the main sugar transporters in plants.

MSTs mediate the transport of a wide range of monosaccharides including glucose, fructose, maltose, raffinose, and sugar alcohols (Turgeon and Medville, 2004; Klepek *et al.*, 2005). In *Arabidopsis*, 53 monosaccharide transporter (-like) genes group into seven subfamilies: (i) STP (plasma membrane monosaccharide transporter), (ii) ERD-like, (iii) VGT (vacuolar glucose transporter), (iv) PLT (polyol

transporter), (v) INT (inositol transporter), (vi) TMT (tonoplast monosaccharide transporter), and (vii) pGlcT (plastid glucose transporter) (Johnson *et al.*, 2006; Buttner, 2007). Most MSTs have 12 membrane-spanning domains separated by a cytoplasmic loop between transmembrane helices 6 and 7. In addition to transport across the plasma membrane, monosaccharides are also transported across organelle membranes, such as the inner plastid envelope (Weber *et al.*, 2000; Niittyta *et al.*, 2004) and tonoplast (Wormit *et al.*, 2006). MSTs on the Golgi apparatus (Wang *et al.*, 2006) and mitochondria (Szarka *et al.*, 2004) have also been reported. The rice genome encodes a total of 65 MST genes that group into the seven known subfamilies as defined by the *Arabidopsis* clades (Johnson and Thomas, 2007). The localization of a few of these transporters are known but, to date, nothing is known about their physiological functions (Toyofuku *et al.*, 2000; Ngampanya *et al.*, 2003; Mamun *et al.*, 2006; Oliver *et al.*, 2007; Wang *et al.*, 2007, 2008; Cho *et al.*, 2010).

In plants, sugars are signalling molecules that regulate plant growth and development and responses to biotic and abiotic stresses (Moore and Sheen, 1999; Rolland *et al.*, 2002, 2006; Gibson, 2005). There is evidence that abiotic stresses affect the expression of plant monosaccharide transporters. The transcript level of *TMT1* increased in response to salt, drought, and cold treatment in *Arabidopsis*, and *TMT1* is involved in vacuolar monosaccharide accumulation during cold stress (Wormit *et al.*, 2006). The expression of *ERD6*, which encoded a putative sugar transporter of *Arabidopsis*, was up-regulated by dehydration and cold treatment (Kiyosue *et al.*, 1998). *AtESL1* was also an abiotic stress-inducible monosaccharide transporter gene in *Arabidopsis* (Yamada *et al.*, 2010). In rice, expression of *OsMST7* and *OsMST8* were affected by cold treatment during male gametophyte development (Mamun *et al.*, 2006; Oliver *et al.*, 2007). Therefore, some MSTs may play a pivotal role during stress responses.

The Golgi-localized hexose transporter SGB1 (Wang *et al.*, 2006) belongs to the pGlcT subfamily in *Arabidopsis*. The gain-of-function mutant *sgb1-1<sup>D</sup>* is a suppressor of *agb1*, a null mutant of the  $\beta$  subunit of G protein. *sgb1* and *agb1* exhibited similar phenotypes with shorter hypocotyls and open hooks. SGB1 may transport glucose for cell wall biosynthesis in *Arabidopsis* during cell division (Wang *et al.*, 2006). To date, SGB1 is the only known monosaccharide transporter localized in the Golgi apparatus in plants.

OsGMST1, encoded by locus Os02g17500 with unknown function, showed the highest sequence identity (61%) with SGB1 by a BLASTP search. The function of OsGMST1, a Golgi-localized monosaccharide transporter in rice, is described here.

## Materials and methods

### Plant materials

Rice plants (*Oryza sativa* L. ssp. *Japonica* cv. Zhonghua 10) were grown under field conditions or in a greenhouse with a 16/8 h, 30/28 °C, light/dark photoperiod.

### Molecular cloning, vector construction, and the generation of transgenic rice

The cDNA fragment of *OsGMST1* was cloned by reverse transcription PCR (RT-PCR) using specific primers (forward primer 5'-ATGCGGTGGAAGCTTAAGTC-3' and reverse primer 5'-TCGCCCTTACTGGTCACA-3'), and then it was ligated into the binary vector pUN1301 digested with *Bam*HI and *Kpn*I in an antisense orientation to enable expression to be driven by the maize (*Zea mays*) ubiquitin promoter. This constructed vector (*Ubi::antiOsGMST1*) was genetically transformed into rice mediated by *Agrobacterium tumefaciens* EHA105 as previously described by Ge *et al.* (2004). The T<sub>2</sub> generations of transgenic plants were used for further functional characterization.

### Heterologous expression of OsGMST1 in yeast

The complete open reading frame of *OsGMST1* was ligated with vector pEX-Tag (Meyer *et al.*, 2000) digested by *Eco*RI in both the sense and antisense orientations, and then the recombinant vectors or empty vector were transformed into the yeast hexose transporter-deficient mutant EBY. VW4000 (Wieczorke *et al.*, 1999), to create strains S1, S2, AS1, and Vector. Transformed cells were pregrown in YEPM medium (1% yeast extract, 2% peptone, 2% maltose) to OD<sub>600</sub> of 1.0. Serial dilutions of cell suspensions were streaked on uracil-deficient solid medium containing 0.67% yeast nitrogen base with ammonium sulphate, supplemented with leucine, tryptophan, histidine, and various sugars, such as maltose, glucose, fructose, mannose, galactose, xylose, and ribose, and were incubated at 30 °C for 2 or 3 d.

### Subcellular localization of the OsGMST1-GFP fusion protein

A transient expression vector 35S::*OsGMST1-GFP* was constructed, in which *OsGMST1* was fused with the 5'-end of *GFP* driven by the 35S promoter. Protoplast transformation was as previously described (Sheen, 2001; Bart *et al.*, 2006). Briefly, leaf sheaths from 10-d-old etiolated rice seedlings were cut and digested by cellulase RS and macerozyme R-10 (Yakult, Japan), and the released protoplast cells were transformed by polyethylene glycol (PEG) mediation and then were incubated in darkness overnight. To observe the auto fluorescence of chloroplasts, transformed protoplast cells were incubated under dim light. Mitochondria were stained with 20 nM fluorescent dye MitoTracker Red CMXRos (Molecular Probes) for 2 min, and then visualized at an excitation wavelength of 543 nm and an emission wavelength of 599 nm. Chlorophyll autofluorescence was used as the chloroplast marker. Then, cells were observed under confocal microscope (LSM 510 Meta, Carl Zeiss).

### Quantitation of mRNA steady-state levels

Total RNA was prepared from seedlings using Trizol reagent (Invitrogen, USA) and first strand cDNA was synthesized with M-MLV reverse transcriptase (Promega, USA) according to the manufacturer's instructions, and PCR was used to clone genes by specific primers. Real-time PCR was performed by using SYBR Green Master Mix PCR reagent (Toyobo, Japan) and the MX3000P detector (Stratagene, USA). In the analysis of relative transcript level, gene-specific primers of *OsGMST1* were used with the rice  $\alpha$ -*TUBULIN* gene (Accession EF575922) as the internal control.

### Tissue expression pattern analysis

Total RNA was extracted from different tissues, and the *OsGMST1* transcript level was analysed by real-time PCR. *OsGMST1::GUS* transgenic plants were generated, and GUS staining of various tissues was performed by the histochemical method as previously described by Jefferson *et al.* (1987). Different organs of *OsGMST1::GUS* transgenic seedlings were incubated in

5-bromo-4-chloro-3-indolyl- $\beta$ -glucuronic acid buffer at 37 °C for 12 h. After staining, the tissues were destained in 70% ethanol several times until chlorophyll was removed and images were visualized on an Olympus SZX9 microscope.

#### Southern blot

Genomic DNA was extracted from 14-d-old seedlings of wild-type and transgenic rice. 20  $\mu$ g of DNA was digested with *Eco*RI or *Hind*III, electrophoresed on a 0.7% agarose gel, and transferred to a nylon membrane (Hybond N<sup>+</sup>; Amersham Pharmacia Biotech, Buckinghamshire, UK) under alkaline conditions. [ $\alpha$ -<sup>32</sup>P]dCTP-labelled *GUS* amplified from pUN1301 was used as a probe for hybridization. Membrane was stored in -70 °C for 2–4 d, and then exposed to X-ray film (Eastern Kodak, Rochester NY).

#### Expression pattern analysis with stress treatment

To detect the transcript level of *OsGMST1*, seedlings of wild-type rice plants were used. Two-week-old seedlings grown in Kimura B nutrient solution (Kato-Noguchi and Ino, 2005; Chen *et al.*, 2006) were treated with 200 mM NaCl or 18% PEG4000, followed by sampling at 0, 3, 5, 7, 12, 24, and 36 h. For the cold stress treatment, soil-grown seedlings were transferred to a chamber at 4 °C with a 12/12 h light/dark photoperiod, and sampled at the same time as the two stresses above.

#### Stress tolerance assays

Seeds of wild-type or transgenic rice plants were soaked in water at 28 °C for 2 d, and germinated seeds were sown in the 96-well plates from which the bottoms were removed. The plate was floated in Kimura B nutrient solution, and seedlings were incubated in a growth chamber with a 30/28 °C, 16/8 h light/dark photoperiod. For the NaCl treatment, 14-d-old or 21-d-old seedlings were treated in culture solution containing 150 mM or 200 mM NaCl for 9–11 d, and the treated seedlings were recovered for 7 or 8 d followed by survival rate analysis. For PEG treatment, 3-week-old seedlings were transferred to culture solutions containing 20% (w/v) PEG4000. For cold treatment, 3-week-old seedlings were incubated at 4 °C in a growth chamber for 4 d, and then removed to normal growth conditions for 7 d. For the seed germination assay, T<sub>2</sub> homozygous transgenic seeds were germinated on 1/2 MS medium supplemented with 0, 150, and 200 mM NaCl for 4–6 d, and were scored for germination based on whether the shoot length exceeded half that of the seed length.

#### Measurement of glucose/fructose/sucrose levels in leaves

Two-week-old seedlings of wild-type and transgenic rice were treated with 0 mM and 150 mM NaCl for 24 h, and then leaves were cut and weighed for fresh weight determination. Sugar extraction from the leaves was performed as described with modifications (Strand *et al.*, 1999). Briefly, leaves were ground to powder in liquid nitrogen, and then extracted with 80% ethanol at 80 °C for 40 min, and this extraction was repeated once. Extracts were evaporated to dryness then dissolved in sterile water. Spectroscopic quantification and calculation of the sugar contents were performed according to the protocol of D-glucose/D-fructose/sucrose kits (Biosentec, France).

## Results

### *OsGMST1* encodes a putative monosaccharide transporter

The *OsGMST1* full-length cDNA is 2196 bp, with an opening reading frame of 1446 bp, encoding a protein of 481 amino acids (Fig. 1A) with a predicted molecular mass

of 52 kDa and an isoelectric point of 8.45. It is a putative monosaccharide transporter, and has the sugar transport proteins signature 1 (Fig. 1A, underlined sequence) by the ExpASy (Expert Protein Analysis System) proteomics server (<http://ca.expasy.org/prosite/>). The plant membrane protein database Aramemnon (<http://aramemnon.botanik.uni-koeln.de/>) predicted 10–12 transmembrane domains, and TopPred (<http://mobyle.pasteur.fr/cgi-bin/portal.py?form=toppred>) predicted 12 hydrophobic transmembrane domains with a hydrophobicity value above 1.0 (Fig. 1A, double-arrow lines) arranged in two sets separated by a central hydrophilic loop (Fig. 1C). The transmembrane topology structure is consistent with the monosaccharide transporters in plants (Bush, 1999), yeast (Bisson *et al.*, 1993), and mammals (Bell *et al.*, 1993).

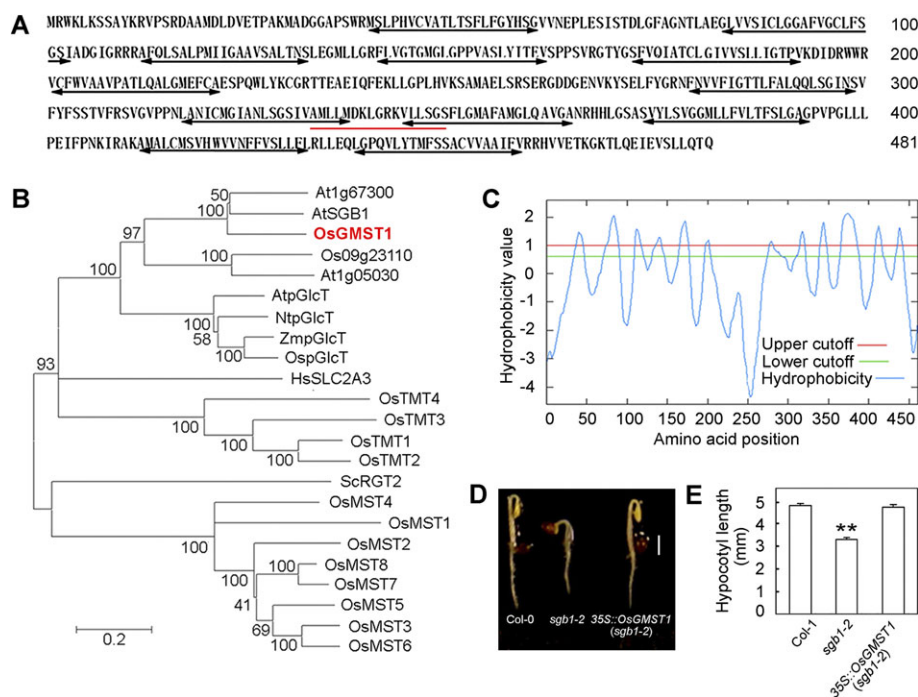
Phylogenetic analysis (Fig. 1B) shows that the putative *Arabidopsis* paralogues to *OsGMST1* are encoded by *At1g67300*, *SGB1*, *At1g05030*, and *AtpGlcT* and the proteins have 67%, 65%, 44%, and 43% identity to *OsGMST1*, respectively. These four MST proteins are in the pGlcT subfamily (Buttner, 2007). *SGB1* (suppressor of G protein beta1) was identified as a Golgi-localized hexose transporter in this subfamily (Wang *et al.*, 2006). Wang and coworkers showed that *SGB1* genetically complemented the phenotype of the *agb1* mutant lacking the  $\beta$  subunit of G protein and that the transcript-null mutant, *sgb1-2*, mimicked the *agb1* 2 d etiolated phenotypes with shorter hypocotyls and open hooks (Wang *et al.*, 2006). It has been proposed that, in rice (*Japonica*), *OsGMST1*, Os09g23110, and Os01g04190 (*OspGlcT*) belong to the pGlcT subfamily (Johnson and Thomas, 2007) with sequence identity approximately 36% (see Supplementary Fig. S1 at *JXB* online). Tobacco pGlcT and maize pGlcT belong to the pGlcT subfamily and are also closely related to *OsGMST1* (36.33% and 36.16% identity, respectively). Yeast RGT2 and human SCL2A are similar to *OsGMST1*.

Expression of *OsGMST1* in *sgb1-2* mutants rescued the short hypocotyl phenotype to the wild type ( $P=0.634$ ) (Fig. 1D, E), indicating that *OsGMST1* is paralogous to *SGB1*. As was the case for *SGB1* (Wang *et al.*, 2006), *OsGMST1* did not rescue the growth phenotype of a yeast strain EBY.VW4000 (Wieczorke *et al.*, 1999) which lacked 22 monosaccharide transporter genes, *HXT1-17*, *GAL2*, *STL1*, *AGT1*, *MPH2*, and *MPH3* (see Supplementary Fig. S2 at *JXB* online).

### *OsGMST1* is localized on the Golgi apparatus

To identify the subcellular localization of *OsGMST1*, a transient expression vector harbouring *OsGMST1*-GFP fusion protein driven by the 35S promoter (*35S::OsGMST1-GFP*) was constructed. Protoplast cells were prepared from leaf sheaths of 7–10 d dark grown rice seedlings, and then transiently transformed with recombinant or empty vector as a control. Fluorescence images were observed under the confocal microscope. *OsGMST1*-GFP fluorescence was distributed in cells in a punctate pattern (Fig. 2A, B), but GFP alone was distributed throughout the cytoplasm (Fig. 2C, D).





**Fig. 1.** Sequence characterization of *OsGMST1* and *OsGMST1* complements the short hypocotyl phenotype of *sgb1-2*. (A) Deduced amino acid sequence of *OsGMST1*; numbers on the right refer to the positions of amino acid residues. Sugar transporter signature 1 predicted for *OsGMST1* is indicated by the red underline. Double-headed arrows show the transmembrane regions with a high hydrophobicity value. (B) Phylogenetic analysis of *OsGMST1* homologues from rice and other organisms by MEGA 4.0. The predicted protein sequence is compared with *OsMST1-8*, *Os09g23110*, *OspGlcT*, and *OsTMT1-4* from *Oryza sativa*, *AtSGB1*, *At1g67300*, *At1g05030*, and *AtpGlcT* from *Arabidopsis thaliana*, *ScRGT2* from *Saccharomyces cerevisiae*, *HsSLC2A3* from *Homo sapiens*, *NtpGlcT* from *Nicotiana tabacum*, and *ZmpGlcT* from *Zea mays*. (C) *OsGMST1* is a membrane protein predicted by the TopPred program, showing 12 putative transmembrane helix domains. The red line and the green line indicate upper and lower cutoff of hydrophobicity, respectively. (D) Etiolated seedlings of Col-0 (WT), *sgb1-2*, and a transgenic line over-expressing *OsGMST1* in the *sgb1-2* mutant background. Bar = 2 mm. (E) The hypocotyl length of etiolated seedlings. A total of 36 hypocotyls were measured for each line (wild-type, mutant, and transgenic). Error bars represent SE. \*\* indicates  $P < 0.01$  by Student's *t* test. (This figure is available in colour at JXB online.)

To test the hypothesis that *OsGMST1* is localized to the Golgi as is *SGB1* (Wang et al., 2006), *OsGMST1*-GFP was co-expressed with the Golgi marker sialyltransferase (ST) tagged red fluorescence protein (RFP). Most of the red and green fluorescence merged (Fig. 2E, F, G, H), indicating that *OsGMST1* was in the Golgi body. By contrast, *OsGMST1*-GFP fluorescence did not co-localize with the mitochondrion fluorescence dye MitoTracker Red (Fig. 2I, J, K, L) or with chlorophyll autofluorescence (Fig. 2M, N, O, P).

#### *OsGMST1* is ubiquitously expressed

Transgenic rice plants expressing  $\beta$ -glucuronidase gene driven by the *OsGMST1* promoter showed that *OsGMST1* was expressed in all developmental stages from germination to flowering (Fig. 3A). Furthermore, quantitative PCR confirmed high levels of *OsGMST1* mRNA in all the organs tested, such as the shoot, root, culm, panicle, leaf, and leaf sheath before and after heading (Fig. 3B), suggesting that *OsGMST1* is ubiquitously expressed.

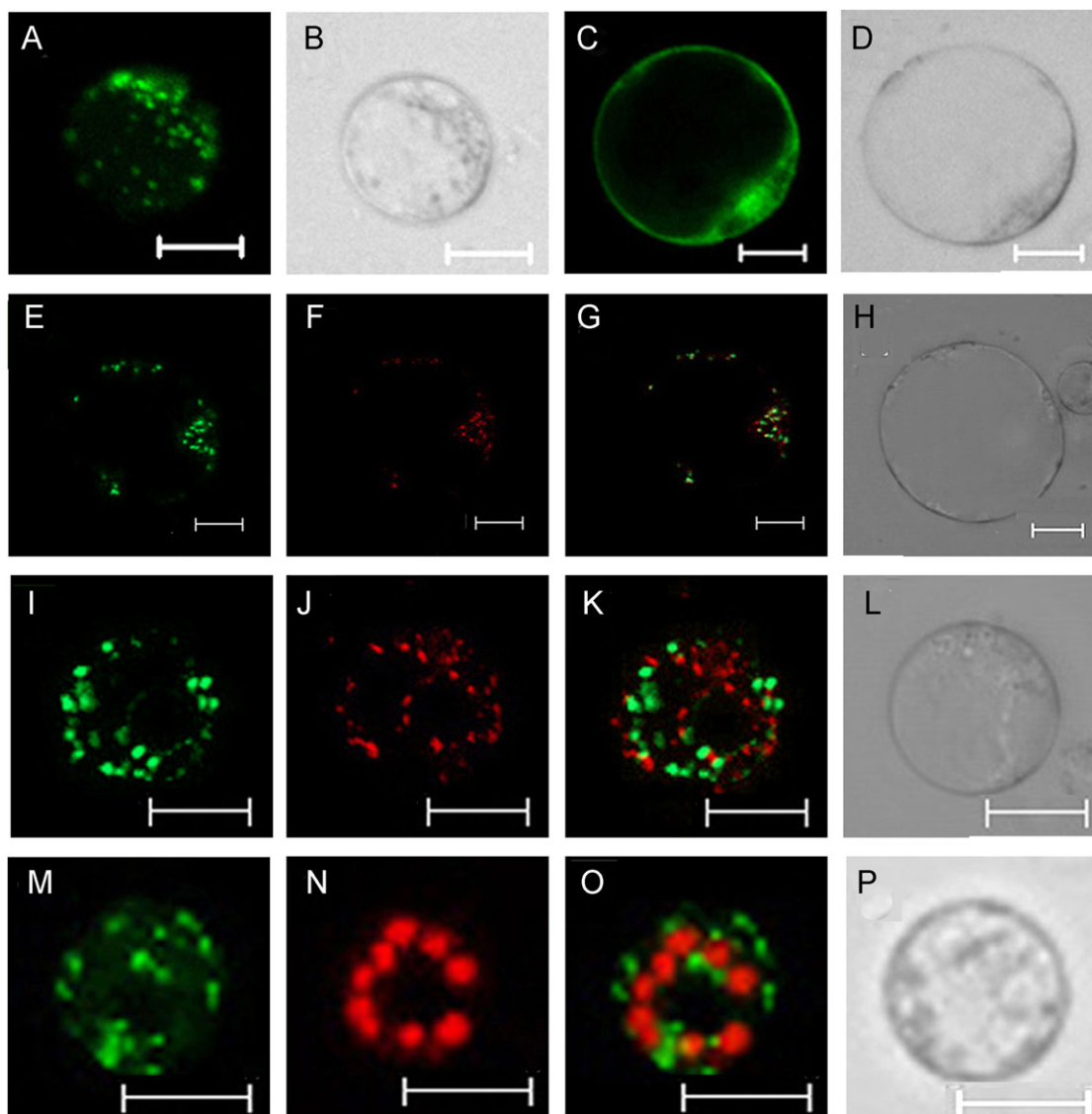
#### *OsGMST1* expression is salt responsive

The *OsGMST1* promoter sequence (1500 bp upstream of the start codon ATG) contains stress-responsive related

*cis*-elements, such as ABRE, a MYB recognition site, a MYC recognition site, and a GCC box (see Supplementary Fig. S3 at JXB online) based on predictions using the PLACE database (<http://www.dna.affrc.go.jp/PLACE>). As shown in Fig. 3C, the transcript level of *OsGMST1* in seedlings was induced by NaCl treatment but unaffected by low temperature (4 °C) and PEG4000. Salt treatment conferred a sustained increase in the steady-state level of the *OsGMST1* mRNA (Fig. 3C).

#### Reduced expression of *OsGMST1* confers hypersensitivity to NaCl

Reduced expression of *OsGMST1* by antisense technology was performed (see Supplementary Fig. S4A at JXB online; see the Materials and methods). Three independent transgenic rice lines, AS-L4, L12, L18 (see Supplementary Fig. S4B at JXB online) were confirmed by quantitative PCR to have reduced expression (see Supplementary Fig. S4C at JXB online). In AS-L12 and AS-L18, the expression of *OspGlcT* (Os01g04190) and *Os09g23110*, which have the highest nucleic acid sequence identity (46.8% and 39.8%) with *OsGMST1* were not affected (see Supplementary Fig. S4D at JXB online). In addition,



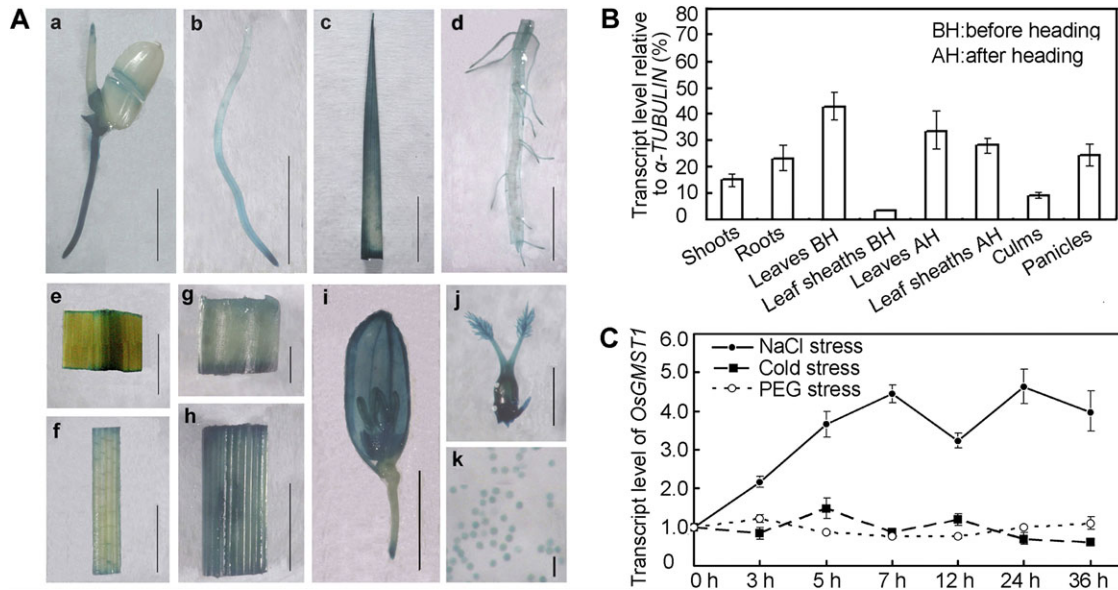
**Fig. 2.** Subcellular localization of OsGMST1-GFP transiently expressed in rice protoplasts. (A, B) A rice protoplast cell expressing OsGMST1-GFP (A) and its DIC image (B), showing a punctuate expression pattern. Bars=10  $\mu$ m. (C, D) A rice protoplast cell expressing GFP (C) as control and its DIC image (D), showing its distribution in nucleus, membrane, and cytoplasm. Bars=10  $\mu$ m. (E–H) A rice protoplast cell expressing OsGMST1-GFP (E), Golgi localized ST-RFP (F), a merged image (G), and its DIC image (H). Bars=10  $\mu$ m. (I–L) A rice protoplast expressing OsGMST1-GFP (I), stained by mitochondria dye MitoTracker Red (J), a merged image (K), and its DIC image (L). Bars=10  $\mu$ m. (M–P) A rice protoplast cell expressing OsGMST1-GFP (M), the chlorophyll autofluorescence (N), a merged image (O), and its DIC image (P). Bars=10  $\mu$ m. This figure is available in colour at *JXB* online.)

the expression of other genes might not be affected in the antisense transgenic lines, as sequence similarity of *OsGMST1* with other genes is lower than 30%, and there is no gene that has high similarity with *OsGMST1* in a short sequence stretch by BLAST search in rice. The development and morphology of the T2 progeny was no different from the wild type (WT) under normal growing conditions (see Supplementary Fig. S4E at *JXB* online).

There were no differences in the seed germination rate between WT and the transgenic lines without NaCl stress (Fig. 4A). Treatment with 150 mM and 200 mM NaCl, however, reduced the germination rate of AS-L12 and AS-L18 (Fig. 4A, B). At 200 mM NaCl, the germination rate of

the wild type was 90%, while the germination rates of the AS-L12 and AS-L18 lines were reduced to 40% and 50%, respectively ( $P < 0.01$ ) (Fig. 4B), and this difference was maintained over several days ( $P < 0.01$ ) (Fig. 4C).

Two-week-old rice seedlings of WT, AS-L12, and AS-L18 were treated with 200 mM NaCl as a supplement in Kimura B nutrient solution (see the Materials and methods) for 9 d, and then recovered in Kimura B nutrient solution alone for 8 d. Reduced expression of OsGMST1 also conferred reduced tolerance to salt stress in young seedlings (Fig. 4D). The survival rate of AS-L12 (40% and 20% in 150 mM NaCl and 200 mM NaCl, respectively) and AS-L18 (50% and 30%) were lower than the WT (70% and 50%)



**Fig. 3.** Expression pattern analysis of *OsGMST1*. (A) GUS histochemical staining in *OsGMST1*-promoter::*GUS* transgenic rice plants, showing a germinating seedling (a), young root (b), young leaf (c), mature root (d), mature leaf (e), leaf sheath (f), node (g), internode (h), panicle (i), pistil (j), and pollen grains (k). (a–f, h, i) Bars=5 mm; (g, j) 1 mm; (k) 0.1 mm. (B) Quantitative real-time PCR analysis of relative *OsGMST1* transcript levels in shoots, roots, leaves, leaf sheaths, culms, and panicles, and data are the percentage of  $\alpha$ -TUBULIN expression. The results are means  $\pm$ SD of triplicate assays. (C) Real time PCR analysis of *OsGMST1* transcript level under salt stress, PEG, and cold treatment with different time-courses. Error bars indicates SD of three replicates. This figure is available in colour at *JXB* online.)

with treatment of 150 mM and 200 mM NaCl ( $P < 0.01$ ) (Fig. 4E). By contrast, the tolerance of *OsGMST1* antisense plants to PEG and cold stress was the same as that of WT plants (Fig. 4F, G). Just as for *SGB1*, increased expression of *OsGMST1* had no effect (see Supplementary Fig. S5 at *JXB* online). The observation that *OsGMST1* overexpression in rice seedlings did not confer increased salt tolerance suggests that *OsGMST1* is not rate limiting.

#### Knockdown expression of *OsGMST1* reduced glucose and fructose content in leaves

Because *OsGMST1* is a putative monosaccharide transporter and its transcript is up-regulated by salt stress, the leaf sugar contents were determined in normal and salt stress conditions. As shown in Fig. 5A, under normal growth conditions, the glucose content was 4.0 mg g<sup>-1</sup> fresh weight in the WT, and 3.0 and 3.3 mg g<sup>-1</sup> fresh weight in the antisense transgenic plants, representing 25% and 20% reduced glucose content in knockdown plants ( $P < 0.01$ ). Fructose content was reduced by 25% and 12% of the WT ( $P < 0.01$ ) (Fig. 5B), whereas sucrose levels in transgenic rice plants were 1.5–2-fold of that in the WT, 10.0–14.0 versus 7.0 mg g<sup>-1</sup> fresh weight, respectively ( $P < 0.01$ ) (Fig. 5C). The data indicated that sugar levels were interrupted in *OsGMST1* knockdown rice, although they showed no visibly different phenotype compared with WT plants.

To analyse the differences in sugar accumulation in plants under NaCl stress further, the sugar levels were quantified after the wild-type and *OsGMST1* knockdown rice were treated with 150 mM NaCl for 24 h. In leaves of WT plants, salt stress caused glucose and fructose contents to increase

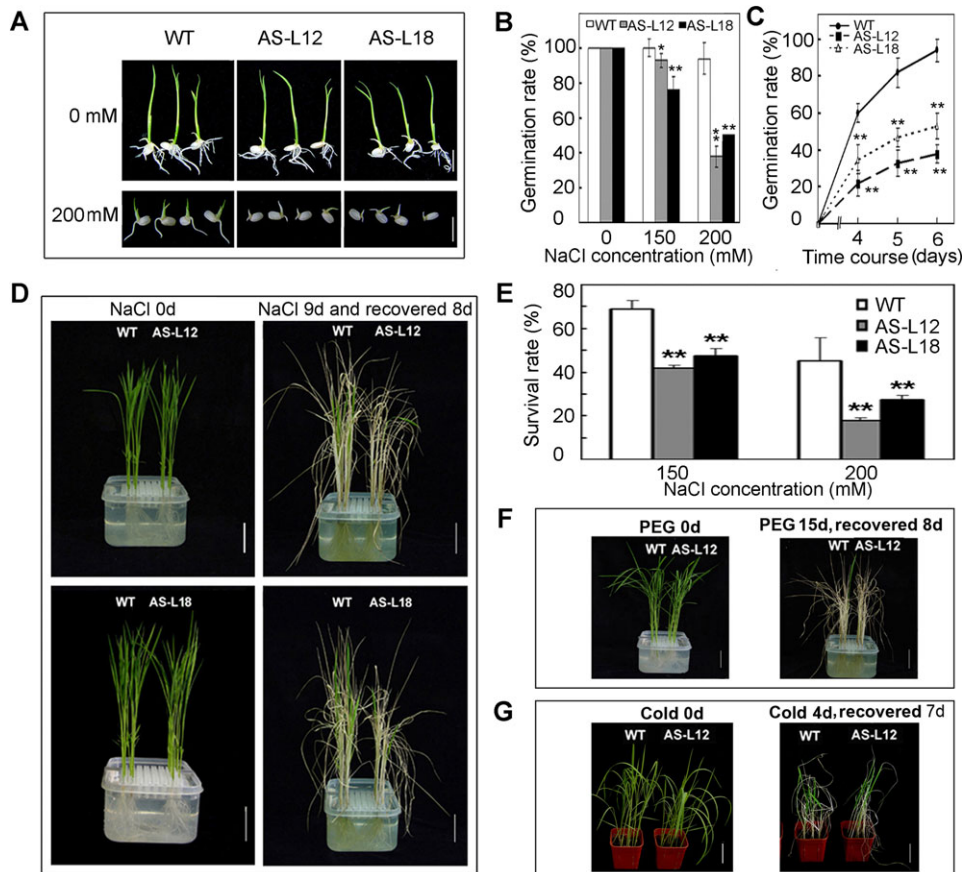
50% and 60% compared with that of the untreated control leaves, respectively ( $P < 0.01$ ) (Fig. 5A, B), and sucrose rose to 3-fold that of the untreated leaves ( $P < 0.01$ ) (Fig. 5C). In the antisense transgenic lines, glucose and fructose levels were not statistically increased by salt stress (Fig. 5A, B). In addition, sucrose contents in antisense plants accumulated only 0.5–1.0-fold higher than the untreated leaves in contrast to the 2-fold accumulation of sucrose in WT plants ( $P < 0.01$ ) (Fig. 5C). These results indicate that accumulation of sugars is, in part, by *OsGMST1*.

## Discussion

### *OsGMST1* is a Golgi-localized monosaccharide transporter in rice

Monosaccharide transporters play a pivotal role in the translocation and distribution of monosaccharides throughout the plant. In rice, 12 MSTs, *Osmst1*–8 and *Osmst1*–4 have been reported. Nevertheless, functional evidence for their roles in rice is not presented. In this study, we identified *OsGMST1* as a Golgi-localized monosaccharide transporter in rice. Although the transport activity of *OsGMST1* has not been characterized, we speculate that it is a sugar transporter based on its sequence characteristics (Fig. 1) and the effect on the sugar content when this gene expression is reduced in rice (Fig. 5). The orthologue of *OsGMST1* in *Arabidopsis* is *SGB1* (Wang et al., 2006), which is a Golgi-localized hexose transporter. Furthermore, expression of *OsGMST1* in the *sgb1-2* mutant can rescue the hypocotyl phenotype (Fig. 1). Therefore, we conclude





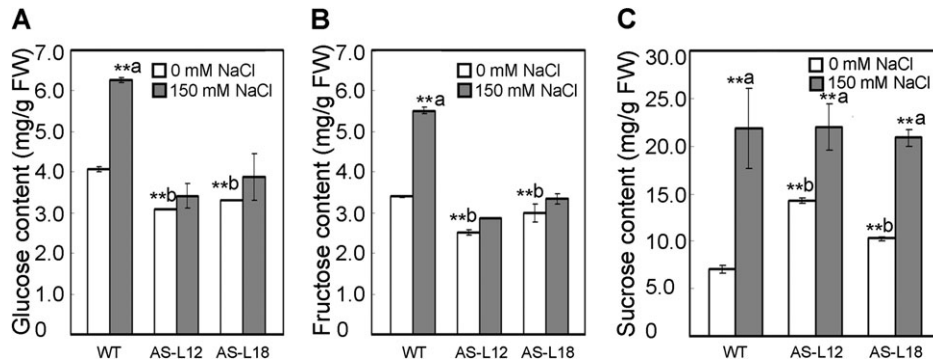
**Fig. 4.** Salt tolerance test of *OsGMST1* antisense transgenic rice plants. (A) Seed germination of WT and *OsGMST1* knockdown transgenic rice plants on MS medium containing 0 mM and 200 mM NaCl after 6 d. (B) Germination rate of WT and *OsGMST1* knockdown transgenic rice seeds with 0, 150, and 200 mM NaCl treatment for 6 d. Data are means  $\pm$ SD of three repeats, 20 seeds in each repeat. \* and \*\* indicate  $P < 0.05$  and  $P < 0.01$ , respectively by Student's *t* test. (C) Seed germination rate of WT and *OsGMST1* knockdown transgenic rice seeds with 200 mM NaCl treatment in 0–6 d. Data are means  $\pm$ SD of three repeats, 20 seeds in each repeat. \*\* indicate  $P < 0.01$  by Student's *t* test. (D) Growth of WT and transgenic seedlings before (left) and after (right) NaCl treatment. 2-week-old seedlings were treated with 200 mM NaCl for 9 d, and then recovered for 8 d. Bars=5 cm. (E) Survival rate of WT and transgenic seedlings after being treated with 150 mM and 200 mM NaCl. 15 seedlings at 2 weeks old were used in each repeat. Error bars are SE of three replicates. \*\* indicates  $P < 0.01$  by Student's *t* test. (F) PEG stress tolerance test of WT and *OsGMST1* knockdown seedlings. Three-week-old seedlings were treated with 20% PEG for 15 d, and then recovered for 8 d. Bars=5 cm. (G) Cold stress tolerance test of WT and knockdown seedlings. 3-week-old seedlings were treated with 4 C for 4 d, and then recovered for 7 d. Bars=5 cm. This figure is available in colour at *JXB* online.)

that the biochemical function of *OsGMST1* is the same or similar to *SGB1* function. Some monosaccharide transporter genes are only expressed in source tissues (Buttner *et al.*, 2000), and in sink tissues (Schneidereit *et al.*, 2003, 2005; Scholz-Starke *et al.*, 2003; Mamun *et al.*, 2006; Oliver *et al.*, 2007), or in both (Wormit *et al.*, 2006; Wang *et al.*, 2007). *OsGMST1* is expressed ubiquitously (Fig. 3A, B), suggesting that it may be involved in sugar translocation in both source and sink tissues of rice.

#### *OsGMST1* may play a role in salt-stress tolerance

Under abiotic stress conditions, soluble sugars derived from starch breakdown accumulate in plants to increase stress tolerance (Yano *et al.*, 2005; Lee *et al.*, 2009; Yamada *et al.*, 2010). The *Arabidopsis* *sex1* mutant, which was unable to accumulate sugars when exposed to freezing stress, displayed

impaired freezing tolerance (Yano *et al.*, 2005). *OsGMST1* knockdown rice seedlings are hypersensitive to NaCl stress compared with the WT (Fig. 4), as the NaCl-induced accumulation of glucose and fructose is impaired in knockdown plants, and the accumulation amount of sucrose is also different from the WT (Fig. 5). Sugars generate adenosine 5'-triphosphate (ATP) and other important metabolites needed for biosynthesis and growth during  $O_2$  deficiency stress of rice (Lee *et al.*, 2009). Sugars can also function as signals that affect gene expression under stress conditions (Hanson and Smeekens, 2009; Hey *et al.*, 2010), so there is cross-talk between sugar signalling and stress. The interaction of stress and sugar signalling is essential for plants to tolerate stress. Sucrose non-fermenting-1-related protein kinases (SnRKs) play a central role in the interaction of the two signalling pathways, which serve as metabolic sensors to adjust energy homeostasis under stress



**Fig. 5.** Effect of NaCl treatment on sugar content in rice leaves. Glucose (A), fructose (B), and sucrose level (C) in the leaves of WT and *OsGMST1* knockdown transgenic rice plants under 0 mM and 150 mM NaCl treatment. Two-week-old seedlings were treated with 0 mM and 150 mM NaCl for 24 h, and then the sugar levels of the leaves ( $\text{mg g}^{-1}$  FW) were measured as described in the Materials and methods. Twelve plants were used in each experiment. Data are the means of three independent repeats  $\pm$ SD. The confidence level for significant differences in sugar content between 0 mM and 150 mM NaCl is indicated by \*\*a ( $P < 0.01$ ); and between WT and knockdown plants under non-salt stress treatment (0 mM NaCl) confidence is indicated by \*\*b ( $P < 0.01$ ).

(Halford *et al.*, 2003; Halford and Hey, 2009). In rice, the SnRK1-dependent sugar-sensing cascade regulates sugar and energy production in order to enable rice growth under flooding stress (Lee *et al.*, 2009). In *OsGMST1* knockdown rice plants, the accumulation of sugars are affected under NaCl stress, suggesting that sugar signal transduction may be interrupted. Consequently, the expression of genes regulated by sugars may not be activated (or depressed) to provide energy for rice.

Expression of tonoplast localized *TMT1* and *TMT2* is induced by cold treatment, and glucose uptake into isolated leaf mesophyll vacuoles of WT plants is promoted by cold stress. In addition, *tmt* knockout lines accumulated less glucose and fructose compared with the WT plants (Wormit *et al.*, 2006), analogous to results found with reduced expression of *OsGMST1*.

*OsGMST1* resides in the Golgi apparatus (Fig. 2) and contributes to monosaccharide transport, and NaCl stress up-regulates the expression of *OsGMST1* transcriptionally (Fig. 3C). One possible explanation is that NaCl stress stimulates the monosaccharide transport activity of *OsGMST1* and, consequently, the activated *OsGMST1* upon salt stress import sugars into the Golgi or export them out of the organelle to keep cytosolic sugar at homeostasis. In conclusion, it is hypothesized that sugar homeostasis is affected in *OsGMST1* knockdown transgenic rice, which putatively results in altered sugar contents, so the seedlings show a NaCl hypersensitive phenotype.

In summary, *OsGMST1* is a novel Golgi-localized monosaccharide transporter that may play a role in salt stress tolerance in rice.

## Supplementary data

Supplementary data can be found at *JXB* online.

Supplementary Fig. S1. Alignment of *OsGMST1* with other members of the rice pGlcT (plastid glucose transporter) subfamily.

Supplementary Fig. S2. Growth of hexose transporter-deficient yeast cells transformed with sense and antisense *OsGMST1* on plates supplemented with sugars.

Supplementary Fig. S3. Distribution of stress related *cis*-elements in the *OsGMST1* promoter region.

Supplementary Fig. S4. Molecular identification and phenotype observation of *OsGMST1* knockdown transgenic rice plants.

Supplementary Fig. S5. Molecular identification and phenotype observation of *OsGMST1* overexpressed transgenic rice plants.

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## References

- Bart R, Chern M, Park CJ, Bartley L, Ronald PC. 2006. A novel system for gene silencing using siRNAs in rice leaf and stem-derived protoplasts. *Plant Methods* **2**, 13–21.
- Bell GI, Burant CF, Takeda J, Gould GW. 1993. Structure and function of mammalian facilitative sugar transporters. *Journal of Biological Chemistry* **268**, 19161–19164.
- Bisson LF, Coons DM, Kruckeberg AL, Lewis DA. 1993. Yeast sugar transporters. *Critical Reviews in Biochemistry and Molecular Biology* **28**, 259–308.
- Bush DR. 1999. Sugar transporters in plant biology. *Current Opinion in Plant Biology* **2**, 187–191.



- Buttner M.** 2007. The monosaccharide transporter(-like) gene family in *Arabidopsis*. *FEBS Letters* **581**, 2318–2324.
- Buttner M, Truernit E, Baier K, Scholz-Starke J, Sontheim M, Lauterbach C, Huss VAR, Sauer N.** 2000. AtSTP3, a green leaf-specific, low affinity monosaccharide-H<sup>+</sup> symporter of *Arabidopsis thaliana*. *Plant and Cell Physiology* **23**, 175–184.
- Chen RF, Shen RF, Gu P, Dong XY, Du CW, Ma JF.** 2006. Response of rice (*Oryza sativa*) with root surface iron plaque under aluminium stress. *Annals of Botany* **98**, 389–395.
- Cho JI, Burla B, Lee DW, et al.** 2010. Expression analysis and functional characterization of the monosaccharide transporters, OsTMTs, involving vacuolar sugar transport in rice (*Oryza sativa*). *New Phytologist* **186**, 657–668.
- Ge L, Chen H, Jiang JF, Zhao Y, Xu ML, Xu YY, Tan KH, Xu ZH, Chong K.** 2004. Overexpression of OsRAA1 causes pleiotropic phenotypes in transgenic rice plants, including altered leaf, flower, and root development and root response to gravity. *Plant Physiology* **135**, 1502–1513.
- Gibson SI.** 2005. Control of plant development and gene expression by sugar signaling. *Current Opinion in Plant Biology* **8**, 93–102.
- Halford NG, Hey S, Jhurreea D, Laurie S, McKibbin RS, Paul M, Zhang Y.** 2003. Metabolic signalling and carbon partitioning: role of Snf1-related (SnRK1) protein kinase. *Journal of Experimental Botany* **54**, 467–475.
- Halford NG, Hey SJ.** 2009. Snf1-related protein kinases (SnRKs) act within an intricate network that links metabolic and stress signalling in plants. *Biochemical Journal* **419**, 247–259.
- Hanson J, Smeekens S.** 2009. Sugar perception and signaling: an update. *Current Opinion in Plant Biology* **12**, 562–567.
- Hey SJ, Byrne E, Halford NG.** 2010. The interface between metabolic and stress signalling. *Annals of Botany* **105**, 197–203.
- Jefferson RA, Kavanagh TA, Bevan MW.** 1987. GUS fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *The EMBO Journal* **6**, 3901–3907.
- Johnson DA, Hill JP, Thomas MA.** 2006. The monosaccharide transporter gene family in land plants is ancient and shows differential subfamily expression and expansion across lineages. *BMC Evolutionary Biology* **6**, 64–83.
- 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.
- Kato-Noguchi H, Ino T.** 2005. Possible involvement of momilactone B in rice allelopathy. *Journal of Plant Physiology* **162**, 718–721.
- Kiyosue T, Abe H, Yamaguchi-Shinozaki K, Shinozaki K.** 1998. ERD6, a cDNA clone for an early dehydration-induced gene of *Arabidopsis*, encodes a putative sugar transporter. *Biochimica et Biophysica Acta* **1370**, 187–191.
- Klepek YS, Geiger D, Stadler R, Klebl F, Landouar-Arsivaud L, Lemoine R, Hedrich R, Sauer N.** 2005. *Arabidopsis* POLYOL TRANSPORTER5, a new member of the monosaccharide transporter-like superfamily, mediates H<sup>+</sup>-symport of numerous substrates, including myo-inositol, glycerol, and ribose. *The Plant Cell* **17**, 204–218.
- Lalonde S, Boles E, Hellmann H, Barker L, Patrick JW, Frommer WB, Ward JM.** 1999. The dual function of sugar carriers. Transport and sugar sensing. *The Plant Cell* **11**, 707–726.
- Lee KW, Chen PW, Lu CA, Chen S, Ho TH, Yu SM.** 2009. Coordinated responses to oxygen and sugar deficiency allow rice seedlings to tolerate flooding. *Science Signaling* **2**, ra61.
- Mamun EA, Alfred S, Cantrill LC, Overall RL, Sutton BG.** 2006. Effects of chilling on male gametophyte development in rice. *Cell Biology International* **30**, 583–591.
- Meyer S, Melzer M, Truernit E, Hummer C, Besenbeck R, Stadler R, Sauer N.** 2000. AtSUC3, a gene encoding a new *Arabidopsis* sucrose transporter, is expressed in cells adjacent to the vascular tissue and in a carpel cell layer. *The Plant Journal* **24**, 869–882.
- Moore B, Sheen J.** 1999. Plant sugar sensing and signaling: a complex reality. *Trends in Plant Science* **4**, 250–250.
- Ngampanya B, Sobolewska A, Takeda T, Toyofuku K, Narangajavana J, Ikeda A, Yamaguchi J.** 2003. Characterization of rice functional monosaccharide transporter, OsMST5. *Bioscience, Biotechnology, and Biochemistry* **67**, 556–562.
- Niittyla T, Messerli G, Trevisan M, Chen J, Smith AM, Zeeman SC.** 2004. A previously unknown maltose transporter essential for starch degradation in leaves. *Science* **303**, 87–89.
- Oliver SN, Dennis ES, Dolferus R.** 2007. ABA regulates apoplastic sugar transport and is a potential signal for cold-induced pollen sterility in rice. *Plant and Cell Physiology* **48**, 1319–1330.
- Rolland F, Baena-Gonzalez E, Sheen J.** 2006. Sugar sensing and signaling in plants: conserved and novel mechanisms. *Annual Review of Plant Biology* **57**, 675–709.
- Rolland F, Moore B, Sheen J.** 2002. Sugar sensing and signaling in plants. *The Plant Cell* **14**, SupplementS185–S205.
- Sauer N.** 2007. Molecular physiology of higher plant sucrose transporters. *FEBS Letters* **581**, 2309–2317.
- Schneidereit A, Scholz-Starke J, Buttner M.** 2003. Functional characterization and expression analyses of the glucose-specific AtSTP9 monosaccharide transporter in pollen of *Arabidopsis*. *Plant Physiology* **133**, 182–190.
- Schneidereit A, Scholz-Starke J, Sauer N, Buttner M.** 2005. AtSTP11, a pollen tube-specific monosaccharide transporter in *Arabidopsis*. *Planta* **221**, 48–55.
- Scholz-Starke J, Buttner M, Sauer N.** 2003. AtSTP6, a new pollen-specific H<sup>+</sup>-monosaccharide symporter from *Arabidopsis*. *Plant Physiology* **131**, 70–77.
- Sheen J.** 2001. Signal transduction in maize and *Arabidopsis* mesophyll protoplasts. *Plant Physiology* **127**, 1466–1475.
- Strand A, Hurry V, Henkes S, Huner N, Gustafsson P, Gardstrom P, Stitt M.** 1999. Acclimation of *Arabidopsis* leaves developing at low temperatures. Increasing cytoplasmic volume accompanies increased activities of enzymes in the Calvin cycle and in the sucrose-biosynthesis pathway. *Plant Physiology* **119**, 1387–1398.

- Szarka A, Horemans N, Banhegyi G, Asard H.** 2004. Facilitated glucose and dehydroascorbate transport in plant mitochondria. *Archives of Biochemistry and Biophysics* **428**, 73–80.
- Toyofuku K, Kasahara M, Yamaguchi J.** 2000. Characterization and expression of monosaccharide transporters (OsMSTs) in rice. *Plant and Cell Physiology* **41**, 940–947.
- Turgeon R, Medville R.** 2004. Phloem loading. A reevaluation of the relationship between plasmodesmatal frequencies and loading strategies. *Plant Physiology* **136**, 3795–3803.
- Wang HX, Weerasinghe RR, Perdue TD, Cakmakci NG, Taylor JP, Marzluff WF, Jones AM.** 2006. A Golgi-localized hexose transporter is involved in heterotrimeric G protein-mediated early development in *Arabidopsis*. *Molecular Biology of the Cell* **17**, 4257–4269.
- Wang Y, Xiao Y, Zhang Y, Chai C, Wei G, Wei X, Xu H, Wang M, Ouwerkerk PB, Zhu Z.** 2008. 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, 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 A, Servaites JC, Geiger DR, Kofler H, Hille D, Groner F, Hebbeker U, Flugge UI.** 2000. Identification, purification, and molecular cloning of a putative plastidic glucose translocator. *The Plant Cell* **12**, 787–802.
- Weschke W, Panitz R, Gubatz S, Wang Q, Radchuk R, Weber H, Wobus U.** 2003. The role of invertases and hexose transporters in controlling sugar ratios in maternal and filial tissues of barley caryopses during early development. *The Plant Journal* **33**, 395–411.
- Wieczorke R, Krampe S, Weierstall T, Freidel K, Hollenberg CP, Boles E.** 1999. Concurrent knock-out of at least 20 transporter genes is required to block uptake of hexoses in *Saccharomyces cerevisiae*. *FEBS Letters* **464**, 123–128.
- Williams LE, Lemoine R, Sauer N.** 2000. Sugar transporters in higher plants: a diversity of roles and complex regulation. *Trends in Plant Science* **5**, 283–290.
- Wormit A, Trentmann O, Feifer I, Lohr C, Tjaden J, Meyer S, Schmidt U, Martinoia E, Neuhaus HE.** 2006. Molecular identification and physiological characterization of a novel monosaccharide transporter from *Arabidopsis* involved in vacuolar sugar transport. *The Plant Cell* **18**, 3476–3490.
- Yamada K, Osakabe Y, Mizoi J, Nakashima K, Fujita Y, Shinozaki K, Yamaguchi-Shinozaki K.** 2010. Functional analysis of an *Arabidopsis thaliana* abiotic stress-inducible facilitated diffusion transporter for monosaccharides. *Journal of Biological Chemistry* **285**, 1138–1146.
- Yano R, Nakamura M, Yoneyama T, Nishida I.** 2005. Starch-related alpha-glucan/water dikinase is involved in the cold-induced development of freezing tolerance in *Arabidopsis*. *Plant Physiology* **138**, 837–846.