Sugar Uptake and Transport in Rice Embryo. Expression of Companion Cell-Specific Sucrose Transporter (*OsSUT1*) Induced by Sugar and Light[1]

Chi-aki Matsukura[2], Toshikazu Saitoh[2], Toshiro Hirose[3], Ryu Ohsugi, Pierdomenico Perata, and Junji Yamaguchi*

Bioscience Center and Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464–8601, Japan (C.M., T.S., J.Y.); Department of Plant Physiology, National Institute of Agrobiological Resources, Tsukuba, Ibaraki, 305, Japan (T.H., R.O.); and Dip. Biologia Patologia Vegetale, University of Bari, Via Orabona 4, I–70125 Bari, Italy (P.P.)

We investigated sugar uptake and transport in rice (*Oryza sativa*) embryo during grain germination. Endogenous sugar levels, accumulation of starch granules, and gene expression of a rice sucrose transporter (*OsSUT1*) were examined using rice embryos germinated with or without exogenous sugar supply. Starch granules remarkably accumulated in the cells around vascular bundles as a consequence of the sugar taken up by the embryos, indicating that the taken-up sugars are transiently converted into starch. In situ detection for *OsSUT1* mRNA indicated its localization in the phloem companion cells. Furthermore, northern-blot and in situ hybridization analyses showed that *OsSUT1* expression is not detectable in embryos subjected to sugar starvation conditions, whereas its expression is enhanced by an increased endogenous sugar level. Overall results indicate that the expression of companion cell-specific sucrose transporter, *OsSUT1* is regulated by the endogenous sugar status as well as light exposure.

Appropriate partitioning of assimilate between individual tissues is essential to sustain a normal growth and development in higher plants. Sugar transport is a fundamental process for the allocation of assimilates, but little is known about the regulation mechanisms of this crucial physiological step. Suc is the primary carbohydrate for long-distance transport of the carbon assimilates through the vascular system in many plant species. Direct evidence for apoplastic loading of Suc was obtained in dicot by the heterologous expression of yeast-derived invertase in leaves from tobacco, potato, and Arabidopsis (von Schaewen et al., 1990; Heineke et al., 1992).

The first Suc transporter was isolated from spinach by heterologous expression in *Saccharomyces cerevisiae* (*SoSUT1*; Riesmeier et al., 1992), which opened the way to investigate the role and localization of Suc transporters and to elucidate the mechanism of phloem loading in higher plants. In addition, other Suc transporters have been cloned from different species, e.g. potato, tomato, Arabidopsis, *Plantago major*, and rice (*Oryza sativa*; Gahrtz et al., 1994; Sauer and Stolz, 1994; Hirose et al., 1997; Kühn et al., 1997), and phloem-specific expression was demonstrated by antisense plant analysis and in situ hybridization in *P. major* (*PmSUC2*) and potato (*StSUT1*; Riesmeier et al., 1993; Stadler et al., 1995; Kühn et al., 1996). In recent years the regulation of expression of the Suc transporter gene (*SUT*) has been investigated; its steady-state level of mRNA is under developmental and hormonal control (Riesmeier et al., 1993; Harms et al., 1994; Truernit and Sauer, 1995). *SUT* mRNA and its protein were highly turned over in a few hours, correlating with diurnal regulation, a possible specific mechanism that controls the number of carriers in plasma membrane and determines Suc transport activity (Kühn et al., 1997). However, these findings are limited to the case of dicot species. It is therefore of importance to elucidate the mechanisms of Suc transport in monocots species including cereals.

In the germinating cereal grain, absorption of sugars derived from starch degradation in the endosperm provides the growing cereal seedling with a source of carbohydrates. Sugar transport in germinating seeds occurs from the apoplast (dead storage endosperm) across the plasma membrane of the embryonic scutellar epithelium cells. The molecular basis of transmembrane carbon transport by the cereal embryo (scutellum) is still obscure. Cereal embryo is also a suitable model system for studying sugar sensing machinery through the analysis of sugar-modulated α-amylase genes (Karrer and Rodriguez, 1992; Perata et al., 1997; Yamaguchi, 1998).

[1] This work was supported by Grants-in-Aid for Scientific Research on Priority Areas (grant nos. 10170215 and 09660004) from the Ministry of Education, Science, Sports and Culture, Japan.

[2] These authors contributed equally to this paper.

[3] Present address: Laboratory of Rice Metabolism, Hokuriku National Agricultural Experiment Station, 1–2–1 Inada, Joetsu, Niigata 943–0193, Japan.

* Corresponding author; e-mail jjyama@agr.nagoya-u.ac.jp; fax 81–52–789–5219.

OsSUT1 was the first isolated cDNA clone encoding a Suc transporter in monocots (Hirose et al., 1997). The cDNA clone encoded an open reading frame of 1,611 bp (537 amino acids) and showed 76.8% to 79.7% similarity at the amino acid level to other Suc transporters of dicot species. The predicted membrane topology of OsSUT1 protein is made up of 12 transmembrane helices, consistent with most of the mono- and disaccharide-transporters pervasively identified. When *OsSUT1* cDNA was introduced into yeast and expressed, the cells rapidly accumulated Suc demonstrating that *OsSUT1* does, in fact, encode a Suc transporter. From the northern analysis, *OsSUT1* has shown *SUT1*-type property, suggesting that it plays a role in phloem loading of Suc (Hirose et al., 1997).

In this study we investigated molecular and physiological basis for sugar uptake and transport using rice embryos and the tissue localization and sugar-dependent expression for *OsSUT1* transcript.

RESULTS AND DISCUSSION

Cellular Sugar Status Is Easily Manipulated Using Isolated Rice Embryos

Cells of dry embryo are poorly hydrated and lack mitotic activity since the embryo enters the quiescent stage during ripening of seeds. Their metabolism is almost completely arrested. However, vascular tissues have already developed and their network is distributed from the epithelium layer to both plumule and radicle (Fig. 1A). Anatomical observation showed that the epithelium of isolated embryo is covered with a fibrous layer to protect it from physical damage and α-amylases are produced from the intact scutellar epithelium cells (Yamaguchi, 1998). When the embryo was imbibed the coleoptile rapidly elongated, but the radicle did not—at least up to 120 h (radicle appeared at the later stage; Fig. 1B). After 36 h from the imbibition the longer plumule from the embryo supplied with 100 mM Glc appeared much faster in comparison with the one not fed with Glc, which was greenish in color.

Temporal changes of sugar contents in isolated rice embryos under sugar supply and embryos dissected from germinating grains are shown in Figure 2. Dry embryos contain their own carbohydrates, i.e. glucose (about 45 nmol), Fru (20 nmol), and Suc (100 nmol; Fig. 2, 0 h). We measured the levels of Glc, Fru, and Suc in the embryos that were imbibed with or without 100 mM Glc on the Gellan Gum plates. Mannitol (Fig. 2B) was used as a control to exclude possible osmotic effects. The embryos imbibed with 100 mM Glc showed an increase in their Glc and Fru content up to 72 h, whereas Suc content is kept constant (Fig. 2A). Similar results were obtained from the embryos that were imbibed with 100 mM Suc instead of Glc (data not shown). The embryos imbibed with 100 mM mannitol (Fig. 2B) and without

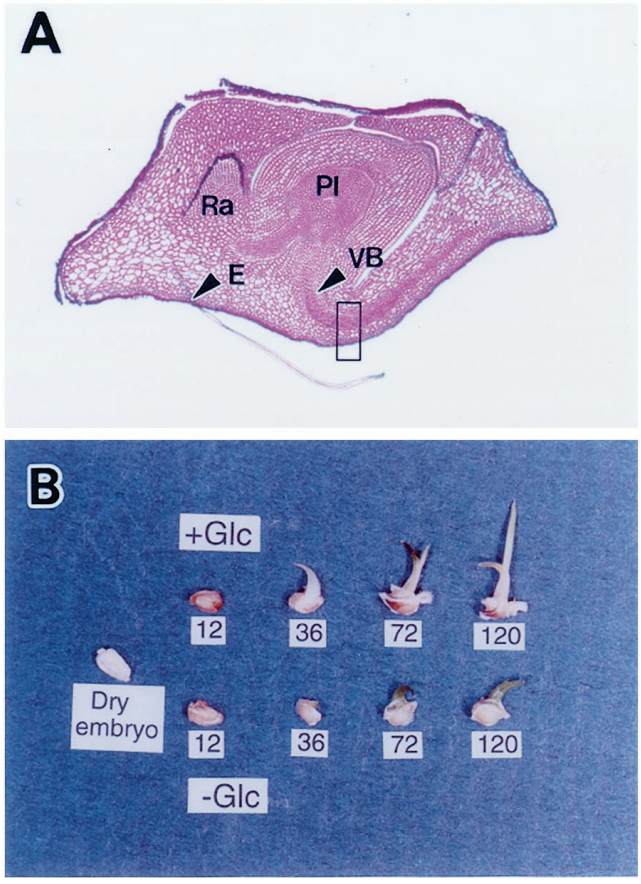

Figure 1. Anatomical structure of dry rice embryo (A) and germination of the isolated embryos (B) with or without application of 100 mM Glc. A, Longitudinal section of dry rice embryo by PAS staining. Scutellar epithelium cells (lowest cells) face the endosperm. Scutellar and vascular tissues in boxed area are also shown in Figure 3. Arrowheads E and VB indicate epithelium cell layer and vascular bundle, respectively. Pl, Plumule; Ra, radicle. B, The isolated embryos were imbibed and harvested after 12, 36, 72, and 120 h.

sugars (Fig. 2C) showed a rapid decrease in all sugars, especially Suc, and almost no sugars were detected 36 h after imbibition. Sugar contents of the embryos dissected from germinating grains (Fig. 2D) showed similar increase to that of the isolated embryos imbibed with 100 mM Glc (Fig. 2A), except for retarded increase of the sugar contents and higher accumulation of Suc content after 72 h. These results indicate that dry rice embryos store carbohydrate mainly as Suc and a relatively lower level of Glc and Fru, and that starvation on the Glc-free medium leads to a dramatic decrease in the endogenous sugar levels. Sugar-uptake assay showed that the isolated embryos have kept high-uptake ability of exogenous Glc through the time-course (Fig. 3A) and the taken-up substrates were partially translocated to shoot tissues at least after 72 h (Fig. 3B), indicating that the increase of the sugar contents shown in Figure 2 was due to the newly uptaken sugars. Thus we concluded that this experimental system is a suit-

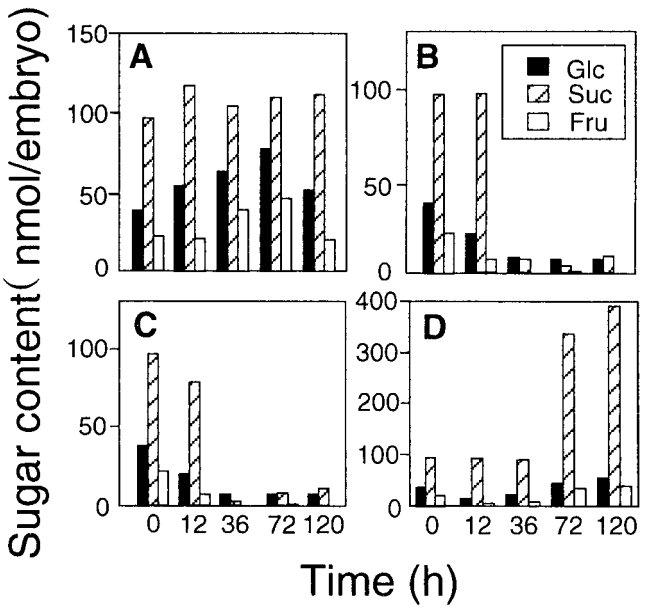

Figure 2. Endogenous levels of sugars in isolated rice embryos imbibed with 100 mM of Glc (A), mannitol (B), control (water; C), and the embryos dissected from germinating rice grains (D).

able model system for further characterization of the sugar transport machinery by manipulating its endogenous sugar levels.

Sugars Taken Up by Rice Embryo Are Transiently Converted to Starch

Temporal changes of starch accumulation in isolated rice embryos under sugar supply are shown in Figure 4. Starch granules in the embryos were visualized by periodic acid Schiff (PAS) staining (Fig. 4A) and the content was quantified (Fig. 4B). No starch granules were detected in the dry embryos, but a limited number of small granules were observed in the scutellar cells after 3 h of imbibition even under sugar starvation conditions. These starch granules disappeared from the embryos at 30 h after sugar starvation (Fig. 4A, −Glc, 30, 72, and 120 h), when no sugars were detectable (see Fig. 2C), and inner scutellar cells expanded longitudinally. In contrast the granules became larger in the embryos treated with Glc (Fig. 4A, +Glc, 30, 72, and 120 h) and were localized in the cells around vascular tissues, as well as in the scutellar cells near to the epithelium in which the remarkable morphological change of the inner scutellar cells was not observed. We speculate that the morphological difference of the scutellar cell treated with or without Glc is mainly caused by a distinct carbon flow, i.e. sugars coming through epithelium versus sugars coming from photosynthetic shoot. In the latter the epithelium cells would have no function as a sugar supplier and might be separated from the shoot by the expanded cells between them. Indeed the shoot from the embryo treated without Glc shows a much faster greening compared with that with Glc (Fig. 1B). Starch was not detected in dry embryos: the newly synthesized granules are therefore derived from sugars taken up by the embryos through the scutellar epithelium (Fig. 3). Similar starch accumulation was also observed in the scutellar cells of the germinating grains (data not shown). Glc is likely the primary sugar taken up by the embryos through the scutellar epithelium. Glc arising from starch degradation is taken up by the embryos in the starch degradation step during germination. The transport machinery for Glc in the epithelium has not been identified, although the peptide transporter was reported in the barley scutellum

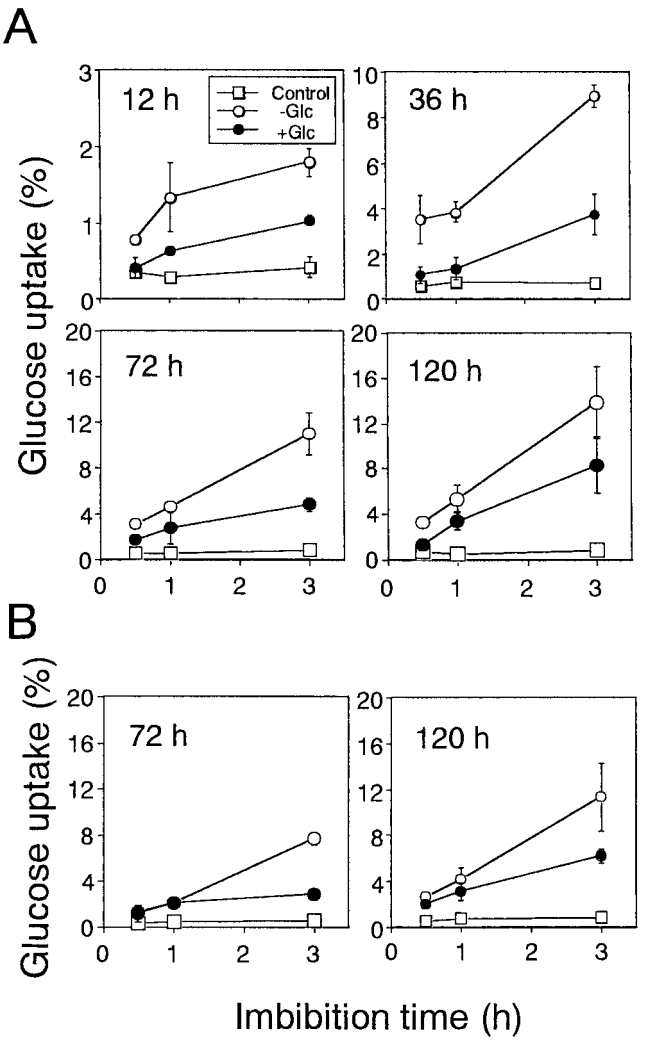

Figure 3. Sugar uptake by isolated rice embryos (A) and translocation to shoots (B). A, Samples for this assay were imbibed and harvested after incubation for 12, 36, 72, and 120 h. Glc uptake was assayed by incubating the three embryos at 30°C in radiolabeled Glc and uptake was measured at the intervals shown. B, After the imbibition the shoots harvested at 72 and 120 h were dissected from the scutellum and separately measured to estimate the translocation of taken up sugars. See "Materials and Methods" for definition of 100% uptake. Data are the means ± SE (n = 3).

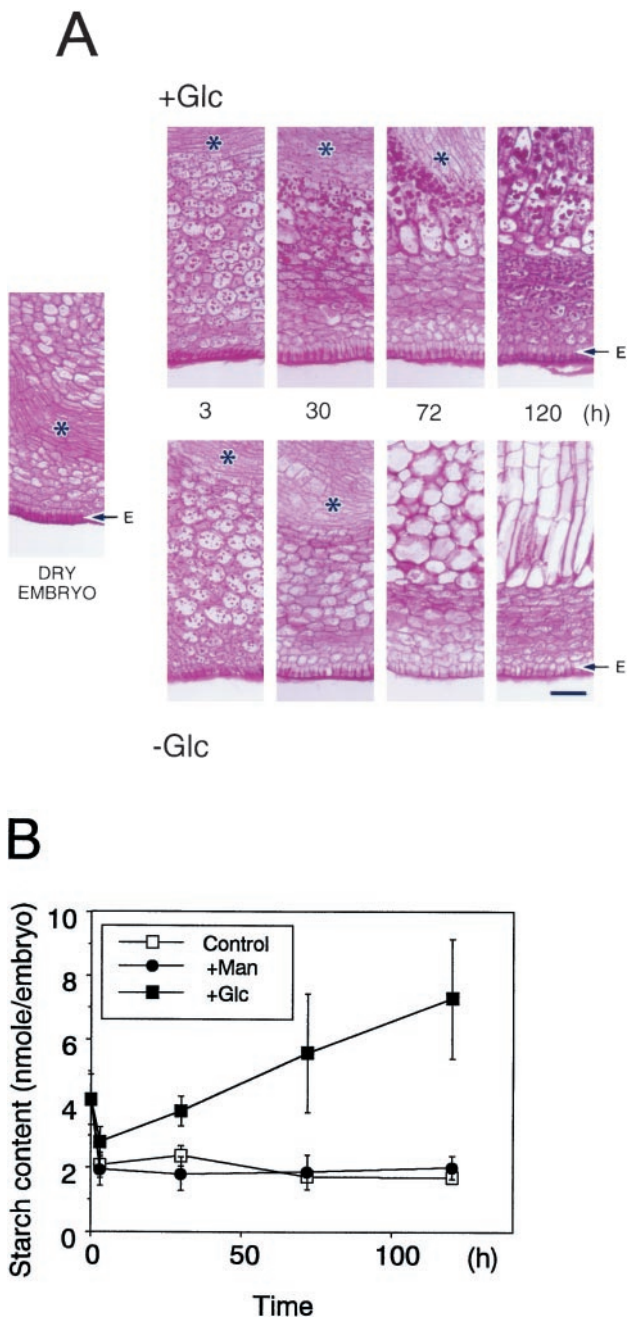

Figure 4. Accumulation of starch granules in embryonic scutellar cells of rice. A, The isolated embryo was imbibed without (−Glc) or with 100 mM Glc (+Glc) and sampled after 3, 30, 72, and 120 h. Longitudinal paraffin sections (10 μm thick) were stained by PAS reaction to visualize the starch granules and cell wall. See Figure 1 to identify the localization of the tissues within the whole embryo section. Asterisks and arrows E indicate vascular tissue from the plumule and epithelium cell layer, respectively. Bar = 0.02 mm. B, Endogenous levels of starch in isolated rice embryos imbibed with 100 mM of Glc, mannitol, and control.

(West et al., 1998). Monosaccharide transporter(s) might be involved in this step.

We have demonstrated that starch accumulation is closely correlated with endogenous sugar levels in the embryos (Figs. 2 and 4). These results indicate that regulation of starch production plays an important role in homeostasis of cellular sugar levels. Similar correlation was also reported in immature kernels of maize (Doehlert and Lambert, 1991) callus-forming embryo, and in leaf sheath in rice (Matsukura et al., 1998; Toyofuku et al., 1998).

Rice Suc Transporter (*OsSUT1*) Is Expressed in Vascular Companion Cells (CCs)

A rice Suc transporter (*OsSUT1*) cDNA has been cloned and characterized previously (Hirose et al., 1997). The cell type-specific expression of *OsSUT1* was analyzed in embryo and leaf sheath by in situ hybridization using a 0.8-kb fragment of 3′-coding region as probe. Figure 5B shows that the localization of Suc transporter mRNA is within the vascular bundle of scutellum at the first leaf stage. PAS staining of the adjacent section to visualize the starch granules showed that the starch accumulates in the cells surrounding the vascular tissue where the Suc trans-

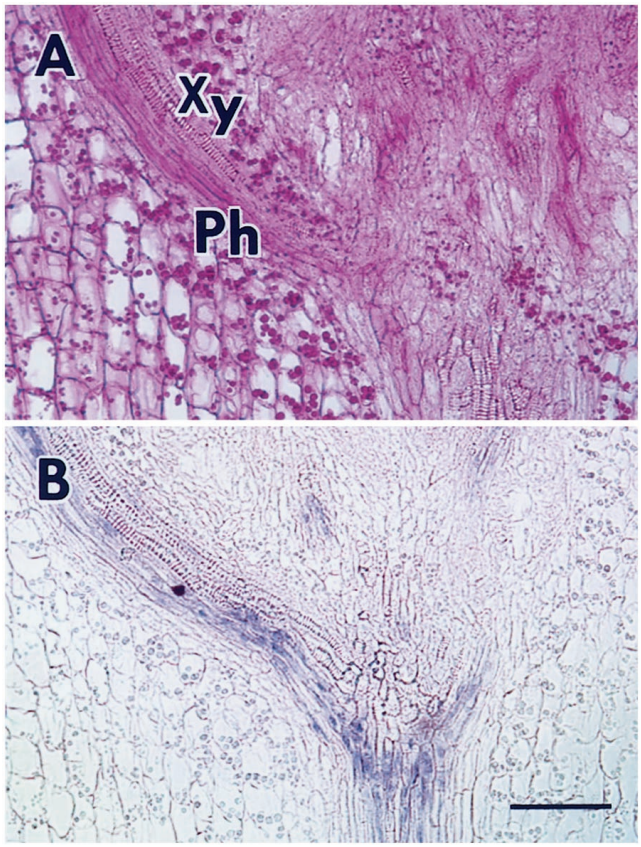

Figure 5. Localization of starch granules (A) and *OsSUT1* mRNA (B) in embryonic scutella of rice at the first leaf stage. PAS staining was used to visualize the starch granules and in situ hybridization with 0.8-kb digoxygenin (DIG)-labeled RNA fragment corresponding to the 3′-coding region of *OsSUT1* as a probe was performed using adjacent longitudinal sections (10 μm thick). Xy, Xylem; Ph, phloem. Bar = 0.1 mm.

porter mRNA is detected (Fig. 5A). Similar accumulation pattern of starch granules was also observed in the scutellar and leaf sheath cells (Figs. 4A, asterisks, and 6, A and C), indicating that the sugars are converted to starch and transiently deposited in the cells around the vascular bundles. To clarify the exact localization of the Suc transporter mRNA in vascular bundles, in situ analysis was also performed in the transversal and longitudinal section of first leaf (Fig. 6). As shown in Figure 6, B and D, *OsSUT1* mRNA was detected in the CCs (indicated by arrowheads and CCs), whereas the starch granules were detected in the cells around the vascular bundles. No signals for the Suc transporter mRNA were detected in other tissues or cells except the phloem CCs in leaf sheath. To evaluate the CC-specific localization of *OsSUT1* mRNA, in situ hybridization using the probe prepared from *RPP13-1* cDNA was performed (Fig. 7C). *RPP13-1* gene encodes thioredoxin h, which is an abundant protein in rice phloem sap and specifically expresses in CCs (Ishiwatari et al., 1995, 1998). Furthermore, to exclude possibilities of cross-hybridization to another *SUT* gene members in rice, identification of the signals was confirmed by in situ hybridization using probes of both *OsSUT1* 3′-coding and non-coding region (Fig. 7, A and B). As shown in Figure 7 the in situ analysis using the adjacent sections of series showed the identical localization of *OsSUT1* and *RPP13-1* mRNAs in the cells in vascular bundle, indicating that this Suc transporter is exclusively expressed in phloem CCs and may play a role in phloem loading in rice.

Although Suc transporter protein is probably localized in plasma membranes of the enucleate sieve elements (SEs) in tobacco, potato, and tomato, *SUT1* mRNA was detectable in both SE-CC complex in potato (Kühn et al., 1997). However, *OsSUT1* mRNA is exclusively localized in phloem CCs (CCs; Figs. 6 and 7) and similar results were also reported in PmSUC2 protein from *P. major* (Stadler et al., 1995). It is likely that Suc transporter is transcribed in CCs in monocots. The antisense repression of *SUT1* transcript with a CC-specific promoter leads to an inhibition of Suc transport in potato (Kühn et al., 1996). We cannot rule out at the present stage the possible localization of a minor amount of *OsSUT1* transcript in the SEs.

Expression of *OsSUT1* Is Regulated by Endogenous Sugar Status and Light

The effect of endogenous sugars on *OsSUT1* expression was investigated by RNA gel-blot analyses (Fig. 8). Total RNA was prepared from isolated embryos imbibed under various sugar conditions as shown in Figure 2, blotted, and hybridized with a

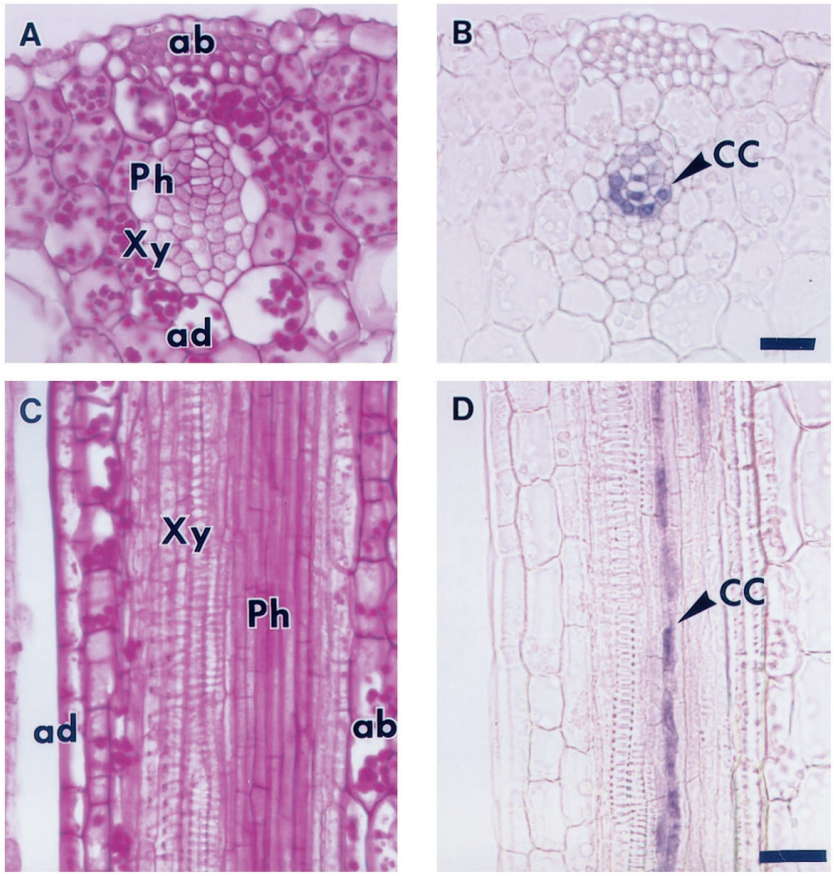

Figure 6. Localization of *OsSUT1* mRNA in leaf sheath of rice. The 5-d-old shoot was embedded in paraffin block and serially sectioned at 10 μm thick. Using the adjacent sections of these series PAS staining was used to visualize starch granules and cell walls (A and C) and in situ hybridization (B and D) were performed. A and B, Adjacent cross section; C and D, adjacent longitudinal section magnified around vertical vascular bundle sheath at the base of first leaf. *OsSUT1* mRNA was specifically localized in phloem CCs (indicated by arrowheads with CCs). ad, Adaxial side; ab, abaxial side; Ph, phloem; Xy, xylem. Bar = 0.02 mm.

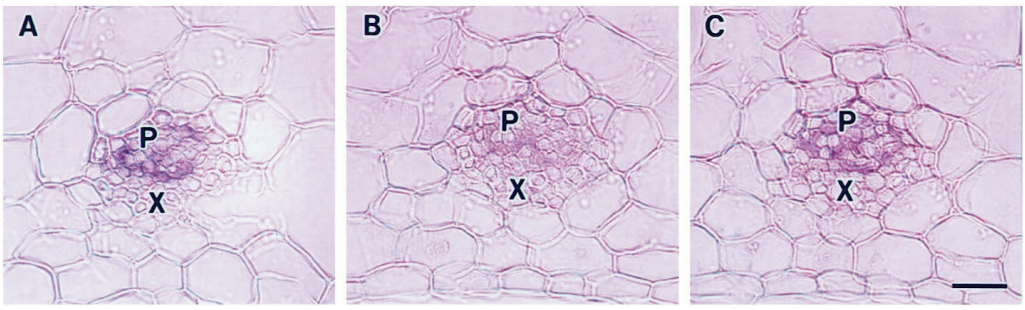

Figure 7. Identical localization of *OsSUT1* (A and B) and *RPP13-1* (C) mRNAs in leaf sheath of rice. The 5-d-old shoot was embedded in paraffin block and serially sectioned at 10 μm thick. Using the adjacent sections of these series in situ hybridization were performed with DIG-labeled RNA fragments corresponding to the 0.8-kb 3′-coding (A) and 0.34-kb non-coding region (B) of *OsSUT1*, and the 0.75-kb 3′-coding region of *RPP13-1* (C) as probe, respectively. P, Phloem; X, xylem. Bar = 0.02 mm.

radiolabeled *OsSUT1* probe (the same fragment used in the in situ analyses shown in Figs. 5B, 6, B and D, and 7A). Under light conditions, *OsSUT1* transcription was rapidly induced in all treatments, although relatively lower levels were detectable in the samples incubated in the absence of exogenous sugars (control in Fig. 8A) and 100 mM mannitol (+Man) compared with those treated with 100 mM Glc (+Glc). Light may affect induction directly through photoreceptors and indirectly through carbohydrate accumulation arising from photosynthesis. To exclude a direct light effect, the embryos were imbibed in darkness and sampled at the indicated times (12, 36, 72, and 120 h; Fig. 8B). The induction by sugars of *OsSUT1* transcription was detectable after 72 h (+Suc and Glc in Fig. 8B), whereas very slight and/or delayed induction was observed when embryos were incubated in the absence of sugars (control) or in the mannitol solution (+Man). In situ mRNA detection also showed that sugar-dependent induction for *OsSUT1* takes place in the vascular CCs (Fig. 8C).

We demonstrated that an increase in endogenous sugar levels (see Fig. 2) enhances the *OsSUT1* expression at the mRNA level (Fig. 8). The mRNA and protein for source-specific Suc transporter are also highly turned over in a few hours, correlating with diurnal regulation (Kühn et al., 1997). Since *OsSUT1* mRNA was detectable in the leaf blade, leaf sheath, and germinating grains by northern analysis (Hirose et al., 1997), this gene product is suggested to play a role in phloem loading. Indeed signals for *OsSUT1* mRNA were detectable in the embryos even without sugar supply when grown under the light conditions (see control in Fig. 8A). This suggests that the embryos without sugar supply produced carbohydrates by photosynthesis in the leaf sheath. Indeed under light, *OsSUT1* is expressed starting at 36 h (Fig. 8A), prior to leaf sheath greening, which occurs at 72 and 120 h as shown in Figure 1B (−Glc) and mRNA for chlorophyll *a/b* binding protein was detectable by northern blot in this stage (data not shown). Complete sugar starvation conditions with no sugar supply and under dark conditions revealed no or slight

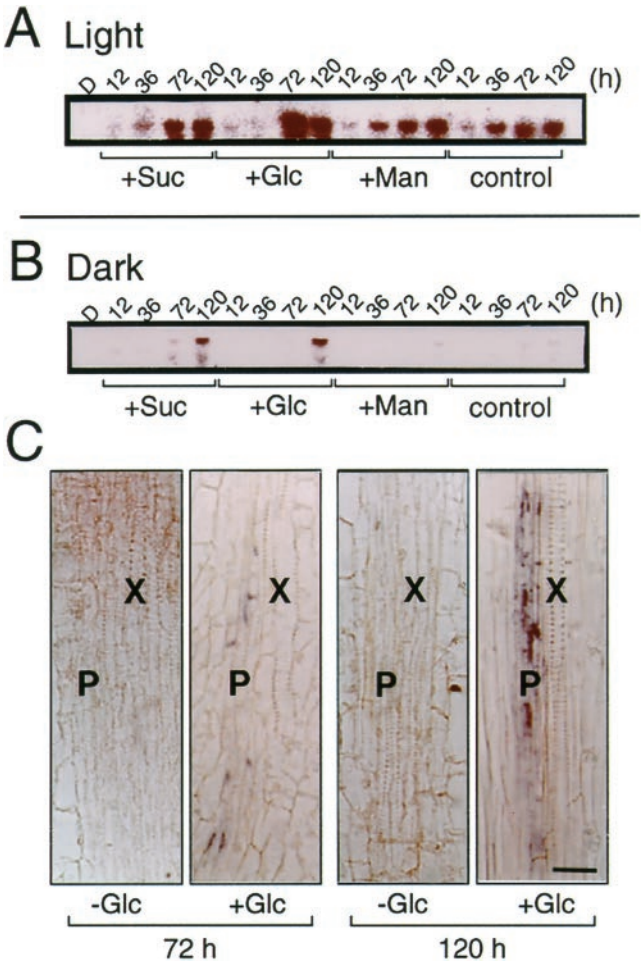

Figure 8. Northern-blot analysis of *OsSUT1* of isolated rice embryos fed with or without sugars. A and B, Accumulation of *OsSUT1* mRNA in isolated rice embryos germinated under light conditions (A) and dark conditions (B) with supplement of various sugars (+Suc; +Glc; +Man, mannitol at 100 mM; control, only distilled water). Mannitol treatment was used as a control of osmotic pressure. C, In situ hybridization using *OsSUT1* antisense probe on the sections from the sample germinated under the same conditions as in B. D, Dry embryo; P, phloem; X, xylem. Bar = 0.02 mm.

induction for *OsSUT1* transcription (control in Fig. 8B). Therefore the production of Suc transporter in the vascular tissues might be regulated by the cellular sugar status.

Examples of sugar-modulation of the expression of the Suc transporter were reported in fava bean (*VfSUT1*; Weber et al., 1997) and sugar beet (*BVSUT1*; Chiou and Bush, 1998). However, the sugars showed suppressive effect in both cases; i.e. *VfSUT1* is expressed in developing embryo of fava bean seed and its transcript level decreases by treatment with high concentration (150 mM) of either Suc or Glc compared with the low concentration (10 mM), whereas *BVSUT1* is mainly expressed in mature leaf of sugar beet and repressed by Suc (100 mM), but not Glc. These discrepancies might be due to the differences between dicots and monocots in their regulation of sugar transporters. However, we could not rule out the possibility that the observed increase in *OsSUT1* mRNA level is simply due to a developmental regulation; that is, the induction of CC-specific Suc transporter might be also regulated by the developmental stage of vascular tissues, i.e. the protein is expressed in the matured tissues. It is likely that the maturation of the tissues is trigger by cellular sugar status, as well as by light.

Sugar Uptake and Transport in Rice Embryo

In this study we used isolated rice embryos as an experimental system to characterize sugar uptake and transport processes. The following steps are involved: (a) Starch degradation products (mainly Glc) are taken up by embryonic epithelium cells through an unknown mechanism(s) (Figs. 2 and 3); (b) sugars in the epithelium cells are transported to the cells around vascular tissues and transiently deposited as starch granules (Fig. 4); (c) the starch is broken down and the resulting compounds are transported and/or finally converted into Suc in the vascular cells; and (d) the Suc molecules are loaded into the phloem by means of the Suc transporter (Figs. 5 and 6), whose expression is under positive regulation by the substrates (sugars; Fig. 8). Since *OsSUT1* mRNA was detectable in the leaf blade, leaf sheath, and germinating grains by northern analysis (Hirose et al., 1997), this gene product is suggested to play a role in phloem loading.

Sugar sensing and gene regulation by sugars in cereal embryos was reported in terms of α-amylase gene expression. Sugar perception for regulation of the *OsSUT1* gene still remains obscure, although hexose kinases might be involved in the processes (Perata et al., 1997; Umemura et al., 1998). Furthermore it has also been reported that sugar (Glc) transporters can be involved in sugar sensing in yeast (Özcan et al., 1996). Further investigations will be needed for identification and characterization of the mechanism(s) for sugar-transport and regulation of the transporter in the embryos including tracer experiments to clarify metabolite conversion.

MATERIALS AND METHODS

Plant Materials

Seeds of japonica rice (*Oryza sativa* L. cv Nipponbare) were obtained from the Nagoya University farm in 1996. To isolate embryos of rice (Yamaguchi, 1998), the seeds were passed through with huller machine (Ohtake, Japan) more than three times. The isolated embryos were manually selected. The embryos were sterilized in 0.25% (v/v) NaClO for 10 min and placed on the 0.25% (w/v) Gellan Gum gel containing 2 mM $CaCl_2$ and 100 mM Glc, Suc, mannitol, or no sugars, as indicated in each figure legend. The embryos were then incubated at 30°C in light or dark conditions. The growing embryos were harvested after 12, 36, 72, and 120 h.

Sugar Assay

Samples for this assay were imbibed and harvested after 12, 36, 72, and 120 h and stored at −80°C after freezing in liquid nitrogen. Samples were grounded in 5.5% (w/v) perchloric acid and extracted as described by Tobias et al. (1992). Glc, Suc, and Fru contents in isolated embryos were assayed by coupled enzymatic assay methods measuring the increase in A_{340} as described in Guglielminetti et al. (1995).

Sugar Uptake Experiments

Samples for this assay were imbibed and harvested after 12, 36, 72, and 120 h. Glc uptake was assayed by incubating the three embryos at 30°C in 2 mM $CaCl_2$ containing 1 mM Glc added with 1 μCi of radiolabeled Glc (D-[U-^{14}C]Glc; Amersham Pharmacia Biotech, Buckinghamshire, UK). Embryos were collected at the indicated times (30 min, 1 h, and 3 h), and washed three times with a 1 mM Glc solution to remove radiolabeled Glc eventually trapped (but not taken up by the cells) in embryos. After the imbibition the shoots harvested at 72 and 120 h were dissected from the scutellum and separately measured to estimate the translocation of taken-up sugars. In addition, incubation in the radiolabeled Glc solution was also performed using 5-min-boiled embryos to estimate the amount of Glc trapped. Uptake was determined by extraction of the embryos followed by scintillation counting. Each value at the designed time was obtained by the three independent experiments. One hundred percent uptake is defined as uptake of an amount of Glc corresponding to the theoretical equilibrium between the Glc concentration in the external medium and that inside of the cell.

Starch Determination

Starch content in embryos was measured by a modified method described in Raskin and Kende (1984). Samples for the assay were harvested after 3, 30, 72, and 120 h. Fifty

embryos per treatment were extracted in 0.5 mL of 80% (w/v) ethanol, boiled for 1 min at 100°C, centrifuged at 12,000*g* for 10 min and resulting pellet was resuspended in 80% (w/v) ethanol. This was repeated once more to remove soluble sugars. The resulting pellet was extracted in 0.3 mL of 200 mM KOH, boiled for 1 min, and centrifuged at 12,000*g* for 10 min. Eight microliters of 18% (w/v) acetic acid was added to 0.1-mL aliquots of supernatant to adjust the pH to around 5.0 and was then added to 0.1 mL of α-amylase solution (10 units of α-amylase in 100 mM sodium-acetate buffer, pH 5.3) and incubated at 37°C for 1 h. Reaction mixture was added to 0.1 mL of amyloglucosidase (1,4-α-D-glucan glucohydrolase from *Aspergillus niger*, Boehringer Mannheim, Basel) solution (1 unit of amyloglucosidase in 100 mM sodium-acetate buffer, pH 4.6), incubated at 55°C for 1 h, boiled for 2 min, and centrifuged at 1,300*g* for 10 min. Resulting Glc in the supernatant was analyzed enzymatically using hexokinase and Glc-6-P-dehydrogenase from Sigma (St. Louis) as described in "Sugar Assay."

RNA Isolation and Northern Blotting

RNA extraction was performed by the aurintricarboxylic acid method, as described by Skadsen (1993). Procedures of northern blots were performed by the standard method as described by Perata et al. (1997). Equal loading was checked by reprobing with an rRNA cDNA probe and ethidium bromide staining. Blots were exposed using a Bio-Imaging analyzer (Fujix BAS 2000, Fuji Photo Film, Tokyo).

In Situ Hybridization and PAS Staining

Isolated rice embryos were fixed in formaldehyde:acetic acid:50% [w/v] ethanol, 1:1:18) for 60 h at 4°C and then dehydrated in a graded 2-methyl-2-propanol series. They were embedded in Paraplast Plus (Fisher Scientific, Loughborough, Leicestershire, UK), serially sectioned by rotary microtome (10 μm thick), and applied on slide glasses treated with 3-aminopropyltrichlorosilane (Shinetsu Chemicals, Tokyo). DIG-labeled RNA probes were prepared from *OsSUT1* and *RPP13-1* cDNA clones, respectively. In situ hybridization and the detection of hybridization signals were performed as described in Kouchi and Hata (1993). Since hybridization signal was not detected when sense probes were used, only results obtained using the antisense probe are shown. PAS staining to visualize starch granules was performed according to Jensen (1962).

Received December 20, 1999; accepted May 5, 2000.

LITERATURE CITED

Chiou T-J, Bush DR (1998) Sucrose is a signal molecule in assimilate partitioning. Proc Natl Acad Sci USA **95:** 4784–4788

Doehlert DC, Lambert RJ (1991) Metabolic characteristics associated with starch, protein, and oil deposition in developing maize kernels. Crop Sci **31:** 151–157

Gahrtz M, Stolz J, Sauer N (1994) A phloem-specific sucrose-H^+ symporter from *Plantago major* L. supports the model of apoplastic phloem loading. Plant J **6:** 697–706

Guglielminetti L, Perata P, Alpi A (1995) Effect of anoxia on carbohydrate metabolism in rice seedlings. Plant Physiol **108:** 735–741

Harms K, Wöhner RV, Schulz B, Frommer WB (1994) Isolation and characterization of P-type H^+-ATPase genes from potato. Plant Mol Biol **26:** 979–988

Heineke D, Sonnewald U, Büssis D, Günter G, Leidreiter K, Wilke I, Raschke K, Willmitzer L, Heldt HW (1992) Apoplastic expression of yeast-derived invertase in potato. Plant Physiol **100:** 301–308

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 Cell Physiol **38:** 1389–1396

Ishiwatari Y, Fujiwara T, McFarland KC, Nemoto K, Hayashi H, Chino M, Lucas WJ (1998) Rice phloem thioredoxin h has the capacity to mediate its own cell-to-cell transport through plasmodesmata. Planta **205:** 12–22

Ishiwatari Y, Honda C, Kawashima I, Nakamura S, Hirano H, Mori S, Fujiwara T, Hayashi H, Chino M (1995) Thioredoxin h is one of the major proteins in rice phloem sap. Planta **195:** 456–463

Jensen W (1962) Botanical Histochemistry. W.H. Freeman, San Francisco

Karrer EE, Rodriguez RL (1992) Metabolic regulation of rice α-amylase and sucrose synthase genes *in planta.* Plant J **2:** 517–523

Kouchi H, Hata S (1993) Isolation and characterization of novel nodulin cDNA representing genes expressed at early stages of soybean nodule development. Mol Gen Genet **238:** 106–119

Kühn C, Franceschi VR, Schulz A, Lemoine R, Frommer WB (1997) Macromolecular trafficking indicated by localization and turnover of sucrose transporters in enucleate sieve elements. Science **275:** 1298–1300

Kühn C, Quick WP, Schulz A, Riesmeier JW, Sonnewald U, Frommer WB (1996) Companion cell-specific inhibition of the potato sucrose transporter SUT1. Plant Cell Environ **19:** 1115–1123

Matsukura C, Itoh S, Nemoto K, Tanimoto E, Yamaguchi J (1998) Promotion of leaf sheath growth by gibberellic acid in a dwarf mutant of rice. Planta **205:** 145–152

Özcan S, Dover J, Rosenwald AG, Wölfl S, Johnston M (1996) Two glucose transporters in *Saccharomyces cerevisiae* are glucose sensors that generate a signal for induction of gene expression. Proc Natl Acad Sci USA **93:** 12428–12432

Perata P, Matsukura C, Vernieri P, Yamaguchi J (1997) Sugar repression of a gibberellin-dependent signaling pathway in barley embryos. Plant Cell **9:** 2197–2208

Raskin I, Kende H (1984) Effect of submergence on translocation, starch content and amylolytic activity in deepwater rice. Planta **162:** 556–559

Riesmeier JW, Hirner B, Frommer WB (1993) Potato sucrose transporter expression in minor veins indicates a role in phloem loading. Plant Cell **5:** 1591–1598

Riesmeier JW, Willmitzer L, Frommer WB (1992) Isolation and characterization of a sucrose carrier cDNA from spinach by functional expression in yeast. EMBO J **11:** 4705–4713

Sauer N, Stolz J (1994) SUC1 and SUC2: two sucrose transporters from *Arabidopsis thaliana*. Expression and characterization in baker's yeast and identification of the histidine-tagged protein. Plant J **6:** 67–77

Skadsen RW (1993) Aleurones from a barley with low α-amylase activity become highly responsive to gibberellic acid when detached from the starchy endosperm. Plant Physiol **102:** 195–203

Stadler R, Brandner J, Schulz A, Gahrtz M, Sauer N (1995) Phloem loading by the PmSUC2 sucrose carrier from *Plantago major* occurs into companion cells. Plant Cell **7:** 1545–1554

Tobias RB, Boyer CD, Shannon JC (1992) Alterations in carbohydrate intermediates in the endosperm of starch-deficient maize (*Zea mays* L.) genotypes. Plant Physiol **99:** 146–152

Toyofuku K, Umemura T, Yamaguchi J (1998) Promoter elements required for sugar-repression of the *RAmy3D* gene for α-amylase in rice. FEBS Lett **428:** 275–280

Truernit E, Sauer N (1995) The promoter of the *Arabidopsis thaliana SUC2* sucrose-H^+ symporter gene directs expression of β-glucuronidase to the phloem: evidence for phloem loading and unloading by SUC2. Planta **196:** 564–570

Umemura T, Perata P, Futsuhara Y, Yamaguchi J (1998) Sugar sensing and α-amylase gene repression in rice embryos. Planta **204:** 420–428

von Schaewen A, Stitt M, Schmidt R, Sonnewald U, Willmitzer L (1990) Expression of a yeast-derived invertase in the cell wall of tobacco and Arabidopsis plants leads to accumulation of carbohydrate and inhibition of photosynthesis and strongly influences growth and phenotype of transgenic tobacco plants. EMBO J **9:** 3033–3044

Weber H, Borisjuk L, Heim U, Sauer N, Wobus U (1997) A role for sugar transporters during seed development: molecular characterization of a hexose and a sucrose carrier in fava bean seeds. Plant Cell **9:** 895–908

West CE, Waterworth WM, Stephens SM, Smith CP, Bray CM (1998) Cloning and functional characterization of a peptide transporter expressed in the scutellum of barley grain during the early stages of germination. Plant J **15:** 221–229

Yamaguchi J (1998) Analysis of embryo-specific α-amylase using isolated mature rice embryos. Breed Sci **48:** 365–370