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The Monosaccharide Absorption Activity of *Arabidopsis* Roots Depends on the Expression Profiles of Transporter Genes under High Salinity Conditions*

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Running title: *Absorption of sugars into* Arabidopsis *roots*

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Background: Transporters to absorb monosaccharides into *Arabidopsis* roots are poorly understood.
Results: *stp1* and *stp13* mutants are insensitive to exogenous monosaccharides. Monosaccharide uptake by STP1 and STP13 was affected by environmental stresses.
Conclusion: Contribution of STP13 to monosaccharide uptake become higher under high salinity conditions in which *STP13* is induced.
Significance: The expression profiles of transporter genes under environmental stresses influence monosaccharide uptake.

SUMMARY
Plant roots are able to absorb sugars from the rhizosphere but also release sugars and other metabolites that are critical for growth and environmental signaling. Reabsorbtion of released sugar molecules could help reduce the loss of photosynthetically fixed carbon through the roots. Although biochemical analyses have revealed monosaccharide uptake mechanisms in roots, the transporters that are involved in this process have not yet been fully characterized. In the present study, we demonstrate that *Arabidopsis* STP1 and STP13 play important roles in roots during the absorption of monosaccharides from the

rhizosphere. Among 14 *STP* transporter genes, we found that *STP1* had the highest transcript level and that STP1 was a major contributor for monosaccharide uptake under normal conditions. In contrast, *STP13* was found to be induced by abiotic stress, with low expression under normal conditions. We analyzed the role of STP13 in roots under high salinity conditions, where membranes of the epidermal cells were damaged, and we detected an increase in the amount of STP13-dependent glucose uptake. Furthermore, the amount of glucose efflux from *stp13* mutants was higher than that from wild type plants under high salinity conditions. These results indicate that STP13 can reabsorb the monosaccharides that are released by damaged cells under high salinity conditions. Overall, our data indicate that sugar uptake capacity in *Arabidopsis* roots changes in response to environmental stresses and that this activity is dependent on the expression pattern of sugar transporters.

INTRODUCTION

In plants, sugars are primary energy sources, substrates for polymer synthesis, storage compounds and carbon precursors for a wide range of anabolic and catabolic reactions. To distribute sugars throughout the entire plant, several transporters are required, not only to move the sugars across biological membranes at the subcellular level, but also for long-distance transport (1,2). In most plant species, the majority of soluble sugars are present in the forms of glucose, fructose and sucrose. Sucrose is the form that is used for transport into sink cells or heterotrophic organs and is taken up by sucrose transporters or hexose transporters, after cleavage into the monosaccharides fructose and glucose by cell wall-bound invertases. During the daytime, many plant species fix carbon and synthesize starch, which is transiently stored in the chloroplasts. The sugars are then mobilized to sink cells or to heterotrophic organs from the source leaves during the night to supply these areas with nutrients (3). However, roots can release the carbohydrates that are supplied from the source organs to the rhizosphere (4). It has been estimated that the amount of carbon lost within root exudates ranges from 1 to 10 percent of the net carbon that is fixed by photosynthesis (5). Moreover, in addition to carbohydrates, root exudates also contain a variety of organic compounds (4), some of which are involved in the interaction of plants with their surrounding environment. For example, root exudates are important sources of nutrients for microbes in the rhizosphere and can also participate in the early colonization of roots by microbes through the induction of chemotactic responses of microbes in the rhizosphere. In fact, a chemotactic response has been demonstrated by endophytic bacteria with rice root exudates that contain several carbohydrates and amino acids (6). Moreover, *Arabidopsis* roots export malic acid to the rhizosphere upon bacterial infection and malic acid recruits a beneficial microbe to the roots, which can induce a systemic resistance to infection in leaves (7). These findings suggest that roots may regulate the amount of carbohydrates in the rhizosphere as part of the maintenance of the surrounding environment.

In addition to providing signals for interacting with the rhizosphere, roots are also able to absorb carbohydrates from surrounding environments. The results of a number of physiological analyses have indicated that the influx of monosaccharides into roots is mediated by monosaccharide/proton symporters and that the external pH conditions influence the influx transport activity of glucose in maize root protoplasts (8). The uptake of glucose in cotton roots has been shown to be significantly inhibited by the addition of carbonyl cyanide m-chlorophenylhydrazone (CCCP), a protonophore that disrupts the proton motive gradient of membranes (9).

The transporters that are responsible for the uptake of monosaccharides in *Arabidopsis* have been extensively studied. STP1, a monosaccharides/proton symporter, is involved in the absorption of monosaccharides in *Arabidopsis* (10). An *stp1* knockout mutant is insensitive to galactose and mannose, and the level of uptake of monosaccharides in *stp1* mutant plants was shown to be lower than in wild type plants (11). However, the precise role of STP1 in the uptake of the monosaccharides in the roots was unclear, because whole plants were used to measure the level of monosaccharide uptake.

In the present study, we identified the transporters that absorb monosaccharides into *Arabidopsis* roots from the surrounding environment. We demonstrated that STP1 and STP13 are involved in the absorption of monosaccharides into roots. Also, environmental stresses were shown to influence the contribution of these transporters. *STP13* transcription was highly induced under abiotic stress conditions, such as high salinity and drought. Our data suggest that STP1 has a major contribution to the uptake of monosaccharides under normal conditions, whereas the contribution of STP13 is higher under high salinity conditions. Finally, our results led to the hypothesis that STP13, which is expressed in the cortex and endodermis, reabsorbs monosaccharides that are leaked from damaged epidermal cells to increase the cellular osmotic pressure or to reduce the loss of nutrients under abiotic stress conditions.

EXPERIMENTAL PROCEDURES

Plant Materials and Growth Conditions

Arabidopsis thaliana (Columbia ecotype) plants were grown on germination medium (GM) agar plates containing 3 % sucrose for 3 weeks under a 16-hour light/8-hour dark cycle, as previously described (12). For the monosaccharide sensitivity assay, plants were grown on GM agar plates containing a monosaccharide without added sucrose. The T-DNA insertion mutants were obtained from the *Arabidopsis* Biological Resource Center (ABRC). Information about the T-DNA insertion mutants was obtained from the website for the Salk Institute Genomic Analysis Laboratory (http://signal.salk.edu/).

RNA Gel Blot Analysis, Quantitative RT-PCR (qRT-PCR) and Abiotic Stress and Hormone Treatments

RNA gel blot analysis and qRT-PCR analysis of 3-week-old *Arabidopsis* plants on GM plates containing 3 % sucrose were conducted

as previously described (13). For the RNA gel blot analysis, 7 μg of total RNA was loaded, and the full-length sequence of each gene was used as a probe. For the qRT-PCR analysis, the cDNA was synthesized from the total RNA using SuperScript III (Invitrogen) with random primers, according to the manufacturer's instructions. The qRT-PCR reactions were performed with a Light Cycler (Roche Diagnostics) using the SYBR Premix Ex Taq Kit (Takara), following the manufacturer's instructions. The *STP* gene coding sequences and an 18S rRNA fragment were amplified with primers for the qRT-PCR. All of the primer sequences that were used for the present study are described in Supplemental Table S1. For the abiotic stress and ABA treatments, the plants were transferred into hydroponic Murashige and Skooge (MS) medium without sucrose 2 days prior to the abiotic stress and hormone treatments. The plants were dehydrated on parafilm or were cultured with 250 mM NaCl or 100 μM ABA.

Functional Analysis of STP transporters using Yeast Cells

The cDNA fragments of *STP1*, *STP4*, *STP7* and *STP13* were inserted into the pVT-102U vector. These constructs were used to transform the hexose uptake-deficient yeast mutant EBY.S7 strain (14). The yeast cells were grown in SD medium containing 2 % maltose. For the radiolabeled sugar uptake assay, the yeast cells (in early log phase) were suspended with wash buffer (50 mM MES-NaOH pH 5.5 containing 2 mM $MgSO_4$). Aliquots (180 μl) of the cells and 20 μl of 10 mM sugar solution containing 7.4 kBq radiolabeled compounds were mixed and incubated for 10 min at 30°C. The reaction was stopped by the addition of 5 ml of cold wash buffer and this was then filtered through a glass fiber filter (GF/B, Whatman). The filters were washed with 10 ml of cold wash buffer and placed in vials containing 2 ml of scintillation cocktail (Clear-sol, nacalai tesqu) and measured with a LS6000 scintillation counter (Beckman).

Sugar Influx/Efflux Analysis

Seedlings that were grown on GM agar plates with 3 % sucrose for 10 days were transferred to 1/2 MS liquid medium that contained 0.5 % MES-KOH (pH 5.7) without sucrose. This medium was replaced with fresh medium after 24 hours of incubation. The seedlings were equilibrated for 1 hour and then 7.4 kBq of the radiolabeled monosaccharide was added to the medium. The total concentration of this monosaccharide was adjusted by adding unlabeled sugars. The non-absorbed labeled sugars were removed by washing 3 times (1 minute each) with fresh liquid medium. After washing, the plants were separated into their shoots and roots using scissors, placed into separate vials with scintillation cocktail (2ml) and radioactivity was measured in the scintillation counter. Any competitors or inhibitors were added 5 minutes prior to the addition of ^{14}C-glucose. For the high salinity treatment, the seedlings were transferred to medium containing 125 mM NaCl 24 hours prior to the addition of the radiolabeled sugars. For the sugar efflux assay, after the pretreatment with ^{14}C-glucose for 10 hours

under normal conditions, the seedlings were transferred into 125 mM NaCl for 24 hours, and the amount of ^{14}C-glucose in the medium was measured.

Histochemical Localization

The *STP1*- and the *STP13*-promoter *GUS* reporter plasmids were constructed by cloning PCR-amplified fragments that contained a 3763-bp or a 2462-bp sequence from the sequence upstream of the initiation codon of *STP1* or *STP13*, respectively. These promoter fragments were ligated to the pGK-GUS vector and GUS activity was detected as previously described (15).

RESULTS

The Absorption of Monosaccharides from the Surrounding Environment

To characterize the uptake of various types of monosaccharides from the surrounding environment into *Arabidopsis* roots, we measured uptake using a competition assay with 1 mM ^{14}C-glucose (Fig. 1*A*). In this assay, we cut roots from whole plants to measure only the ^{14}C-glucose accumulation within the roots. The uptake of ^{14}C-glucose was significantly reduced by the addition of non-labeled glucose, galactose or mannose in the *Arabidopsis* roots, whereas the addition of non-labeled fructose or mannitol resulted in a weak inhibition of uptake (Fig. 1*A*).

We next sought to determine which type(s) of transporters were involved in the absorption of monosaccharides in *Arabidopsis* roots. There are 53 monosaccharide transporter genes in *Arabidopsis* that are classified into 7 families (Supplemental Fig. S1) (16). Sugar transporters are classified into two types, the facilitated-diffusion transporters and secondary active transporters. The ERD6-like family is a facilitated diffusion transporter for monosaccharides, including glucose (13). Although the transport system of the pGlcT family has not yet been defined, SGB1, which is a member of the pGlcT family, has been shown to transport glucose (17). Other families of monosaccharide transporters have been reported to be secondary active transporters. All of the previously characterized secondary active sugar transporters in plants have been shown to be dependent on proton-motive energy of the membrane. For example, the TMT family and VGT family are glucose/proton antiporters (18, 19), whereas the INT family, PMT family and STP family are monosaccharide/proton symporters (20, 21). Among the latter group of transporters, the STP family transports monosaccharides (e.g., glucose, fructose and mannose), the INT family members are myo-inositol-specific transporters and the PMT family transports monosaccharides and polyols (e.g., sorbitol and xylitol). To identify the transporters that are involved in the uptake of monosaccharides in roots, a competition assay was performed (Fig. 1*A*). We observed that neither *myo*-inositol nor sorbitol was able to inhibit the uptake of ^{14}C-glucose (Fig. 1*A*), indicating that the INT and PMT family transporters were not involved in the absorption of monosaccharides in *Arabidopsis* roots.

We also investigated whether disruption of the membrane proton gradient,

with the application of a protonophore, CCCP, could affect the uptake of glucose (Fig. 1*B*). The reduction of the proton gradient by the addition of CCCP caused a strong inhibition of the glucose uptake into the roots. These results indicate that the absorption of monosaccharides from the environment surrounding *Arabidopsis* roots is mediated by monosaccharide/proton symporters. Taken together, these findings suggest that the transporters that absorb monosaccharides in *Arabidopsis* roots belong to the STP family.

Functional Characterization of Candidate STP Transporters

To identify which transporters in the STP family are involved in the absorption of monosaccharides in *Arabidopsis* roots, we measured the mRNA expression levels of the 14 *STP* transporters (Fig. 2*A*). In roots, the expression levels of the *STP1* and *STP4* genes were higher than those of the other *STP* genes. We also found that the *STP7* and *STP13* genes were expressed in roots, although their expression levels were much weaker than those of the *STP1* and *STP4* genes. Furthermore, publicly available expression data revealed that the expression levels of *STP1, STP4, STP7* and *STP13* were higher than those of the other *STP* genes in these roots (Supplemental Fig. S2).

The majority of the previously characterized STP transporters exhibit a wide range of substrate specificity for monosaccharides, but a few transporters exhibit narrow substrate specificity. For example, STP14 and STP9 have been identified as galactose- and glucose-specific transporters, respectively (22, 23). Thus, we characterized the transport activity of these candidate transporters (i.e., STP1, STP4, STP7, and STP13) to investigate whether their substrate specificities correlated with the results of the competition assay shown in Fig. 1*A*. We transformed *STP1, STP4, STP7* and *STP13* into hexose transporter-deficient yeast cells, which grow on disaccharides (e.g., maltose) but not monosaccharides. All of the transformed yeast cells grew on maltose (Supplemental Fig. S3*A*). The yeast cells expressing *STP1* or *STP13* grew on glucose, fructose, galactose or mannose (Fig. 2*B*), whereas the cells expressing *STP4* grew on glucose, galactose or mannose but not fructose (Fig. 2*B*). In contrast, the cells expressing *STP7* did not grow on any of these monosaccharides (Fig. 2*B*).

Next, we analyzed the subcellular localization of these transporters in yeast cells. The transporters that we investigated were localized to the plasma membrane (Supplemental Fig. S3*B*). These results indicated that the subcellular localization did not influence transport activity of STP7 in yeast cells and that the substrates of STP7 might be monosaccharides other than glucose, fructose, galactose and mannose. The transport activity of STP1, STP4 and STP13 in yeast cells was weak when the cells were grown on 100 mM glucose (Fig. 2*B*). STP1 activity is repressed at high glucose concentrations (24), and our results suggest that a high concentration of glucose similarly inhibits the activity of other STP transporters (Fig. 2*B*). To further investigate the substrate specificity of STP1, STP4 and STP13, we

measured the uptake rates of several monosaccharides in yeast cells (Fig. 2*C*). *STP1*-expressing cells exhibited a higher level of glucose uptake than galactose or fructose. In addition, glucose uptake in the *STP4*-expressing cells was comparable with the uptake of galactose, whereas the uptake of fructose in these cells was low. Conversely, *STP13*-expressing cells exhibited similar levels of uptake for glucose, fructose and galactose. These results were in accordance with the results of the complementation assay (Fig. 2*B*) and from previous reports (10, 25, 26). Furthermore, the substrate specificities of STP1 and STP4 in yeast cells were in agreement with the results of the competition assay in *Arabidopsis* roots shown in Fig. 1*A*.

STP1 and STP13 are Involved in the Absorption of Exogenous Monosaccharides

The above results indicated that STP1, STP4 and STP13 are candidate transporters for the absorption of monosaccharides by roots from the rhizosphere. To investigate the physiological roles of STP1, STP4 and STP13, we obtained T-DNA insertion mutants from the ABRC (Supplemental Fig. S4). *STP1* expression was not detected in the *stp1-1* and *stp1-3* homozygote mutants; however, the expression of *STP1* was detected by RT-PCR analysis in the *stp1-2* mutant at a similar level to that of wild type (Supplemental Fig. S4). *STP13* expression was not detected in either the *stp13-1* or *stp13-2* mutants. We were not able to obtain mutants with a T-DNA insertion in the coding region of *STP4.* We obtained one mutant line, *stp4-1*, which contains a T-DNA insertion in the 5' untranslated region and found that the level of *STP4* expression in this mutant was similar to that of wild type (Supplemental Fig. S4).

In order to quantify the short-term uptake of monosaccharides into the roots of the *stp1* and *stp13* single- and double-knockout mutants, *stp1-1, stp1-3, stp13-1*, *stp13-2* and *stp1-1 stp13-1,* we used a radiolabeled monosaccharide uptake assay (Fig. 3). The uptake quantities of galactose, glucose and fructose were significantly reduced in the *stp1* single mutants, compared to wild type plants. Although the amounts of galactose taken up by *stp13* single mutants were similar to that of wild type, the amounts of both glucose and fructose taken up by *stp13* mutants were lower than those in wild type plants. Furthermore, the uptake amounts of glucose and fructose in the *stp1 stp13* double mutants were less than those in the single mutants. These data indicate that *STP1* and *STP13* are involved in the absorption of exogenous sugars into the roots. In particular, STP1 had a major contribution to the short-term absorption of these sugars, as the absorption by the *stp1* mutants was significantly lower than that for the wild type and *stp13* mutant plants.

To investigate the long-term absorption of monosaccharides, we employed sugar sensitivity tests using the *stp1* and *stp13* mutants. Monosaccharides are known to inhibit plant growth, and different monosaccharides inhibit different developmental steps. We first examined the growth of the *stp1* and *stp13* mutants on 6 % glucose, but we could not detect any

difference between wild type and mutants. We next investigated sensitivity to other monosaccharides, such as galactose and mannose, in these mutants. Galactose inhibits root elongation (Fig. 4, *A* and *B*), but it does not affect seed germination (Fig. 4C). In addition, mannose strongly inhibits seed germination and post-germination growth (27). It has been previously reported that the *stp1* mutant exhibits insensitivity to galactose and mannose (11). We also observed that the root lengths of the *stp1* mutants were longer than those of wild type plants under high galactose conditions (Fig. 4*B*), which is in agreement with the previous report. The *stp13* mutants also exhibited insensitivity to galactose (Fig. 4*B*); the root lengths of the *stp13* mutants were the same as those of the *stp1* mutants grown in 10 mM galactose. However, the roots of the *stp13* mutants were longer than those of the *stp1* mutants in 50 mM or 100 mM galactose. Moreover, the *stp1-1 stp13-1* double mutant plants exhibited hyper-insensitivity to galactose (Fig. 4*B*). We did not detect any root growth-related galactose insensitivity of the *stp4-1* mutant (Supplemental Fig. S5).

We next investigated the mannose sensitivity of the *stp1* and *stp13* mutants with germination assays. The germination rates of the *stp1* mutants on mannose-containing medium were higher than those of wild type (Fig. 4*C*). The *stp13* mutants also exhibited insensitivity to mannose, but the germination rates of the *stp13* mutants were lower than those of the *stp1* mutants. Moreover, the germination rate of the *stp1-1 stp13-1* double mutant was slightly higher than those of the *stp1* single mutants on medium containing 4 mM mannose. Although the germination rates of the *stp13* mutants were less than those of the *stp1* mutants on the medium containing mannose, the *stp13* mutants grew to be larger than the *stp1* mutants (Fig. 4*D*). Furthermore, the rosette size of the *stp1-1 stp13-1* double mutant grown on mannose-containing medium was much larger than that of either of the single mutants. The insensitivity of this double mutant to galactose and mannose was an additive phenotype of the *stp1-1* and *stp13-1* single mutants. Based on these findings, STP1 and STP13 appeared to have distinct functions. However, STP1 and STP13 might work synergistically because the uptake quantities of glucose and fructose into the *stp1 stp13* double mutants were lower than those into the *stp1* and *stp13* single mutants (Fig. 3). These results indicate that STP13 plays a major role in long-term absorption and that the role of STP1 is greater than STP13 for short-term absorption under our experimental conditions.

STP13 Absorbs Monosaccharides into Roots under Abiotic Stress Conditions

We analyzed the expression levels of 14 *STP* genes using publicly available data. The results of our analysis indicated that *STP13* is the only osmotic- and salt-stress-inducible gene among these 14 *STP* genes (Supplemental Fig. S6*A*). We then analyzed the expression level of the 14 *STP* genes under high salinity conditions. The expression levels of *STP13* in roots after NaCl treatment were much higher than those in the non-treatment conditions (Fig. 2*A* and Supplemental Fig.

S6*B*). We performed RNA gel blot hybridization to monitor the expression level of *STP13* under 10 mM and 100 mM galactose conditions because insensitivity to galactose in the *stp13* mutants might indicate the induction of the *STP13* gene in wild type plants under high galactose conditions. However, *STP13* was not induced under 10 mM or 100 mM galactose conditions (Supplemental Fig. S7). The 100 mM galactose treatment might not represent an osmotic stress to plants because *RD29A*, which is an osmotic stress marker gene, was also not induced under 100 mM galactose (Supplemental Fig. S7). Next, we investigated the expression profiles of *STP1* and *STP13* under drought and high salinity stress conditions and ABA treatments using RNA gel blot hybridization (Fig. 5*A*). The expression of *STP13* was induced within 1 hour of exposure to drought and high salinity stresses or ABA, whereas the expression level of *STP1* was not changed by drought and high salinity stress or ABA treatment. We also examined changes in the expression levels in leaves and roots under high salinity and exogenous ABA conditions using qRT-PCR (Fig. 5*B*) and found that the expression of *STP13* in the leaves and roots was highly induced.

The identification of glucose and fructose as major components of root exudates (6, 28, 29) prompted us to test the uptake of these sugars in wild type and mutant plants under high salinity conditions (Fig. 6, *A* and *B*). The wild type and mutant plants were first pretreated for 24 hours with 125 mM NaCl to induce *STP13* expression. The wild type and mutant plants were harvested after 10 hours of incubation with ^{14}C-glucose. The amount of ^{14}C-glucose that was taken up into the wild-type roots under high salinity conditions was slightly higher than the amount taken up under normal conditions (Fig. 6*A*). Moreover, the amount of ^{14}C-glucose that was taken up into *stp1-1* roots was significantly higher under high salinity compared to normal conditions. However, the amount of glucose uptake in the *stp1-1 stp13-1* double mutant was lower than in either of the single mutants, and no increase in uptake under the high salinity conditions was detected in the double mutant. Similar results were obtained for the uptake of fructose (Fig. 6*B*). Taken together, these results suggest that the increase in the amount of sugars that was taken up under high salinity was dependent on the induction of *STP13* expression.

We also investigated the uptake of monosaccharides under high salinity conditions using the modified glucose-conjugating fluorescent dye 2-NBDG. This dye is absorbed by plants as a non-metabolized glucose (Fig. 6*C*) (30). Because this dye is not metabolized by plant cells, it can be used to detect the sites where glucose accumulates. After a 5-hour treatment with 2-NBDG, the fluorescence of the 2-NBDG was observed mainly in the epidermal layer. Under high salinity conditions, the fluorescence was partially detected in the epidermal cells but was mainly detected at the stele. Dead root cells under the high salinity conditions were detected using propidium iodide (PI). Because the cellular membrane is non-permeable to PI, the PI stains the cell walls of the living cells

and the nuclei of the dead cells. After treatment with NaCl, the nuclei of the root epidermal and cortex cells were stained with PI and 2-NBDG did not accumulate in these damaged cells (Fig. 6*C*). These results indicated that the dead cells were unable to absorb monosaccharides.

Because the use of PI indicated that some cells were no longer intact, we postulated that monosaccharides might leak from these cells under high salinity conditions. Accordingly, we measured the amount of glucose that leaked from the plants under high salinity conditions (Fig. 6*D*). After 24 hours of incubation with ^{14}C-glucose, the plants were transferred to a glucose-free medium with or without 125 mM NaCl. The amount of glucose efflux from wild type plants was reduced under the high salinity conditions compared to the non-treatment conditions, although the epidermal cells were damaged. In contrast, the ^{14}C-glucose efflux from the *stp13-1* mutants increased under the high salinity condition compared to the non-treatment condition. These data indicate that a possible function of STP13 under high salinity conditions is the reabsorption of monosaccharides that have leaked from damaged cells.

Tissue Specificities of STP1 and STP13

The tissue specificities of transporters are related to their physiological functions. To better understand the physiological functions of *STP1* and *STP13*, we examined the tissue specificities of these two genes using publicly available expression data. We found that *STP1* was expressed in the epidermis and stele and that *STP13* was expressed in the endodermis (Supplemental Fig. S2). We then generated *STP1*pro:*beta-glucuronidase* (*GUS*) and *STP13*pro:*GUS* transgenic plants. It has been previously reported that *STP1* expression was detected in stomatal guard cells using *in situ* hybridization (29). We also detected GUS staining in the stomatal guard cells of the *STP1*pro:*GUS* plants (Fig. 7*A*), whereas the GUS staining in the leaves of the *STP13*pro:*GUS* plants was mainly detected in veins (Fig. 7*B*). In the roots of the *STP1*pro:*GUS* plants, GUS staining was detected mainly in the epidermal tissues, whereas the endodermis or stele stained strongly in the roots of the *STP13*pro:*GUS* plants. These differences in the tissue specificities might influence the physiological functions of these transporters.

DISCUSSION

Plant roots release various metabolites including carbohydrates to modify and provide chemoattractive signals in their immediate environment. However, roots might also attempt to reduce carbon loss through the reabsorption of these metabolites (32). Although the biological significance of the absorption of sugars is a matter of continuing debate, it is clear that roots are able to uptake sugar from the rhizosphere. In the present study, we analyzed the root uptake system for monosaccharides from the surrounding environment. Our data showed that monosaccharide transport activity in *Arabidopsis* roots changed under conditions of high salinity as a result of changes in the expression level of transporter genes. It has

been reported previously that STP1 is involved in the uptake of monosaccharides from the surrounding environment (11). In the present study, we demonstrated that STP13 also plays an important role in the uptake of monosaccharides in *Arabidopsis* roots. Moreover, we showed that *stp13* mutants were more insensitive to exogenous monosaccharides than *stp1* mutants (Fig. 4). However, the amount of monosaccharides that was taken up by the *stp1* mutants was much lower than by the *stp13* mutants. According to the results of our promoter GUS reporter analysis and publicly available expression data, *STP1* was strongly expressed in epidermal cells, and *STP13* was expressed in the endodermis (Fig. 7 and Supplemental Fig. S2). It has been demonstrated that NIP5.1 and SULTR1, which transport exogenous boron and sulfate ions, respectively, are expressed in the epidermal layer of roots (33, 34). The results of the competition assay that are reported in the present paper also indicated that STP1 is a major contributor to the uptake of exogenous monosaccharides (Fig. 1*A* and 2*C*). Taken together, our data suggest that the STP1 transporter, which is expressed in epidermal cells, was the major contributor to the uptake of monosaccharides from the surrounding environment. The data from our experiment using 2-NBDG suggested that the monosaccharides that were absorbed into the epidermal cells were mainly accumulated in the vacuole of the epidermal cells (Fig. 6*C*). This finding suggested that the majority of the monosaccharides absorbed by STP1 are retained in epidermal cells. Conversely, the monosaccharides that were absorbed by STP13 could be transported to other tissues. This might be one of the reasons why the *stp13* mutants were more insensitive to exogenous monosaccharides than the *stp1* mutants, although the uptake of the radiolabeled monosaccharides by the *stp1* mutants was significantly lower than by the *stp13* mutants.

The contribution of STP13 to the uptake of monosaccharides increased under conditions of high salinity as compared with normal conditions. STP13 is the only STP transporter that has been described as an abiotic stress-inducible gene (26). The increase in monosaccharide uptake under high salinity was dependent on the induction of STP13 because this increase was not detected for *stp1-1 stp13-1*. Moreover, the monosaccharide uptake by *stp13-1* was reduced under high salinity conditions (Fig. 6, *A* and *B*). We speculate that this decrease in uptake occurred because the epidermal cells, where *STP1* is expressed, were damaged under high salinity. The endodermis or stele, where *STP13* is expressed, might become the boundary between the root and the surrounding environment under conditions (e.g., high salinity) in which the epidermal layer is damaged. This suggests that the role of STP13 might be more important under high salinity than under normal conditions. Our current data and those from previous reports have demonstrated that STP13 has broader substrate specificity than the other STP family transporters (26). Various types of monosaccharides might leak from roots when the cell membrane is broken under conditions of high salinity, and STP13 may reabsorb

these monosaccharides more efficiently than other transporters.

A lack of *STP1* or *STP13* did not result in any obvious growth or morphological changes under our normal growth or high salinity conditions (data not shown). We previously characterized another abiotic stress-inducible monosaccharide transporter, ESL1 (13). We hypothesized that because tissues in which *STP13* was expressed were similar to tissues in which *ESL1* was expressed, STP13 might have a redundant or cooperative function with ESL1. Thus, we established an *esl1-1 stp13-1* double mutant. However, we did not observe any difference in the growth of the double mutant compared with wild type plants under our normal and high salinity conditions (data not shown). It is possible that additional transporters may be functionally redundant with STP13 or ESL1 or that other molecules (e.g., ions) may substitute for monosaccharides under abiotic stress conditions. In particular, STP4 may play a role in the absorption of monosaccharides in roots because *STP4* is expressed in the root at the same level as *STP1* and may also be expressed in epidermal cells (Fig. 2 and Supplemental Fig. S2). Moreover, the substrate specificity of STP4 was in agreement with the results of the competition assay in roots (Fig. 1*A* and 2*C*). It will be interesting to determine the contribution of STP4 to monosaccharide uptake from the environment surrounding the roots. An *stp4* knock-down mutant in an *stp1-1 stp13-1* background would provide information about the function of the *STP* genes in the absorption of monosaccharides.

Previous biochemical results have indicated that the release of sugars into the rhizosphere by roots is mediated by a facilitated-diffusion transporter (9), and we have shown previously that ESL1 is a facilitated-diffusion transporter for monosaccharides (13). ERD6, which is a homolog of ESL1, is expressed at the epidermal layer of roots (13); therefore, ERD6 might be involved in the release of monosaccharides from the epidermal layer. Recently, other facilitated-diffusion transporters, such as the SWEETs, have been isolated (35). Because several of these SWEETs are induced by pathogens, it has been proposed that pathogens may commandeer these efflux transporters to obtain sugars from plants. However, according to the data that are available in public databases, the influx transporters in this study, *STP1* and *STP13*, are also induced upon pathogen infection and by the addition of elicitors (Supplemental Fig. S8). It is possible that these transporters might take up the monosaccharides that the facilitated-diffusion transporters have exported to the apoplast to prevent pathogens from obtaining nutrients. In the present study, we focused on the relationship between monosaccharide uptake and abiotic stress responses. It is possible that STP1 and STP13 may be additionally involved in responses to other environmental factors.

In conclusion, our data suggest that STP1 and STP13 are involved in the root absorption of monosaccharides from the surrounding environment. STP1 has a major role in monosaccharides uptake under normal

conditions, whereas the uptake of monosaccharides by STP13 was more significant under high salinity conditions. Furthermore, our data indicated that monosaccharide absorption activity was influenced by changes in the expression of transporter genes. Therefore, we hypothesize that STP13 reabsorbs monosaccharides that leak from damaged root epidermal cells to increase the cellular osmotic pressure or to reduce the loss of nutrients for the adaptation against conditions of osmotic stress.

REFERENCES

1. Williams L. E., Lemoine R., Sauer N. (2000) *Trends Plant Sci.***5**, 283-290
2. Lalonde S., Wipf D., Frommer W. B. (2004) *Annu. Review Plant Biol.***55**, 341-372
3. Smith A. M., Zeeman S. C., Smith S. M. (2005) *Annu. Review Plant Biol.***56**, 73-98
4. Shaw L. J., Morris P., Hooker J. E. (2006) *Environ. Microbiol.***8**, 1867-1880
5. Kuzyakov Y., Jones D. L. (2006) *Soil Biol. Biochem.***38**, 851-860
6. Bacilio-Jimenez M., Aguilar-Flores S., Ventura-Zapata E., Perez-Campos E., Bouquelet S., Zenteno E. (2003) *Plant Soil***249**, 271-277
7. Rudrappa T., Czymmek K. J., Pare P. W., Bais H. P. (2008) *Plant Physiol.***148**, 1547-1556
8. Lin W., Schmitt M. R., Hitz W. D., Giaquinta R. T. (1984) *Plant Physiol.***76**, 894-897
9. Sacchi G. A., Abruzzese A., Lucchini G., Fiorani F., Cocucci S. (2000) *Plant Soil***220**, 1-11
10. Sauer N., Friedlander K., Gramlwicke U. (1990) *EMBO J.* **9**, 3045-3050
11. Sherson S. M., Hemmann G., Wallace G., Forbes S., Germain V., Stadler R., Bechtold N., Sauer N., Smith S. M. (2000) *Plant J.***24**, 849-857
12. Osakabe Y., Maruyama K., Seki M., Satou M., Shinozaki K., Yamaguchi-Shinozaki K. (2005) *Plant Cell***17**, 1105-1119
13. Yamada K., Osakabe Y., Mizoi J., Nakashima K., Fujita Y., Shinozaki K., Yamaguchi-Shinozaki K. (2010) *J. Biol. Chem.***285**, 1138-1146
14. Wieczorke R., Dlugai S., Krampe S., Boles E. (2003) *Cellular Physiol. Biochem.***13**, 123-134
15. Mizuno S., OsakabeY., Maruyama K., Ito T., Osakabe K., Sato T., Shinozaki K., Yamaguchi-Shinozaki K. (2007) *Plant J.***50**, 751-766
16. Büttner M. (2007) *FEBS Lett.***581**, 2318-2324
17. Wang HX., Weerasinghe RR., Perdue TD., Cakmakci NG., Taylor JP., Marzluff WF., Jones AM. (2006) *Mol. Biol. Cell.*17, 4257-69.
18. Wormit A., Trentmann O., Feifer I., Lohr C., Tjaden J., Meyer S., Schmidt U., Martinoia E., Neuhaus H. E. (2006) *Plant Cell***18**, 3476-3490
19. Aluri S., Buttner M. (2007) *Proc. Natl. Acad. Sci. USA***104**, 2537-2542
20. Reinders A., Panshyshyn J. A., Ward J. W. (2005) *J. Biol. Chem.***280**, 1594-1602
21. Schneider S., Beyhl D., Hedrich R., Sauer N. (2008) *Plant Cell***20**, 1073-1087
22. Schneidereit A., Scholz-Starke J., Buttner M. (2003) *Plant Physiol.***133**, 182-190

23. Poschet G., Hannich B., Buttner, M. (2010) *Plant Cell Physiol.***51**, 1571-1580
24. Sherson S. M., Alford H. L., Forbes S. M., Wallace G., Smith S. M. (2003) *J. Exp. Bot.***54**, 525-531
25. Truernit E., Schmid J., Epple P., Illig J., Sauer N. (1996) *Plant Cell***8**, 2169-2182
26. Norholm M. H. H., Nour-Eldin H. H., Brodersen P., Mundy J., Halkier B. A. (2006) *FEBS Lett.***580**, 2381-2387
27. Pego J. V., Weisbeek P. J., Smeekens S. C. M. (1999) *Plant Physiol.***119**, 1017-1023
28. Kamilova F, Kravchenko LV, Shaposhnikov AI, Azarova T, Makarova N, Lugtenberg B. (2006) *Mol Plant Microbe Interact.***19**, 250-6.
29. Kamilova F, Kravchenko LV, Shaposhnikov AI, Makarova N, Lugtenberg B.(2006) *Mol Plant Microbe Interact*.19, 1121-6.
30. Etxeberria E., Gonzalez P., Tomlinson P., Pozueta-Romero J. (2005) *J. Exp. Bot.***56**, 1905-1912
31. Stadler R., Buttner M., Ache P., Hedrich R., Ivashikina N., Melzer M., Shearson S. M., Smith S. M., Sauer N. (2003) *Plant Physiol.***133**, 528-537
32. Farrar J. F., Jones D. L. (2000) *New Phytologist***147**, 43-53
33. Takahashi H., Watanabe-Takahashi A., Smith F. W., Blake-Kalff M., Hawkesford M. J., Saito K. (2000) *Plant J.***23**, 171-182
34. Takano J., Wada M., LudewigU., Schaaf G., von Wiren N., Fujiwara T. (2006) *Plant Cell***18**, 1498-1509
35. Chen L. Q., Hou B. H., Lalonde S., Takanaga H., Hartung M. L., Qu X. Q., Guo W. J., Kim J. G., Underwood W., Chaudhuri B., Chermak D., Antony G., White F. F., Somerville S. C., Mudgett M. B., Frommer W. B. (2010) *Nature***468**, 527-U199

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FOOTNOTES

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[3] The abbreviations used are: qRT, quantitative reverse transcription; GFP, green fluorescent protein; CCCP, carbonyl cyanide m-chlorophenylhydrazone; ABA, abscisic acid; GUS, beta-glucuronidase

FIGURE LEGENDS

FIGURE 1. The characterization of transporters in the root absorption of glucose from the surrounding environment. The inhibition ^{14}C-glucose (1 mM) uptake by various monosaccharides or polyols (*A*) or CCCP (*B*) in *Arabidopsis* roots. Competitors or CCCP were added to the medium 5 minutes prior to the addition of ^{14}C-glucose. Plants were grown on agar plates with 3 % sucrose for 10 days and were transferred into liquid medium without sucrose 24 hours before adding ^{14}C-glucose.

FIGURE 2. The expression level and biochemical characterization of STP1, STP4, STP7 and STP13. *A*. The expression level of the 14 *STP* transporter genes in roots (right) or leaves (left) was determined using qRT-PCR. The value of *STP1* expression levels was set to 1.0 and the expression values of the other genes were displayed relative to *STP1*. The expression of 18S RNA was used as an internal control. *B*. Growth complementation of the hexose uptake-deficient yeast mutant by STP1, STP4, STP7 or STP13. *C*. Relative uptake rates in the yeast cells of different monosaccharide substrates at an initial outside concentration of 1 mM.

FIGURE 3. The uptake amounts of monosaccharides into the roots of *stp1* and *stp13* mutants. After incubation with 1 mM of each monosaccharide for 2 hours, the plants were harvested and the levels of radioactivity in the roots were measured. Plants were grown on agar plates with 3% sucrose for 10 days and were then transferred into liquid medium without sucrose 24 hours before adding ^{14}C-labeled sugars. $^{**}P < 0.01$, $^{*}P < 0.05$ significant differences between Col and mutants.

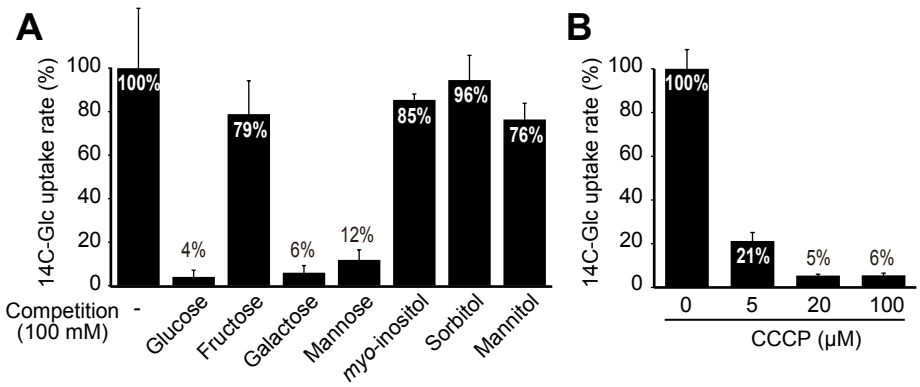
FIGURE 4. The sugar sensitivity of the *stp1* and *stp13* mutants. *A* and *B*. The effects of galactose on the *stp1* and *stp13* mutants. Seeds were germinated on medium containing galactose and the root lengths of the plants were measured at 3 weeks. *C*. The germination rates on galactose or mannose. *D*. Plants grown on mannose for 2 weeks. All of the plants were grown under continuous light. No sucrose is contained in the medium. $^{*}P < 0.01$ significant differences between Col and mutants.

FIGURE 5. The expression of *STP1* and *STP13* under abiotic stress conditions. Total RNA from whole plants, leaves and roots were used for northern blots (*A*) and qRT-PCR analyses (*B*), respectively. The value of the control (leaf) was set to 1.0, and those of the other sections were expressed as relative values of the control (leaf). To normalize the expressions, 18S rRNA was amplified as an internal control. Plants were grown on agar plates for 3 weeks and were then

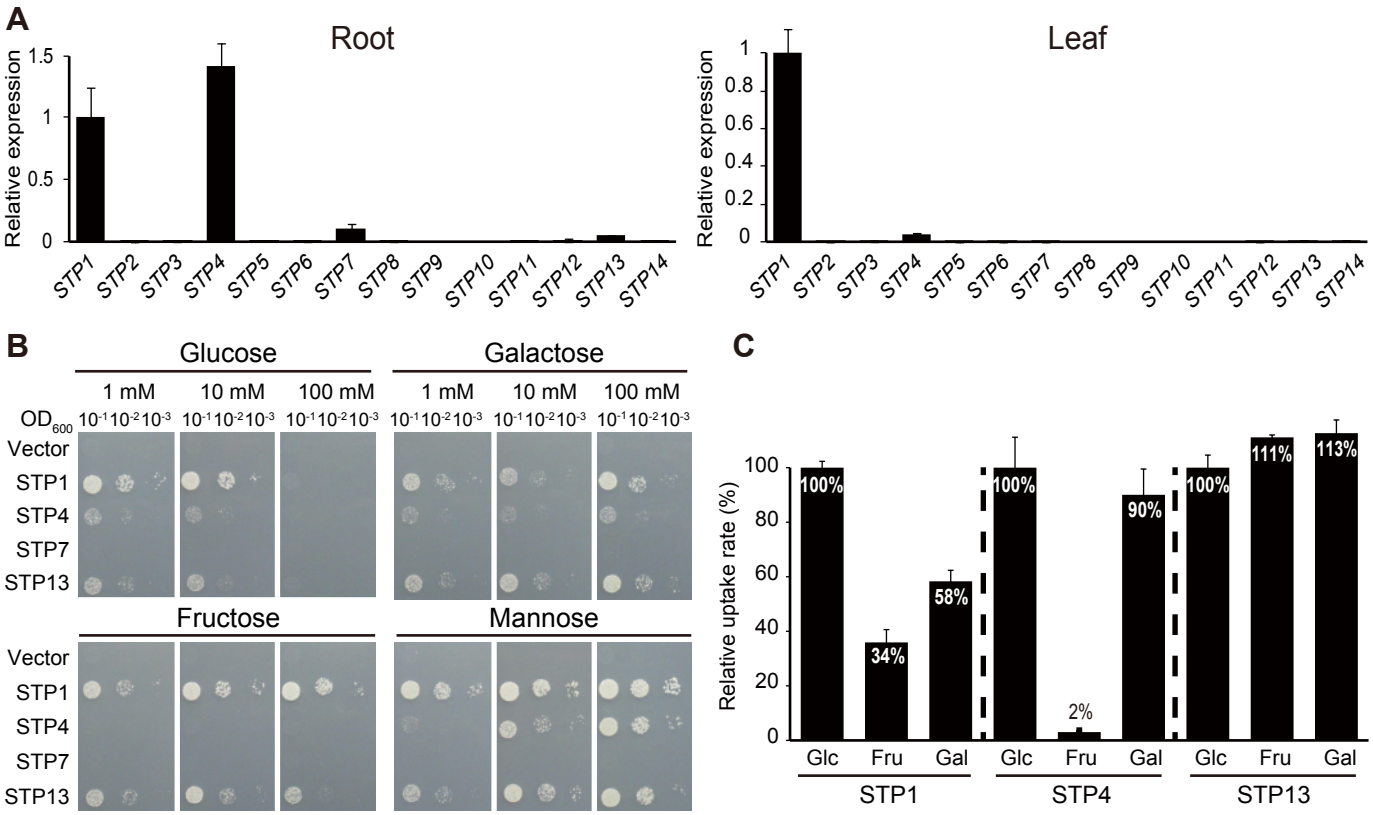
transferred into liquid medium without sucrose 2 days before treatment.

FIGURE 6 . The uptake and efflux of monosaccharides into roots under high salinity conditions. After pretreatment with 125 mM NaCl for 24 hours, ^{14}C-glucose (*A*) or ^{14}C-fructose (*B*) was added. After the incubation with each monosaccharide for 10 hours, the plants were harvested, and the radioactivity was measured. *C.* The accumulation of 2-NBDG in roots under high salinity. After pretreatment with 125 mM NaCl for 24 hours, treated (NaCl) or non-treated (Control) plants were incubated with 200 μM 2-NBDG for 5 hours. The nuclei of dead cells were stained with propidium iodide (PI). *D.* The efflux of glucose under high-salinity conditions. After pre-incubation with 1 mM ^{14}C-glucose for 10 hours, the plants were transferred to glucose-free medium containing 125 mM NaCl for 24 hours, followed by measurement of radioactivity in the medium. Each value without NaCl was set to 100. Plants were grown on agar plates with 3% sucrose for 10 days and were then transferred into liquid medium without sucrose 24 hours before 125 mM NaCl treatment. **P* < 0.05 significant differences between control and high salinity conditions.

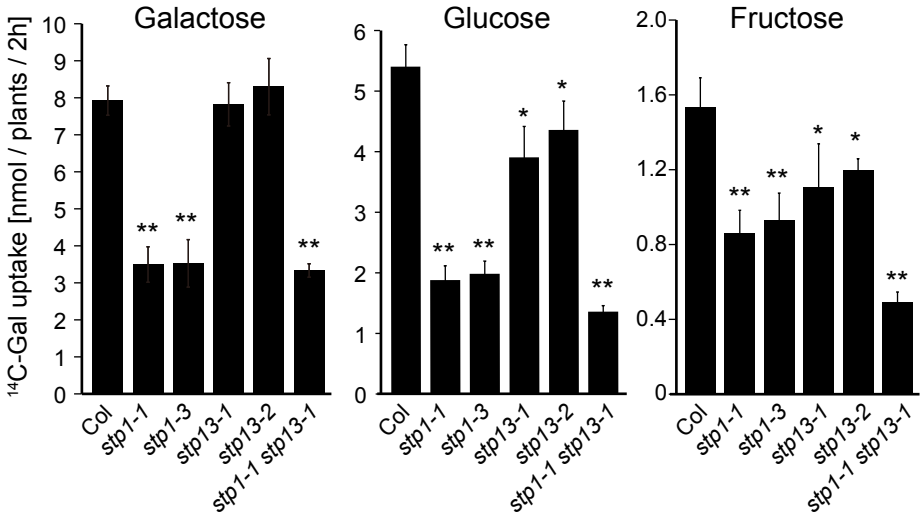
FIGURE 7. The histological analysis of *STP1* and *STP13* expression. GUS staining of the *STP1*pro:*GUS* plants (*A*) and the *STP13*pro:*GUS* plants (*B*) which were grown on agar plates with 3 % sucrose for 3 weeks.



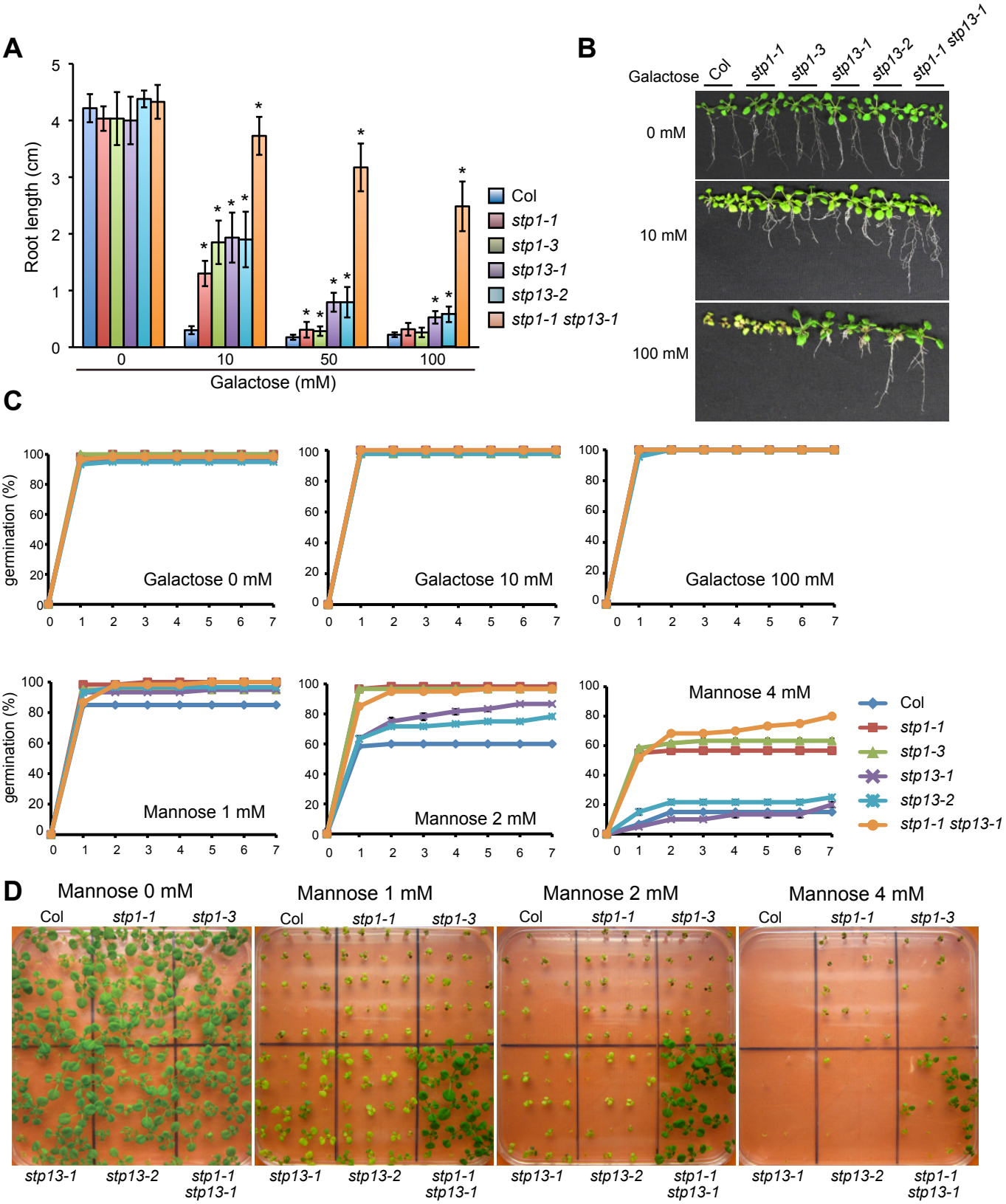
Yamada et al. Figure 1



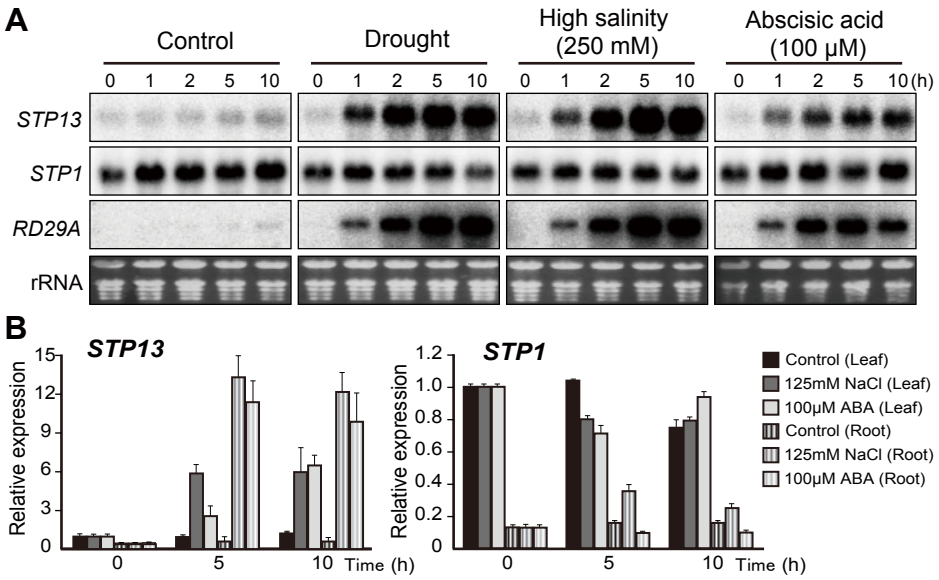
Yamada et al. Figure 2



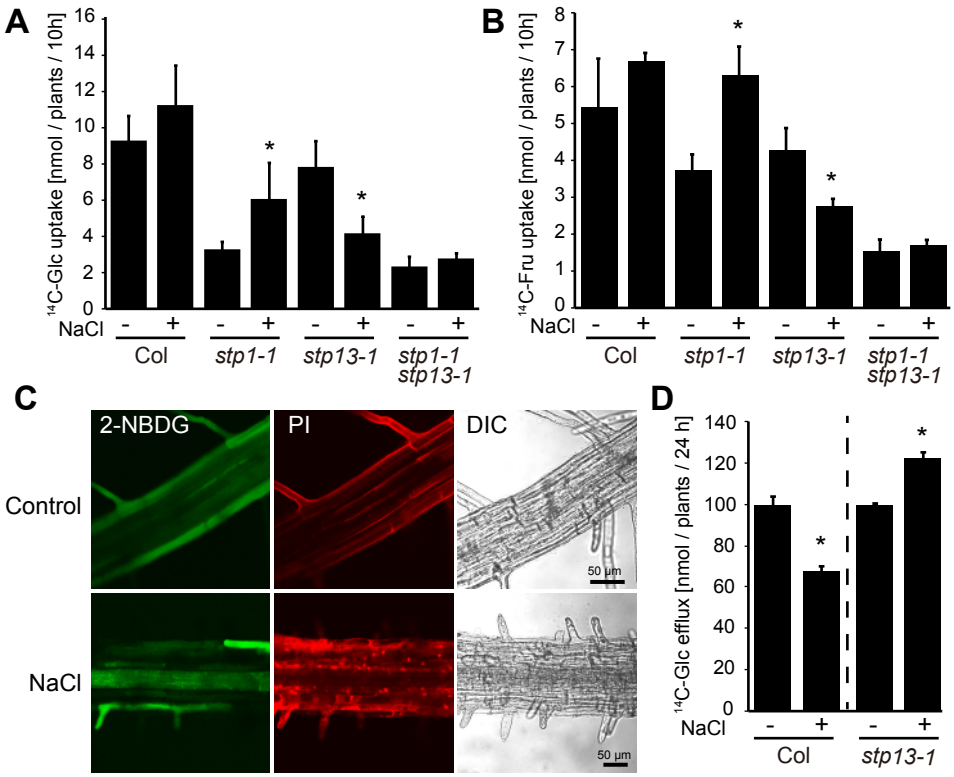
Yamada et al. Figure 3



Yamada et al. Figure 4

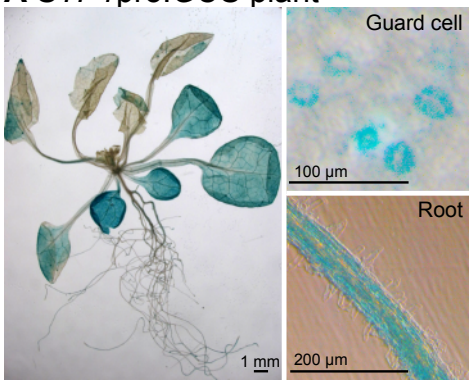


Yamada et al. Figure 5

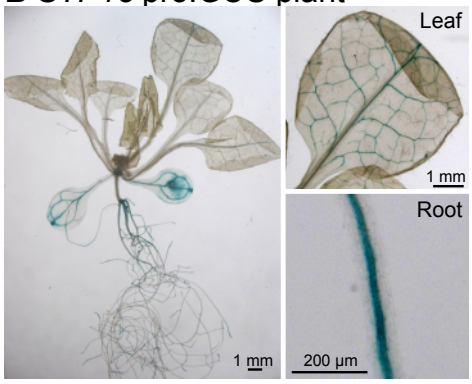


Yamada et al. Figure 6

A *STP1*pro:*GUS* plant



B *STP13* pro:*GUS* plant



Yamada et al. Figure 7

The Monosaccharide Absorption Activity of *Arabidopsis* Roots Depends on the Expression Profiles of Transporter Genes under High Salinity Conditions

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