A genomic clone and the corresponding cDNA of a new Arabidopsis monosaccharide transporter AtSTP9 were isolated. Transport analysis of the expressed protein in yeast showed that AtSTP9 is an energy-dependent, uncoupler-sensitive, high-affinity monosaccharide transporter with a $K_m$ for glucose in the micromolar range. In contrast to all previously characterized monosaccharide transporters, AtSTP9 shows an unusual specificity for glucose. Reverse transcriptase-polymerase chain reaction analyses revealed that AtSTP9 is exclusively expressed in flowers, and a more detailed approach using AtSTP9 promoter/reporter plants clearly showed that AtSTP9 expression is restricted to the male gametophyte. AtSTP9 expression is not found in other floral organs or vegetative tissues. Further localization on the cellular level using a specific antibody revealed that in contrast to the early accumulation of AtSTP9 transcripts in young pollen, the AtSTP9 protein is only found weakly in mature pollen but is most prominent in germinating pollen tubes. This preloading of pollen with mRNAs has been described for genes that are essential for pollen germination and/or pollen tube growth. The pollen-specific expression found for AtSTP9 is also observed for other sugar transporters and indicates that pollen development and germination require a highly regulated supply of sugars.

Pollen development and pollen tube growth represent an essential stage in plant reproduction (Zheng and Yang, 2000). Because pollen tissue is a photosynthetically inactive “sink,” a constant supply with carbohydrates has to be provided during these processes. The main transport form of photoassimilates, Suc, is synthesized in green “source” tissues, loaded into the phloem stream by a specific Suc/H$^-$/H$_{11001}$ symporter (Riesmeier et al., 1992; Stadler et al., 1995; Stadler and Sauer, 1996; Kühn et al., 1997) and is then delivered to the various sink organs. Here, the Suc is symplastically unloaded from the phloem into the surrounding mesophyll cells and from there into the apoplastic space. The pollen itself represents a symplastically isolated sink without plasmodesmatal connections to the surrounding tissue. Therefore, the sugars have to be taken up from the apoplast by specific transporters either directly as Suc or, after cleavage by an apoplastic invertase, as hexoses. Gene products involved in this sink-loading process have already been isolated from different plants. Pollen-specific Suc transporters have been identified in tobacco (Nicotiana tabacum; Lemoine et al., 1999) and Arabidopsis (Stadler et al., 1999). In Petunia sp., a putative monosaccharide transporter, Pmt1, was found to be expressed specifically in the male gametophyte, and mRNA levels accumulate in mature and germinating pollen (Ylstra et al., 1998). At the same time, the presence of cell wall-bound invertase activity was found in an in vitro germination assay (Ylstra et al., 1998). Furthermore, the expression of a putative apoplastic invertase in potato (Solanum tuberosum) was shown to be restricted to pollen by promoter/β-glucuronidase (GUS) analysis (Maddison et al., 1999).

The recent completion of the Arabidopsis genome revealed the existence of more than 50 genes homologous to sugar transporters. Within this superfamily, we have identified a family of 14 highly homologous monosaccharide transporters called AtSTPs (Büttner et al., 1999; Büttner and Sauer, 2000). Most of the so far characterized AtSTPs show a sink-specific expression profile (Truernit et al., 1996, 1999; Sherson et al., 2000; Stadler et al., 2003), and three of them, AtSTP2, AtSTP4, and AtSTP6, are expressed in pollen. The high-affinity monosaccharide transporter AtSTP2 was localized in AtSTP2 promoter-GUS Arabidopsis plants showing reporter activity during pollen maturation (Truernit et al., 1999). Further analysis using in situ hybridization and immunolocalization revealed that both AtSTP2 mRNA and AtSTP2 protein are first seen at the beginning of callose degradation and microspore release from the tetrads and are no longer detectable after the mitotic divisions and the gametophyte formation or in mature pollen grains or pollen tubes (Truernit et al., 1999). AtSTP6 expression is confined to the latest stage in pollen development (Scholz-Starke et al., 2003). AtSTP4 was also found to

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be expressed in pollen by promoter/GUS analysis (Truernit et al., 1996), but in contrast to AtSTP2 and AtSTP6, AtSTP4 mRNA and AtSTP4 protein are not detectable at the same time during pollen development. AtSTP4 mRNA was found during early pollen development by in situ hybridization, whereas a specific antibody could detect AtSTP4 protein only in mature pollen grains and after pollen germination in the pollen tube (R. Stadler, unpublished data). AtSTP1, the first monosaccharide transporter isolated from higher plants (Sauer et al., 1990), shows a low substrate specificity in heterologous expression systems (baker’s yeast; Sauer et al., 1990; Xenopus sp. oocytes; Boorer et al., 1992, 1994). In accordance, a broad spectrum of monosaccharide substrates was determined for AtSTP2, AtSTP4, and AtSTP6 by heterologous expression (Truernit et al., 1996, 1999; Scholz-Starke et al., 2003).

Here, we present the characterization of a new Arabidopsis monosaccharide transporter AtSTP9, which is expressed specifically in pollen and shows an unusual selectivity for Glc. Potential functions of monosaccharide transporters in pollen development and germination are discussed.

RESULTS

Isolation of AtSTP9 Sequences

Isolation of the Genomic Clone

Screening 250,000 plaque forming units of a genomic Arabidopsis library with a radiolabeled AtSTP9-specific 204-bp PCR fragment led to the isolation of six positive clones. Southern analyses with the same 204-bp probe identified a 3,386-bp EcoRI fragment in all six positive clones (data not shown). This 3,386-bp EcoRI fragment (of the 13-kb phage insert) was cloned into the vector pUC19 yielding the construct pAS91. Determining the complete sequence of insert) was cloned into the vector pUC19 yielding the

AtSTP9 containing 1,095 bp of the

AtSTP9

sequence of the genomic Arabidopsis library with a radiolabeled AtSTP9-specific 204-bp PCR fragment led to the isolation of six positive clones. Southern analyses with the same 204-bp probe identified a 3,386-bp EcoRI fragment in all six positive clones (data not shown). This 3,386-bp EcoRI fragment (of the 13-kb phage insert) was cloned into the vector pUC19 yielding the clone pAS91. Determining the complete sequence of the 3,386-bp fragment identified the AtSTP9 gene containing 1,095 bp of the AtSTP9 promoter region and the complete AtSTP9 gene sequence (2,123 bp). This sequence is identical to the chromosome locus At1g50310. Computer analysis for splice site predictions (GENSCAN; NetGene2) and comparison with other AtSTPs suggest an AtSTP9 open reading frame of 1,551 bp, interrupted by three putative introns.

Isolation of the cDNA Clone

The sequence information obtained with the isolation of the genomic AtSTP9 clone was used to generate specific primers directed against the N- and C-terminal sequences of the AtSTP9-coding region. By reverse transcriptase (RT)-PCR using these primers and total RNA from flowers of different developmental stages, a 1,550-bp fragment was isolated and subcloned into pGEM-T easy (Promega, Madison, WI), yielding the construct pLEX13. Sequencing of the 1,550-bp AtSTP9 fragment in pLEX13 and comparison with the genomic sequence reconfirmed the absence of PCR artifacts and the position of the three introns. The encoded AtSTP9 protein is 517 amino acids long and has a calculated molecular mass of 56.3 kD and an pl of 6.4. Furthermore, the AtSTP9 protein sequence has two consensus sequence motives for potential N-glycosylation at positions 153 to 156 (NQST) and 430 to 433 (NVSV). As shown in Figure 1, the AtSTP9 protein shows a high degree of homology to the so far characterized Arabidopsis monosaccharide transporters AtSTP1, AtSTP2, AtSTP3, AtSTP4, and AtSTP6 (65%–76% similarity and 49%–64% identity). In addition, the positions of introns are strongly conserved (marked in Fig. 1) with AtSTP6 having introns at positions 1 and 5, AtSTP3 at positions 1 and 2, and AtSTP1, AtSTP2, AtSTP4, and AtSTP9 at positions 1, 2, and 5 (Böttner and Sauer, 2000). The determined AtSTP9 cDNA sequence (GenBank accession no. AJ344336) verifies the predicted annotation for the AtSTP9 gene locus At1g50310 on chromosome one.

Functional Characterization of AtSTP9 by Heterologous Expression in Yeast

The sequence of AtSTP9 shows a high degree of homology to the other members of the Arabidopsis AtSTP family. Therefore, we wished to investigate whether AtSTP9 is also a functional monosaccharide transporter by expressing the AtSTP9 gene in the heterologous yeast system and studying the transport characteristics. To this end, we cloned the cDNA sequence of AtSTP9 into the yeast expression vector pEX-Tag (Meyer et al., 2000) in both sense and antisense orientation, yielding clones pACH91s and pACH91a, respectively. With these constructs, the yeast mutant strain EBY.VW4000 (Wieczorke et al., 1999) was transformed. EBY.VW4000 is a hxt1 null mutant, which does no longer grow on monosaccharides because all genes for hexose transporters (Hxt1–17) and for the Gal transporter (Gal2) are deleted but can be grown on maltose. Expression of the AtSTP9 gene in antisense orientation in this null mutant background (ScLEX12) did not restore growth on Glc-containing medium (data not shown) nor the uptake of [14C]Glc (Fig. 2A). However, expression of the AtSTP9 sense construct (ScLEX8) restored growth on 0.2% and 2% (w/v) Glc (data not shown). Furthermore, ScLEX8 regained the ability to accumulate [14C]Glc (concentration equilibrium was 0.07 nmol μL−1 packed cells), which was not seen in the antisense control ScLEX12 (Fig. 2A). ScLEX8 was further used to determine the substrate specificities for AtSTP9. All AtSTPs characterized so far (AtSTP1, AtSTP2, AtSTP3, AtSTP4, and AtSTP6) transport a broad spectrum of monosaccharides at comparable rates. In contrast, AtSTP9 shows an unusual preference for Glc, whereas other monosaccharides are...
transported with much lower rates if at all (Gal, 25%, and Man and Xyl below 5% with respect to Glc uptake; Fig. 2B). The $K_m$ of AtSTP9 for Glc was measured to be 84 ± 2.5 μM (Fig. 2A, inset) and is similar to the values determined for previously characterized AtSTPs (Sauer et al., 1990; Truernit et al., 1996, 1999; Büttner et al., 1999; Scholz-Starke et al., 2003). Glc accumulation was maximal at pH 5.5, which is in agreement with the pH optima determined for the other AtSTPs (data not shown). Furthermore, low concentrations of the proton uncouplers carbonyl cyanide m-chlorophenyl-hydrazone and 2,4-Dinitrophenol decrease Glc uptake significantly (Fig. 2B), suggesting that sugar uptake via AtSTP9 is driven by a proton gradient across the plasma membrane. Taken together, heterologous expression in the yeast system suggests that AtSTP9 is an energy-dependent, high-affinity monosaccharide/H$^+$ symporter specific for Glc.

### Tissue-Specific Expression of the AtSTP9 Gene

The majority of the AtSTPs characterized so far in Arabidopsis are specifically expressed in sink tissues (Sauer et al., 1990; Truernit et al., 1996, 1999; Büttner et al., 1999; Stadler et al., 2003). To determine the spatial and developmental expression profile of the AtSTP9 gene, we first tried to detect the AtSTP9 transcript in different...
types of sink and source tissues using RT-PCR. Total RNA was isolated from 5- to 10-d-old seedlings, from leaves, stems, and roots of 3- to 6-week-old plants, and from flowers and fruits of mature plants. First-strand cDNA synthesis was performed using a poly(dT) primer and equal amounts of RNA from each tissue. With this cDNA, a PCR reaction was run with AtSTP9-specific primers. As shown in Figure 3A, only with cDNA from flowers an AtSTP9-specific PCR product was detected (confirmed by direct sequencing of the PCR product). As a control that equal amounts of cDNA were used, a PCR reaction with Actin1-specific primers was run in parallel (Fig. 3B). The fact that PCR products from contaminations with genomic DNA are much stronger for AtSTP9 than for Actin is probably due to differences in the expression rates of both genes. AtSTP9 is only weakly expressed, and 40 PCR cycles had to be run before an amplified product could be seen. Actin on the other hand is highly expressed in all tissues, and the majority of template is cDNA rather than genomic DNA.

The AtSTP9 Promoter Drives Pollen-Specific Expression of Reporter Genes

To examine the AtSTP9 expression profile in more detail, transgenic Arabidopsis plants were generated, which drive reporter gene expression under the control of the AtSTP9 promoter. To this end, a fragment containing 1,085 bp of the AtSTP9 promoter sequence and 1,147 bp of the AtSTP9 structural gene sequence was cloned in front of the uidA gene (GUS) in the plant transformation vector pGPTV-BAR (Becker et al., 1992), yielding construct pLEX22 (Fig. 4A). The N-terminal part of the AtSTP9 structural gene used in pLEX22 encodes 232 amino acids representing the first six transmembrane domains of AtSTP9, so that expression of this construct should result in a membrane-bound AtSTP9-GUS fusion protein with the GUS portion facing the cytosol. In parallel, a second construct was generated, in which the 1,085-bp fragment of the AtSTP9 promoter without the AtSTP9 structural gene sequence was cloned into a pGPTV-BAR derivative, in which the uidA gene was replaced by the green fluorescent protein (GFP) gene, yielding construct pLEX23 (Fig. 4B). Both constructs were used to transform Arabidopsis, and for each construct 30 independent lines were analyzed for reporter gene expression. As shown in Figure 5, GUS expression under the control of the AtSTP9

Figure 2. Transport activity of the AtSTP9 monosaccharide transporter. A, Uptake of [14C]Glc was measured in yeast strains ScLEX8 (expressing AtSTP9 sense) and ScLEX12 (expressing AtSTP9 antisense control) using an initial outside concentration of 100 μM Glc at pH 5.5. Data of ScLEX8 and ScLEX12 represent mean values of four and three independent experiments, respectively (bars represent the se); inset, the uptake rates for increasing concentrations of [14C]Glc were determined and used to calculate the Km value according to Lineweaver-Burk. Data represent mean values of two independent measurements. The AtSTP9 transporter drives Glc-uptake with a K_m of 84 ± 2.5 μM and a maximum uptake rate (V_max) of 31 ± 3.2 μmol h^{-1} mL^{-1} packed cells (p.c.), B, Uptake rates of different monosaccharide substrates at an initial outside concentration of 100 μM and inhibition of Glc-uptake by uncouplers (final concentration 50 μM) in AtSTP9-expressing yeast cells.

Figure 3. RT-PCR Analysis of AtSTP9 expression in various tissues. A, AtSTP9-specific RT-PCR products in various Arabidopsis tissues (L, leaf; F, flower; Si, silique; Se, seedling; St, stem; R, root). Arrows indicate the size of PCR products derived from genomic DNA contaminations (white arrow) and from reverse-transcribed mRNA (black arrow). A specific product derived from AtSTP9 cDNA can only be detected in RNA preparations from flowers. B, Control with Actin1-specific primers.
promoter was only found in pollen. GUS activity first appeared in flowers of stage 10 (Bowman, 1994) and was detectable throughout pollen maturation up to its germination on the stigma (Fig. 5C). An identical expression profile could be observed in the AtSTP9 promoter-GFP plants (Fig. 5, D–J). GFP expression in pollen started when flowers reached the developmental stage 10 and could be detected in isolated pollen grains (Fig. 5H) and in germinating pollen on the stigma (Fig. 5G). To further investigate the AtSTP9 promoter activity in germinating pollen, we used an in vitro pollen germination assay. Here, the GFP fluorescence was strongly visible in pollen grains and in pollen tubes (Fig. 5J).

Localization of AtSTP9 Protein in Pollen with an AtSTP9-Specific Antibody

To detect the AtSTP9 protein on the cellular level, a specific antibody was raised against the last 31 amino acids of the AtSTP9 protein, representing one of the most variable regions within the AtSTP proteins (Fig. 1). To this end, the AtSTP9 C terminus was expressed in Escherichia coli as a fusion to the maltose-binding protein and was used to inject rabbits. Finally, the generated antiserum was affinity-purified against AtSTP9 full-length protein expressed in yeast. To exclude cross-reactivity with other AtSTP proteins, the purified anti-AtSTP9 antiserum was tested for specificity on western blots. As shown in Figure 6, a specific antibody reaction can only be observed in extracts from yeast cells expressing AtSTP9 in sense orientation. The observed 43-kD band differs in size from the calculated mass of 56 kD for the AtSTP9 protein, which is due to the hydrophobic nature of membrane proteins and has also be seen for previously characterized sugar transport proteins (Beyreuther et al., 1980; Sauer and Tanner, 1984; Sauer and Stadler, 1993; Truernit et al., 1999). No signals can be seen in extracts from yeast cells ex-
pressing AtSTP9 in antisense orientation or other AtSTP genes (including all AtSTP genes expressed in pollen) in sense orientation (Fig. 6), reconfirming that the purified antiserum specifically recognizes AtSTP9 protein. This antiserum was further used to detect AtSTP9 protein in flowers of various developmental stages. As shown in Figure 7, AtSTP9 protein is only found in germinating pollen with the strongest signals in the pollen tube. The staining in cross sections of pollen tubes clearly demonstrates that the AtSTP9 monosaccharide transporter localizes to the plasma membrane and not to internal membranes (Fig. 7B, inset). No AtSTP9 protein was detectable in other flower organs. In some cases, we could also see AtSTP9-specific immunofluorescence in mature pollen grains attached to the stigma. Presumably, this pollen had already germinated, and the appendant pollen tube could not be seen in the thin section; or pollen germination was just initiated. However, AtSTP9 protein was absent in pollen of stage 10 flowers at the time when AtSTP9 promoter activity started in GUS and GFP reporter plants. This suggests that pollen grains are preloaded with AtSTP9 mRNA during maturation but that translation does not start until the pollen germinates on the stigma.

**DISCUSSION**

In the results presented here, we describe the functional characterization and in planta localization of AtSTP9, a new member of the Arabidopsis monosaccharide transporter family. We have isolated the genomic clone and the cDNA of AtSTP9, which enabled us to study AtSTP9 transport activity in the differences from wild-type plants. The AtSTP9 knockout mutants were fertile and produced normal seeds, and light microscopic analyses could not detect any differences in pollen development, pollen size or pollen number, and in vitro pollen germination when compared with wild-type anthers (data not shown), suggesting that the Atstp9 mutation does not interfere significantly with pollen viability, with pollen tube growth, or with pollen fertility.

**Identification and Analysis of a T-DNA Insertion Mutant of AtSTP9**

To further analyze the physiological role of AtSTP9 in Arabidopsis pollen development, a knockout mutant line was isolated from a T-DNA mutagenized population of Arabidopsis (Knockout Facility at the University of Wisconsin, Madison). By DNA sequencing of PCR-amplified fragments, the insertion site of the T-DNA was determined at position +1,631 within the AtSTP9 gene. Plants homozygous for the Atstp9 allele were identified by PCR, and the absence of AtSTP9 transcript was verified by RT-PCR (data not shown). We extensively analyzed this homozygous AtSTP9 knockout line but could not find any
heterologous yeast system and to follow AtSTP9 expression in promoter/reporter plants. Considering the high degree of homology to the characterized AtSTPs (Fig. 1) and the similarities of their hydrophathy profiles (data not shown), AtSTP9 (At1g50310) clearly is a new member of the AtSTP family.

**AtSTP9 Is a Putative Monosaccharide/H\(^+\) Symporter**

Analysis of the transport activity in yeast demonstrates that AtSTP9 is an active, energy-dependent monosaccharide transporter. The observed \(pH\) dependence as well as the inhibitory effect of uncouplers indicate that AtSTP9 allows substrate accumulation by a proton symport mechanism. AtSTP9 shows a \(K_m\) value for Glc of 84 \(\mu\)M, which is in the same micromolar range as the values found for the other characterized AtSTPs. However, AtSTP1, AtSTP2, AtSTP3, AtSTP4, and AtSTP6 transport a broad spectrum of monosaccharides. In contrast, AtSTP9 is the first Arabidopsis transporter that shows a high selectivity for Glc. It is not clear whether this Glc specificity of AtSTP9 is of relevance for its in planta function during pollen development and/or germination.

**AtSTP9 Is Expressed Exclusively in Pollen**

The presented set of data clearly demonstrates that AtSTP9 is exclusively expressed in pollen. RT-PCR analysis showed that the AtSTP9 transcript is only found in flowers but not in other tissues like leaves (sink or source), stems, roots, or siliques or in seedlings. A more detailed expression profile was obtained by analyzing transgenic Arabidopsis plants expressing AtSTP9-promoter/GUS or AtSTP9-promoter/GFP fusions. In these plants, GUS activity or GFP fluorescence was exclusively found in pollen starting in stage 10 flowers and continuing until pollen germination. In an in vitro germination assay, GFP expression was also found in the pollen tube. Finally, to detect the AtSTP9 protein, we generated an AtSTP9-specific antibody and examined thin sections of Arabidopsis flowers. In contrast to the early activity of the AtSTP9 promoter found in promoter/reporter plants, the AtSTP9 protein was not or only very weakly detectable in pollen grains but was very abundant in pollen tubes. A similar translational control has been found earlier for another monosaccharide transporter expressed in pollen, AtSTP4 (R. Stadler, unpublished data). Also, there are other examples showing that mature ungerminated pollen contains a store of presynthesized mRNAs that are translated only upon germination (for review, see Mascarenhas, 1989). This store presumably comprises mRNAs that have to be available immediately at the rapid onset of metabolism upon contact with the papillae and rehydration. One example is a calcium-dependent calmodulin-independent protein kinase from maize (Zea mays; Estruch et al., 1994), which was shown to be required for normal germination and pollen tube growth. Similarly, preloading of pollen with AtSTP mRNAs suggests an important function during the process of pollen germination. Like all AtSTPs characterized so far, AtSTP9 is targeted to the plasma membrane. This was already suggested by the heterologous expression analyses in yeast and could be verified by immunolocalization in cross sections of pollen tubes (Fig. 7B, inset). Thus, the AtSTP family is clearly distinguished to the recently identified plastidic Glc transporter pGlcT (Weber et al., 2000), to which the AtSTPs show only little homology.

**Possible Function of AtSTP9 during Pollen Development**

Pollen development and maturation seem to require sugar supply during distinct phases. In early stages, AtSTP2 facilitates the uptake of hexoses released during callose degradation within the pollen sac. Both AtSTP2 mRNA and AtSTP2 protein are only detectable at the beginning of callose degradation and microspore release from the tetrades and are no longer detectable after the mitotic divisions and the gametophyte formation or in mature pollen grains or pollen tubes (Truernit et al., 1999). AtSTP6 expression is only found in stages 11 and 12 of pollen grain development (Scholz-Starke et al., 2003). Although the AtSTP9 transcript can already be found in developing pollen grains, the AtSTP9 protein is only detectable in pollen tubes, suggesting an important function of AtSTP9 for the sugar supply of the growing pollen tube. Whether the Glc specificity of AtSTP9 plays a role in germination or growth of pollen tube is not clear. However, in vitro germination experiments demonstrate that pollen germination is strictly dependent on Suc in the medium. Suc could directly be taken up by AtSUC1, a Suc/H\(^+\) symporter, which was also identified in pollen tubes (Stadler et al., 1999). Alternatively, the Suc can be cleaved by a pollen-specific invertase, and the resulting hexoses might be imported. Such a pollen-specific invertase was recently identified in tomato (Lycopersicon esculentum; Goetz et al., 2001). Interestingly, the addition of Glc to germinating pollen leads to an immediate burst of the pollen tubes (Lemoine et al., 1999). This could be due to a lack of stabilizing surrounding tissue but could also indicate that disaccharides and monosaccharides have different functions during germination, where one serves as a signal (e.g. in pollen tube guidance) and the other as metabolite. One example for such a regulation mediated by the supply of different sugars is the seed development in broad bean (Vicia faba). Here, it was shown that hexoses are required for the division phase, whereas Suc initiates the storage phase (Weber et al., 1997). In this respect, the expression of two monosaccharide transporters, AtSTP4 and...
AtSTP9, in pollen tubes suggest that sugar uptake into this cell type is a highly regulated process. The expression of several sugar transporter genes in pollen and the absence of phenotypes in single knockouts of these genes (AtSTP6; Scholz-Starke et al., 2003; AtSTP9; this work) further stress the importance of sugar supply during this essential stage in plant reproduction. Future experiments involving the analysis of double/multiple knockout mutants will be directed toward unraveling the distinct role of monosaccharide transporters during pollen development, germination, and pollen tube growth.

MATERIALS AND METHODS

Strains and Growth Conditions

*Escherichia coli* strain DH5α (Hanahan, 1983) was used for cloning, Brewer’s yeast (Saccharomyces cerevisiae) strain EBY.VW4000 (kindly provided by E. Boles, University of Düsseldorf, Germany) was used for heterologous expression. The *kst* null mutant EBY.VW4000 was grown on maltose, and yeast transformation was performed as described (Gietz et al., 1992). Arabidopsis C24 was grown in the greenhouse in potting soil or on agar medium in growth chambers under a 16-h-light/8-h-dark regime at 22°C and 55% relative humidity as previously described (Truernit and Sauer, 1995). Transformations of Arabidopsis were performed with *Agrobacterium tumefaciens* strain GV3101 (Holsters et al., 1980).

Isolation of AtSTP9 Genomic and Full-Length cDNA Sequences

Screening of 250,000 plaque forming units of a genomic library from Arabidopsis (strain Columbia wild type in AGE111) generated by J.T. Mulligan and R.W. Davis, Department of Biochemistry, Stanford, CA; supplied by the European Economic Community Arabidopsis stock center, Köln, Germany) was used to isolate the desired *AtSTP9*-specific 204-bp PCR fragment encoding bases +1,222 to +1,425 of the coding region. The 3,386-bp EcoRI fragment of the Lambda insert containing the *AtSTP9* promoter and gene sequences was cloned into the vector pUC19 yielding the clone pAS91. A full-length *AtSTP9* cDNA clone was isolated by RT-PCR using primers STP9cs5 (5’-CTA GGA ATT CAT GCC TGG AGG AGC CTT TGT ATC AGA AGG) and STP9cs3 (5’-CTA GGC CCC GGC GCC TCT TCA AAC CCT CTT GAC G-3’) and total RNA from flower tissues. The PCR product was cloned into the vector pGEM-T Easy (Promega), yielding the plasmid pLEX13.

Functional Characterization of AtSTP9 by Heterologous Expression

The insert of the *AtSTP9* cDNA clone pLEX13 was ligated into the unique Ncol site of the Brewer’s yeast/E. coli shuttle vector pEX-Tag (Meyer et al., 2000), in both sense and antisense orientation, yielding constructs pACH91s and pACH91a, respectively. Constructs pACH91s and pACH91a were then used for transformation of Brewer’s yeast strain EBY.VW4000 that carries mutations in all 18 endogenous hexose transporter genes (Wieczorke et al., 2000), in both sense and antisense orientation, yielding constructs pACH91s and 0.5 μg of total RNA were incubated at 42°C for 2 min, 1 μL (200 units) of Moloney murine leukemia virus reverse transcriptase was added, and after further incubation for 50 min at 42°C, the reaction was heat-inactivated at 70°C for 15 min. The cDNA (0.5 μL) was then used as a template for amplification in PCR with primers STP9cs5 (CTA GGA ATT CAT GCC TGG AGG AGC CTT TGT ATC AGA AGG) and pas91-6 (GCA ACA ATC TAC CAA CGA TGA GC) to detect the *AtSTP9* transcript or with primers AtACT1-5’ (GGC TCC TCC AGC CAT GTT TAT TTA TTC TTC-3’) and STP9startNcoI-rec (5’-GGC TCC TCC AGC CAT GTT TAT TTA TTC TTC-3’). The PCR product was digested with BamHI and NcoI and was cloned into pEP/pUC19 (Imlau et al., 1999) in which the *AtSTP9* promoter fragment had been removed, yielding plasmid pLEX7. After verification of the sequence, a 1,222-bp fragment containing the *AtSTP9* promoter and the GFP gene was used to replace the GUS gene sequence in the vector pGPTV-BAR (Becker et al., 1992), yielding plasmid pLEX23. Arabidopsis plants were transformed with *A. tumefaciens* harboring the constructs pLEX22 or pLEX23 using the floral dip method according to the protocol of Clough and Bent (1998). Transformation of Arabidopsis with pLEX22 and pLEX23 resulted in 30 and 35 independent basta-resistant transformants, respectively. Reporter gene activity was studied in all of these plants in the T1 generation. For GFP analysis in pollen tubes, in vitro germination of pollen was as described by Stadler et al. (1999).

Computer Analysis

Splice site prediction was performed with the programs GENSCAN (http://genome.dkrz-heidelberg.de/cgi-bin/GENSCAN/genesis cgi) and NetGene2 (http://www.cbs.dtu.dk/services/NetGene2) using default parameters.

Identification of a T-DNA-Tagged AtSTP9 Knockout Line of Arabidopsis

A population of 60,400 independent kanamycin-resistant T-DNA insertion lines was screened at the Arabidopsis Knockout Facility at the University of Wisconsin Biotechnology Center (http://www.biotech.wisc.edu/Arabidop-
s) following the method described by Krysan et al. (1996, 1999). PCR reactions were performed with the T-DNA border primer JL-270 (5′-CAT TTT ATA ATA ACG CTC CCG ACA TAC ACG-3′) and the gene-specific primers AtSTP9-816f (5′-ATA ACG CAG CCA CAT GAG AAG ATC TGC AGG-3′) and AtSTP9-816r (5′-CTA GGG TTT CCT GGC TGG AGG AGC-3′). The sequence of hybridizing PCR products was determined using the T-DNA border primer JL-270 (5′-TGT CAT ATT GAC CAT CAT ACT CAT G TG-3′) to reconfirm the T-DNA insertion. Plants homozygous for the T-DNA allele were identified because they did not show a PCR product with genomic DNA and primers flanking the insertion site (AtSTP9-816f and AtSTP9-816r). To verify the absence of AtSTP9 mRNA in these AtSTP9 knockout lines, RT-PCR analysis was performed with total RNA from flowers of knockout and wild-type lines (ecotype Wassilewskija) using AtSTP9-specific primers AtSTP9F5 and AtSTP9R5 to reconfirm the T-DNA insertion. Plants homozygous for the T-DNA allele were identified because they did not show a PCR product with genomic DNA and primers flanking the insertion site (AtSTP9-816f and AtSTP9-816r).

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LITERATURE CITED


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