The Companion Cell-Specific Arabidopsis Disaccharide Carrier AtSUC2 Is Expressed in Nematode-Induced Syncytia

Katja Juergensen, Joachim Scholz-Starke, Norbert Sauer, Paul Hess, Aart J.E. van Bel, and Florian M.W. Grundler


Cyst nematodes induce a metabolically highly active syncytial cell complex in host roots. The syncytia are symplastically isolated. Because they form a strong sink, assimilates must be imported via the apoplast, thus suggesting that specific membrane-bound sugar transport proteins are expressed and activated. To identify possible candidate genes, transgenic Arabidopsis plants expressing different reporter genes under the control of different promoters from Arabidopsis sugar transporter genes were infected with the beet cyst nematode (Heterodera schachtii). With polymerase chain reaction, 13 additional sugar transporters were tested for their presence in the syncytia through the use of a syncytium-specific cDNA library. Analysis of the infected roots showed that the promoter of the sucrose (Suc) transporter AtSUC2 gene that codes for a companion cell-specific Suc transporter in noninfected plants was found to be expressed in syncytia. Its expression patterns in β-glucuronidase and green fluorescent protein plants were monitored. Syncytium-specific gene expression was confirmed by reverse transcriptase-polymerase chain reaction. Results support the idea that AtSUC2 mediates the transmembrane transfer of Suc. AtSUC2 is the first disaccharide carrier described to be activated by pathogens.

Sedentary nematodes are a group of economically important plant parasites that cause profound changes in root anatomy and plant physiology. The so-called cyst nematodes induce syncytial feeding sites in the root vascular cylinder. The nematode juveniles invade the roots and migrate through the cortical tissue by piercing cells with a stylet and releasing cell wall-degrading enzymes (Smant et al., 1998; Popeijus et al., 2000). Eventually, the juveniles enter the vascular cylinder searching for procambial or pericycle cells. In Arabidopsis, the cellular events during feeding site induction and expansion by the beet cyst nematode (Heterodera schachtii) are well described. Initiating from one single cell, a feeding site is induced by releasing secretions from the hollow stylet that is inserted into the initial cell without perforating the plasma membrane (Golinowski et al., 1996). The secretions are supposed to affect the selected cell by inducing a number of changes that finally lead to the fusion of the cell with its neighboring cells. This process continues until a syncytial feeding cell is formed that includes several hundred strongly hypertrophied cells. The syncytium provides the nutritional basis of the developing nematodes, which are strictly biotrophic parasites. Although syncytia are greatly enlarged cell systems, the developing nematodes were calculated to take up several times the syncytium content (Sijmons et al., 1991). Nematode feeding is a highly sophisticated behavioral program that meets the demands of the nematode without destroying the affected host cells (Wyss, 1992). With the aid of so-called feeding tubes, which are most probably the product of stylet secretions, nutrients are taken from the syncytial cytoplasm in a highly specific manner (Sobczak et al., 1997) so that only certain compounds are withdrawn from the syncytial cytosol (Böckenhoff and Grundler, 1994) and probably also from the syncytial endoplasmic reticulum (Sobczak et al., 1999).

Because of their high metabolic activity and the permanent withdrawal of syncytial compounds by the parasites, syncytia act as major sinks for phloem-derived solutes within the roots. Previous studies indicated that syncytia induced by the cyst nematode are symplastically isolated from surrounding host cells. Plasmodesmata to neighboring cells are rare and not functional because of deposited cell wall material (Grundler et al., 1998). The absence of a...
symplasmic pathway between syncytia and the surrounding tissue could also be visualized by microinjection of low-\(M_c\) weight fluorescent dyes into the syncytium. In no cases were the fluorochromes able to spread from the syncytium (Böckenhoff and Grundler, 1994). These results strongly indicated a symplasmic isolation of the syncytium and, in conclusion, an apoplastic transfer of assimilates from the phloem into the syncytial complex. Thus, Suc has to be unloaded from the phloem into the apoplast and then to be imported into the syncytia either directly via a syncytial Suc carrier or, after extracellular hydrolysis by cell wall-bound invertases, by a syncytial monosaccharide transporter.

Numerous genes and cDNAs encoding such sugar transporters have been cloned from different plant species during the last decade (Lalonde et al., 1999; Böttner and Sauer, 2000; Williams et al., 2000). Higher plants possess large families of both monosaccharide and disaccharide transporters and even Arabidopsis has at least 14 genes for monosaccharide transporters—the AtSTP gene family—and eight genes for disaccharide transporters—the AtSUC gene family (Williams et al., 2000). Suc transporters were found both in the phloem, where they catalyze the energy-dependent accumulation of Suc (Stadler et al., 1995; Stadler and Sauer, 1996; Kühn et al., 1997), and in sink tissues, where they catalyze the import of Suc primarily for storage (Weber et al., 1997; Lemoine et al., 1999; Stadler et al., 1999). In leaves, the transporters are supposed to perform the import of Suc from the apoplastic space into the sieve element/companion cell complexes of the minor veins (van Bel, 1993). They are also discussed to be responsible for the retrieval of Suc, which permanently leaks from the sieve tubes into the surrounding apoplast (Stadler and Sauer, 1996). The expression of monosaccharide transporter genes, however, is strictly confined to sink tissues such as pollen (Truernit et al., 1999), the embryo (Weber et al., 1997), or seedling roots (Sher-son et al., 2000), where monosaccharides are imported primarily for fueling the cellular metabolism. Several monosaccharide transporter genes were shown to be induced in response to stress, such as wounding, elicitor treatment, or pathogen infection (Harrison, 1996; Truernit et al., 1996; Böttner et al., 1999).

The function of the transporters is dependent on \(H^+\)-ATPases generating the energy for the secondary active transport. In Arabidopsis, the plasma membrane \(H^+\)-ATPases are encoded by a multigene family (DeWitt et al., 1991; DeWitt and Sussmann, 1995).

To date, nothing is known about the occurrence and function of sugar carriers in plant-nematode interactions. Especially in cases where specific feeding sites are formed, mediated sugar transport may play a crucial role in these interactions. Therefore, in the present study, seven transgenic Arabidopsis lines that had been transformed with fusion constructs of reporter genes and promoters from different sugar transporter genes were infected with beet cyst nematode. Our aim was to identify transport proteins that catalyze the import of carbohydrates from the apoplast into the nematode-induced syncytium.

### RESULTS

**Promoter-gus Lines**

Six lines of transgenic Arabidopsis plants containing different promoter/reporter gene constructs were screened for \(\beta\)-glucuronidase (GUS) activity in syncytia. The promoters were derived from the Arabidopsis monosaccharide transporter genes \(AtSTP2\), \(AtSTP3\), \(AtSTP4\), and \(AtSTP6\) and from the Arabidopsis disaccharide transporter genes \(AtSUC1\) and \(AtSUC2\). The characteristics of gus expression in these lines are summarized in Table I. Syncytial GUS staining was only found in plants harboring the \(AtSUC2\) promoter-gus fusion. No GUS staining was found in the syncytia of those lines driving gus expression under the control of the \(AtSUC1\) promoter or under the control of one of the \(AtSTP\) promoters.

**Analysis of \(AtSUC2\) Promoter-gus Plants**

The \(AtSUC2\) gene encodes a plasma membrane Suc/H\(^+\) symporter (Sauer and Stolz, 1994). In addition to the companion cell-specific expression observed for this gene in uninfected plants (Truernit

### Table I. Identification of GUS activity in various tissues of plants from six different, nematode-infected promoter-gus lines of Arabidopsis

<table>
<thead>
<tr>
<th>Expression</th>
<th>Syncytia*</th>
<th>Root</th>
<th>Stem</th>
<th>Leaf</th>
<th>Flower</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>(AtSTP2)-gus</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>Truernit et al. (1999)</td>
</tr>
<tr>
<td>(AtSTP3)-gus</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Böttner et al. (1999)</td>
</tr>
<tr>
<td>(AtSTP4)-gus</td>
<td>–</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>Truernit et al. (1996)</td>
</tr>
<tr>
<td>(AtSTP6)-gus</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Scholz-Starke et al. (2003)</td>
</tr>
<tr>
<td>(AtSUC1)-gus</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>Stadler et al. (1999)</td>
</tr>
<tr>
<td>(AtSUC2)-gus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Truernit and Sauer (1995)</td>
</tr>
</tbody>
</table>

*+, GUS activity; –, No GUS activity.
and Sauer, 1995; Stadler and Sauer, 1996), in this study, the AtSUC2 promoter turned out to be active in syncytia induced by beet cyst nematode. Twelve-day-old plants were inoculated and GUS-positive syncytia were examined at 2, 4, and 7 d after infection (dai).

GUS staining in syncytia was generally strong and could easily be detected under the dissecting microscope. Two days after infection (Fig. 1A), the staining was located in a diffuse zone within and around the developing syncytia. Later, at 7 dai, the GUS staining was more restricted to the syncytia (Fig. 1C). For a more detailed inspection, histological sections of syncytia were examined. In cross sections of specimens taken at 4 dai, the GUS staining was found in syncytia but, to a lower level, also in almost all other cells of the central cylinder (Fig. 1B). In roots of uninfected control plants, phloem cells were intensely stained but a faint blue stain was also found in cells bordering the phloem (Fig. 1D). This faint staining may be because of a diffusion of the dye before crystallization.

To find out whether the expression pattern of AtSUC2 in syncytia is specific for these structures, the AtSUC2 promoter-gus plants were infected with the sedentary root-knot nematode. This nematode also induces a strong metabolic sink with its feeding cells in the central cylinder. However, the feeding cells differ considerably from syncytia induced by cyst nematodes. They consist of a number of hypertrophied, multinucleate giant cells that conserve their character as single cells and remain separated throughout the nematode life cycle. On the other hand, they are interconnected by plasmodesmata in numerous pit fields (Hussey and Grundler, 1998). Developing nematodes and their giant cells are embedded by strongly proliferating gall tissue that is formed upon infection. A strong gus expression was observed at the phloem in the central cylinder above the induced feeding site (2 dai; not shown). The staining had increased in intensity and expansion but never occurred in the feeding cells themselves (7 dai; Fig. 1E).

Analysis of AtSUC2 Promoter-gfp Plants

Because feeding cell induction and expansion is a dynamic process, reporter gene expression was determined at different time points during syncytium development. The gus reporter gene does not allow the continuous monitoring of the promoter activity in a single plant. Therefore, a transgenic Arabidopsis line was taken, in which the AtSUC2 promoter was fused to the open reading frame of the gfp gene. Like the gus gene, gfp is specifically expressed in the companion cells of uninfected Arabidopsis or tobacco (Nicotiana tabacum) plants, when expressed under the control of the promoter pAtSUC2 (Imlau et al., 1999).

As expected from the GUS data presented in Figure 1, a through D, GFP fluorescence was also detected inside the syncytia of nematode-infected Arabidopsis plants. However, GFP fluorescence was only observed in syncytia induced by female nematodes (Fig. 1, G and H) and usually not in those induced by males (Fig. 1, I and J). Only when several male juveniles were associated to a single syncytial complex that is formed by fusion of several syncytia, visible GFP fluorescence could also be induced by male nematodes (not shown).

The percentage of GFP-positive syncytia strongly correlated with syncytium development. GFP fluorescence was not detected before 6 dai (Fig. 2). Between 6 and 20 dai, the percentage increased to a maximum level of 100% GFP-positive syncytia. After that, the frequency slowly decreased during the completion of the parasitic stage and the simultaneous degradation of syncytia.

Fluorescent syncytia were viewed with an epifluorescence microscope (Fig. 1, G and H) and with a CLSM (Fig. 1F). The conventional micrographs and the optical sections made by CLSM both showed the GFP fluorescence inside the syncytium and not in neighboring cells.

The analyses of nematode-infected roots from both pAtSUC2-gus plants and pAtSUC2-gfp plants clearly suggest that expression of the AtSUC2 gene is induced in syncytia and that this induction is regulated by factors associated with syncytium development.

Identification of AtSUC2 mRNA in Wild-Type Syncytia by Reverse Transcriptase (RT)-PCR

The analysis of promoter activities using reporter gene constructs is indirect and usually performed with not more than 2,000 bp from the 5′-flanking sequence of the gene of interest. Therefore, we analyzed the expression of the AtSUC2 gene in nematode-infected wild-type plants using RT-PCR for the identification of AtSUC2 mRNA in syncytia. For these analyses, samples of cytoplasm were drawn from syncytia with a special micro-aspiration method between 5 and 7 dai (P.S. Puzio, P. Voss, and F.M.W. Grundler, unpublished data). After RNA isolation and synthesis of cDNA, PCRs were performed with oligonucleotide primers specific for the AtSUC2 coding sequence. Using the syncytial cDNA as template the PCR analyses yielded only one single band with the expected length of 661 bp (Fig. 3). This size is exactly the length of the corresponding fragment from the AtSUC2 mRNA. As a control, PCR was also performed using the same pair of primers under the same conditions with genomic DNA as template. The resulting band had a length of 1,182 bp, which is because of an intron in the genomic sequence. This result confirms the specificity of the PCR reaction and provides additional evidence that AtSUC2 mRNA is present in syncytia of nematode-infected roots from wild-type plants.
Figure 1. (Legend appears on facing page.)
PCR Detection of Sugar Transporter Genes in a Syncytia-Specific cDNA Library

With PCR, 11 additional sugar transporters were tested for their presence in syncytia through the screening of a syncytia-specific cDNA library. Gene-specific primers were used from five monosaccharide transporters (*AtSTP7*, *AtSTP9*, *AtSTP10*, *AtSTP11*, and *AtSTP13*) and six disaccharide transporters (*AtSUC3*, *AtSUC4*, *AtSUC5*, *AtSUC6*, *SUC8*, and *SUC9*). As control, primers of *AtSUC2* were also tested. The results are summarized in Table II. As expected from the GUS and GFP data presented in Figure 1, a specific PCR product could be detected using primers of *AtSUC2*. No amplification products with cDNA from syncytia as template were detectable with gene-specific primers of *AtSUC3, 5, 6, 8, and 9* and *AtSTP7, 9, 10, 11, and 13*. However, the screening of the cDNA library with gene-specific primers of *AtSUC4* yielded a positive PCR product with the expected length of 461 bp. These examinations gave a first indication that another Suc transporter, *AtSUC4*, may also be present in syncytia of beet cyst nematode.

DISCUSSION

The nematode-induced syncytium is a metabolically highly active structure that has a high demand for water and assimilates (Graudel and Böckenhoff, 1997). Detailed ultrastructural (Graudel et al., 1998) and physiological (Böckenhoff and Graudel, 1994; Böckenhoff et al., 1996) analyses revealed that syncytia are symplastically isolated from surrounding tissues including the phloem. Functional plasmodesmata could not be found, nor could a transition of injected fluorescent markers into neighboring cells be observed. A lack of symplastic connections between the phloem of Arabidopsis uninfected roots and the adjacent cells was also shown in studies analyzing the phloem trafficking of the low-M₉ compound carboxy fluorescein (Oparka et al., 1994) or the 27-kD GFP (Imlau et al., 1999). Neither of the two compounds were able to traffic out of the root phloem into the adjacent procambial and pericyclic cells that are usually selected by the nematodes for feeding site induction. Unloading of both substances from the phloem was observed only in the very root tips.

![Figure 1](image1.png)

**Figure 1.** A, Line p*AtSUC2-gus* 2 dai; *gus* is expressed in a syncytium and in the surrounding tissue. B, Transverse section of a line *AtSUC2* root with a syncytium 4 dai. The syncytium and almost all cells of the central cylinder show *gus* expression. C, Line p*AtSUC2-gus* 7 dai; *gus* is solely expressed in a syncytium, no staining is visible in neighboring cells. D, Transverse section of a noninfected root of line *AtSUC2* at the same age and stage as the specimen shown in B. *Gus* expression is seen in the phloem. A faint blue staining is visible in cells around the phloem. E, Root galls induced by the root knot nematode (*Meloidogyne incognita*) 7 dai in Arabidopsis plants carrying a p*AtSUC2-gus* fusion. Intense GUS staining accumulates at the phloem above the induced feeding sites proximal to the root base. Neither gall tissue nor feeding cells show *gus* expression. F, Confocal laser scanning microscope (CLSM); picture of a syncytium in p*AtSUC2-gfp* roots (8 dai). Green fluorescent protein (*GFP*) is exclusively in the syncytium. The red stain [N-(4-sulfobutyl)-4-(4-(4-dibutylamino)phenyl)butadienylnitripyridinium inner salt (RB-160)] is specific to non-charged structures. G, Third stage beet cyst nematode female juvenile (8 dai) associated to its syncytium in p*AtSUC2-gfp* roots. The central cylinder is strongly hypertrophied because of syncytium formation. Below the infected root, an uninfected root is depicted for comparison. H, Same specimen as shown in G with blue-light excitation (455 nm). GFP is seen in the syncytium and the phloem of the infected and uninfected root. I, Third stage beet cyst nematode male juvenile (8 dai) associated to its syncytium. J, Same specimen as shown in I with blue-light excitation. GFP is only seen in the phloem but not in the syncytium of the infected root. The bright-yellow fluorescence is caused by cells damaged during nematode invasion. K, Proliferating gall tissue containing the feeding cells of developing nematode juveniles; S, syncytium; n, nematode; arrow, root tip. B and D, Bars = 50 μm; A, C, E, I, and J, bars = 200 μm; F through H, bars = 500 μm.

![Figure 2](image2.png)

**Figure 2.** Percentage of GFP-positive female syncytia of beet cyst nematode in roots of nematode-infected p*AtSUC2-gfp* plants at different stages after infection. Columns show the percentage of GFP-positive female syncytia in relation to all female syncytia analyzed. For each time point, 22 infected single plants were screened. Vertical bars indicate means ± s.s.
The present study was performed to test and prove the concept of active sugar import into syncytia via plasma membrane-localized sugar transport proteins. Screening of a collection of promoter-gus lines and the search for sugar transporter transcripts in syncytial extracts from wild-type plants were used to identify potential candidates. The idea was that nematode infections trigger the expression of one or several transporter genes, which normally are expressed at other locations or developmental stages of Arabidopsis plants. The data presented in this paper show that the expression of the gene encoding the phloem-specific AtSUC2 Suc transporter is induced in syncytia, and that both AtSUC2 mRNA and AtSUC2 protein are synthesized.

To elucidate the temporal and developmental regulation of the AtSUC2 promoter in syncytia, analyses were performed using pAtSUC2-gfp plants. The product of the reporter gene was clearly identified within syncytia. The number of fluorescent syncytia increased with time, reflecting the expanding size and activity of the syncytia. The highest percentage of GFP-positive syncytia was observed at 20 dai at a developmental stage of the nematode life cycle when syncytia have reached maximum expansion. At that time, mature females start egg production and take up the highest amounts of nutrients. Weak fluorescence was still seen at more than 60 dai (Fig. 2), when some females are still alive.

Interestingly, GFP fluorescence was only seen in syncytia associated with females nematodes and in those cases where the syncytia of several males had been fused to one single syncytium. In contrast, GUS staining could be observed in syncytia induced by male and female nematodes. It has been shown that GUS activity in general is detectable at a lowest limit of GUS of less than 100 molecules per cell (Köhler, 1998), whereas more than 10,000 molecules per cell are necessary to visualize GFP expression in CLSM (Patterson et al., 1997). This fact seems to limit detection of GFP activity to female syncytia and syncytial complexes of several males.

The RT-PCR analyses of AtSUC2 mRNA clearly revealed a specific gene activation. As with other genes induced in syncytia, it is difficult to evaluate whether this is a process directly induced by the nematode or whether it is an effect that accompanies the development and metabolic activity of syncytia. The nematodes are able to induce a modified programming in the affected cells that leads to the induction of AtSUC2 expression.

Figure 4 presents a concept that reflects our model of sugar transport at nematode infection sites. We were able to detect transcriptional activation of AtSUC2 and the occurrence of the gene product in syncytia. The import of Suc from the apoplast is the basis of the high metabolic activity of syncytia and the continuous uptake of metabolites by the nematodes. We conclude that AtSUC2 is responsible for a

<table>
<thead>
<tr>
<th>Suc Transporters</th>
<th>PCR ±</th>
<th>Monosaccharide Transporters</th>
<th>PCR ±</th>
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</thead>
<tbody>
<tr>
<td>AtSUC2 (At1g22770)</td>
<td>+</td>
<td>AtSTP7 (At4g02050)</td>
<td>−</td>
</tr>
<tr>
<td>AtSUC3 (At2g02860)</td>
<td>−</td>
<td>AtSTP9 (At1g050310)</td>
<td>−</td>
</tr>
<tr>
<td>AtSUC4 (At1g09960)</td>
<td>+</td>
<td>AtSTP10 (At3g19940)</td>
<td>−</td>
</tr>
<tr>
<td>AtSUC5 (At1g71890)</td>
<td>−</td>
<td>AtSTP11 (At5g23270)</td>
<td>−</td>
</tr>
<tr>
<td>AtSUC6 (At5g43610)</td>
<td>−</td>
<td>AtSTP13 (At5g26340)</td>
<td>−</td>
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<td>AtSUC8 (At2g14670)</td>
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<td>−</td>
</tr>
<tr>
<td>AtSUC9 (At5g06170)</td>
<td>−</td>
<td></td>
<td>−</td>
</tr>
</tbody>
</table>

*+, Positive PCR reaction; −, negative PCR reaction.
Potential apoplastic transport mechanisms of sugar between se/cc complex and the syncytium

![Diagram showing transport mechanisms]

**Figure 4.** Concept of the putative functions of sugar transport proteins at nematode infection sites. In healthy roots, AtSUC2 retrieves Suc leaking from the phloem. At infection sites, the phloem is unloaded and AtSUC2 transports Suc into the syncytium. In addition or alternatively, assimilates could also be supplied as Glc and Fru after being processed by an invertase. Appropriate hexose transporters have not yet been identified.

specific import and maintenance of a high Suc level in syncytia because infection assays with the root-knot nematode showed the promoter is not activated in its feeding cells. This indicates that the mechanism of gene activation in syncytia is specific and not related generally to the formation of pathological sink tissue. In the healthy Arabidopsis root, AtSUC2 retrieves Suc, which leaks from the phloem because of the high concentration gradient. Alternatively, a role of AtSUC2 in the unloading of Suc from the phloem has been discussed (Truernit and Sauer, 1995). In leaves, where AtSUC2 catalyzes the Suc accumulation in companion cells (Stadler and Sauer, 1996), it is assumed to be functionally linked to the companion cell-specific H\(^+\)-ATPase AHA3 (DeWitt et al., 1991; DeWitt and Sussmann, 1995), which is likely to provide the proton motive force for Suc import into the phloem. Because the expression pattern of AtSUC2 matches the phloem-specific expression of the AHA3 gene, AHA3 and AtSUC2 may represent a pair of primary and secondary active transporters also in the plasma membrane of syncytial cells. With RT-PCR, it was shown that the companion cell-specific H\(^+\)-ATPase AHA3 was present in syncytial RNA (data not shown). Thus, it can be assumed that AHA3 is generating the proton gradient and the membrane potential needed by AtSUC2 for the import of Suc into the syncytium.

At the infection sites, a specific phloem unloading of C\(^{14}\)-labeled Suc was shown by Böckenhoff et al. (1996). It is still unclear whether this enhanced unloading is because of a specific inhibition of the retrieval mechanism or the H\(^+\)-ATPase, or whether an active unloading mechanism is involved. Future investigations on the pH in the phloem of healthy roots and at infection sites should give information on the role of the H\(^+\)-ATPase.

AtSUC2 is the first disaccharide carrier described to be activated by a plant pathogen and it is tempting to find out why it is employed in syncytia. The physiological relevance of the sugar transport into syncytia via AtSUC2 is not yet clear because other Suc transporters as well as hexose transporters may also contribute to the assimilate supply of syncytia. None of the five hexose transporter promoters tested so far showed activity in the syncytium, but AtSUC4, a disaccharide carrier (AtSUC4 = AtSUT4 as published by Weise et al., 2000) was detected in the syncytium-specific CDNA library. The promoter of AtSUC4 has been shown to be active in minor veins in source leaves and AtSUC4 is also expressed in sink tissues like sink leaves, flowers, and fruits. In sink tissue, AtSUC4 is supposed to have a function in Suc uptake into sink cells (Weise et al., 2000). Further studies will be performed to determine the detailed time course of AtSUC4 activity.

**MATERIALS AND METHODS**

**Plant and Nematode Culture**

For the experiments, plants of Arabidopsis wild-type C-24 and seven transgenic Arabidopsis lines with promoter of sugar transporters (AtSUT2, 3, 4, and 6 and AtSUC1 and 2) were used. The plants expressed the gus gene or the gene of the gfp under the control of the promoter of the corresponding sugar transporter gene. Cloning of the promoters and transformation of Arabidopsis were described previously (Truernit and Sauer, 1995; Truernit et al., 1996; Imlau et al., 1999). In each case, three independently transformed gus lines were tested. For AtSUC2, two independent promoter-gfp fusion lines were examined. Because the different lines gave nearly the same results, we present only one set of data for each case.

Seeds of Arabidopsis were surface sterilized for 10 min in 5% (w/v) calcium hypochlorite and for 5 min in 70% (v/v) ethanol and washed subsequently three times in sterile water. Sixteen sterilized seeds were placed in two lines into sterile petri dishes on a modified Knop nutrient medium and kept at 4°C for 3 d and then under a 16-h-light/8-h-dark regime at 25°C. The petri dishes were placed tilted slightly to promote unidirectional root growth. Plants for microscopic detection of GFP fluorescence were grown singly on a thin layer of agar on a glass coverslip (Böckenhoff and Grundler, 1994). After growth for 12 d, roots were inoculated with batches of about 50 freshly hatched beet cyst nematode (Heterodera schachtii) second stage juveniles, obtained from sterile agar stock cultures (Grundler, 1989).

**Histochemical GUS Assay**

Infected plants were stained on plates by adding 7 mL of staining solution on top of the agar layer (50 mM Na\(_2\)PO\(_4\) [pH 7.0], 5 mM EDTA [pH 8.0], 0.05% (v/v) Triton X-100, 0.5 mM K\(_3\)Fe[CN]\(_6\), 0.5 mM K\(_3\)Fe[CN]\(_6\)3H\(_2\)O, and 1 mM 5-bromo-4-chloro-3-indolyl-β-D-glucuronidase). GUS staining was carried out at 37°C for 16 h. Chlorophyll was removed by incubation of the samples in 70% (v/v) ethanol. Plants were examined for the presence of GUS activity 2, 4, 7, 10, and 21 dai. For each time point, 12 petri dishes were prepared. The percentage of GUS-positive syncytia was determined for each petri dish.

**Sectioning and Microscopic Analysis**

To prepare sections of fresh GUS-stained tissue a vibrating blade microtome VT 1000 S (Leica, Heidelberg) was used. Samples were collected by dissecting segments of roots containing blue-stained syncytia and subsequently embedded in 4% (w/v) low-melting agarose. Cross sections were cut with a frequency of the knife between 70 and 80 Hz, knife advance of 0.20 mm s\(^{-1}\), and an amplitude of 0.6 mm. Sections with a thickness of 40 μm were examined an Axioskop light microscope (Zeiss, Oberkochen, Germany).
Microscopic Detection of GFP Fluorescence

Roots growing on the coverslip were observed using an IMT-2 inverted fluorescence microscope (Olympus, Tokyo) equipped with a broad-band blue filter combination (IMT2-DMB) supplemented by a B460 barrier filter and an appropriate 455-nm excitation filter. GFP fluorescence was checked at an interval of 2 to 4 d. Inspection intervals were expanded to 8 to 12 d when nematodes had completed their development. Twenty-two plants per line were tested. Pictures were taken on Elitechrome 100 daylight film (Eastman Kodak, Rochester, NY).

Syncytia were also viewed with a Leica TCS 4D CLSM. For membrane staining, 25 mg of RH-160 was solubilized in 1 mL of ethanol. Two microliters of the stock was added to the bathing medium. For two-channel scans with GFP in the one and RH-160 in the other, the 488- and 564-nm lines of the argon/krypton laser (Omnitechrome, Chino, CA) were used simultaneously for excitation. A 561-nm beam splitter passed the light to the two detectors, and the appropriate emission filters were selected for imaging. Micrographs were recorded in a digitized format (TIFF).

RNA Isolation and RT-PCR

With the aid of a microcapillary and a micromanipulator, the cytoplasm was extracted from syncytia without contaminations from noninfected root cells or nematodes (P.S. Puzio, P. Voss, and F.M.W. Grundler, unpublished data). Samples of cytoplasm were collected between 5 and 7 d after nematode infection. Because syncytia yield only a small quantity of starting material, RNA was isolated from 100 micro-aspirated syncytia by using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions (P.S. Puzio, P. Voss, and F.M.W. Grundler, unpublished data).

To synthesize first strand cDNA, SUPERSCRIPT II RNase H-Reverse Transcriptase (Life Technologies/Gibco-BRL, Cleveland) was used. For the reaction, 11.5 µL of dissolved RNA, 40 pmol oligo(dT)18 primer, and 10 pmol SUC2-reverse primer (5’-CCCCAGCTACCCGCACTGGGC-3’) and SUC2-forward (5’-GACCTAAGTCCACGCTCAGCGTAC-3’), respectively, were incubated for 10 min at 70°C, then chilled quickly on ice. Subsequently, 4 µL of 5X first strand buffer, 2 µL of 0.1 M dithiothreitol, and 250 µM dNTPs were added. After incubation for 2 min at 42°C, 200 µL of SUPERSCRIPT II was added and the tube was incubated for 50 min at 42°C. The reaction was inactivated by heating at 70°C for 15 min.

PCR was performed with the oligonucleotide primers SUC2-forward (5’-GATCCGTGGTGCCTCCCTCC-3’) and SUC2-reverse or AHA3-forward (5’-GACCTAAGTCCACGCTCAGCGTAC-3’), respectively, and 50 ng of the primary DNA library as template, 100 pmol of each primer, 2.5 mM dNTPs, 250 µM MgCl2, 200 units of SUPERSCRIPT II, and 2.5 µL of dissolved RNA, 40 pmol oligo(dT)18 primer, and 10 pmol SUC2-reverse primer (5’-GCCAGCCCTTCTTGTTGCACTA-3’) and AHA3-forward (5’-GGAGTCAGAGCTGGTGCTTG-3’), respectively. For all pairs of primers, the same temperature program was followed, except that the annealing temperatures were 60°C (AISUC2, AISUC3, AISUC4, AISUC6, AISUC8, AISTP7, AISTP9, AISTP11, and AISTP13) and 56°C (AISUC9), respectively. The cycle order was as follows: denaturation for 5 min at 95°C; 1 min at 92°C, 1 min at 56°C, and 2 min at 72°C; and a final extension at 72°C for 10 min. A total of 30 cycles was used. In cases without PCR products, different annealing temperatures were tested accordingly.

Screening of a Syncytia-Specific cDNA Library

A cDNA library from syncytia-specific RNA was constructed using a PCR-based SMART cDNA library construction kit (CLONTECH Laboratories, Palo Alto, CA) according to the manufacturer’s instructions (P.S. Puzio, P. Voss, and F.M.W. Grundler, unpublished data).

The library was screened by PCR, using specific primers of 11 additional sugar transporters. PCR amplifications were performed with the following gene-specific primers: AISUC2 (At1g22710), 5’-GATCCGTGGTGCCTCCCTCC-3’ and 5’-GATCCGTGGTGCCTCCCTCC-3’; AISUC8 (At5g06170), 5’-GATTGGACTTGAGGACAGGAGGAGGCC-5’ and 5’-GATTGGACTTGAGGACAGGAGGAGGCC-5’; AISUC9 (At5g06170), 5’-GATTGGACTTGAGGACAGGAGGAGGCC-5’ and 5’-GATTGGACTTGAGGACAGGAGGAGGCC-5’; AISUC10 (At3g19940), 5’-GCTTGGCCACTGACAGACCTGGGC-3’ and 5’-GCTTGGCCACTGACAGACCTGGGC-3’; AISUC11 (At5g06170), 5’-GCTTGGCCACTGACAGACCTGGGC-3’ and 5’-GCTTGGCCACTGACAGACCTGGGC-3’; AISTP1 (At3g19940), 5’-GCTTGGCCACTGACAGACCTGGGC-3’ and 5’-GCTTGGCCACTGACAGACCTGGGC-3’; AISTP7 (At3g0310), 5’-GCTTGGCCACTGACAGACCTGGGC-3’ and 5’-GCTTGGCCACTGACAGACCTGGGC-3’; AISTP9 (At5g26304), 5’-GCTTGGCCACTGACAGACCTGGGC-3’ and 5’-GCTTGGCCACTGACAGACCTGGGC-3’; and AISTP13 (At5g26304), 5’-GCTTGGCCACTGACAGACCTGGGC-3’ and 5’-GCTTGGCCACTGACAGACCTGGGC-3’.

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