RESEARCH PAPER

Sucrose supply to nematode-induced syncytia depends on the apoplastic and symplasmic pathways

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Abstract

The plant parasitic nematode Heterodera schachtii induces syncytial feeding structures in the roots of host plants. Nematode-induced syncytia become strong sink tissues in the plant solute circulation system as the parasites start withdrawing nutrients. In the present work, the expression pattern of the phloem-specific sucrose transporter AtSUC4 (also described as AtSUT4) is analysed in syncytia induced by H. schachtii and it is compared with that of AtSUC2, another phloem-specific sucrose transporter, which is expressed in syncytia. The temporal expression pattern was monitored by GUS-tests and real-time RT-PCR, while the localization within the syncytia was performed using in situ RT-PCR. In this context, the concentration of sucrose in infection sites was also analysed and, in fact, an increase in response to syncytium development was found. Silencing of the AtSUC4 gene finally resulted in a significant reduction of female nematode development, thus demonstrating a function for this gene for the first time. It is therefore concluded that AtSUC4 plays a significant role in the early phase of syncytium differentiation when functional plasmodesmata to the phloem are not yet established. It is further concluded that, during syncytium establishment, transporters are responsible for sucrose supply and, at a later stage, when a connection to the phloem is established via plasmodesmata, transporters are required for sucrose retrieval.

Key words: AtSUC2, AtSUC4, Heterodera schachtii, nematode, plasmodesmata, sucrose transporter.

Introduction

The sedentary endoparasitic cyst nematode Heterodera schachtii evolved a highly specific and complex host–pathogen interaction which leads to the formation of a syncytial feeding site in the plant root. In Arabidopsis thaliana, infective second-stage juveniles (J2) invade the roots and penetrate the central cylinder intracellularly where they select a single cell for feeding-site induction (Wyss et al., 1992). Starting from this initial cell, neighbouring root cells are incorporated by local cell wall dissolutions, thus forming a syncytial cell complex (Golinowski et al., 1996; Grundler et al., 1998). Females and males differentiate in the third juvenile stage (about 7 d after inoculation) depending on nutrient supply, whereby females require higher amounts of nutrients than males. High metabolic activity of the syncytia and continuous withdrawal of nutrients by the juveniles turn the feeding cells into major sinks within the host plants. The sink pattern and the uptake of carbohydrates were shown by loading experiments using 14C-labelled sucrose. The radioactive signal could be monitored from source leaves of A. thaliana plants, to which the labelled sucrose was applied, along the roots, within syncytia, and in the associated nematodes (Böckenhoff et al., 1996).

In most plant species, sucrose is the major carbohydrate translocated in the phloem from source to sink tissues. In Arabidopsis, the phloem is loaded both apoplasmically and symplasmically (Haritatos et al., 2000; Kühn, 2003) and, so far, two phloem-specific sucrose transporters—AtSUC2 and AtSUC4 (also described as AtSUT4)—have been characterized (Truemper and Sauer, 1995; Stadler and Sauer, 1996; Barker et al., 2000; Weise et al., 2000). While AtSUC4 was suggested to be responsible primarily...
Materials and methods

Plant and nematode culture

Arabidopsis seeds were surface-sterilized, first for 10 min in 5% (v/w) calcium hypochlorite, secondly, for 5 min in 70% EtOH, and then immediately washed with distilled water three times. Sterile seeds were planted on 0.2% Knop medium and grown with 16 h light/8 h dark at 25 °C. After 12 d, each plant was inoculated with 50 freshly hatched J2 H. schachtii (Sijmons et al., 1991), obtained from sterile agar stock cultures. Inoculated plants were kept in darkness overnight and put back into the growth chamber the next day. For sugar analysis, plants were grown on soil/sand culture (1:2, v/v) in 24-well plates. In each well, 5–10 plants were grown that were inoculated after 12 d with approximately 500 J2 per well.

Histochemical GUS assay

Histochemical assay for GUS expression was performed by screening nine Petri dishes each with seven Arabidopsis plants containing a pAtSUC4-gus fusion construct (Weise et al., 2000). At 2, 4, 6, 8, 10, 12, 14, 17, 20, 25, and 30 dai GUS-tests were performed by incubating the whole plates with X-Gal (5-bromo-4-chloro-3-indolyl-β-d-galactopyranoside) (PEQLAB Biotechnologie GMBH, Erlangen, Germany) at 37 °C overnight in darkness and washing them the next day with 70% EtOH. GUS expression in roots and syncytia was monitored microscopically, blue syncytia were counted, and their percentage was calculated from the total number of syncytia. Pictures were taken by using a stereomicroscope (Stemi 2000, Zeiss) and a digital camera (Coolpix995, Nikon).

In situ RT-PCR

Syncytia at 10 dai were dissected from root tissue, carefully removed from the growth media, and immediately put into cold fixation solution as described in Koltai et al. (2001). Syncytia were embedded in 4% low-melting agarose and sectioned into slices (25 μm thick) using a vibratome (VT 1000, Leica, Germany). In situ RT-PCR was carried out as described previously (Koltai and Bird, 2000; Urbanczyk-Wochniak et al., 2002; Wieczorek et al., 2006). Treated cross-sections were screened and photographed under an inverted microscope (Axiovert 200M, Zeiss, Hallerbergmoos, Germany) containing an integrated camera (AxioCam MRc5; Zeiss).

RNA isolation

Total RNA was isolated from syncytia of H. schachtii and equivalent root parts of non-infected plants. Syncytia were dissected from the roots, taking care not to lose their contents by damaging them and at the same time keeping the amount of non-infected root tissue to the minimum so as not to dilute the effects. The plant material was immediately flash-frozen in liquid nitrogen. RNA was isolated using the RNase Plant Mini Kit (Qiagen, Hilden, Germany), according to the producer’s instructions, including a DNase I digest (Qiagen, Hilden, Germany).

Real-time RT-PCR

RNA samples of roots and syncytia were analysed and quantified by an Agilent 2100 bioanlyser (Agilent Technologies, Palo Alto, CA, USA). RNA was transcribed into cDNA using random primers [ olig(dN)4] and the SuperScript III reverse transcriptase (Invitrogen, Carlsbad, CA, USA) according to the producer’s instructions. For quantitative RT-PCR, primers and probes were selected by using the Primer Express v2.0 Software (Applied BioSystems, Foster City, CA, USA) and checked for gene specificity within the Arabidopsis genome compared with the gene database. The
following sequences were used: 18S RNA, primer forward -TGACACGGGAGGTAGTGACA-, primer reverse -AGTCTGGTAATGGAGATGG-, probe -TTAATAACATACCCGGCCT-, E = 0.97, R² = 0.99; AtSUC2 (At1g22710), primer forward -GATCAAGATCG; reverse, CACGAGAAGCACGCT), primer reverse -TTTCCC-TAGGGTTTCTGAAATGTC-, probe -CCCAAGCCATTGTTA-, E = 0.96, R² = 0.99; and AtSUC4 (At1g09960), primer forward -CTTATTACATGTGGGTCCTCATT-, reverse -ACGGAGAAT; probe -CGCCGACCGAAGGGACAAGCCGA). The PCR was carried out at 95 °C for 5 min, 95 °C for 10 min, and was followed by 40 cycles at 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 60 s. After each run a dissociation curve was performed to rule out the formation of primer dimers. PCR efficiencies were checked for AtSUC4 (E = -0.95, R² = 0.99) and for 18S RNA (E = 0.92, R² = 0.996).

Nematode infection test

Plants were grown under sterile conditions and inoculated with J2 as described. Wild-type Col and ppyk20-AtSUC4 RNAi lines were grown on Knop medium without sugars added and with 25% nitrogen compared with normal Knop medium in order to ensure that the nematodes obtain sugars only from the plants.

Before inoculation, root length was estimated as described by Juergensen (2001). Fifteen days after inoculation, when males and females could be differentiated, the number of nematodes was counted. Data were collected at three independent time points.

Loading experiments with CF

CF for loading experiments was prepared as described in Wright and Opara (1997). At different dai the dye was loaded on leaves of infected plants by tweaking them with tweezers. Plates were kept in complete darkness for 8 h after CF was loaded. Syncytia were then counted under an inverted microscope (Axiovert 200M, Zeiss, Hallerbergmoos, Germany) using UV light and an FITC filter. Plates were returned to the growth chamber and kept there until females and males could be differentiated. All experiments were performed in three separate series.

Statistical analysis

In order to test significant differences between single sampling events, one-way ANOVA (analysis of variance) was conducted using the LSD (least significant difference) test. The LSD test was appropriate, as the sample number remained constant in all tests. Statistical analysis was carried out with StatGraphics Plus 4.0 software (Statistical Graphics Inc.).

Results

Temporal and spatial expression profile of sucrose transporter

Previously, the temporal expression profile of AtSUC2 in syncytia was performed using pAtSUC2–gfp and pAtSUC2–gus lines (Juergensen, 2001; Juergensen et al., 2003). In the present work, GUS assays were performed with infected pAtSUC4–gus lines at different dai. Staining was intensive and easily visible under the dissecting microscope in the phloem of roots and above-ground tissues in all tests. In the first days after inoculation a few syncytia showed a blue staining, increasing to >70% at 8 dai (Fig. 1). An intensive GUS signal was observed in syncytia or side roots originating from infected root areas (Fig. 2A–D). In the following days the percentage of GUS-stained syncytia slightly decreased and remained at a plateau level for the following days. The number of

Sucrose supply to nematode-induced syncytia 1593

Sugar analysis by HPLC

Ten and 15 dai, soil was washed from the roots of infected and non-infected plants and control roots and syncytia were dissected as described. Soluble sugars of three independent sampling events, each consisting of 18–127 mg of fresh root material, were extracted in vacuo using a Dionex ICS3000 chromatography system. To analyse sucrose, the column was thermostated at 30 °C and eluted with 200 mM NaOH at a flow rate of 0.5 ml min⁻¹.

Gene silencing by RNAi

Gene silencing of AtSUC2 was performed based on the RNAi approach. Therefore the gateway vector system pENTR11 was used as the entry vector and pK7GWlWG2(II) as the destination vector. A 178 bp fragment was isolated by PCR (forwards, GCTACTTCC-GATCAAGATCG; reverse, CAGGAGAAGACACGCT) attaching the attB sites (ACTTTGTACAAAAAAGCAGGCT), and was cloned into the pENTR11 vector using BspI1407 restriction sites. Further, the syncretum-specific promoter ppyk20 was cloned into the destination vector to obtain localized gene silencing. After performing the LR reaction and sequencing, A. thaliana wild-type col was transformed by floral dip. Transgenic seedlings were performing the LR reaction and sequencing, and eluted with

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GUS-positive syncytia decreased drastically at 17 dai (Fig. 1). In a few syncytia, GUS staining was still observed at 30 dai but was often restricted to an area close to the head of the nematodes (Fig. 2D).

In situ RT-PCR was applied to study the spatial expression of AtSUC2 and AtSUC4 in detail. A specific signal was observed for both transporter genes in cross-sections of 10-d-old syncytia (Fig. 3A, B). Staining was restricted to syncytia and never appeared in adjacent root cells. Negative controls of the in situ RT-PCR performed without polymerase (Fig. 3C), without reverse transcriptase, without primers, or DIG (not shown) never showed specific staining.

**Quantitative RT-PCR: relative expression of AtSUC2 and AtSUC4**

In the GUS experiment, a clear temporal effect of the pAtSUC4 activity was observed. In order to follow and quantify the expression of AtSUC2 and AtSUC4 during nematode development real-time RT-PCR was applied. For quantifying the relative changes in gene expression in

**Fig. 1.** GUS expression in syncytia of pAtSUC4–gus Arabidopsis plants. Columns show the percentage of GUS-stained syncytia at different time points after nematode inoculation. At every time point 63 single plants, each inoculated with 50 freshly hatched J2 of H. schachtii, were analysed. Letters show significant differences at P <0.05 (one-way ANOVA, LSD). Values are means ±SE.

**Fig. 2.** Histochemical GUS-assay of pAtSUC4–gus lines infected with J2 of H. schachtii: (A) local GUS activity in a nematode-induced syncytium and in the phloem of non-infected roots at 4 dai; (B) syncytia and roots arising from the syncytia are stained blue at 6 dai; (C) at 14 dai there is still a strong GUS signal in a syncytium of a female juvenile; (D) an adult female is feeding on a syncytium where GUS expression is restricted to the area close to the nematode’s head (20 dai). dai, Days after inoculation, S, syncytium, N, nematode, U, non-infected root. Scale bars=400 μm.

**Fig. 3.** In situ RT-PCR on cross-sections of syncytia (25 μm) 10 d after inoculation for (A) AtSUC2, (B) AtSUC4, and (C) negative control without polymerase. In (A) and (B) the staining is restricted to syncytia, whereas in (C) no staining is visible. S, Syncytium, X, xylem, E, endodermis. Scale bars=20 μm.
syncytia, 18S RNA was found to be an appropriate internal reference. Equal amounts of cDNA of three independent root and syncytium samples were analysed. Ct-values of 18S RNA expression in a single sample (all tested in triplicate) are compared in Fig. 4A. There were no differences in 18S RNA expression in control roots and dissected syncytia. Due to the small differences in Ct-values (Fig. 4A) and the result of the calculation $2^{-\Delta\Delta Ct}=1.03$ (comparing roots and syncytia) (Livak and Schmittgen, 2001), it was concluded that 18S RNA was an appropriate internal reference for relative quantification of gene expression in syncytia.

Syncytia and sections of coeval control roots were tested at 5, 10, and 15 dai to follow the expression profiles of AtSUC2 and AtSUC4 during nematode development. Fold changes in the expression of syncytium-enriched root tissue compared with control roots were calculated with the $2^{-\Delta\Delta Ct}$ method and are presented in Fig. 4B. The expression of AtSUC4 was not changed in syncytium-enriched root tissue at 5 dai. However, at 10 dai it was >2-fold up-regulated, but only slightly induced at 15 dai. The transcript level of AtSUC2 was found to be down-regulated in syncytium-enriched root tissue at 5 dai. However, at 10 dai, a 2-fold induction was measured as found for AtSUC4 (Fig. 4B). At 15 dai no more specific induction could be observed.

Sucrose is highly increased in nematode-induced syncytia

The amounts of sucrose in roots of non-infected plants and dissected syncytia were analysed by HPLC-PAD at 10 dai and 15 dai. Levels were significantly increased in the samples of syncytia tested compared with control roots (Fig. 5).

Symplasmic connectivity of nematode-induced syncytia to the phloem

Nematode-induced syncytia were described as being symplasmically connected to the phloem after about 12 dai by large GFP-permeable plasmodesmata (Hofmann and Grundler, 2006). In the present experiment, CF, which is a small (460 Da), phloem-mobile dye frequently used for studying the presence of functional plasmodesmata, was employed. At different times after inoculation the phloem of infected plants was loaded as described in the Materials and methods. In the first days after inoculation no symplasmic connections to the phloem could be found (Fig. 6). After 14 dai the majority of treated syncytia took up the fluorescent dye (Fig. 6). In these cases, CF was unloaded from the phloem into the syncytia and later taken up by the nematodes (Fig. 7A, B), while in other cases unloading was not observed and the dye remained in the strongly fluorescing phloem elements (Fig. 7C, D). With this experiment, similar results obtained with GFP transported in the phloem are confirmed (Hofmann and Grundler, 2006). Most symplasmic connections to the phloem were opened to syncytia induced by female juveniles. In syncytia of male juveniles the dye was rarely found and those syncytia were mostly fused with ones induced by females (data not shown).

Nematode development relies on AtSUC4 expression

In order to study the importance of the sucrose transporter AtSUC4 for nematode development, infection assays were performed with lines containing an AtSUC4 RNAhp fragment, to facilitate RNAi. For gene silencing the ppyk20 promoter was chosen, as it was described as highly up-regulated in nematode-induced syncytia (Heinen, 2001). Further the promoter was shown to be active.

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**Fig. 4.** (A) Real time RT-PCR of 18S RNA control. Gene expression was analysed in three independent root and syncytium samples. Ct-values of root tissues, syncytia tissues, and both tissues tested combined are shown. Values are means ± SE (n=3). (B) Relative real-time RT-PCR of AtSUC2 and AtSUC4 in syncytia. Values are given in fold change in syncytium-enriched root tissues compared with control roots. Values are means ± SE (n=3)

**Fig. 5.** Levels of sucrose in non-infected roots and syncytia at 10 dai and 15 dai. Syncytial samples show highly elevated values. Values are means ± SE (n=3).
in cotyledons, root tips, and hydatodes, and in leaves 1–5 h after they had been mechanically injured.

Gene silencing was studied in cotyledons, root tips, and leaves 5 h after wounding them with tweezers (see Materials and methods). This seemed to be the most appropriate approach as the number of developing syncytia was very limited and silencing in developing syncytia is probably not effective. None of the lines generated showed an altered phenotype and all of them were grown successfully on medium containing antibiotics. To test gene silencing and to perform the nematode infection test, a representative ppyk20-AtSUC4 RNAi line was chosen. In two independently tested cotyledon samples of the ppyk20-AtSUC4 RNAi lines, AtSUC4 expression was 56% and 61% that of wild-type plants, in root tips 85%, and in leaves 5 h after wounding 46% and 105%. Obviously promoter activity differs between the tissues tested, and the silencing effect after wounding is difficult to monitor due to its dynamic nature.

For infection assays, RNAi and wild-type plants were grown on media without added sugar in order to ensure the nematode’s sugar supply was fully dependent on the plant. Although plant growth was reduced under these conditions, seedlings were able to develop well, and were successfully inoculated with nematode juveniles. Determining nematode development at 15 dai, there was a significant reduction in female development in the AtSUC4 gene silencing line compared with the wild type, although overall infection was not reduced (Fig. 8). Generally, there was a strong shift from female to male development and several plants developed without one single female juvenile. Although the induced gene silencing of AtSUC4 seemed to be relatively moderate, it had a significant effect on nematode development.

Discussion

Host–pathogen interactions are often characterized by nutrient translocation towards the infection site, whereby carbohydrates play the most important role in energy supply of the pathogens (André et al., 2005). Accordingly, plant sugar transporters were reported in a number of plant–microbe interactions. Altered transcription levels of sugar transporters were found in plants after infection with pathogenic fungi (Truernit et al., 1996; Fotopoulos et al., 2003; García-Rodríguez et al., 2005), mycorrhiza (García-Rodríguez et al., 2005), root nodule development (Flemetakis et al., 2003), treatment with bacterial elicitors

![Fig. 6. Percentage of fluorescing syncytia after loading with CF on different days after inoculation (dai). At 4 dai no syncytia showed fluorescence whereas at later stages the percentage increased to >90%. Values are means ±SE (n=3).](https://academic.oup.com/jxb/article-abstract/58/7/1591/512067)

![Fig. 7. Loading experiments with CF. (A) Transmission light image of a female nematode and its syncytium. (B) Fluorescence microscopic view of the same specimen. CF has been unloaded from the phloem to the syncytium and taken up by the nematode. (C) Transmission light image of a female stage-4 juvenile and its syncytium. (D) Fluorescence microscopic view of the same specimen as shown in (C). CF is retained in the phloem and not translocated into the syncytium. N, Nematode, S, syncytium. Scale bars=200 µm.](https://academic.oup.com/jxb/article-abstract/58/7/1591/512067)
Sucrose supply to nematode-induced syncytia

During nematode development the associated syncytia undergo dramatic changes in anatomy, physiology, and gene expression. Numerous reports of altered gene expression in syncytia have been published (Niebel et al., 1996; Escobar et al., 2003; Juergensen et al., 2003; de Almeida Engler et al., 2004; Favery et al., 2004; Mazarei et al., 2004; Hammes et al., 2005) and, step by step, a more complete picture of the processes involved can be composed.

Within the first 24 h after nematode infection the second-stage juveniles induce the first syncytial cells, start feeding, and thereby induce nutrient import into syncytia (Golinowski et al., 1996). In the early stages of nematode development, pAtSUC4 is already active in >20% of the established syncytia (Fig. 1) but gene expression of AtSUC4 is not considerably changed compared with the control. However, as secondary plasmodesmata to the phloem are not yet formed (Hofmann and Grundler, 2006; results of the present study) the expression level should be high enough to import the required amount of sucrose actively. In the following days, juveniles undergo their second moult and enter their major growth period that ends at about 10 dai. It is accompanied by vigorous expansion of the associated syncytia. Hence, energy and nutrient demands of the pathogens rise, and the sucrose concentration in syncytia is dramatically increased. At this stage, the first few functional plasmodesmata were found to connect syncytia to the phloem (Hofmann and Grundler, 2006; results of the present study). At the same time, a strongly increased expression of AtSUC4 was observed at the promoter and the mRNA level, indicating an active sugar import into syncytia. At 8 dai the AtSUC4 promoter was active in most but not all syncytia (75%).

AtSUC2, the second sucrose transporter monitored here, has reached the same expression level as AtSUC4, at 10 dai. As soon as syncytia and phloem are connected through functional plasmodesmata, AtSUC2 activity might be necessary to retrieve sucrose leaking from the highly enriched syncytia.

Entering the fourth juvenile stage at 2 weeks after inoculation, syncytia reach their final expansion. In grafting experiments, all syncytia of females showed fluorescence at 18 dai due to GFP uptake, but in loading experiments with CF never 100% of the syncytia gave a signal.

GFP is a very stable protein that, once imported into the syncytia, will continue to fluoresce for a couple of days. Further, it is permanently expressed in the leaves of the grafted scion and continuously transported through the phloem. In comparison, CF is unstable and gives information about the transport dynamics and syncytium status in a short period. Accordingly, translocation of GFP indicates a large exclusion limit of the plasmodesmata, while the differing results with CF indicate a transport regulation that may be based on a changing ‘gate open/gate closed’ status of plasmodesmata. Further observations will be needed to reveal whether the actual status is related to the feeding behaviour of the nematode.

At 15 dai, when symplasmic connectivity was observed between the majority of the syncytia and the phloem, the expression of AtSUC4 was reduced but was still detectable. Its ongoing expression in syncytia may be due to continuous transport and retrieval of sucrose in parallel to the symplasmic import, as suggested by Hofmann and Grundler (2006).

Differences between AtSUC2 and AtSUC4 expression

AtSUC4 is reported to be a low-affinity/high-capacity sucrose transporter (Weise et al., 2000). The high capacity transporter has a weaker affinity for sucrose but facilitates the movement of large quantities. Thus, it is responsible for supplying sink tissues with sucrose and determining their sink strength. While gene expression of AtSUC4 is either up-regulated or not changed during nematode...
development, AtSUC2 is down-regulated in root sections containing syncytia at 5 dai (Fig. 4B). AtSUC2 is a high-affinity/low-capacity transporter associated with phloem loading processes and retrieval of diffused sucrose into sieve elements. A model is presented here, in which down-regulation of AtSUC2 in root sections with syncytia is due to a reduced expression in the phloem companion cells, thus lowering sucrose retrieval into the phloem and increasing sucrose in the apoplast. This model is supported by the concept of Patrick (1990) suggesting that localized phloem unloading might increase apoplasmic nutrient concentrations, due to an inhibition of sucrose retrieval back into the phloem. The resulting high apoplasmic concentrations would facilitate sucrose supply of young syncytia by AtSUC4. Increasing apoplasmic sugar concentrations were also studied in developing legume seeds (Weber et al., 1995; Delrot et al., 2000; Aldape et al., 2003). There, sucrose/hexoses are released into the apoplast to be retrieved by the cotyledons’ transporters. In both cases the plant responds to the formation of sink tissue with a high demand for carbohydrates.

Localization of sucrose transporter expression

Changes of gene expression in syncytia were often studied in dissected syncytia containing surrounding root material or even whole infected roots compared with control roots (Puthoff et al., 2003; Hammes et al., 2005). Changes may occur in the whole root or in the area surrounding nematode feeding sites and, in both cases, a detailed differentiation between genes activated in syncytia or elsewhere in the sample is not possible. Therefore, histological analyses such as in situ RT-PCR or in situ hybridization are essential to verify molecular genetic studies. GUS analyses of intact roots alone are usually not sufficient for an exact cellular localization.

In situ RT-PCR has previously been used to localize gene expression in various plant tissues such as pollen (Lee and Tegeder, 2004), buds (Urbanczyk-Wochniak et al., 2002), siliques (Mølhøj et al., 2004), and nematode-induced feeding sites (Koltai et al., 2001; Gal et al., 2006; Wieczorek et al., 2006).

In the present study, transcripts of both transporters could be clearly localized in syncytia. Hoth et al. (2005) discussed whether AtSUC2 is expressed in syncytia or if RNA is passively translocated from the phloem. In order to clarify this problem, they used constructs coding for mobile and membrane-bound GFP. Membrane-bound GFP was expressed in the phloem but not in syncytia, and they concluded that there is no pAtSUC2 activity in syncytia and thus no expression of AtSUC2. Following the conclusions of Hoth et al. (2005), these transcripts should be produced in phloem companion cells and then be translocated via plasmodesmata into syncytia. However, if transcripts and not proteins were the mobile elements both mobile and immobile GFP should be expressed in syncytia. Another important argument is the fact that the expression pattern of both sucrose transporters in syncytia does not correlate with the connectivity of syncytia to the phloem. On the contrary, sucrose transporters were expressed in an early developmental phase when only very few syncytia were symplasmically connected to the phloem. Thus, symplasmic transport of protein or RNA leading to the translation of transporter protein may be ruled out. Detection of AtSUC2 at the protein level has still to be clarified as there are controversial reports by Scholz-Starke (2003) and Hoth et al. (2005).

Function of sucrose transporters in syncytia

According to their genuine function, sucrose transporters play an important role for sucrose import into syncytia as shown by silencing AtSUC4 expression. Especially in the early stages of juvenile development, sufficient nutrient availability by transporters is crucial for female development. The data presented clearly show that a reduced expression of AtSUC4 leads to a significant reduction of females and a shift towards male development. As the total amount of developing nematodes stays unchanged, it can be concluded that the infection process is successful in the gene silencing line compared with the wild type, but sexual differentiation is affected due to a limited carbohydrate supply. The moderate gene silencing of AtSUC4 in cotyledons, root tips, and wounded leaves, and the strong effect on nematode development may be explained by different promoter activities.

In later stages of juvenile development the associated syncytia concentrate sucrose at high levels (Fig. 5), leading to a steep gradient towards the surrounding apoplast and adjacent cells. The resulting sucrose leakage by diffusion has to be compensated by the activity of transporters. Similar effects were observed in root galls induced by the root-knot nematode Meloidogyne incognita. The sucrose transporter AtSUC1 was found to be up-regulated in galls compared with control roots in Arabidopsis (Hammes et al., 2005), while loading experiments with CF in tomato indicate that nematode-induced galls are also connected to the phloem by plasmodesmata. Symplasmic loading resulted in fluorescing galls, whereas after apoplasmic loading no dye was observed inside the galls (Dorhout et al., 1993).

Syncytia form a new symplasmic domain

In principle, plant cells are connected by plasmodesmata, but during plant development and organ specification ‘symplasmic domains’ (according to Erwee and Goodwin (1985) ‘cell aggregates complete isolation to their surrounding’, or ‘symplasmic fields’ (according to Rinne and Van der Schoot (1998) partially isolated cell complexes] become established. This phenomenon was shown during
cotton fibre elongation (Ruan et al., 2004), in the shoot apex during the onset of flower induction (Gisel et al., 1999), and in embryos of Arabidopsis at the torpedo stage of their development (Kim et al., 2002). Symplasmic isolation is often accompanied by an increase in turgor pressure induced by enhanced sucrose transporter activity and higher sucrose levels. In cotton fibres, elongation is achieved by turgor pressure against the cell wall combined with its loosening due to expansin activity (Ruan et al., 2004). As soon as elongation is terminated plasmodesmata were shown to open again. This concept is very similar to the formation of nematode-induced syncytia of H. schachtii. The initial feeding cell, as part of the central cylinder, is connected to its surrounding cells. As soon as the cell is pierced by the nematode’s stylet, plasmodesmata close and neither CF (Fig. 7C) nor GFP (Hofmann and Gründler, 2006) can enter. In the following days turgor pressure increases (Böckenhoff, 1995) and sucrose transporters (Fig. 4) and expansins are expressed (Wieczorek et al., 2006). Finally, syncytia reach their final expansion and plasmodesmata to the phloem may open.

With this work, it was possible to show the distinct expression of AtSUC2 and AtSUC4 in syncytia and follow their temporal expression profile during nematode development. It was proved that sucrose concentrations in syncytia compared with control roots increase dramatically before the majority of syncytia is symplasmically connected to the phloem. Further, silencing of AtSUC4 expression of AtSUC4 in syncytia and follow their temporal expression profile during nematode development. It was proved that sucrose concentrations in syncytia compared with control roots increase dramatically before the majority of syncytia is symplasmically connected to the phloem. Further, silencing of AtSUC4 expression resulted in a significant reduction in female development. This is the first demonstration of a function for AtSUC4. It leads to the conclusion that juveniles are dependent on sucrose supply by transporters during feeding-site induction and establishment. At later stages, when syncytia are connected to the phloem via plasmodesmata, transporters may be active in sucrose retrieval and transport needed for specific metabolic processes.

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References


