Transcriptional regulation of host enzymes involved in the cleavage of sucrose during arbuscular mycorrhizal symbiosis

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Received 17 July 2006; revised 18 October 2006
doi: 10.1111/j.1399-3054.2007.00873.x

To investigate plant carbon metabolism in arbuscular mycorrhizas, we have analyzed expression of the tomato invertase (EC 3.2.1.26) gene family members and the sucrose synthase (EC 2.4.1.13) gene TOMSSF in roots of non-mycorrhizal, Glomus mosseae- and Glomus intraradices-colonized plants. Furthermore, root soluble carbohydrate contents have been determined. Gene expression analyses showed that the cell wall invertase Lin6, the vacuolar invertase TIV1 and TOMSSF were upregulated in mycorrhizal roots and that this effect was caused by a direct effect of the colonization by the arbuscular mycorrhizal (AM) fungi and not mediated by an improved phosphorus nutrition. This study shows for the first time upregulation of a cell wall invertase gene in an AM association, which supports the general assumption that carbon transfer across the symbiotic interface requires host sucrose hydrolysis by a cell wall invertase. Transcriptional upregulation of sucrose-splitting enzymes during early colonization development agrees with the decreased levels of sucrose detected in these roots. Mycorrhizal plants had lower root glucose and fructose concentrations, which indicate consumption of the products of sucrose breakdown. The promoter sequences of Lin6, TIV1 and TOMSSF were analyzed in silico to get insights into the causes of their transcriptional activation in mycorrhizal roots. Upregulation of Lin6, TOMSSF and TIV1 expressions by salicylic acid and of TOMSSF and TIV1 by abscisic acid suggest that these compounds might mediate upregulation of these genes in mycorrhizal roots.

Introduction

Arbuscular mycorrhizal (AM) fungi belong to the phylum Glomeromycota (Schübler et al. 2001) and form mutualistic symbioses with the roots of the majority of terrestrial plants. These fungi biotrophically colonize the cortex of the root to obtain carbon compounds from the host plant while assisting the plant with the supply of phosphate and other mineral nutrients that the external mycelium takes up from the soil (Harley and Smith 1983). AM fungi form asexual spores able to germinate in the absence of any host plant and able to produce a limited mycelium at the expenses of the spore reserves; however, they can only complete their life cycle after colonization of a host root (Azcón-Aguilar et al. 1998). Although the reasons for the obligate biotrophy of AM fungi are unknown, it is believed that the fungus, during the long evolution of its symbiotic relationship with the host plant (more than 450 million years), lost some of the carbon acquisition capabilities needed for saprophytic growth and became completely dependent on the host plant

Abbreviations – ABA, abscisic acid; AM, arbuscular mycorrhizal; P, phosphorus; PCR, polymerase chain reaction; pl, isoelectric point; RT, reverse transcription; RT–PCR, reverse transcription–polymerase chain reaction; SA, salicylic acid.
for fixed carbon supply. Therefore, the AM symbiosis represents an additional carbon demand to the photosynthetic organs, which adds to the demand of the root sink. Measurements of carbon flux indicate that mycorrhizal plants direct from 4 to 20% more photoassimilates to the root system than non-mycorrhizal plants (Graham 2000, Jakobsen and Rosendahl 1990, Pearson and Jakobsen 1993).

Utilization of sucrose, the major long-distance transport form of fixed carbon in higher plants, as a source of carbon and energy by sink tissues depends on its cleavage into hexoses either by a sucrose synthase (EC 2.4.1.13) or an invertase (EC 3.2.1.26). Sucrose synthase is a cytoplasmic enzyme that catalyzes the reversible hydrolysis of sucrose to yield UDP-glucose and fructose, and two or more closely related isoforms of this enzyme have been identified in most plants (Sturm et al. 1999). This enzyme is assumed to be important for synthesizing sugar, a component of specific plant growth phases (Hohnjec et al. 2003). Based on their solubility, subcellular localization, optimum pH and isoelectric point (pl), three different types of invertase isoforms can be distinguished: vacuolar (acidic pH – optimum, neutral pl and vacuolar localization), cell-wall-bound (acidic pH – optimum, basic or neutral pl and apoplastic localization) and cytoplasmic invertases (neutral or alkaline pH – optimum, neutral pl and cytoplasmic localization) (Avigad 1982). Extracellular and vacuolar invertase isoforms are key metabolic enzymes that are involved in regulating carbohydrate partitioning, developmental processes, hormone responses, biotic interactions and responses to abiotic stresses; however, the function of the cytosolic isoform is currently unknown (Roitsch and Gonza´lez 2004, Sturm 1999).

Materials and methods

Plant materials

Tomato seeds (Solanum lycopersicum, formerly Lyco-persicon esculentum Mill, cv 76R; Peto Seed Company, Woodland, CA) were surface sterilized and sown in autoclaved vermiculite. Plantlets were transplanted to 500-ml pots when the first true leaf was expanded. Pots contained a sterile mixture of soil and sand (1:2, v/v). Plants were grown in a greenhouse with 16-h photoperiod, 25/18°C day/night temperature and 60% relative humidity. They were watered three times per week with half-strength Hoagland nutrient solution (Hoagland and Arnon 1938), with different phosphorus content.

The treatments applied were as follows: non-inoculated control plants fed with nutrient solution containing 20, 100 or 500 μM KH2PO4 and plants inoculated with the AM fungus G. mosseae (Nicol. and Gerd.) Gerd and Trappe BEG119 or G. intraradices Smith and Schenck BEG123. All treatments had five replicates. Plants inoculated with the AM fungi were watered with nutrient solution containing 20 μM KH2PO4. Mycorrhizal inoculation was performed as described by Benabdellah et al. (1999) using a sand–vermiculite-based inoculum of the AM fungi. Control plants received an aliquot of a filtrate (<20 μm) of both AM inocula to provide the microbial populations accompanying the mycorrhizal inocula but free from AM propagules. Plants were harvested 4 and 6 weeks after inoculation with the AM fungi. After fresh weight determination of shoots and roots, root samples were frozen in liquid nitrogen and stored at –80°C. Mycorrhizal development was estimated in root samples after trypan blue staining (Phillips and Hayman 1970), using the Giovannetti and Mosse (1980) method.

For treatments with phytohormones and sugars, the standard protocol and concentrations described in the literature were used (Rausch and Grenier 2004, Sinha et al. 2002). Briefly, 25-day-old seedlings grown in sterile vermiculite and watered with half-strength Hoagland nutrient solution were transferred to Falcon tubes.
containing nutrient solution supplemented with H$_2$O (control), 100 μM abscisic acid (ABA), 100 μM salicylic acid (SA), 40 mM glucose or 20 mM sucrose. Roots were harvested 12 and 24 h after the treatment, frozen in liquid nitrogen and stored at $-80^\circ$C.

**RNA isolation and gene expression analyses**

Total RNA was isolated from frozen roots of 4- and 6-week-old plants and of hormone- and sugar-treated plantlets by phenol/chloroform extraction (Kay et al. 1987). Lin5 (accession number X91389), Lin6 (AB004583), Lin7 (X91391), Lin8 (X91392), TIV1 (AF4656131) and TOMSSF (L19762) gene expressions were studied by real-time reverse transcription–polymerase chain reaction (RT–PCR) using iQ-Cycler (Bio-Rad, Hercules, CA). cDNAs were obtained from 1 μg of total DNase-treated RNA from the different treatments in a 20-μl reaction containing 200 U SuperScript II RNase H$^+$ Reverse Transcriptase (Invitrogen, Groningen, The Netherlands), 5 μM random hexamer primers, 0.5 mM each dNTP, 20 U RNase inhibitor and 1X RT buffer. Each 25-μl polymerase chain reaction (PCR) reaction contained 1 μl of a dilution 1:10 of the cDNA, 200 mM dNTPs, 200 nM each primer, 3 mM MgCl$_2$, 2.5 μM SYBR Green (Molecular Probes, Eugene, OR) and 0.5 U Platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA) in 1X PCR buffer (20 mM Tris–HCl pH 8.4, 50 mM KCl). The gene-specific primers used for the different genes are listed in Table 1. The PCR program consisted of 5 min of incubation at 95°C to activate the hot-start recombinant Taq DNA polymerase, followed by 35 cycles of 30 s at 95°C, 45 s at 58°C and 45 s at 70°C, where the fluorescence signal was measured. The specificity of the PCR amplification procedure was checked with a heat dissociation protocol (from 70°C to 100°C) after the final cycle of the PCR. The efficiency of each primer set was evaluated by performing real-time PCR on several dilutions of tomato genomic DNA. In all cases, the results obtained for the different treatments were standardized to the 18S rRNA levels, which were amplified with the tomato-specific primers R1 and R2 (Table 1). Real-time PCR determinations were performed on three independent biological replicates. Three technical replicates for each biological sample were included. The relative levels of transcription were calculated using the $2^{-ΔΔCt}$ method (Livak and Schmittgen 2001). In all RT–PCR reactions, a non-RT control was used to detect any possible DNA contamination.

**Carbohydrate analysis**

Carbohydrates were extracted from approximately 1 g of frozen roots as described by Bligh and Dyer (1959). The methanolic extract was evaporated to dryness and re-dissolved in sterile water. The contents of sucrose, glucose and fructose were estimated by enzyme-coupled reactions using the Sucrose/D-Glucose/D-Fructose kit (r-biopharm, Darmstadt, Germany), as described by the manufacturer.

**Isolation of TOMSSF promoter**

To isolate the 5′ non-coding sequence of TOMSSF, four GenomeWalker libraries were constructed using the GenomeWalker kit (Clontech, Palo Alto, CA) according to the manufacturer’s manual. Tomato genomic DNA was extracted from frozen tomato leaves (Murray and Thompson 1980) and digested to blunt ends with Dral, EcoRV, PvuI and Stul. Each pool of DNA fragments was then ligated to the GenomeWalker Adaptor, and upstream genomic regions were amplified from each library using a nested adaptor primer and the gene-specific primer PSS (Table 1). PCR products were cloned in the TA Cloning kit (Invitrogen, Carlsbad, CA) and sequenced. Promoter sequences were screened for the presence of cis-regulatory elements using PLACE (Higo et al. 1999) and PlantCARE programs (Lescot et al. 2002).

**Statistical analyses**

Plant growth data were subjected to ANOVA, followed by the Fisher’s protected least significant test when appropriate.
Results

AM colonization and plant growth

Mycorrhizal colonization and growth of the tomato plants used for gene expression analyses are shown in Table 2. Although mycorrhizal colonization was low 4 weeks after inoculation, all characteristic fungal structures were present in both G. mosseae- and G. intraradices-colonized tissues. Two weeks later, the percentage of root colonization was considerably higher, reaching 30 and 38% for G. mosseae- and G. intraradices-colonized plants, respectively. To make realistic comparisons between mycorrhizal and non-mycorrhizal plants, it is important to verify that the different sets of plants have comparable growth characteristics. As shown in Table 2, growth of non-mycorrhizal plants was clearly limited by phosphorus supply. Four weeks after inoculation, G. mosseae-colonized plants performed slightly, but significantly, better than G. intraradices-colonized plants; however, no significant differences were found between both mycorrhizal plants 2 weeks later. At both harvest times, growth of G. mosseae- and G. intraradices-colonized plants were more similar to those of non-mycorrhizal plants fed with 500 μM P than with lower phosphorus levels. Therefore, gene expression levels in mycorrhizal plants were compared with those of non-mycorrhizal plants fed with 500 μM P.

Regulation of tomato invertase genes by the AM symbiosis

Expression of the five tomato invertase genes Lin5, Lin6, Lin7 and Lin8 encoding cell wall isoymes (Godt and Roitsch 1997) and TIV1 encoding a vacuolar invertase (Klann et al. 1992) was assessed by real-time RT–PCR in RNA isolated from non-mycorrhizal and mycorrhizal roots. All primer sets used in this study amplified tomato genomic DNA. However, Lin6 was the only cell wall invertase gene detected in cDNA from mycorrhizal and non-mycorrhizal tomato roots (data not shown), which agrees with previous observations by Godt and Roitsch (1997) in roots of non-mycorrhizal tomato plants. Lin6 mRNA levels were higher in symbiotic than in control roots at both harvest times. Upregulation of Lin6 gene expression was similar in G. mosseae- than in G. intraradices-colonized roots (Fig. 1A).

As shown in Fig. 1B, TIV1 gene expression was regulated by the development of the symbiosis. Four weeks after inoculation, TIV1 mRNA levels were similar in control and in G. mosseae- or G. intraradices-colonized roots. However, 2 weeks later, upregulation of TIV1 mRNA levels was observed in mycorrhizal roots, with the increase being significantly higher in G. mosseae-colonized (4.4-fold) than in G. intraradices-colonized (2.2-fold) roots.

Expression analysis of a tomato sucrose synthase gene

The relative transcript abundance of the tomato sucrose synthase gene TOMSSF (Wang et al. 1993) was also investigated in mycorrhizal and non-mycorrhizal roots. In relation to non-mycorrhizal roots, the transcript levels of TOMSSF were slightly but consistently increased in both G. mosseae- and G. intraradices-colonized roots at the first harvest time (4 weeks after inoculation). Two weeks later, 5.7- and 4.5-fold increases in TOMSSF mRNA levels were detected in the roots colonized by G. intraradices or G. mosseae, respectively (Fig. 2).

<table>
<thead>
<tr>
<th>Plant treatments</th>
<th>First harvest</th>
<th>Second harvest</th>
</tr>
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<tbody>
<tr>
<td>Phosphorus (μM)</td>
<td>Fresh weight (g)</td>
<td>Mycorrhizal colonization (%)</td>
</tr>
<tr>
<td>20</td>
<td>Shoots: 0.50 a, Roots: 0.20 a</td>
<td>Mycorrhizal colonization: 0 a</td>
</tr>
<tr>
<td>100</td>
<td>Shoots: 1.35 b, Roots: 0.57 b</td>
<td>Mycorrhizal colonization: 0 a</td>
</tr>
<tr>
<td>500</td>
<td>Shoots: 5.14 c, Roots: 2.03 cd</td>
<td>Mycorrhizal colonization: 0 a</td>
</tr>
<tr>
<td>Mycorrhizal</td>
<td>G. mosseae</td>
<td>Shoots: 7.35 d, Roots: 2.79 d</td>
</tr>
<tr>
<td>G. intraradices</td>
<td>Shoots: 5.60 c, Roots: 1.51 c</td>
<td>Mycorrhizal colonization: 14 b</td>
</tr>
</tbody>
</table>

Table 2. Shoot and root fresh weight and mycorrhizal colonization of tomato plants subjected to different phosphate (20, 100 or 500 μM P) and mycorrhizal (20 μM P and inoculated with G. mosseae or G. intraradices) treatments after a growing period of 4 (first harvest) or 6 (second harvest) weeks after transplanting. Data in the same column sharing a letter in common do not differ significantly (P ≤ 0.05) according to Fisher’s least significant difference test.
Regulation of tomato invertase and sucrose synthase genes by phosphorus

To determine if regulation of the investigated genes was because of a direct effect of the colonization of the roots by the AM fungi or because of an indirect effect through an improvement in phosphorus nutrition, expression level of these genes was determined in roots of non-mycorrhizal plants fed with 100 or 500 μM P. As shown in Figs 1 and 2, Lin6, TIV1 and TOMSSF mRNA levels showed no significant changes in response to phosphate. This indicates that the observed increase in Lin6, TIV1 and TOMSSF mRNA levels in mycorrhizal roots is not mediated by an improved phosphorus nutrition.

Effect of mycorrhizal colonization on root soluble carbohydrate content

The levels of sucrose, glucose and fructose in control and mycorrhizal roots were determined to try to correlate the observed changes in gene expression of the analyzed sucrose-cleaving enzymes with the carbohydrate status of the roots. Four weeks after inoculation, root sucrose concentration was lower in mycorrhizal plants (colonized either with G. mosseae or G. intraradices) than that in control plants. However, no statistically significant differences in sucrose levels were found between G. mosseae-colonized and non-mycorrhizal plants 2 weeks later (Fig. 3). At the two time points analyzed, the concentrations of glucose and fructose were significantly lower in both G. mosseae- and G. intraradices-colonized roots than in those of non-mycorrhizal plants.

Possible regulatory elements in the promoter sequences of Lin6, TIV1 and TOMSSF

To get some insights into the causes of the transcriptional activation of Lin6, TIV1 and TOMSSF in mycorrhizal plants, their promoter sequences were screened for the
presence of regulatory elements with similarity to known cis-acting elements via sequence alignments. For this purpose, the promoter sequences of Lin6 (accession number AF506004) and TIV1 (Z12027) were retrieved from Genbank, and the putative TOMSSF promoter (AM408346) was isolated by PCR amplification from an adapter-ligated genomic tomato library. Comparison of this genomic sequence (3395 bp upstream of the translation initiator ATG) with TOMSSF cDNA showed the presence of a long (1573 bp) leader intron in the 5′ untranslated region, which is a typical feature of sucrose synthase genes (Fu and Park 1995). The 5′-flanking regions of Lin6, TIV1 and TOMSSF contained several core elements identical to the root-specific element ATATT (Elmayan and Tepfer 1995), the consensus sequence motifs of the organ-specific elements OSE1 (AAAGAT) and OSE2 (CTCTT) characteristic of promoters activated in infected cells of root nodules (Stougard et al. 1987) and several motifs with similarity to hormone-specific elements. The Wbox (TTGAC) found in the promoters of SA-responsive genes (Yu et al. 2001) was identified in the 5′-flanking regions of the three genes. ABA-response elements were also found in the promoter sequences of TIV1 and TOMSSF (Abe et al. 2003, Hattori et al. 1995). While the ABA cis-acting element MYCR (CACATG) was found in both promoters, the ABRE motif (ACGTG) was only identified in TOMSSF. Lin6 and TOMSSF promoters also contained the sucrose-responsive element SURE1 (AATAGAAAA), recognized as a conserved motif among a number of sucrose-responsive genes (Grierson et al. 1994).

Regulation of tomato invertase and sucrose synthase genes by sugars and hormones

To validate the in silico analysis of Lin6, TIV1 and TOMSSF promoters, expression of these genes was analyzed in roots of tomato plantlets exposed for 12 and 24 h to glucose, sucrose, ABA or SA, substances that have been shown to be involved in signal transduction pathways in plants and in mycorrhiza formation. Application of 100 μM ABA resulted in an elevated level of mRNA for the sucrose synthase TOMSSF and for the vacuolar invertase TIV1. In contrast, the mRNA levels for the cell wall invertase Lin6 were not changed in response to ABA (Fig. 4). External application of SA and glucose increased the expression level of the three analyzed sucrose-cleaving enzymes, while the application of sucrose only increased the expression level of Lin6 and TOMSSF. These data are in agreement with the in silico analysis of the 5′-flanking regions of these genes.

Discussion

In this article, we report that development of the AM symbiosis induces upregulation of genes encoding the cell wall invertase Lin6, the vacuolar invertase TIV1 and the sucrose synthase TOMSSF in tomato roots.

Plants have evolved a small family of acid invertase genes, the members of which are expressed independently at specific times and in specific tissues during plant development. The finding that of the four genes encoding tomato cell wall invertases, only Lin6, the isoform constitutively expressed in tomato roots, was detected in mycorrhizal roots indicates that development of the symbiosis does not induce the synthesis de novo of a previously reported cell wall invertase isoform (Godt and Roitsch 1997). Upregulation of Lin6 mRNA levels in mycorrhizal roots is consistent with the results of Wright et al. (1998), who reported higher cell wall invertase activity in mycorrhizal clover plants compared with non-mycorrhizal plants. In a previous study, Blee and Anderson (2002) failed to detect increased transcript levels of a cell wall invertase in mycorrhizal carrot roots. However, it is necessary to keep in mind that cell wall invertases are encoded by multigene families, the members of which present an organ-, developmental- and environmental-specific regulation.

Cell wall invertases have been implicated in the regulation of the sink strength of plant tissues (Goetz et al. 2000). Therefore, activation of Lin6 gene expression in mycorrhizal roots could represent a mechanism contributing to the sink strength arising from mycorrhizal colonization (Graham 2000). As the intraradical structures of AM fungi cannot take up sucrose (Shachar-Hill...
et al. 1995; Solaiman and Saito 1997), upregulation of Lin6 mRNA in mycorrhizal roots also suggests that the protein encoded by this gene could be responsible for the hydrolysis of the sucrose delivered to the apoplast of the symbiotic interface to release the hexoses that will be then taken up by the fungal partner for growth and metabolism. Alternatively, hexoses released from sucrose hydrolysis at the apoplast could be taken up by the host root cells to support their increased metabolic activity. This hypothesis is supported by the observation by Harrison (1996) that the formation of the AM symbiosis is accompanied by upregulation of a hexose transporter that potentially functions to supply sugars to root cells critically involved in the symbiotic association.

In this study, we show for the first time induction of a cell wall invertase in an AM association, which supports the general assumption that carbon transfer across the symbiotic interface requires host sucrose hydrolysis by a cell wall invertase (Ferrol et al. 2002). The importance of host cell wall invertases for supplying the fungal partner with hexoses has been shown in ectomycorrhizas. Heterologous expression of highly active yeast invertase in poplar hybrids had a profound effect on the availability of hexoses and, in consequence, on fungal metabolism and development after mycorrhiza formation (Guttenberger 1998).

Vacuolar invertases determine the level of sucrose stored in the vacuole and play an important role in the remobilization of sucrose for metabolic processes (Roitsch and González 2004). Upregulation of the mRNA levels for the vacuolar invertase isozyme TIV1 in tomato mycorrhizal roots agrees with previous observations of Blee and Anderson (2002) who found an accumulation of transcripts for a vacuolar invertase in cortical cells containing arbuscules in Phaseolus vulgaris. These data suggest that TIV1 could provide hexoses to support the elevated metabolic activity and respiration rates of the colonized tissues in a mycorrhizal root (Gianinazzi-Pearson 1996). Differential regulation of TIV1 in roots colonized either by G. mosseae or G. intraradices suggests that carbon utilization in tomato roots probably depends on the species of the AM fungus involved in the symbiosis.

Activation of a sucrose synthase gene during AM symbiosis in tomato roots is in agreement with previous observations in other plant species, such as P. vulgaris (Blee and Anderson 2002), Medicago truncatula (Hohnjec et al. 2003) and Zea mays (Ravnskov et al. 2003), and with the increased sucrose synthase activity found in mycorrhizal clover and soybean roots (Schubert et al. 2003, Wright et al. 1998). In situ hybridization experiments in P. vulgaris showed that transcripts for sucrose synthase were located in phloem tissues and in cortical

**Fig. 4.** Effect of glucose, sucrose, SA and ABA on (A) Lin6, (B) TIV1 and (C) TOMSSF gene expressions. RNAs were extracted from roots of tomato plantlets exposed for 12 and 24 h to H2O (control), 100 µM ABA, 100 µM SA, 40 mM glucose or 20 mM sucrose. RNAs were reverse transcribed, and the expression levels were assayed by quantitative real-time RT–PCR using gene-specific primers for Lin6, TIV1, TOMSSF and 18S rRNA. The relative levels of transcription were calculated by the 2−ΔΔCt method. Data represent the means of three independent biological replicates. Error bars represent SD.
Therefore, upregulation of roots (Danneberg et al. 1992, Meixner et al. 2005). Phytohormone ABA has been reported in mycorrhizal the signaling compound SA (Blilou et al. 1999) and of these genes in the symbiotic roots. An accumulation of glucose and fructose found in mycorrhizal roots (Trouverie et al. 2004). However, the lower concentrations previously reported in other plant species (Roitsch et al. 1995, Trouverie et al. 2002). Upregulation of hexoses or sucrose in the control of metabolic enzymes supports previous observations for a central role of these genes, reflecting increased catabolism and utilization of the AM symbiosis upregulates sucrose metabolism than in providing carbon compounds to the fungus.

Transcriptional upregulation of sucrose-splitting enzymes in roots of the 4-week mycorrhizal plants agrees with the decreased levels of sucrose detected in these roots. At the second harvest, sucrose content increased in mycorrhizal roots, suggesting that more sucrose was being translocated to the roots of the mycorrhizal plants probably to compensate for the extra cost as a result of the presence of the fungus. The decrease in the glucose and fructose contents in mycorrhizal roots can be interpreted as a result of their conversion into fungal-specific compounds such as trehalose and lipids.

Regulation of Lin6, TOMSSF and TIV1 genes by sugars supports previous observations for a central role of hexoses or sucrose in the control of metabolic enzymes in higher plants (Rolland et al. 2002). Upregulation of these genes in roots of tomato plantlets exposed to glucose suggests that the higher hexose concentration resulting from sucrose hydrolysis induces their expression by a positive feedback regulation, as it has been previously reported in other plant species (Roitsch et al. 1995, Trouverie et al. 2004). However, the lower concentrations of glucose and fructose found in mycorrhizal roots suggest an alternative mechanism for the regulation of these genes in the symbiotic roots. An accumulation of the signaling compound SA (Blilou et al. 1999) and of the phytohormone ABA has been reported in mycorrhizal roots (Danneberg et al. 1992, Meixner et al. 2005). Therefore, upregulation of Lin6, TOMSSF and TIV1 by SA and of TOMSSF and TIV1 by ABA suggest that these compounds might mediate regulation of these genes in mycorrhizal roots.

In conclusion, all these data indicate that development of the AM symbiosis upregulates sucrose metabolism genes, reflecting increased catabolism and utilization of sucrose in mycorrhizal tomato roots. Future detailed promoter analyses using GUS fusions and analysis of plants with knock outs for Lin6, TIV1 and TOMSSF by RNA interference will shed light on their specific functions in the symbiosis and on the regulatory elements that are required for their transcriptional activation in mycorrhizal roots.

Acknowledgements – We thank Dr José Miguel Barea for helpful discussions and Ms Custodia Cano and Ascensión Valderas for excellent technical assistance. S. G-R. was supported by an I3P fellowship from the Spanish Council for Scientific Research. This research was supported by CICYT (AGL2003-01551), Spain.

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Edited by H. Usuda