Plasma membrane ATPase and $H^+$ transport activities in microsomal membranes from mycorrhizal tomato roots

Karim Benabdellah, Concepción Azcón-Aguilar and Nuria Ferrol

Departamento de Microbiología del Suelo y Sistemas Simbióticos, Estación Experimental del Zaidín (CSIC), Profesor Albareda 1, 18008, Granada, Spain

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Abstract
ATPase activity, ATP-dependent $H^+$ transport and the amount of antigenic tomato plasma membrane $H^+$-ATPase have been analysed in membrane vesicles isolated from *Glomus mosseae* or *Glomus intraradices*-colonized roots and from non-mycorrhizal tomato roots. Microsomal protein content was higher in mycorrhizal than in control roots. The specific activity of the plasma membrane $H^+$-ATPase was not affected by mycorrhizal colonization, although this activity increased in membranes isolated from mycorrhizal roots when expressed on a fresh weight basis. Western blot analysis of microsomal proteins using antibodies raised against the *Arabidopsis thaliana* plasma membrane $H^+$-ATPase showed that mycorrhizal colonization did not change the relative amount of tomato plasma membrane ATPase in the microsomes. However, on a fresh weight basis, there was a greater amount of this protein in roots of mycorrhizal plants. In addition, mycorrhizal membranes showed a higher specific activity of the vanadate-sensitive ATP-dependent $H^+$ transport than membranes isolated from control roots. These results suggest that mycorrhiza might regulate the plasma membrane ATPase by increasing the coupling efficiency between $H^+$ transport and ATP hydrolysis. The observed effects of mycorrhizal colonization on plasma membrane $H^+$-ATPase were independent of the AM fungal species colonizing the root system.

Key words: Arbuscular mycorrhiza, *Lycopersicon esculentum*, microsomes, plasma membrane $H^+$-ATPase.

Introduction
Arbuscular mycorrhizal (AM) fungi are able to establish mutualistic symbioses with the roots of most terrestrial plants, including many agricultural important crop species (Barea et al., 1993). The 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 fungal mycelium takes up from the soil (Harley and Smith, 1983). The transfer mechanisms between symbionts are of considerable interest and are likely to involve specific alterations in membrane transport processes. Woolhouse was the first to suggest that the controlled transfer of nutrients required for a functional mycorrhizal symbiosis is carried out by means of active transport processes (Woolhouse, 1975).

In many cellular systems, transport of solutes into and out of the cell involves processes driven by the $H^+$ electrochemical gradient generated by the plasma membrane $H^+$-ATPase, which pumps protons out of the cell (Serrano, 1989). Therefore, the hypothesis of an active transport in mycorrhizal symbioses requires the presence of a $H^+$ pumping activity. This hypothesis has been supported by cytochemical, electrophysiological and biochemical data. In particular, cytochemical studies (Marx et al., 1982; Smith and Smith, 1990; Gianinazzi-Pearson et al., 1991) have shown that in arbuscular mycorrhizas ATPase activity is associated with both plant and fungal membranes of the arbuscular interface, which is consistent with active transport processes occurring at this location. ATPases represent proton pumps that establish proton motive forces and drive the uptake of $P_i$ or hexose against their electrochemical gradients, from the interfacial apoplast into the root or the fungal cell. In addition, because ATPase activity was also detected in the plasma membrane of the intercellular hyphae, it has been hypothesized that these structures could also have a role in carbon uptake from the host apoplast, suggesting a certain spatial separation of $P_i$ and $C$ transfer (Gianinazzi-Pearson et al., 1991).
Recently, a phosphate transporter (GvPT), which is operated by proton-coupled symport, has been cloned from the AM fungus *Glomus versiforme*, and its expression was found to be located in the external hyphae of the fungus during mycorrhizal association (Harrison and van Buuren, 1995). However, little is known about the molecular mechanisms involved in the transfer processes between the two symbionts.

Electrophysiological studies have also shown a H⁺ pump activity in the root cells of mycorrhizal *Allium porrum* (Fieschi et al., 1992). The electrical potential of the cells was significantly more negative in mycorrhizal than in non-mycorrhizal roots, indicating an increased H⁺ pump activity in mycorrhizal roots. In addition, an enhanced plasma membrane ATPase activity has been found in microsomal membranes from mycorrhizal potato, onion and sunflower roots (McArthur and Knowles, 1993; Bago et al., 1997), and a gene homologous to H⁺-ATPase was shown to be up-regulated during the establishment of the mycorrhizal symbiosis between *Hordeum vulgare* and *Glomus intraradices* (Murphy et al., 1997).

The present work was undertaken to gain new insights into the relevance of the plasma membrane H⁺-ATPase concerning membrane transport processes in a functional mycorrhizal symbiosis. For this purpose, parameters such as ATPase activity, ATP-dependent H⁺ pumping and the amount of antigenic plant H⁺-ATPase were determined in microsomal membranes from non-mycorrhizal tomato roots and mycorrhizal with either of two species of AM fungi, *Glomus mosseae* or *Glomus intraradices*, differing in their ability to colonize the root system.

**Materials and methods**

**Biological material and growth conditions**

Tomato seeds (*Lycopersicon esculentum* cv. Earlymech) were surface-sterilized in 10% NaClO for 3 min, rinsed thoroughly with sterile distilled water and pre-germinated for 7 d in sterile vermiculite in the dark at 22 °C. Plants were transplanted into pots (2 plants per pot) containing 1 kg of a sterile mixture of sand-vermiculite (1/1, v/v). Mycorrhizal treatments were performed using two different AM species: *Glomus mosseae* Nicol. and Gerd. (BEG 12) or *Glomus intraradices* Smith and Schenck (BEG 72). The inoculum consisted of thoroughly mixed rhizosphere samples containing spores, hyphae and mycorrhizal root fragments. Control pots received a filtrate (<20 μm) of the AM inocula in order to provide the general microbial populations accompanying AM fungi, but free from AM propagules. Plants were maintained in a growth chamber (25/18 °C day/night temperature, 70% relative humidity and 16 h photoperiod at 400 μmol m⁻² s⁻¹) and watered 3 times per week with Long Ashton nutrient solution (Hewitt, 1952), containing a reduced level of phosphorus (25%). Plants were harvested 4 weeks after transplanting. At harvest, shoot and root weights were determined, and 1 g of the root system was stained by the trypan blue method (Phillips and Hayman, 1970) to quantify AM colonization by using the gridline intersection method (Giovannetti and Mosse, 1980). To check the possibility of a cross-reaction between plant ATPase antibodies and the fungal ATPase, extraradical mycelia of *G. intraradices* were collected from mycorrhizal plants under a dissecting microscope and submitted to Western blot analyses.

**Membrane isolation**

Membranes were obtained according to the method of De Michielis and Spanswick with minor modifications (De Michielis and Spanswick, 1986). Excised roots (10 g) were chopped and homogenized (1/3, w/v) with a chilled pestle and mortar in a cold grinding medium containing 25 mM TRIS-HCl pH 8.0, 250 mM sucrose, 2 mM MgSO₄, 2 mM ATP, 10% glycerol, 2% bovine serum albumin (BSA), 2 mM dithiothreitol (DTT), 2 mM phenylmethylsulphonyl fluoride (PMSF), 2 mM ethylene glycol-bis ([β-aminoethyl ether) N,N,N′,N′-tetraacetic acid (EGTA), and 10% (w/v) polyvinylpyrrolidone (PVPP). The homogenate was filtered through four layers of cheesecloth and centrifuged at 13,000 g for 15 min. The supernatant fraction was centrifuged at 80,000 g for 35 min, and the microsomal pellet was resuspended in a medium containing 2 mM TRIS-HCl pH 8.0, 250 mM sucrose, 10% glycerol, 1 mM DTT, 1 mM PMSF, and 20 μg ml⁻¹ chymostatin. Aliquots of the membranes were stored at −80 °C until assayed.

**Protein content of the isolated microsomes was determined by the method of Bradford using BSA as a reference standard (Bradford, 1976).**

**ATPase activity determination**

ATP hydrolytic assays were performed using the procedure of Galier et al. (Galier et al., 1988). For the basal activity (the activity only of inside-out vesicles), the assay medium contained 25 mM 1,3-bis[(tris(hydroxymethyl)methylamino] propane (BTP)-HCl pH 6.5, 100 mM KCl, 0.1 mM Na₂MoO₄, 3 mM MgSO₄, and 3 mM ATP-BTP pH 6.5, in a final volume of 0.5 ml. The amount of membrane protein routinely used was 8 μg per assay. Enzyme activity was assayed for 30 min at 30 °C, and triplicate samples were run for each assay. For measurement of total ATPase activity (activity of both inside-out and right-side-out vesicles), 20 μg ml⁻¹ of lysophosphatidylcholine (LPC) was added to permeabilize the membranes to ATP. The latent activity (the activity of right-side-out vesicles) is the difference between the activities measured with and without LPC, and the latency is the latent activity expressed as a percentage of the activity in the presence of LPC.

The plasma membrane Mg-ATPase activity was defined as the vanadate-sensitive, molybdate-insensitive, nitrate-insensitive, and azide-insensitive Mg-ATP hydrolysis (Δ vanadate); the tonoplast Mg-ATPase activity as the nitrate-sensitive and molybdate-insensitive (Δ nitrate) and the mitochondrial as the azide-sensitive and molybdate-insensitive (Δ azide), using sodium orthovanadate at 0.25 mM, KNO₃ at 100 mM and NaN₃ at 1 mM as the corresponding inhibitors (Ferrol et al., 1993).

**H⁺ transport assays**

ATP-dependent proton transport across membrane vesicles was measured by 9-amino-6-chloro-2-methoxyacridine (ACMA) fluorescence quenching (Bennett and Spanswick, 1983), using a Shimadzu RF-540 spectrofluorometer at excitation and emission wavelengths of 418 and 485 nm, respectively. The assay medium contained 50 mM BTP-MES pH 6.5, 250 mM sucrose, 100 mM KNO₃, 2 mM NaN₃, 0.1 mM valinomycin, 1 μM ACMA, 3 mM ATP-BTP pH 6.5, and 200 μg membrane protein. After incubating for 10 min at 25 °C, the reaction was started by addition of...
3 mM MgSO₄. The plasma membrane ATP-dependent H⁺ transport was defined as the activity sensitive to vanadate but resistant to nitrate and azide. The initial rate of fluorescence quenching was determined and used as a measure of the rate of the ATP-dependent H⁺ pumping. These experiments were carried out in the presence of valinomycin, a potassium ionophore, to prevent the formation of a membrane potential that could inhibit H⁺ influx. For the calibration of the fluorescence, acid-interior pH gradients were imposed by the addition of aliquots of 1 N NaOH and the ΔpH was calculated by measuring the pH in the cuvette before and after the addition of NaOH (Perlin et al., 1986). Buffering capacity was determined as described by Maloney (Maloney, 1979) and was considered in the estimation of the H⁺/ATP stoichiometry.

To determine the passive permeability to H⁺, 1 mM glucose and 42 units ml⁻¹ yeast hexokinase (type VI Sigma) were added at the steady-state pH gradient, and the passive permeability to H⁺ was estimated by the time for half dissipation of the gradient (t₁/₂). Intravesicle volume was estimated as described previously (Bennett and Spanswick, 1983).

**Gel electrophoresis and immunoblotting techniques**

SDS-PAGE was performed according to the method of Laemmli (Laemmli, 1970). Microsomal proteins (100 µg) were precipitated with trichloroacetic acid, and pellets were resuspended in SDS-PAGE buffer containing 50 mM TRIS-HCl pH 6.8, 2% SDS, 10% glycerol, 5% mercaptoethanol, 2 mM PMSF, and 100 µg ml⁻¹ chymostatin and incubated for 30 min at room temperature before electrophoresis (Ferrol and Bennett, 1996). Proteins were separated on 10% SDS-polyacrylamide gels on a BioRad (Richmond, CA, USA) Protein II, and electro-transferred to nitrocellulose membranes at 100 V for 1.5 h in a BioRad transfer cell with a buffer consisting of 10 mM 3-cyclohexylamino-1-propane sulphonic acid-NaOH, pH 11.0, and 10% (v/v) methanol.

Blots were immunodetected with polyclonal antibodies raised against the plasma membrane H⁺-ATPase of Arabidopsis thaliana and Saccharomyces cerevisiae (kindly supplied by Dr Serrano, Universidad Politécnica de Valencia, Spain), at dilutions 1:1000 and 1:100, respectively, using goat-anti-rabbit IgG-alkaline phosphatase conjugate as the second antibody. Relative quantities of antigenic proteins on nitrocellulose blots were determined by densitometric scanning with a Shimadzu CS-9000 densitometer.

**Results**

Growth responses of tomato plants to mycorrhizal colonization were different depending on the AM fungus involved. Plants inoculated with *G. mosseae* had a significantly greater shoot and root fresh weight than controls, while no significant differences were found for plants inoculated with *G. intraradices* (Table 1). In control plants, the shoot/root ratio was similar than in those inoculated with *G. mosseae* but lower than in *G. intraradices*-inoculated plants. Mycorrhizal colonization was also quantitatively and qualitatively different depending on the AM fungus involved. Root colonization by *G. intraradices* was higher than by *G. mosseae*, reaching 50% and 37% of the total root length, respectively (Table 1). Moreover, colonization by *G. mosseae* consisted mainly of arbuscules and hyphae, whereas plants inoculated with

<table>
<thead>
<tr>
<th>Control</th>
<th>Mycorrhizal treatments</th>
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<tbody>
<tr>
<td>Shoot FW (g plant⁻¹)</td>
<td>10.25 a</td>
</tr>
<tr>
<td>Root FW (g plant⁻¹)</td>
<td>4.06 a</td>
</tr>
<tr>
<td>Shoot/root</td>
<td>2.52 a</td>
</tr>
<tr>
<td>Mycorrhizal colonization (%)</td>
<td>0</td>
</tr>
</tbody>
</table>

Data in the same row sharing the same letter do not differ significantly (P ≤ 0.05) according to Duncan's test.

**Table 1. Shoot and root fresh weight, shoot/root ratio and mycorrhizal colonization of tomato plants inoculated with the AM funguses Glomus mosseae or Glomus intraradices or maintained as uninoculated controls**

G. *intrapradices* also contained a high number of lipid-rich vesicles (data not shown).

Microsomal protein content, expressed as mg protein g⁻¹ of root fresh weight, was higher for mycorrhizal than for control roots, and the protein content was higher in *G. intraradices-* than in *G. mosseae*-colonized plants (Table 2). The results of ATPase specific activity measured in the absence (basal activity) and the presence of LPC (total activity), as well as the membrane latency values are also shown in Table 2. These ATPase activities and the latency of the membranes were quite similar in microsomes isolated from the three treatments under study.

To measure plasma membrane, tonoplast and mitochondrial ATPase activities in the isolated microsomes, specific inhibitors for these enzymes were used in the ATPase assays. These experiments were carried out in the

**Table 2. Microsomal protein content, ATPase activity assayed in the absence (basal activity) and presence (total activity) of LPC, and latency values of microsomal membranes isolated from control and Glomus mosseae- or Glomus intraradices-inoculated tomato roots**

Activities are expressed as specific activities (µmol P₄ g⁻¹ protein h⁻¹) and on a fresh weight basis (µmol P₄ g⁻¹ root FW h⁻¹). Results are expressed as mean values of three independent experiments with the activities determined in triplicate.

<table>
<thead>
<tr>
<th>Protein content</th>
<th>Control</th>
<th>Mycorrhizal treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg g⁻¹ FW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal ATPase</td>
<td>0.19 a</td>
<td>0.23 b</td>
</tr>
<tr>
<td>µmol P₄ g⁻¹ protein h⁻¹</td>
<td>18.51 a</td>
<td>17.21 a</td>
</tr>
<tr>
<td>µmol P₄ g⁻¹ root FW h⁻¹</td>
<td>3.52 a</td>
<td>3.96 a</td>
</tr>
<tr>
<td>Total ATPase</td>
<td>36.94 a</td>
<td>37.98 a</td>
</tr>
<tr>
<td>µmol P₄ g⁻¹ protein h⁻¹</td>
<td>7.02 a</td>
<td>8.74 b</td>
</tr>
<tr>
<td>µmol P₄ g⁻¹ root FW h⁻¹</td>
<td>49.89 a</td>
<td>54.68 a</td>
</tr>
</tbody>
</table>

Data in the same row sharing the same letter do not differ significantly (P ≤ 0.05) according to Duncan’s test.
The specific activity of the different ATPases assayed was not affected by mycorrhizal colonization (Table 3). Because several authors have pointed out that the comparison of enzymatic activities mg\(^{-1}\) protein in mycorrhizal and control roots could be biased by the presence of fungal compounds in the colonized roots (Franken and Gnadinger, 1994), these activities have also been calculated g\(^{-1}\) root fresh weight. On a fresh weight basis, the plasma membrane and mitochondrial ATPase activities were higher in membranes isolated from inoculated roots than in those isolated from controls; however, the tonoplast ATPase activity was not affected by mycorrhizal colonization (Table 3).

In microsomes isolated from mycorrhizal roots, membranes from the root and the fungus should be present. Since the fungal and plant ATPases respond similarly to the specific inhibitors tested (Serrano, 1985), and to try to distinguish between the fungal and the plant plasma membrane ATPases and to quantify them, immunoblot analysis of microsomal proteins using antibodies raised against the Arabidopsis thaliana and Saccharomyces cerevisiae plasma membrane H\(^{+}\)-ATPases was carried out. It is known that the Arabidopsis antibody cross-reacts with the tomato, but not with the yeast plasma membrane H\(^{+}\)-ATPase, while the yeast antibody does not cross-react with the plant H\(^{+}\)-ATPases (Palmgren and Christensen, 1993; Ferrol and Bennett, 1996). To determine whether or not these antibodies could recognize the plasma membrane H\(^{+}\)-ATPase of AM fungi, protein extracts from the external mycelium of Glomus intraradices were also analysed. Antibodies raised against the Saccharomyces plasma membrane H\(^{+}\)-ATPase did not react either with microsomal proteins nor with mycelium protein extracts (data not shown). On the other hand, the antibody against the Arabidopsis plasma membrane H\(^{+}\)-ATPase did not cross-react with the G. intraradices proteins (Fig. 1, lane 4), although it recognized the plant ATPase present in the microsomal extracts (Fig. 1, lanes 1, 2, 3).

Equal amounts of microsomal proteins from the different treatments were loaded on the gel, in order to evaluate the amount of tomato plasma membrane H\(^{+}\)-ATPase in the Western blot analysis. Figure 1 shows that there was no apparent change in the relative amount of the antigenic protein in mycorrhizal microsomes (Fig. 1, lanes 2, 3) relative to the control ones (Fig. 1, lane 1). However, on a fresh weight basis there was a 1.6-fold increase in the amount of tomato plasma membrane H\(^{+}\)-ATPase in roots colonized by G. mosseae or G. intraradices, in comparison to the controls.

Microsomal vesicles were also assayed for plasma membrane ATP-dependent H\(^{+}\) pumping activity by measuring the initial rate of ATP-dependent quenching of ACMA fluorescence (Fig. 2). The rate of proton pumping activity expressed mg\(^{-1}\) protein was simulated about 1.6-fold in membrane vesicles isolated from both colonized roots, compared with those isolated from control roots. To determine if the increase in plasma membrane H\(^{+}\) pumping activity was due to an activation of the H\(^{+}\)-ATPase, or to changes in membrane vesicle properties, H\(^{+}\) passive membrane permeability and intravesicular volume were also determined. The time for half dissipation of the gradient (\(t_{1/2}\)) was similar in control and both mycorrhizal root membranes (Table 4; Fig. 2), which indicates that changes in H\(^{+}\) transport activity were not due to changes in H\(^{+}\) leakage. Moreover, neither the latency (Table 2) nor the intravesicular size (data not shown) were significantly affected by mycorrhizal colonization. These results all together indicate that mycorrhizal colonization activates the plasma membrane H\(^{+}\)-ATPase.

### Table 3. Plasma membrane (Δ Vanadate), tonoplast (Δ Nitrate) and mitochondrial (Δ Azide) ATPase activities assayed in the presence of LPC of microsomal membranes isolated from control and Glomus mosseae- or Glomus intraradices-colonized tomato roots

Activities are expressed as specific activities (μmol P\(_{i}\) mg\(^{-1}\) protein h\(^{-1}\)) and on a fresh weight basis (μmol P\(_{i}\) g\(^{-1}\) root FW h\(^{-1}\)). Results are means of three independent experiments with the activities determined in triplicate.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Mycorrhizal treatments</th>
<th>Glomus mosseae</th>
<th>Glomus intraradices</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\Delta) Vanadate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>μmol P(_{i}) mg(^{-1}) protein h(^{-1})</td>
<td>22.24 a</td>
<td>23.15 a</td>
<td>21.53 a</td>
<td></td>
</tr>
<tr>
<td>μmol P(_{i}) g(^{-1}) root FW h(^{-1})</td>
<td>4.22 a</td>
<td>5.32 b</td>
<td>6.03 b</td>
<td></td>
</tr>
<tr>
<td>(\Delta) Nitrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>μmol P(_{i}) mg(^{-1}) protein h(^{-1})</td>
<td>4.59 a</td>
<td>4.71 a</td>
<td>4.23 a</td>
<td></td>
</tr>
<tr>
<td>μmol P(_{i}) g(^{-1}) root FW h(^{-1})</td>
<td>0.87 a</td>
<td>1.08 a</td>
<td>1.18 a</td>
<td></td>
</tr>
<tr>
<td>(\Delta) Azide</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>μmol P(_{i}) mg(^{-1}) protein h(^{-1})</td>
<td>7.82 a</td>
<td>9.03 a</td>
<td>7.24 a</td>
<td></td>
</tr>
<tr>
<td>μmol P(_{i}) g(^{-1}) root FW h(^{-1})</td>
<td>1.48 a</td>
<td>2.07 b</td>
<td>2.02 b</td>
<td></td>
</tr>
</tbody>
</table>

Data in the same row sharing the same letter do not differ significantly (\(P \leq 0.05\)) according to Duncan’s test.
that bidirectional flow occurs (Cooper, 1984; Pearson and Jakobsen, 1993), little is known about the mechanisms involved. During the last few years much attention has been focused on $\text{H}^+\text{-ATPases}$ as the master enzymes in energizing cell membranes (Serrano, 1989; Michelet and Boutry, 1995), and it is well known that the study of these enzymes using membrane vesicles has the advantage of allowing the measurement of both the enzyme activity and the generation of the proton gradient in a single preparation. For this reason, in the present work membrane vesicles were isolated from control and mycorrhizal plants inoculated with either of two AM fungi differing in their ability to colonize the root system.

Membrane vesicles isolated from mycorrhizal roots contained a higher amount of proteins, which could be due to an increase of plant membrane proteins and/or to the presence of proteins from the fungal partner. These results were obtained for plants colonized by either of the AM fungi tested. Similar data have been reported Fig. 2.

Table 4. Plasma membrane ATP-dependent $\text{H}^+$-pumping, basal ATPase activities and passive $\text{H}^+$ permeability of membranes isolated from control and $G$. mosseae- or $G$. intraradices-colonized tomato roots.

The ratio of $\text{H}^+$ pumping to basal ATP hydrolysis activity is also shown. Data are means of two independent experiments with the activity determined in duplicate.

<table>
<thead>
<tr>
<th>Control</th>
<th>Mycorrhizal treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$G$. mosseae</td>
</tr>
<tr>
<td>$\Delta V_{\text{a}}$ $\text{H}^+$ pumping activity</td>
<td>0.130 a</td>
</tr>
<tr>
<td>(nmol $\text{H}^+$ mg$^{-1}$ protein min$^{-1}$)</td>
<td></td>
</tr>
<tr>
<td>$\Delta V_{\text{a}}$ basal ATPase activity</td>
<td>0.181 a</td>
</tr>
<tr>
<td>(nmol P$_i$ mg$^{-1}$ protein min$^{-1}$)</td>
<td></td>
</tr>
<tr>
<td>$\text{H}^+$/ATP</td>
<td>0.72 a</td>
</tr>
<tr>
<td>$t_{1/2}$ (min)</td>
<td>2.23 a</td>
</tr>
</tbody>
</table>

Data in the same row sharing the same letter do not differ significantly ($P \leq 0.05$) according to Duncan’s test.

Discussion

One of the central features of the AM symbiosis is the bidirectional transfer of nutrients that occurs between the fungus and its host plant. This balanced exchange is beneficial to both symbionts and is crucial in enabling the association to be maintained for extended periods of time. While numerous studies have unequivocally shown
higher ATPase activity found in mycorrhiza, on a root fresh weight basis, is a consequence of a higher amount of the plant plasma membrane H\textsuperscript{+}-ATPase. In this context, an enhanced ATPase activity in mycorrhizal roots, mainly at the interfaces between the arbuscules and the surrounding plant cell plasma membrane, has been shown by cytochemical staining (Gianinazzi-Pearson et al., 1991). It has been hypothesized that the promotion of ATPase activity on a root fresh weight basis is a direct consequence of the enhanced plasma membrane surface area of cells due to arbuscular growth (McArthur and Knowles, 1993).

In terms of specific activity, this study found that AM colonization induced a 1.6-fold increase in the rate of H\textsuperscript{+} transport with no change in ATP hydrolysis. This increase in plasma membrane H\textsuperscript{+} pumping activity did not seem to be due to changes in vesicle latency, passive H\textsuperscript{+} permeability or intravesicle volume. It is not clear how mycorrhizal symbiosis can enhance H\textsuperscript{+} pumping activity without a proportional increase in phosphohydrolase activity. Recently, it has been shown that a change in coupling ratio (ATP hydrolysis/H\textsuperscript{+} pumping activity) of yeast plasma membrane H\textsuperscript{+}-ATPase is induced by glucose under in vivo conditions (Venema and Palmgren, 1995). A change in this coupling ratio has also been described for a tobacco plasma membrane ATPase mutant (Morsomme et al., 1996), and it has been suggested that intrinsic uncoupling is an important mechanism for the regulation of pump activity. Although the uncoupling mechanisms of H\textsuperscript{+}-ATPases remain to be elucidated, a certain intrinsic uncoupling can result from the spontaneous dephosphorylation of the phosphorylated intermediate without simultaneous H\textsuperscript{+} translocation (slippage). It might be hypothesized that, in mycorrhizal plants, plasma membrane ATPase can be specifically regulated by increasing coupling efficiency between H\textsuperscript{+} pumping and ATP hydrolysis, consequently decreasing ATPase slippage. As it has been suggested for H\textsuperscript{+}-ATPase in glucose-activated yeasts, activation of H\textsuperscript{+} pumping activity in mycorrhizal roots could involve phosphorylation reactions (Venema and Palmgren, 1995). The tightly coupled high activity state induced by the mycorrhizal symbiosis might be essential for the formation of the very steep H\textsuperscript{+} gradients required for efficient solute uptake in a functional mycorrhiza.

The use of two AM fungi differing in their ability to colonize the root system has shown that the effect of mycorrhizal colonization on ATP hydrolysis and H\textsuperscript{+} pumping activities was independent of the fungal species colonizing the root system. However, the stimulation of the H\textsuperscript{+} pumping activity and, probably, of the transport processes taking place consequently in root membranes of mycorrhizal plants correlates with the increased growth of tomato plants colonized by G. mosseae, but not with those colonized by G. intraradices. As suggested by Peng et al. (Peng et al., 1993), growth depression or lack of stimulation, as in the present case, in plants colonized by G. intraradices, which is a quite aggressive AM fungus producing a high number of lipid-rich vesicles, could be due to the carbon cost associated with the production and maintenance of the fungal biomass.

In conclusion, it is suggested that mycorrhiza might regulate plasma membrane ATPase by increasing coupling efficiency between H\textsuperscript{+} pumping activity and ATP hydrolysis. Molecular genetic studies of plant plasma membrane H\textsuperscript{+}-ATPase have shown that this protein is encoded by a large family of genes (Michelet and Boutry, 1995). It has been also hypothesized that the array of H\textsuperscript{+}-ATPase isoforms is expressed in a cell-, tissue-, and developmental stage-specific manner, which allows for local changes in H\textsuperscript{+} transport capacity (Sussman, 1994). Seven LHA (Lycopersicon H\textsuperscript{+}-ATPase) genes, which are expressed differentially through the plant, have been cloned in tomato (Ewing and Bennett, 1994). Because immunodetection of ATPases does not distinguish between different isoenzymes, gene expression analysis must be done to determine whether a specific isoenzyme is induced in tomato roots by the establishment of AM symbiosis.

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References


