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SHORT COMMUNICATION

Arbuscular mycorrhizal symbiosis regulates plasma membrane H+-ATPase gene expression in tomato plants

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Abstract

Regulation by arbuscular mycorrhizal symbiosis of three tomato plasma membrane H+-ATPase genes (LHA1, LHA2 and LHA4) has been analysed in wild-type and mycorrhiza-defective tomato plants. Expression of these genes was differentially regulated in leaves and roots of both tomato phenotypes after inoculation with *Glomus mosseae*.

Key words: Arbuscular mycorrhizas, mycorrhiza-defective tomato mutants, *Phytophthora parasitica*, plasma membrane H+-ATPases.

Introduction

Arbuscular mycorrhizas (AM) are mutualistic symbioses formed between certain soil fungi belonging to the Glomales (Zygomycota) and over 80% of land plant species (Barea and Jeffries, 1995). Root colonization by the fungus and the establishment of an active association involves a complex sequence of interactions between the fungal hyphae and the plant root cells (Bonfante and Perotto, 1995; Harrison, 1999). These interactions result in the specific recognition by the fungal hyphae of the root surface, their differentiation to form appressoria, the penetration and growth of the hyphae within the root, and their further differentiation into the cortical cells to form intracellular branched haustoria, termed arbuscules. Root colonization is accompanied by the development of a network of hyphae external to the root, which increases the absorption and translocation of slow-diffusing nutrients, mainly phosphate, from the soil to the plant (Smith and Read, 1997; Jackobsen, 1999). In exchange, the fungus, which is an obligate biotroph, obtains all its carbon from the host plant (Jennings, 1995; Douds *et al.*, 2000). This bidirectional nutrient exchange between the plant and the fungus is the main basis for the mutualism of the association, and it is believed to occur over the arbuscule/cortical cell interface.

Phosphate uptake from the soil by AM fungal hyphae and its subsequent translocation to the root cortex has been well documented (Jackobsen, 1999); however, the mechanisms underlying phosphate movement within the symbiosis are still largely unknown. A high-affinity transporter, likely to be responsible for the initial phosphate uptake by the extraradical hyphae, has been cloned from the AM fungus *Glomus versiforme* (Harrison and van Buuren, 1995). Recently, Rosewarne *et al.* (1999) have identified a tomato phosphate transporter that may be involved in the uptake of phosphate that is effluxed across the fungal plasma membrane of the arbuscule. Moreover, cytochemical and immunocytochemical localization of an H+-ATPase, the enzyme that generates the electrochemical gradient of protons that drives active secondary transport systems, in the plant plasma membrane surrounding arbuscular hyphae suggests that the phosphate released across the fungal plasma membrane into the interfacial apoplast is actively taken up into the cortical root cells (Gianinazzi-Pearson *et al.*, 1991, 2000). Biochemical studies using membrane vesicles have provided further evidence that plasma membrane H+-ATPase activity is regulated by mycorrhizal colonization (Bago *et al.*, 1997; Benabdellah *et al.*, 1999).

Plasma membrane H+-ATPases are encoded by multigene families which include at least seven genes in tomato (Ewing and Bennett, 1994), 10 in *Arabidopsis thaliana* (Harper *et al.*, 1994), nine in *Nicotiana plumbaginifolia* (Ouffatole *et al.*, 2000), two in *Saccharomyces cerevisiae* (Schlesser *et al.*, 1988), and five in *Glomus mosseae* (Ferrol *et al.*, 2000a). The enzyme isoforms seem to have a tissue-specific expression, showing the highest expression

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levels in cells where intense active transport is expected to occur (Palmgren and Harper, 1999). Since neither polyclonal antibodies used for ATPase localization nor biochemical ATPase assays distinguish between isoforms, molecular approaches have been adopted to assess H⁺-ATPase regulation in the AM symbiosis (Ferrol *et al.*, 2000*b*). Murphy *et al.* (1997) first reported up-regulation of an H⁺-ATPase gene during AM development in barley roots. Recently, Gianinazzi-Pearson *et al.* (2000), using the *gusA* reporter gene system, have shown that promoters of two (*pma1* and *pma2*) out of eight different H⁺-ATPase genes from *N. plumbaginifolia* were induced in arbuscule-containing cells of mycorrhizal tobacco roots. To assess the regulation of plasma membrane H⁺-ATPases by the establishment of AM symbiosis further, the expression of the three H⁺-ATPase genes, that have been shown to be active in tomato tissues (LHA1, LHA2 and LHA4) in wild-type and mycorrhiza-defective tomato mutants inoculated and non-inoculated with *Glomus mosseae* has been analysed. Moreover, the effect induced by the mycorrhizal fungus on root H⁺-ATPases has been compared with the effect induced by the root pathogen *Phytophthora parasitica*.

Materials and methods

Plant material and inoculation conditions

Mycorrhizal inoculation of wild-type (*Lycopersicon esculentum* Mill, cv. 76R; Peto Seed Company, CA, USA) and of a mutant tomato line showing a reduced mycorrhizal colonization phenotype (*rmc* plants, kindly supplied by Drs Barker and Smith, Adelaide, Australia) was performed as described by Benabdellah *et al.* (1999) using a soil–sand-based inoculum of the AM fungus *G. mosseae* (Nicol. and Gerd.) Gerd. and Trappe (BEG119). Control plants received a filtrate (<20 μm) of the AM inoculum. Plants infected by the root pathogen *Phytophthora parasitica* were prepared by watering 4-week-old wild-type and *rmc* control plants with a suspension of *P. parasitica* mycelium as previously decribed by Pozo *et al.* (1999).

Plants from all treatments were grown in a constant environment (25/18 °C day/night temperature, 60% relative humidity, 16 h photoperiod, 400 μmol photons m⁻² s⁻¹), watered three times per week with a low-phosphorus-content (25%) Long Ashton nutrient solution (Hewitt, 1952) and harvested 6 weeks after inoculation with *G. mosseae* and 2 weeks after inoculation with *P. parasitica*. Mycorrhizal development was estimated in root samples after trypan blue staining (Phillips and Hayman, 1970) using the gridline intersect method (Giovannetti and Mosse, 1980). The spread of *P. parasitica* was estimated as the percentage of root length with necrotic zones (Pozo *et al.*, 1999).

Semi-quantitative RT-PCR

RNA was extracted from frozen tissues following the method of Logemann *et al.* (1987), treated with DNase and reverse transcribed with Avian Myeloblastosis virus reverse transcriptase (Finnzymes Oy, Finland) and random hexamer primers. The synthesized cDNAs were amplified using the LHA1-, LHA2- and LHA4-specific pair of primers designed by Mito *et al.* (1996). Primers were annealed at 60 °C, and DNA was synthesized at 72 °C for 1 min for 18 cycles, a time point where the linearity of the three genes was achieved. Since RNA extracts from mycorrhizal roots contain plant and fungal RNAs, the specificity of each set of primers was further analysed by PCR amplification of *G. mosseae* genomic DNA. No amplification products were obtained when the PCR was conducted with 100 ng of *G. mosseae* genomic DNA and either the LHA1-, LHA2- or LHA4-specific set of primers. Tomato cDNAs were calibrated by PCR amplification of the 18S rRNA using the specific set of primers, R1 (5′-AAAAGGTCGACGCGGGCT-3′) and R2 (5′-CGACAGAAG-GGACGAGAC-3′), that amplify the tomato, but not the *G. mosseae*, ribosomal gene. R1 and R2 primers were annealed at 55 °C for 30 s and DNA was amplified at 72 °C during 30 s for 10 cycles. Amplified cDNAs were subjected to electrophoresis and blotted to nylon membranes. Hybridizations were carried out using the terminal C-regions of the corresponding H⁺-ATPase genes and with the amplified region of the tomato 18S rRNA, labelled with DIG-11-dUTP by random priming. Hybridizations and chemiluminescent detection of digoxigenin-labelled probes were performed following the manufacturer's instructions (Roche Mannheim, Germany). Hybridization signals were quantified with the Quantity-One Quantification Software (Bio-Rad).

Results and discussion

Mutant plants that are unable to form complete mycorrhizas are a powerful tool to identify mechanisms controlling the symbiosis. In the present study, wild-type and mycorrhiza-defective tomato mutants have been used to analyse the effect of mycorrhizal symbiosis on host H⁺-ATPase gene expression. At harvest, more than 40% of the root length of the wild-type plants was colonized by *G. mosseae*, and all characteristic fungal structures of an Arum-type colonization were present, including appressoria on the root surface, intercellular hyphae and intracellular arbuscules. However, colonization by the AM fungus was blocked at an early stage in the mutant plants. As previously reported by Barker *et al.* (1998), in the mutant plants few fungal hyphae grew along the root surface, few complex branching appressoria were formed and the fungus did not penetrate the root epidermis.

Analysis of H⁺-ATPase gene expression in roots of both AM-inoculated and non-inoculated tomato phenotypes showed that expression of two out of the three analysed H⁺-ATPase genes was regulated only in the wild-type plants after inoculation with *G. mosseae* (Fig. 1). In roots of wild-type plants, mycorrhizal colonization induced down-regulation of LHA1, up-regulation of LHA2 and no changes were observed in the accumulation level of LHA4 transcripts. However, in roots of the mutant plants, in which development of the symbiosis was stopped at the stage of appressoria formation, AM fungal inoculation did not induce changes in the expression of any of these genes. These data suggest that regulation of LHA1 and LHA2 transcripts during the successful mycorrhizal interaction may be associated with the internal growth of the fungus and/or with the functioning of the symbiosis.
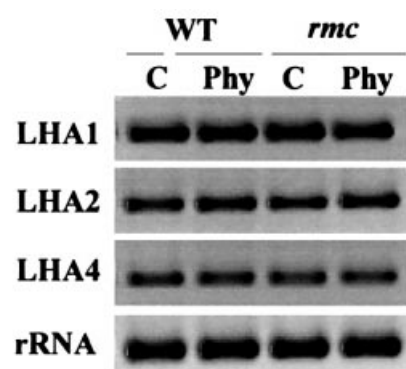
Based on the selective down-regulation of LHA1 transcripts in roots of wild-type plants it can be hypothesized that the H⁺-ATPase isoform encoded by the
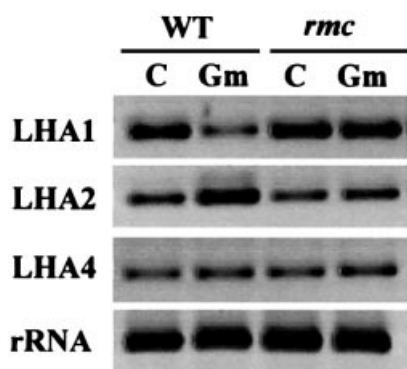
Fig. 1. Effect of mycorrhizal colonization on LHA1, LHA2 and LHA4 gene expression in tomato roots. Gene expression was analysed by RT-PCR. RNA isolated from control (C) and *G. mosseae*-inoculated (Gm) roots of wild-type (WT) and mycorrhiza-defective tomato mutant (rmc) plants was reverse transcribed and PCR amplified with LHA1, LHA2 and LHA4 specific primers. PCR products were detected by hybridization with the corresponding probes. In each case, 18S rRNA was used as a control of gene expression.

Fig. 2. Effect of *P. parasitica* on LHA1, LHA2 and LHA4 gene expression in tomato roots. Gene expression was analysed by RT-PCR. RNA isolated from control (C) and *P. parasitica*-infected (Phy) roots of wild-type (WT) and mycorrhiza-defective tomato mutant (rmc) plants was reverse transcribed and PCR amplified with LHA1, LHA2 and LHA4 specific primers. PCR products were detected by hybridization with the corresponding probes. In each case, 18S rRNA was used as a control of gene expression.

LHA1 gene could be involved in the generation of the proton gradient necessary for phosphate uptake by epidermal cells in the non-mycorrhizal situation, similar to the two high affinity phosphate transporter genes reported to be down-regulated in mycorrhizal *Medicago truncatula* roots (Liu *et al.*, 1998; Chiou *et al.*, 2001). This hypothesis is consistent with the observation that, in some cases, phosphate uptake from the soil by the epidermal root cells becomes almost inactive during the mycorrhizal symbiosis (Pearson and Jackobsen, 1993). Up-regulation of LHA2 transcripts in the successful mycorrhizal interaction is in agreement with the recent report that in arbuscule-containing cells *Glomus fasciculatum* induces activation of two out of the four ATPase genes that can be active in tobacco roots (Gianinazzi-Pearson *et al.*, 2000). Selective induction of LHA2 transcripts in roots of wild-type plants could be explained if the LHA2 protein contributes to the energization of the periarbuscular membrane necessary to drive host absorption of the phosphate that has been transported from the soil by the fungus and released into the symbiotic interface of cortical root cells through the action of the H⁺-phosphate symporter (LePT) that has been already localized in arbuscule-containing cells of tomato mycorrhizal roots (Rosewarne *et al.*, 1999).

Although the main physiological role of the plasma membrane H⁺-ATPase is to generate the driving force for solute uptake, this enzyme is also involved in regulation of intracellular pH, and it has been proposed that the H⁺-ATPase can act as an intermediate in certain signal transduction pathways (Xing *et al.*, 1996). It has been shown that inhibition and activation of the H⁺-ATPase in tomato suspension cultures differentially regulate plant defence responses to pathogens and wounding (Schaller and Oecking, 1999). In this context, regulation of LHA1 and LHA2 mRNAs during the successful mycorrhizal interaction could also be a component of the signal transduction pathway that modulates plant defence responses to AM fungi. Down-regulation of LHA1 and up-regulation of LHA2 in mycorrhizal wild-type plants could be related to the repression of a general defence reaction, but the induction of weak, transient and localized plant defence responses elicited by AM fungi during the development of the symbiosis (Blee and Anderson, 1996; Gianinazzi-Pearson *et al.*, 1996; Lambais and Mehdy, 1998).

To assess whether LHA1, LHA2 and LHA4 gene expression could be involved in the elicitation of the general plant defence response to a pathogen attack, expression of these genes was also analysed in roots of tomato plants infected with the root pathogen *P. parasitica*. Infection of tomato roots by *P. parasitica* did not induce changes in the abundance of LHA1, LHA2 and LHA4 mRNAs in either wild-type or mycorrhiza-defective tomato mutants (Fig. 2). This suggests that regulation of LHA1 and LHA2 transcripts during the mycorrhizal interaction was a mycorrhizal-specific effect. Although these data indicate that neither of these genes are involved in the elicitation of the tomato defence response to a pathogen attack, it can not be ruled out that inhibiton of LHA1 and induction of LHA2 transcripts in mycorrhizal roots are involved in the signal transduction pathways that trigger or modulate the defence response in a successful mycorrhizal interaction, since plant defence reactions elicited by AM fungi and fungal pathogens have been reported to differ in mycotrophic plants (Gianinazzi-Pearson *et al.*, 1996; Pozo *et al.*, 1998). The analysis of
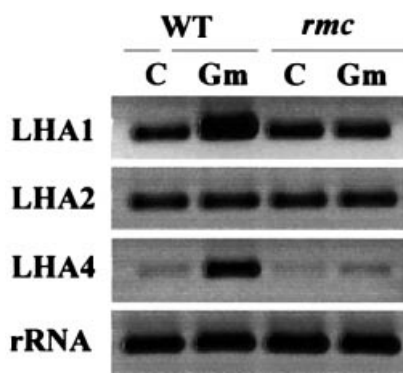
Fig. 3. RT-PCR expression analysis of LHA1, LHA2 and LHA4 genes in tomato leaves. RNA isolated from leaves of control (C) and *G. mosseae*-inoculated (Gm) wild-type (WT) and mycorrhiza-defective tomato mutant (rmc) plants was reverse transcribed and PCR amplified with LHA1, LHA2 and LHA4 specific primers. PCR products were detected by hybridization with the corresponding probes. In each case, 18S rRNA was used as a control of gene expression.

plant defence responses in mycorrhiza-defective (myc^{-1}) pea mutants, which present a phenotype concerning AM formation similar to the tomato mutants used in this study when interacting with *G. mosseae*, has shown that the induction by AM of plant defence responses, which are similar to those elicited by pathogens, prevents further development of the appressorium on the root surface (Gollotte *et al.*, 1993; Dumas-Gaudot *et al.*, 1994). The observation in the present study that the accumulation of LHA1, LHA2 and LHA4 transcripts was not affected in roots of the mycorrhiza-defective phenotype in response to the AM fungus (Fig. 1), provides further evidence that none of these genes were involved in the induction of the defence response that could prevent the development of a successful symbiosis in the mutant plants. Further studies are needed to elucidate the precise role of each H$^+$-ATPase isoform, as a primary transporter and/or as an intermediate in certain specific signal transduction pathways within the symbiosis.

Analysis of LHA1, LHA2 and LHA4 mRNA levels in leaves of mycorrhizal and non-mycorrhizal plants indicated that development of the symbiosis also regulated leaf plasma membrane H$^+$-ATPase gene expression. Figure 3 shows that accumulation of LHA2 mRNA was not affected in leaves of either plant phenotype after inoculation with the AM fungus. LHA1 and LHA4 transcript levels were higher in leaves of mycorrhizal wild-type plants than in those of control plants. However, inoculation of the mutants with *G. mosseae* did not induce significant changes in leaf LHA1 and LHA4 mRNAs. Previous studies have shown that leaf H$^+$-ATPases are involved in the active loading into the phloem of the reduced carbon compounds produced through photosynthesis to support growth of heterotrophic (sink) tissues (DeWitt *et al.*, 1991;

Villalba *et al.*, 1991). AM fungi, as obligate symbionts, depend for their growth and activity on the supply of carbon compounds by the photosynthetic partner (Jennings, 1995), and it has been shown that AM colonization results in an enhanced sink demand for carbon in roots (Douds *et al.*, 1988; Wright *et al.*, 1998). Measurements of carbon flux have shown that up to 20% of photosynthetically fixed carbon may be allocated to the roots during a mycorrhizal interaction (Graham, 2000). Up-regulation of leaf LHA1 and LHA4 transcritps during the successful mycorrhizal interaction could be related to the increased carbon partitioning that occurs in mycorrhizal associations.

In conclusion, these data provide evidence that development of the AM symbiosis regulates plasma membrane H$^+$-ATPase gene expression in roots and leaves of the host plants. Differential regulation of the three analysed tomato H$^+$-ATPase genes in roots and leaves of mycorrhizal plants reflects the complexity of the interactions taking place during the mycorrhizal association and of the mechanisms regulating plasma membrane H$^+$-ATPase gene expression.

Acknowledgements

References

Bago B, Donaire JP, Azcón-Aguilar C. 1997. ATPase activities of root microsomes from mycorrhizal sunflower (*Helianthus annuus*) and onion (*Allium cepa*) plants. *New Phytologist* **136,** 305–311.

Barea JM, Jeffries P. 1995. Arbuscular mycorrhizas in sustainable soil plant systems. In: Hock B, Varma A, eds. *Mycorrhiza: structure, function, molecular biology and biotechnology*. Berlin: Springer-Verlag, 521–560.

Barker SJ, Stummer B, Gao L, Dispain I, O'Connor PJ, Smith SE. 1998. A mutant in *Lycopersicon esculentum* Mill. with highly reduced VA mycorrhizal colonization: isolation and preliminary characterisation. *The Plant Journal* **15,** 791–797.

Benabdellah K, Azcón-Aguilar C, Ferrol N. 1999. Plasma membrane ATPase and H$^+$ transport activities in microsomal membranes from mycorrhizal tomato roots. *Journal of Experimental Botany* **337,** 1343–1349.

Blee KA, Anderson AJ. 1996. Defense-related transcript accumulation in *Phaseolus vulgaris* L. colonized by the arbuscular mycorrhizal fungus *Glomus intraradices* Schenck and Smith. *Plant Physiology* **110,** 675–668.

Bonfante P, Perotto S. 1995. Strategies of arbuscular mycorrhizal fungi when infecting host plants. *New Phytologist* **130,** 3–21.

Chiou TJ, Liu H, Harrison M. 2001. The spatial expression patterns of a phosphate transporter (MtPT1) from *Medicago truncatula* indicate a role in phosphate transport at the root/soil interface. *The Plant Journal* **25,** 281–293.

DeWitt ND, Harper J, Sussman MR. 1991. Evidence for a plasma

membrane proton pump in phloem cells of higher plants. *The Plant Journal* **1**, 121–128.

Douds Jr DD, Johnson CR, Koch KE. 1988. Carbon cost of the fungal symbiont relative to net leaf P accumulation in a split-root VA mycorrhizal symbiosis. *Plant Physiology* **86**, 491–496.

Douds Jr DD, Pfeffer PE, Shachar-Hill Y. 2000. Carbon partitioning, cost, and metabolism of arbuscular mycorrhizas. In: Kalpunik Y, Douds Jr DD, eds. *Arbuscular mycorrhizas: physiology and function.* The Netherlands: Kluwer Academic Publishers, 107–130.

Dumas-Gaudot E, Guillaume P, Tahiri-Alaoui A, Gianinazzi-Pearson V, Gianinazzi S. 1994. Chitinase isoforms in roots of various pea genotypes infected with arbuscular mycorrhizal fungi. *Plant Science* **99**, 27–37.

Ewing NN, Bennett AB. 1994. Assessment of the number and expression of P-type H^{+}-ATPase genes in tomato. *Plant Physiology* **106**, 547–557.

Ferrol N, Barea JM, Azcón-Aguilar C. 2000*a*. The plasma membrane H^{+}-ATPase gene family in the arbuscular mycorrhizal fungus *Glomus mosseae*. *Current Genetics* **37**, 112–118.

Ferrol N, Barea JM, Azcón-Aguilar C. 2000*b*. Molecular approaches to study plasma membrane H^{+}-ATPases in arbuscular mycorrhizas. *Plant and Soil* **226**, 219–225.

Gianinazzi-Pearson V, Arnould C, Oufattole M, Arango M, Gianinazzi S. 2000. Differential activation of H^{+}-ATPase genes by an arbuscular mycorrhizal fungus in root cells of transgenic tobacco. *Planta* **211**, 609–613.

Gianinazzi-Pearson V, Dumas-Gaudot E, Gollotte A, Tahiri-Alaoui A, Gianinazzi S. 1996. Cellular and molecular defence-related root responses to invasion by arbuscular mycorrhizal fungi. *New Phytologist* **133**, 45–57.

Gianinazzi-Pearson V, Smith SE, Gianinazzi S, Smith FA. 1991. Enzymatic studies on the metabolism of vesicular-arbuscular mycorrhizas. V. Is H^{+}-ATPase a component of ATP-hydrolysing enzyme activities in plant–fungus interfaces? *New Phytologist* **117**, 61–79.

Giovannetti M, Mosse B. 1980. An evaluation of techniques for measuring vesicular-arbuscular infection in roots. *New Phytologist* **84**, 489–500.

Gollotte A, Gianinazzi-Pearson V, Giovannetti M, Sbrana C, Avio L, Gianinazzi S. 1993. Cellular localization and cytochemical probing of resistance reactions to arbuscular mycorrhizal fungi in a 'locus a' myc-mutant of *Pisum sativum*. *Planta* **191**, 112–122.

Graham JH. 2000. Assessing costs of arbuscular mycorrhizal symbiosis in agroecosystems. In: Podila GK, Douds Jr DD, eds. *Current advances in mycorrhizae research.* St Paul: APS Press, 127–140.

Harper JF, Manney L, Sussman MR. 1994. The plasma membrane H^{+}-ATPase gene family in *Arabidopsis*: genomic sequence of AHA10 which is expressed primarly in developing seeds. *Molecular General Genetics* **244**, 572–587.

Harrison MJ. 1999. Molecular and cellular aspects of the arbuscular mycorrhizal symbiosis. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**, 361–389.

Harrison MJ, van Buuren ML. 1995. A phosphate transporter from the mycorrhizal fungus *Glomus versiforme*. *Nature* **378**, 625–629.

Hewitt EJ. 1952. *Sand and water culture methods used in the study of plant nutrition.* In: Technical communication, Vol. 22. Farnham Royal, Bucks, UK: Commonwealth Agricultural Bureaux.

Jackobsen I. 1999. Transport of phosphorus and carbon in arbuscular mycorrhizas. In: Varma A, Hock B, eds. *Mycorrhiza: structure, function, molecular biology and biotechnology.* Berlin: Springer Verlag, 305–332.

Jennings DH. 1995. *The physiology of fungal nutrition.* Cambridge, UK: Cambridge University Press.

Lambais MR, Mehdy MC. 1998. Spatial distribution of chitinases and β-1,3-glucanase transcripts in bean arbuscular mycorrhizal roots under low and high soil phosphate conditions. *New Phytologist* **134**, 531–538.

Liu H, Treu AT, Blaylock LA, Harrison MJ. 1998. Cloning and characterization of two phosphate transporters from *Medicago truncatula* roots: regulation in response to phosphate and colonization by arbuscular mycorrhizal (AM) fungi. *Molecular Plant-Microbe Interactions* **11**, 14–22.

Logemann J, Schell J, Willmitzer L. 1987. Improved method for the isolation of RNA from plant tissues. *Analytical Biochemistry* **163**, 16–20.

Mito N, Wimmers LE, Bennett AB. 1996. Sugar regulates mRNA abundance of H^{+}-ATPase gene family members in tomato. *Plant Physiology* **112**, 1229–1236.

Murphy PJ, Langridge P, Smith SE. 1997. Cloning plant genes differentially expressed during colonization of roots of *Hordeum vulgare* by the vesicular-arbuscular mycorrhizal fungus *Glomus intraradices*. *New Phytologist* **135**, 291–301.

Ouffatole M, Arango M, Boutry M. 2000. Identification and expression of three new *Nicotiana plumbaginifolia* genes which encode isoforms of a plasma-membrane H^{+}-ATPase, and one which is induced by mechanical stress. *Planta* **210**, 715–722.

Palmgren MG, Harper JF. 1999. Pumping with plant P-type ATPases. *Journal of Experimental Botany* **50**, 883–893.

Pearson JN, Jackobsen I. 1993. The relative contribution of hyphae and roots to phosphorus uptake by arbuscular mycorrhizal plants, measured by dual labelling with ^{32}P and [33]P. *New Phytologist* **124**, 489–494.

Phillips JM, Hayman DS. 1970. Improved procedure for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions British Mycology Society* **55**, 158–160.

Pozo MJ, Azcón-Aguilar C, Dumas E, Barea JM. 1998. Chitosanase and chitinase activities in tomato roots during interactions with arbuscular mycorrhizal fungi or *Phytophthora parasitica*. *Journal of Experimental Botany* **49**, 1729–1739.

Pozo MJ, Azcón-Aguilar C, Dumas-Gaudot E, Barea JM. 1999. β-1,3 glucanase activities in tomato roots inoculated with arbuscular mycorrhizal fungi and/or *Phytophthora parasitica* and their possible involvement in bioprotection. *Plant Science* **141**, 149–157.

Rosewarne GM, Barker SJ, Smith SE, Smith FA, Schachtman DP. 1999. A *Lycopersicon esculentum* phosphate transporter (*LePT1*) involved in phosphorus uptake from a vesicular–arbuscular mycorrhizal fungus. *New Phytologist* **144**, 507–516.

Schaller A, Oecking C. 1999. Modulation of plasma membrane H^{+}-ATPase activity differentially activates wound and pathogen defense responses in tomato plants. *The Plant Cell* **11**, 263–272.

Schlesser A, Ulaszewski S, Ghislain M, Goffeau A. 1988. A second transport ATPase gene in *Saccharomyces cerevisiae*. *Journal of Biological Chemistry* **263**, 19480–19487.

Smith SE, Read DJ. 1997. *Mycorrhizal symbiosis.* London: Academic Press.

Villalba JM, Lützelschwab M, Serrano R. 1991. Immunocytolocalization of plasma membrane H^{+}-ATPase in maize coleoptiles and enclosed leaves. *Planta* **185**, 485–461.

Wright DP, Read DJ, Scholes JD. 1998. Mycorrhizal sink strength influences whole plant carbon balance of *Trifolium repens* L. *Plant, Cell and Environment* **21**, 881–891.

Xing T, Higgins VJ, Blumwald E. 1996. Regulation of plant defense response to fungal pathogens: two types of protein kinase in the reversible phosphorylation of the host plasma membrane H^{+}-ATPase. *The Plant Cell* **8**, 555–564.