Mycorrhiza (2011) 21:473–493
DOI 10.1007/s00572-010-0352-0

ORIGINAL PAPER

Arbuscular mycorrhizal symbiosis elicits proteome responses opposite of P-starvation in SO4 grapevine rootstock upon root colonisation with two *Glomus* species

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Received: 11 August 2010 / Accepted: 9 December 2010 / Published online: 6 January 2011

Abstract Although plant biotisation with arbuscular mycorrhizal fungi (AMF) is a promising strategy for improving plant health, a better knowledge regarding the molecular mechanisms involved is required. In this context, we sought to analyse the root proteome of grapevine rootstock Selection Oppenheim 4 (SO4) upon colonisation with two AMF. As expected, AMF colonisation stimulates plant biomass. At the proteome level, changes in protein amounts due to AMF colonisation resulted in 39 differentially accumulated two-dimensional electrophoresis spots in AMF roots relative to control. Out of them, 25 were co-identified in SO4 roots upon colonisation by *Glomus irregulare* and *Glomus mosseae* supporting the existence of conserved plant responses to AM symbiosis in a woody perennial species. Among the 18 proteins whose amount was reduced in AMF-colonised roots were proteins involved in glycolysis, protein synthesis and fate, defence and cell rescue, ethylene biosynthesis and purine and pyrimidine salvage degradation. The six co-identified proteins whose amount was increased had functions in energy production, signalling, protein synthesis and fate including proteases. Altogether these data confirmed that a part of the accommodation program of AMF previously characterized in annual plants is maintained within roots of the SO4 rootstock cuttings. Nonetheless, particular responses also occurred involving proteins of carbon metabolism, development and root architecture, defence and cell rescue, anthocyanin biosynthesis and P remobilization, previously reported as induced upon P-starvation. This suggests the occurrence of P reprioritization upon AMF colonization in a woody perennial plant species with agronomical interest.

Keywords *Vitis* sp. · *Glomus mosseae* · *G. irregulare* · Two-dimensional electrophoresis · MALDI TOF-MS

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Introduction

The benefits of arbuscular mycorrhizal (AM) symbiosis on plant fitness are widely known, including improved mineral nutrition in nutrient-poor soils and increased capability to

overcome biotic and abiotic stresses (Azcón-Aguilar and Barea 1996; Smith and Read 2008). Alleviation of damages caused by soil-borne pathogens, including those that are difficult to control by chemical and physical treatments, has been reported in various mycorrhizal plants (St-Arnaud and Elsen 2005; St-Arnaud and Vujanovic 2007; Pozo and Azcón-Aguilar 2007). Sustainable agriculture and ecosystem conservation have challenged scientists to consider the management of arbuscular mycorrhizal fungi (AMF) as biofertilisers and/or bioprotectors as an alternative to standard fertilizers and pesticides (Whipps 2001; Harrier and Watson 2004).

On a worldwide basis, grapes (*Vitis* species) are both the most widely cultivated and economically important fruit crop, encompassing approximately 8 million ha of arable land (Vivier and Pretorius 2002). Due to *Phylloxera* attacks, this perennial woody plant species is grafted onto resistant rootstocks. The Selection Oppenheim 4 (SO4) rootstock, a hybrid between *Vitis berlandieri* and *Vitis riparia*, is commonly used for its ability to adapt to different types of soil and environmental conditions (Reynier 2002). It is tolerant to wet soils and especially suited for fertile soils containing high organic matter. This rootstock produces a large amount of roots and is very vigorous, allowing high productivity, high fruit quality and regular production (Giovannini 1999). However, it is susceptible to pathogen attacks, and thus, the production of high-quality cuttings, resistant/tolerant to root pathogens, is of crucial importance.

AMF form a symbiosis spontaneously with the roots of grapevines and are present in most of the commercial vineyards evaluated (Menge et al. 1983; Schubert and Cravero 1985; Linderman and Davis 2001; Cheng and Baumgartner 2004). Several authors have reported beneficial effects of mycorrhizal inoculation of grapevines (Nikolaou et al. 2003; Aguin et al. 2004; Cheng and Baumgartner 2006; Karagiannidis et al. 2007; Camprubí et al. 2008) although it is also established that different mycorrhizal species may show distinct responses in growth and nutrient uptake (Karagiannidis et al. 1995). This property of AMF is now exploited for inoculating plants to be transferred to micronutrient-deficient soils (Bavaresco and Fogher 1992, 1996). The beneficial effect of AMF inoculation in grapevine tolerance to pathogens has been reported (Waschkies et al. 1994), but even if polyamines have been suggested as playing a role in signalling tolerance processes (Nogales et al. 2009), the cellular and molecular mechanisms involved are yet largely unknown.

Recently, extensive insights into the AM symbiotic program have been gained through the use of global approaches that revealed genes or subsets of genes/gene products which are essential to cell programs involved in the different phases of the plant–AMF interactions (Liu et al. 2003, 2007; Wulf et al. 2003; Brechenmacher et al. 2004; Güimil et al. 2005; Hohnjec et al. 2005; Balestrini and Lanfranco 2006 and references therein; Massoumou et al. 2007; Recorbet and Dumas-Gaudot 2008 and references therein; Fiorilli et al. 2009; Guether et al. 2009). Since the model plant *Arabidopsis* is recalcitrant to AMF colonisation, most knowledge on genes/proteins involved in arbuscular mycorrhizal interaction, as listed above, came up from studies performed on either models or agronomically relevant annual plant species. It was therefore highly pertinent to undertake similar investigations in a perennial woody plant species such as grapevine. In addition, the release of the complete genome of the model plant *Vitis vinifera* (Jaillon et al. 2007) and the subsequent implementation of protein database have open opportunities for identifying proteins with confidence by using mass finger printing (PMF) following matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF MS). To our knowledge, there are a limited number of proteomic studies on grapevines (Sarry et al. 2004; Carvalho et al. 2005; Castro et al. 2005; Vincent et al. 2006, 2007; Wang et al. 2009; Basha et al. 2010) with only one report that has paid attention to the root proteome (Marsoni et al. 2005) and yet nothing dealing with the root proteome responses to AMF colonisation. Therefore, the aim of the current work was to analyse the interaction between AMF associated to SO4 (*V. berlandieri* × *V. riparia*) rootstock cuttings under pot culture conditions. Since development and functionality of AMF may vary depending on the fungal strain, we compared the SO4 rootstock responses to two AMF, i.e. *Glomus irregulare* and *Glomus mosseae*, which are among the more frequently utilized AMF. Plant biomass and AMF colonisation were measured and subsequent proteomic profiling based on two-dimensional electrophoresis (2-DE) coupled to MALDI-TOF MS was carried out for uncovering the root proteome of SO4 rootstock cuttings upon AMF colonisation.

Material and methods

Biological material, growth conditions and experimental design

SO4 rootstock (*V. berlandieri* × *V. riparia*) 30-day old cuttings were transplanted into 400 ml pots containing a sterile mix (2:1 *v*/*v*) of terragreen (Agsorb, Oil Dry Corporation, IMC Imcore) and a neutral clay loam soil (pH 7.8, 28 ppm Olsen P; Epoisses soil). Mycorrhizal inoculation was performed by replacing 40 ml of Epoisses soil with a soil-based inoculum (spores, roots and hyphae) of *G. irregulare* (Stockinger et al. 2009) [formerly *Glomus intraradices* N. C. Schenck & G. S. Smith (DAOM 181602)] or *G. mosseae* (BEG 12). Non-inoculated and AMF-inoculated plants were watered daily (10 ml) with

demineralised water and twice a week with Long Ashton nutrient solution (Hewitt 1966) containing reduced phosphorous (0.13 mM Na_2HPO_4). Cuttings were grown under controlled conditions (16-h photoperiod, 23:18°C day/night, 60% relative humidity, 220 µE m^{-2} s^{-1} photon flux density) for 5 weeks. At harvest, pots were immersed in tap water to carefully remove the roots from the soil mix. Roots were then gently rinsed with running tap water and then with deionized water to eliminate any remaining soil particles. Roots were immediately frozen in liquid nitrogen and stored at −80°C until protein extraction. Plant shoots in each treatment were pooled, and P content was measured after nitroperchloric digestion (Johnson and Ulrich 1959). The biological experiment consisted of three treatments (non-mycorrhizal inoculation (Nm), inoculation with *G. irregulare* or *G. mosseae*).

Mycorrhizal colonisation

The level of mycorrhizal colonisation was estimated on five plant replicates for each treatment. At harvest, small parts of mycorrhizal plants were randomly collected from the whole root systems and stained with Shaeffer's dye blue after clearing with potassium hydroxide (Vierheilig et al. 1998). Mycorrhizal colonisation parameters were estimated under light microscopy as described by Trouvelot et al. (1986). Three parameters of mycorrhizal development were calculated with the MycoCalc program (http://www.dijon.inra.fr/mychintec/Mycocalc-prg/download.html): frequency of mycorrhiza in the root system (*F*%), intensity of the mycorrhizal colonisation in the root system (*M*%) and arbuscule abundance in mycorrhizal parts of root fragments (*A*%).

Protein extraction and two-dimensional electrophoresis

For protein analyses, four replicates of non-mycorrhizal and mycorrhizal roots were submitted to protein extraction and separation by 2-DE. Fresh material (1 g roots) was transferred into a chilled mortar and ground to a fine powder with liquid nitrogen. Phenolic protein extraction and solubilisation were carried out according to Bestel-Corre et al. (2002). Protein content was quantified by the method of Bradford as modified by Ramagli and Rodriguez (1985) using BSA as a standard. Samples were stored at −80°C until electrophoresis. All reagents for gel electrophoresis, including 2-DE molecular markers, were from Bio-Rad (Marnes-La-Coquette, France). Isoelectric focusing (IEF) was performed on immobilized 18 cm non-linear pH 3–10 pH gradient (IPG) strips (Amersham Biosciences (AB), Uppsala, Sweden), in a Multiphor II unit (AB). IEF strips were rehydrated overnight with 350 µl of buffer (8 M urea; 4% *w/v*, 3-[(3-cholamidopropyl) dimethylammonio]-1-propane-sulphonate; 20 mM dithiothreitol; 2% *v/v*, IPG buffer, pH 3–10 and bromophenol blue). Proteins (500 µg) were loaded onto the strips and electrofocused at 20°C according to Dumas-Gaudot et al. (2004). After isoelectric focusing, IPG strips were either stored at −80°C or immediately equilibrated (Görg et al. 1987). Strips were then transferred onto 12%, pH 8.8, sodium dodecyl sulphate (SDS)–polyacrylamide gels (Hoefer DALT, AB). Separations were carried out overnight at 10°C and 90 V. Gels were stained with colloidal Coomassie blue G250 (Bio-Rad, Hercules, CA, USA; Mathesius et al. 2001).

Image analysis

Images were acquired using the Odyssey Infrared Imaging System (LI-COR Biosciences, GmbH, Germany) at 700 nm with a resolution of 169 µm. Image analyses were carried out with the Progenesis SameSpots version 2.0 software (Nonlinear Dynamics) according to manufacturer's instructions. Spot volumes were normalized to the total spot volume with a multiplication factor of 100. Means of spot volumes between treated versus control samples were compared using the ANOVA statistical package included in SameSpots. Only differences with $p<0.01$ for random occurrence were considered.

In-gel protein digestion, MALDI-TOF mass spectrometry and peptide mass fingerprinting

Following extensive gel washing with water, spots of interest were manually excised into small pieces with tips, dried and stored at room temperature before mass spectrometry analyses. In-gel digestion was performed with the Progest system (Genomic Solution). Briefly, gel pieces were washed twice by successive baths of 10% acetic acid, 40% ethanol and acetonitrile (ACN). They were then washed twice with successive baths of 25 mM NH_4CO_3 and ACN. According to spot size, a solution containing 25 to 75 ng of porcine-modified trypsin (Promega, France) dissolved in 20% methanol and 20 mM NH_4CO_3 was added, and digestion was performed overnight at 37°C. Peptides were extracted successively with 2% trifluoroacetic acid (TFA) and 50% ACN and then with ACN. Peptide extracts were dried in a vacuum centrifuge and suspended in 20 µl of 0.05% TFA, 0.05% HCOOH and 2% ACN.

Peptide masses from digested proteins were obtained using a MALDI-TOF MS equipped with a N_2 laser (337 nm, 20 Hz, 3 ns impulsion; Applied Biosystems, Voyager DE super STR). Samples were irradiated in a matrix (α-cyano-4-hydroxycinnamic acid 4 mg/ml), and spectra were acquired in reflectron mode within a 700- to 3,500-Da mass range and a 130-ns delay extraction time. Internal calibration was performed using trypsin peptide masses within a 500- to 5,000-Da mass range. PMF search

was performed on *V. vinifera* database locally downloaded from Uniprot (54411entries) using the protein prospector software (http://prospector.ucsf.edu/ucsfhtml4.0/msfit.htm) and with Aldente on TrEMBL database. Parameters for peptide matching were a minimum of three matches with a tolerance of 30 ppm, a maximum of one missed cleavage and trypsin digestion, cys carboxyamidomethylation and met oxidation were set to enzymatic cleavage, static and possible modifications, respectively. When PMF search does not allow functional protein identification, homology searches were performed using MS BLAST at EMRL (http://dove.embl-heidelberg.de/Blast2/msblast.html).

Statistical analyses

For shoot and root biomass, mycorrhizal colonisation and normalized 2-DE spot volumes, means of each treatment were compared using one-way analysis of variance (ANOVA, $p<0.05$) using SAS software (Statistical Analysis System 9.1; SAS Institute Inc., Cary, NC, USA). This ANOVA was performed with non-transformed data after ensuring conformity of these data with ANOVA assumptions. Percentage values of root colonisation were transformed using arcsin function. Tukey's test at $p<0.05$ was used as a post hoc test when ANOVA showed significance. Clustering was performed using GENESIS software (version 1. 7. 2; Graz University of Technology; Institute for Genomics and Bioinformatics, http://genome.tugraz.at). For that purpose, quantitative variations in protein abundance between treatments were represented by Log2 ratios of normalized volume obtained by SameSpots image analysis. Euclidian distances were then computed for all spots to build the similarity matrix, and clustering was performed.

Results and discussion

Plant biomass and mycorrhizal parameters

Graphical representation showed that shoot biomass was significantly higher ($p<0.05$) in AM-colonised (*G. irregulare* or *G. mosseae*) plants than in those non-colonised (Nm; Fig. 1a), while root biomass was only significantly higher relative to control in *G. mosseae*-colonised plants (Fig. 1b). P content was 2.6 mg g^{-1} in mycorrhizal plants and 1.6 mg g^{-1} in non-mycorrhizal plants, which correspond to deficient and sufficient levels, respectively (Robinson et al. 1997).

Although frequency of infection was similar for the two AMF (F=96.8±3% and 91.6±6% for *G. irregulare* and *G. mosseae*, respectively), both intensity of root cortex AM colonisation (M%) and abundance of arbuscules (A%) were significantly higher ($p<0.05$) in *G. irregulare*- than in *G. mosseae*-colonised plants (Fig. 2 a, b).

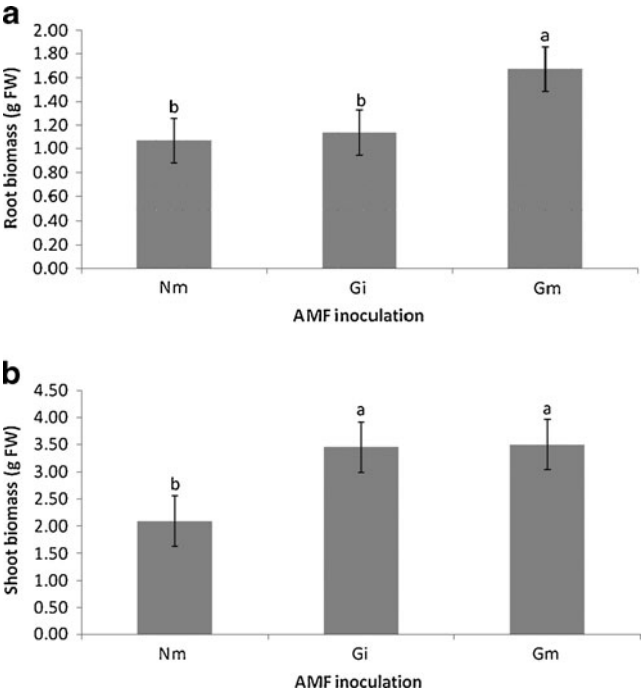

Fig. 1 Effect of mycorrhizal inoculation treatment on **a** shoot biomass and **b** root biomass (g of fresh weight), 5 weeks after inoculation with *G. irregulare* or *G. mosseae*. Means and standard errors are shown for five replicates. Means followed by *the same letter* are not significantly different according to Tukey's test

The shoot biomass increase in AMF-colonised plants is consistent with previous study carried out with grapevine SO4 rootstock inoculated by two other AMF, *Gigaspora margarita* and *Scutellospora heterogama* (Souza et al. 2004). In the present work, rootstock cuttings were analysed 5 weeks after inoculation, corresponding to a functional AM symbiotic stage, as judged by the percentages of arbuscules observed (A=76±4% for *G. irregulare*- and A=46±8% for *G. mosseae*-inoculated plants, respectively).

Proteome changes in SO4 grapevine rootstock

Root tissues of grapevines are well-known for their high content in interfering compounds including polysaccharides, pigments and phenolics, which, together with their hardness, make their proteins challenging to extract and resolve by 2-DE gels. The phenol-based method we used showed a high efficiency for protein extraction and resolution as previously reported for other recalcitrant plant material (Saravanan and Rose 2004) as well as for the *V. vinifera* cv. Cabernet Sauvignon grapevine (Marsoni et al. 2005). Indeed, after phenol extraction, 2-DE gel separation of 500 μg of root proteins of the SO4 grapevine rootstock and Coomassie blue staining, more than 600 well-resolved spots were consistently detected in the whole set of gels. Protein profiles corresponding to all treatments, analysed using SameSpots software, resulted in spots that were

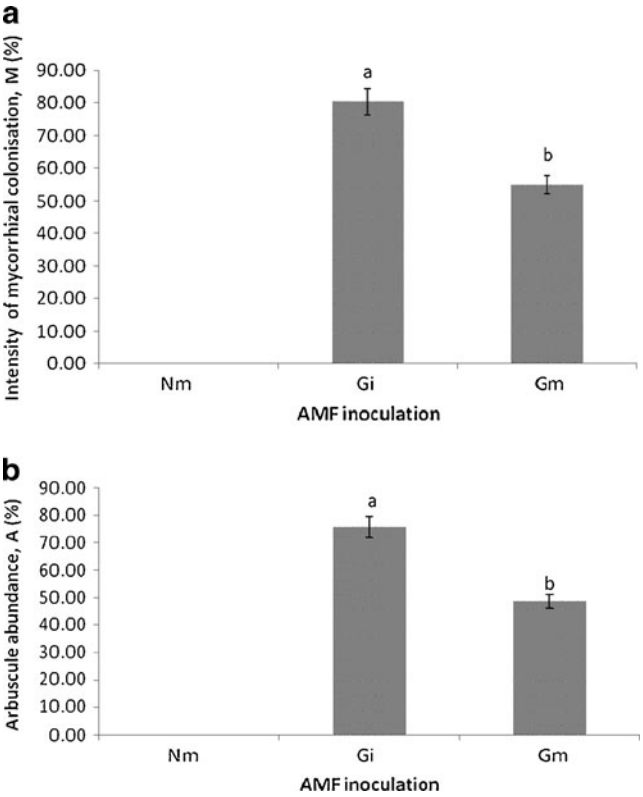

Fig. 2 Comparison of **a** intensity of the mycorrhizal colonisation in the root system (*M*%) and **b** arbuscule abundance in mycorrhizal parts of root fragments (*A*%) as affected by AMF inoculation (non-mycorrhizal inoculation, *G. irregulare* and *G. mosseae* inoculation). Means are shown for five replicates. Means followed by *the same letter* are not significantly different according to Tukey's test

automatically numbered on a virtual reference 2-DE map (data not shown). We detected a total of 52 protein spots whose accumulation was significantly different for at least one treatment (Nm, *G. irregulare*, *G. mosseae*). They were all sampled and analysed by MALDI TOF/MS for peptide PMF identification. Out of these, 39 spots, arbitrarily named 1 to 39 in Table 1, gave positive hits following PMF search when queries were performed on *V. vinifera* or TrEMBL database. Most of the hits matched to *V. vinifera* uncharacterized proteins or "chromosome" annotations without known functions. However, following MS BLAST search, all the proteins had known or predicted roles and were therefore functionally classified using the FunCat annotation scheme (Ruepp et al. 2004; Table 1). Figure 3 shows a representative Coomassie blue-stained 2-DE profile of SO4 grapevine rootstock root proteins, on which are featured the 39 identified plant proteins.

The groups of proteins responding to *G. irregulare* or *G. mosseae* inoculation relative to control plants were further compared by a tree clustering method using Genesis software (Fig. 4). When roots were colonised with *G. mosseae* or *G. irregulare*, a similar trend can be noticed in spot volume variations with six and 18 spots significantly up- and down-accumulated in response to root colonisation regardless of the AMF (Fig. 4 lanes Gi/Nm and Gm/Nm). The 39 proteins which were identified belonged to ten functional categories, i.e. carbon metabolism (spots 3, 15, 16, 19, 22, 24, 25, 28, 29, 34, 35, 39), protein synthesis and fate (spots 5, 6, 11, 14, 23, 30, 32, 36, 37, 38), energy (spots 13, 21, 26), defence and cell rescue (spots 7, 8, 27), signalling (spots 17, 18), transport (spot 20), cell cycle (spot 33), ethylene biosynthesis (spot 10), lignin and anthocyanin biosynthesis (spots 2, 4) and, finally, miscellaneous metabolisms (spots 1, 9, 12, 31; Table 1 and Fig. 4).

Carbon metabolism

The abundances of several proteins that participate in carbon metabolism showed obvious differences in response to AMF colonisation of SO4 roots. These proteins were involved in TCA cycle, pentose phosphate pathway and glycolysis. Thus, spot 24, a glucose 6-phosphate dehydrogenase, an enzyme of the pentose phosphate pathway, and spot 39, a precursor of a plastidic isoform of NAD-dependent malate dehydrogenase, were the only ones significantly up-accumulated in *G. irregulare*-colonised plants. Among the down-accumulated spots, several enzymes of the glycolysis pathways were misrepresented, including two fructose biphosphoaldolases (spots 22, 35), two isoforms of a phosphoglyceromutase (spots 3, 25) and a putative pyruvate dehydrogenase E1 alpha subunit (spot 28). Pyruvate dehydrogenase (E1) is the first component enzyme of pyruvate dehydrogenase complex, which contributes to transform pyruvate into acetyl-CoA by pyruvate decarboxylation, linking the glycolysis metabolic pathway to the TCA cycle. In agreement with the apparent down-regulation of the glycolysis pathway we noticed, the down-accumulation of a mature mitochondrial malate dehydrogenase (spot 29) involved in the TCA cycle was also recorded. An alcohol dehydrogenase-like protein (spot 34) was down-accumulated in response to both AMF. Alcohol dehydrogenase is one of the anaerobic proteins catalysing the reduction of pyruvate to ethanol, resulting in continuous NAD^+ renewal. Roots are frequently submitted to anaerobic growth conditions (Chung and Ferl 1999), and the down-accumulation of this protein that occurred in SO4 roots upon their AMF colonisation could be interpreted as having a protective effect of the AM symbiosis. A phosphoserine amino transferase protein (spot 15) was recorded as down-accumulated in SO4 roots either colonised with *G. irregulare* or *G. mosseae*. Phosphoserine amino transferase acts in the production of phosphoserine to yield L-serine, located at a crossroad of protein synthesis, but might also operate as a potential donor of carbon for glycine and NH_2 in purin biosynthesis. Spot 16 identified a formate dehydrogenase (FDH), an enzyme located in mitochondria that catalyses the oxidation of

Table 1 Root proteins of the SO4 grapevine rootstock cuttings whose accumulation was modified in response to *Glomus* fungi colonization relative to non-treated plants identified following peptide mass fingerprinting analyses

Spot[a]	Coverage (%)	Accession[b]	pI[c] exp/theo	Mr[c] exp/theo	Peptides	Identification	Function[d]
1	21	Q43087	5.6/5.5	36.8/36	FQLDDVVEAQQFDR GYLMATLFYEPSTR LSFESAMR GETLEDTIR TVEGYSDLIVLR HAVIMHPLPR	Aspartate carbamoyltransferase 2	Metabolism (pyrimidine biosynthesis)
2	47	A7P9Q5/P42495	5.6/5.5	45.1/40.0	MDYVNTALER FVVDVAGSKLDQ GGILGLGGVGHMGVK AMGHHVTVISSSDR DPSGVLSPYTYTLR EEAMDHLGADDYLVSSDSTR IITGSFIGSMKETEEMLEFCK	Probable cinnamyl alcohol dehydrogenase	Metabolism (secondary)
3	36	A7Q9U1/Q42908	5.8/5.4	70.0/60.1	KFHQVR VHILTDGR IYEGEGFK IDQLQLLLK GVDAQIASGGGR YENDWEVVKR EKGVDAQIASGGGR FGHVTFFWNGNR SLEYEDFDKFDR LPNHYLVSPPEIER DVLDGSSVGFVETLENDLAK IDQLQLLLKGASENGAKRIR IQILTSHTLQPVPIAIGGPGLAAGVR	Putative phosphoglycerate mutase	Energy production (glycolysis)
4	24	A8I4W9/Q8LP73	5.9/5.6	46.4/40.1	KINYYPK ILSVLSLGLGLEEGR IILKPLPETVSETEPPLFPPR EYIRPQEELTSIGNVFEEEKK	Anthocyanidin dioxygenase	Metabolism (flavonoids)
5	42	A5B729/Q6YW66	6.0/6.1	35.7/36.6	VFDGRK YVGPAPR NSAVLLR THVYKR RPSLPPR YTFTALSK RPSLPPRR GGETPFVPSFR GWEDTFGTSDR GDVLVLDNLALLHGR	Putative signal peptidase	Protein synthesis and fate

				WEKGDVLVLDNLALLHGR			
				NAEDFNQIVEAFGWDDIR			
6	38	A5ADJ5/Q9LKA4	7.0/7.2	41.9/45.4	GYGFVTFK	Putative RNA-binding protein	Signalling
					GFALFVYK		
					HVDGALLALK		
					HLDVLDAIR		
					GLGWDTTTEGLR		
					TAEAAQAALVDPTK		
					QLFSAYGELEEAVVILDK		
					LLAHFSSYGEIEEGPLGFDK		
					IIEPFGHDQLLEILQNAAVR		
7	54	A7QNI7/Q94IB7	5.6/7.7	66.0/53.3	GYLFPLDK	Monodehydroascorbate reductase	Defence and cell rescue
					VEHVDHAR		
					VFEYEGSPR		
					VEHVDHARR		
					IATFWIDSGK		
					IGGNLPGVHYIR		
					EAFAPYERPALTK		
					EFVTVGGGNAAGYAAR		
					RYEEFYQENGVK		
					THLYDYLPYFYSR		
					VGINTTVGGIQVDGQFR		
					TSVPGIFAIGDVAAFPLK		
					LDTTIIFPEDHLLQR		
					GVLLESGSPEEFQLLPK		
					LENGSTIEADTIIIGIGAKPAVSPFER		
8	33	Q8S568	7.7/6.7	58.1/57.0	FSTVIHER	Catalase	Defence and cell rescue
					FAYSDTQR		
					WVETLSDPR		
					SHIQEYWR		
					GSPETIRDPR		
					APGVQTPVIVR		
					FPDVIHAFKPNPK		
					GPVLLEDYHLVEK		
					LGPNYLQLPVNAPK		
					TWPEDILPLQPVGR		
					VGGTNHSHATQDLYDSIK		
					EGNFDMVGNNFPVFFIR		
9	38	A7QJV8/Q84JU8	5.6/5.9	65.1/45	LPNYVVIEQR	Putative phosphoesterase	Metabolism
					VPAILISPWIER		
					LGVRVPAILISPWIER		
					YIDNFHQFDLDFKR		
					LLPANDDHPSHDVSQGQK		
					GTVLHGPSGPYPTSEFEHSSIPATVK		

Table 1 (continued)

Spot[a]	Coverage (%)	Accession[b]	pI[c] exp/theo	Mr[c] exp/theo	Peptides	Identification	Function[d]
10	26	A7NVI3/Q84L58	5.4/5.4	39.5/35.2	LLNELVEK SIASFYNPPLK ATIAPAPQLVEK AFNDGEGDNAFFGTK AHTDAGGVILLFQDDK	1-Aminocyclopropane-1-carboxylic acid oxidase	Metabolism (ethylene biosynthesis)
11	46	A5BFM5/Q9SF16	6.3/6.0	61.3/61.2	VTEEDLQR TASYLAIEK TTLGPRGMDK SEKENAEIR LLDIVHPAAK SQLFINSYAK IKELAVSIEGK DSFLVNGVAFK GGADQFIEEAER TFSYAGFEQQPK LAIGDLATQYFADR EAKPFIEDGVHPQNLIR SQDSEVGDGTTTVVLLAGEFLK	Putative T-complex protein 1	Protein synthesis and fate
12	26	A5AHG5/Q8H6B5	5.3/5.6	44.3/43.8	ATVPVWAK AVHPIALGK DWTGDGFVK AAWEELIDR AFDEGWGAVIAK ILIASIMEEYDK QGLQSDKDWTGDGFVK GASLEYFTT HTDLVQR	Putative dehydrogenase	Metabolism (UMP and pyrimidine biosynthesis)
13	38	A5AY42/Q6L460	5.4/5.3	20.2/19.8	KFDDEIR LIVTEEAR FDALLVELK LIVTEEARK AFDEVNSTLQTK YVDTVTPQYKPK RAFDEVNSTLQTK FSQEPEPIDWEYYR	Putative ATP synthase D chain	Energy production
14	26	A7PDA4/Q40436	4.4/4.7	27.5/27.0	YGEVIEAR VRVNYATDR DDDDEPDDYANR LFIGGLSYSTDDTSLR GFGFVTFTSSEEASSAIQALDGQDLHGR	RNA-binding glycine-rich protein	Signalling
15	23	A7R128/Q9SHP0	7.1/6.6	40.5/36.8	FGLIYAGAQK SLMNVPFTLEK NVGPSGVTIVIVR	Phosphoserine aminotransferase	Metabolism (serine biosynthesis)

					ASIYNAMPLAGVEK		
					IPSFDGLEQNPDAK		
					AQILYDAIDESNGFYR		
					YLHICANETIHGVEFK		
					KAQILYDAIDESNGFYR		
16	43	A7PMA5/Q07511	6.7/6.5	42.0/40.5	DHPWR ILILVR	Formate dehydrogenase	Metabolism (carbohydrate)
					GVLIVNNAR		
					KGVLIVNNAR		
					MPGVLIVVSHWK		
					NLQLLLTAGIGSDHIDLK		
					AKNLQLLLTAGIGSDHIDLK HIPDLHVL ISTPFHPAYVTAER		
					MDPELENQIGAKFEEDVDVMLPK		
17	64	A7NXX2/Q676Z5	7.0/8.7	17.9/22.6	HIPVIDDK SIAGIITER	CBS1 (fragment)	Signalling
					LITVSPNTK		
					LNAYIQGGY		
					AVVTEHREELDR		
					MEEHGFESTTIADVLK		
18	38	A5AL04/Q94KS2	7.7/7.2	36.1/36.0	AIWGPLNR ILQEEIGGVK	TGF-beta receptor-interacting protein 1	Signalling
					GHERPLTFLK		
					TIISAGEDAIVR		
					SFSSGGEDGYVR		
					LHHFDPDYFNVK		
					FYDKILQEEIGGVK		
					DHTPTVWFADNGER		
					LHHFDPDYFNVKI		
					GHFGPINALAFNPDGK		
					IARDPSQQTAESALVIK		
					DPSQQTAESALVIKGPQGR		
19	19	A5JPK5/Q93VR3	6.1/6.0	45.2/43.0	VVGTQAPVQLGSLR FHNIYGPFGTWK	GDP-mannose-3′,5′-epimerase	Metabolism (carbohydrate)
					NLPIHHIPGPEGVR		
					ISITGAGGFIASHIAR		
					SFTFIDECVEGVLR		
20	17	A7Q8L2/O49600	5.5/5.3	62.2/58.1	FVEFLVR VVIDHGAVPIFVK	Importin alpha-like protein	Transport
					SPPIEEVIQSGVVPR		
					LVELLLHPSPSVLIPALR		
					EGLQAQQFPAAAHASTVEK		
					GKPQPPFDQVKPALPALER		
21	55	A7PH19/O82722	6.2/6.6	27.0/59.4	TDHFLPIHR VVDLLAPYQRR	ATP synthase subunit beta	Energy production
					IINVIGEPIDER		

Table 1 (continued)

Spot[a]	Coverage (%)	Accession[b]	pI[c] exp/theo	Mr[c] exp/theo	Peptides	Identification	Function[d]
					RPISRSVLSNPR		
					AHGGFSVFAGVGER		
					VGLTGLTVAEHFR		
					VLNTGSPITVPVGR		
					FTQANSEVSALLGR		
					DAEGQDVLLFIDNIFR		
					QISELGIYPAVDPLDSTSR		
					EAPSFVDQATEQQILVTGIK		
					IPSAVGYQPTLATDLGGLQER		
					FDEGLPPILTALEVLDNSIR		
22	34	A5B118/Q9LF98	7.4/8.0	38.7/38.6	EGGVLPGIK GILAADESTGTIGK VSPEVVAEYTVR	Fructose-bisphosphate aldolase	Energy production (glycolysis)
					LASINVENVEGNR		
					RLASINVENVEGNR LASINVENVEGNRR		
					YHDELIANAAYIGTPGK		
					IGPTEPSELAIHENAYGLAR		
					GTVVLTGTDGETTTQGLDGLGER		
23	51	A5ALB2/Q9XG77	6.0/5.9	26.9/27.3	GSGGGYDR TDSPAFR	Proteasome subunit alpha type-6	Protein synthesis and fate
					TLVQQAR		
					NEAAEFR		
					ASGITSIGVR		
					LFQVEYAFK		
					HITIFSPEGR		
					EQEAINFLEK		
					YGYEMPVDVLAR		
					FLGLLATGMTADAR		
					LLDQTSVTHLFPITK		
					VLSTEEIDEHLTAISERD		
24	29	A7QUV1/Q43793	6.8/6.0	64.6/59.9	NFGTDLDR TLTCRIDK	Glucose-6-phosphate 1-dehydrogenase	Energy production (pentose phosphate pathway)
					HVPGNLYNR		
					LLLDAIEGER		
					SNLNLHYAAR		
					ELVENLSVLR		
					GGYFDNYGIIR		
					QYLAEDQIFR		
					IIPEYYPHGSR		
					FSNLIFEPLWSR		
					NVQFIFSEDFGTEGR		
					DSLTPTFAAAALFIDNAR		

25	28	A7Q9U1/Q42908	5.7/5.4	70.5/60.1	AVEIAEK TSGEYLVHNGVR YENDWEVVKR FGHVTFFWNGNR SLEYEDFDKFDR LPNHYLVSPPEIER DVLDGSSVGFVETLENDLAK IQILTSHTLQPVPIAI GGPGLAAGVR	Phosphoglyceromutase	Energy production (glycolysis)
26	41	A5AYU8/O82722	5.3/5.8	58.8/59.9	IGLFGGAGVGK TREGNDLYR TDHFLPIHR VVDLLAPYQR IINVIGEPIDER RPISRSVLSNPR AHGGFSVFAGVGER VGLTGLTVAEHFR VLNTGSPITVPVGR FTQANSEVSALLGR LVLEVAQHLGMVR DAEGQDVLLFIDNIFR QISELGIYPAVDPLDSTSR EAPSFVDQATEQQILVTGIK IPSAVGYQPTLATDLGGLQER FDEGLPPILTALEVLDNSIR	ATP synthase subunit beta	Energy production
27	33	A7PXI4/Q9ZNZ5	6.4/6.2	43.5/35.0	GFDFIER DGTPNLTLR LGFYGESCPK KHIPNAPSLAAALIR DSIVVTGGPFWNVPTGR SVVEAECPGIVSCADILALVAR GLDLNDLVLLSGAHTIGVSHCSSFSNR	Peroxidase precursor	Defence and cell rescue
28	19	P52902	6.4/6.7	40.6/39.4	EVLGFFR RGDYVPGLK TRDEISGVR DHCIYLGR RDCIITAYR GFGIEVAGADRK NGPIILEMDTYR LILSHELSTEAELK YHGHSMSDPGSTYR	Putative pyruvate dehydrogenase E1 alpha subunit	Energy production (glycolysis)
29	39	A7Q1T9/Q5ZFR7	6.5/6.2	37.6/35.5	ALGQVSER TPAGEKPVR LNVQVSDVK EFAPSIPEK LDLTAQELSEEK	Malate dehydrogenase	Energy production (TCA cycle)

Table 1 (continued)

Spot[a]	Coverage (%)	Accession[b]	p*I*[c] exp/theo	Mr[c] exp/theo	Peptides	Identification	Function[d]
					VLVVANPANTNALILK		
30	42	A7QGQ2/Q7XZJ4	5.5/5.3	46.2/41.6	RGILTLK AGFAGDDAPR IKVVAPPER GEYDESGPAIVHR IWHHTFYNELR LDLAGRDLTDALMK SYELPDGQVITIGAER LAYVALDYEQELETAK VAPEEHPVLLTEAPLNPK	Putative actin	Cytoskeleton
31	26	A7PI27/Q9LZG0	5.4/5.3	44.3/38.0	LAGVNVHYR VKSFPVILLPK RPENWALVEK AGCYASHVIIQR VHGWETDNVEEIAIK YNVEYIAGGATQNSIR ALPYMDYVFGNETEAR	Adenosine kinase (adenosine 5′-phosphotransferase 2)	Metabolism (purine salvage)
32	13	A7PIK8/P93204	4.8/5.0	91.5/74.9	DFPSPR GPNPITR VEPGVLSFK AIVLGPPELSIR SFHPTWSPSAIK NELAPFVVSFSSR DSEGHGSHTASTAAGNLVGGASLLGIGTGTAR	Subtilisin-like protease	Protein synthesis and fate
33	12	A7QBR7/Q9SS94	5.1/6.2	72.6/72.8	EGFSTIPNVR EIALGVPDESAR ISATEVVSGVSGASEENIR	Putative cell division control protein	Cell cycle
34	32	A7PKL2/Q8LDF7	7.1/7.1	44.0/42.2	AYQDLDQGK LAETGIFNLK KCEFEEVGK VFGASHTVNALK AFGASDIIAVDVNDEK GQDDLCEDFFAYNR VFGASHTVNALKEDVADK AVMIGLTKSGAVGEIDINR	Alcohol dehydrogenase-like protein	Metabolism (carbohydrate)
35	21	A5B118/Q9SJQ9	7.6/8.0	39.9/38.6	YYAAGAR VSPEVVAEYTVR LASINVENVEGNR KVSPEVVAEYTVR RLASINVENVEGNR LASINVENVEGNRR IGPTEPSELAIHENAYGLAR	Fructose-bisphosphate aldolase	Energy production (glycolysis)

					GTVVLTGTDGETTTQGLDGLGER		
36	24	A7QDW0/O81340	4.6/4.5	44.3/47.0	NGDYSPTR VSMEEERAR VLVTPTSDLGK IIVFAGSPVKYDK DLLASMQNESESQQK EGEQQSSSQDATMTEHANVAASDADK	26S proteasome regulatory subunit S5A	Protein synthesis and fate
37	46	A7NXQ8/Q8H6U4	5.5/8.0	66.9/33.0	APGFGENRK GVEELADAVR SVAAGMNAMDLR NVVLEQSFGAPK VGKEGVITISDGK GYISPYFITNQK GISMAVDAVVTNLK CELENPLVLIHEK TLYNELEVVEGMKLDR MISTSEEIAQVGTISANGER QVANATNDVAGDGTTCATVLTR	Chaperonin 60	Protein synthesis and fate
38	81	A7PJG9/Q8W0I1	6.4/5.5	14.5/15.7	WSPALQIR SNEAEAVETAK DKWSPALQIR LLSEPAPGISASPSEENMR TVLLSIQALLSAPN PDDPLSENIAK	Ubiquitin carrier protein	Protein synthesis and fate
39	23	A7PMW6/Q9XQP4	5.9/5.3	36.8/31.7	LFGVTTLDVVR GVDVVVIPAGVPR DDLFNINANIVK TKPSVSFTDEEVEELTVR	Malate dehydrogenase	Energy production (TCA)

[a] Spots numbered according to Fig. 3

[b] Obtained accessions when database searches were performed against *V. vinifera* database imported from Uniprot or the Nr protein database limited to Viridiplantae/accession after MS BLAST search

[c] Experimental/theoritical p*I* and Mr. Experimental p*I* and Mr as measured by SameSpots using Precision Plus™ Standards (Bio-Rad) and theoretical p*I* and Mr as determined using the Compute pI/Mr tool from ExPASy

[d] Identified proteins were functionally classified according to Ruepp et al. (2004)

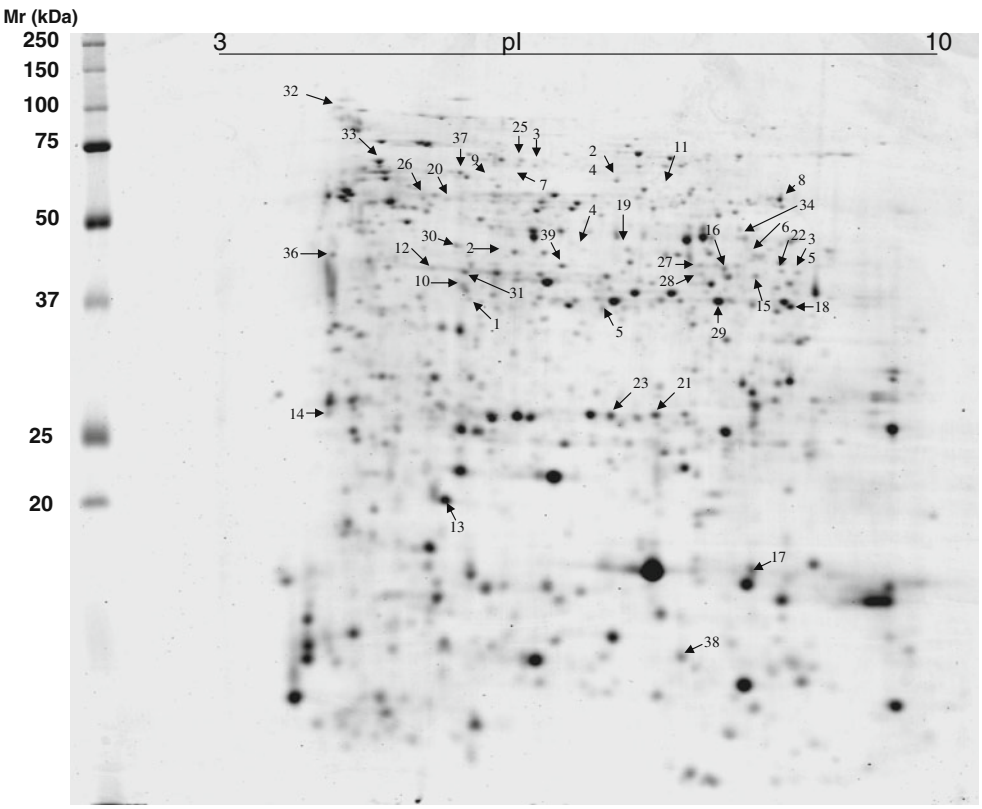

Fig. 3 Representative image of a Coomassie blue-stained 2-DE gel showing the 39 spots differentially accumulated in response to AMF inoculation in 5-week-old SO4 rootstock roots that where identified following PMF search. Five hundred micrograms of total proteins were separated on 18-cm non-linear pH 3–10 IPG strips for the first dimension and on 12% SDS vertical gel for the second dimension. Molecular markers are indicated on the *left*

formate to CO_2 together with reduction of NAD to NADH. FDH plays a crucial role in the biosynthesis of compounds involved in energetic metabolism and in stress-induced signal transduction pathways. Synthesis of FDH, as well as over-expression of the corresponding transcripts, dramatically increases under various stresses such as low temperature, drought and hypoxia (Hourton-Cabassa et al. 1998) and even in some root symbioses (Krüger et al. 2004; Andreadeli et al. 2009), a result that was interpreted as linked to the generation of reducing equivalents, contributing to support cellular metabolism under stress conditions and relative hypoxia in the respective tissues. Thus, the down-accumulation of FDH reported here in *G. irregulare*-colonised SO4 roots could again illustrate the bioprotective effect of the AM symbiosis by decreasing the accumulation of a stress-related protein. Concomitantly, there was a down-accumulation of spot 19 that corresponded to a GDP-3′5′-epimerase-like (spot 19) which, by converting GDP-*d*-mannose to GDP-*l*-galactose, is considered to be a central enzyme of the major ascorbate biosynthesis pathway in higher plants. The antioxidant role played by L-ascorbic acid makes this small molecule a crucial component of the plant response to different stress agents (Valpuesta and Botella 2004).

Protein synthesis and fate

Several proteins playing roles in protein synthesis and fate were differentially accumulated in response to AMF colonisation. Among the up-accumulated spots were two proteins that corresponded to a subtilisin-like protease (spot 32) and a putative signal peptidase (spot 5). Signal peptidases are proteases that cleave away the amino-terminal signal peptide from a translocated pre-protein. Their role in vivo is to release exported proteins from the membrane so they reach their correct cellular or extra-cellular locations (Palma et al. 2002). Since the first report on serine protease isoform activities related to the AM symbiosis (Slezack et al. 1999), activation of serine protease genes upon AM colonisation has consistently been reported (Liu et al. 2003; Güimil et al. 2005). The expression patterns and function of two AM-induced subtilase genes have been recently investigated in *Lotus japonicus*, demonstrating the involvement of the gene products during the development of arbuscules (Takeda et al. 2009). The up-accumulation of two proteases reported in this study is therefore in good agreement with the above-cited works. An ubiquitin carrier protein (spot 38) was significantly co-accumulated with either AMF isolate. Ubiquitin carrier proteins (also called ubiquitin-conjugating enzymes, E2) belong to the ubiquitin/proteasome system in which they catalyse the transfer of the activated ubiquitin moiety to a member of the ubiquitin ligase family (Zeng et al. 2006). An ubiquitin carrier protein was recently reported as up-accumulated in response to *G. irregulare* colonisation of *Medicago truncatula* roots (Aloui et al. 2009). In the present work, two other proteins involved in the proteasome protein

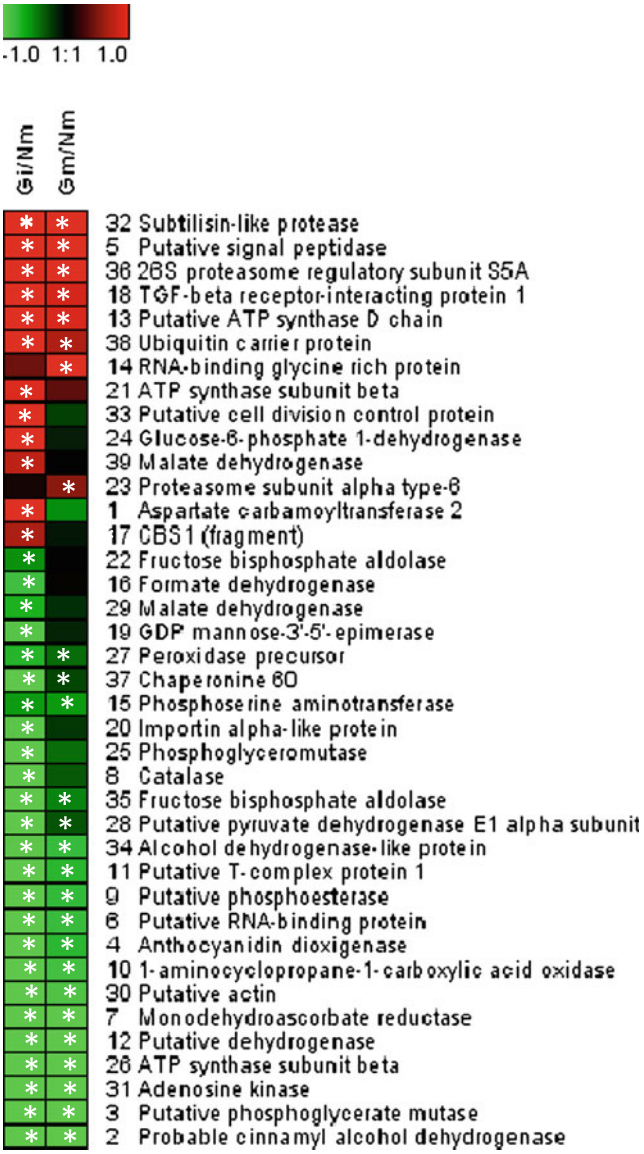

Fig. 4 Clustered abundance pattern using GENESIS software of the 39 proteins identified, as represented by the Log2 transformation of the mean (n=4) of each spot volume ratio relative to control plants (*Nm*). *Each row of coloured boxes* is representative of a single spot, and each mean ratio per treatment is represented using a single column. The *red colour* (+1) indicates the highest up-accumulation and the *green colour* (−1) indicates the highest down-accumulation. *Dark boxes* (0) indicate no changes in protein abundance compared to control. After Tukey's test, significant differences in protein abundance of each treatment (*G. irregulare*, *G. mosseae*) relative to control are indicated by *asterisks*. Protein spots are numbered according to Table 1 and Fig. 3

complex (spot 36, a 26S proteasome subunit, and spot 23, a proteasome subunit alpha type-6) were recorded as up-accumulated, although for the latter with significant volume variation only in response to *G. mosseae* colonisation, reflecting a profound protein turnover occurring upon AM colonisation process. The only protein significantly over accumulated in *G. mosseae*-inoculated roots was a glycine-rich, RNA-binding protein (spot 14) while a putative RNA-binding protein (spot 6) was recorded as significantly down-accumulated upon colonization with both AMF. RNA-binding proteins (RBPs) play key roles in post-transcriptional control of RNAs, which, along with transcriptional regulation, is a major way to regulate patterns of gene expression during development. Transcript over-expression of glycine-rich RBPs have been reported to occur in AM symbiosis of *Ligustrum japonicum* and *M. truncatula* roots in response to *G. mosseae* or *G. margarita* colonisation (Grunwald et al. 2004; Hohnjec et al. 2005; Deguchi et al. 2007). Two proteins belonging to the chaperonin family (spot 11, a putative T-complex protein 1, and spot 37, a chaperonin 60) displayed significant reduced amounts upon SO4 root colonisation with both AMF. Chaperonin 60, usually located in mitochondria and plastids, acts on the folding and assembly of imported proteins, while the cytosol TCP-1 complex has a role in the folding of actin and tubulin. Interestingly, we also observed the down-accumulation of actin (spot 30). In AM symbiosis, both tubulin organised in microtubules and actin filaments, major components of the plant cytoskeletal system, become closely associated with intracellular hyphae (Genre and Bonfante 1998). Based on a time course analysis in relation to mycorrhizal colonisation of tomato, Timonen and Smith (2005) stated that the contribution of actin was less important compared to that of tubulin in AM symbiosis. However, these authors used an actin antibody directed against both plant and fungal antigens. The decrease of actin we observed in well-colonised grapevine roots might therefore be closer to what happens in mature mycorrhizas.

Energy

The up-accumulation of two proteins, a putative adenosine triphosphate (ATP) synthase delta chain (spot 13) and a mature ATP synthase beta subunit (spot 21), belonging to the mitochondrial F1-ATP synthase complex was recorded. This complex synthesizes ATP from adenosine diphosphate and inorganic phosphate (P_i) by using electrons generated through the respiratory chain. Because ATP is the common "energy currency" of cells, ATP synthases are of crucial importance in all organisms. With respect to its Mr, spot 26 corresponded the precursor of a ATP synthase subunit beta, and thus, the down-accumulation reported here is in agreement with the up-accumulation of spot 21, the mature form of the ATP synthase beta subunit.

Defence and cell rescue

The amounts of proteins having role in defence mechanisms and cell rescue also showed clear differences in response to AMF colonisation. Most of them were noted as down-

accumulated. This is the case for a peroxidase precursor (spot 27), a catalase (spot 8) and a monodehydroascorbate reductase MDAR (EC 1.6.5.4; spot 7) classified in the defence and cell rescue protein category. MDAR is a component of the glutathione-ascorbate cycle, which is one of the major antioxidant systems of plant cells for protection against the damages produced by reactive oxygen species. The lower abundance of several proteins involved in plant defence reported here in response to AMF colonisation of a ligneous plant species is thus in good agreement with the modulation of plant defence mechanisms upon AMF colonisation that has been largely documented (Dumas-Gaudot et al. 2000; Garcia-Garrido and Ocampo 2002; Pozo and Azcón-Aguilar 2007; St-Arnaud and Vujanovic 2007).

Signalling

Two proteins belonging to the signalling category (spot 18, a TGF-beta receptor-interacting protein 1, and spot 17, a CBS1 fragment) were recorded as up-accumulated in mycorrhized SO4 roots. Plant TGF-beta receptor-interacting proteins, which share high homology with the TGF-β family of polypeptide growth factors of the animal kingdom playing prominent roles in development and homeostasis of organisms, were also suggested to be required for the normal execution of several plant developmental programs (Jiang and Clouse 2001). An increased accumulation of such a protein has been recently reported (Aloui et al. 2009). Spot 17 matched to a CBS1 fragment that corresponds to an evolutionarily conserved protein domain, i.e. the cystathionine-*b*-synthase (CBS) present in the proteome of archaebacteria, prokaryotes and eukaryotes. Depending on the protein in which they occur, CBS domains have been proposed to affect multimerization and sorting of proteins, channel gating and ligand binding. CBS domains can bind adenosine-containing ligands such ATP, AMP or *S*-adenosylmethionine and may function as sensors of intracellular metabolites (Ignoul and Eggermont 2005). Compared to control roots, the cystathionine-*b*-synthase CBS1 fragment displayed an increased amount, although only significant upon *G. irregulare* root colonisation, a fact that could be related to the higher root colonisation level which was reached with this AM fungus.

Transport

Within the protein transport category, only one protein (spot 20) was recorded as differentially accumulated in mycorrhized roots. Its amount was significantly reduced in SO4 roots only in response to *G. irregulare* colonisation. It corresponded to an importin alpha-like protein acting as an adaptor protein to help protein transport through the nuclear membrane, which is supposed to have an important role in plant signal transduction (Meier 2007).

Cell cycle

A putative cell division control protein (spot 33) involved in cell cycle was identified as up-accumulated only in response to *G. irregulare* colonisation of SO4 roots. Although cell division is not occurring in response to AMF colonisation, cell differentiation might be associated with DNA amplification and polyploidization, a process well documented in AM symbiosis (Berta and Fusconi 1997). The specific up-regulation of a cullin gene, mediating the control of cell cycle in mycorrhizal tomato roots, was thus proposed to be related to the endoreduplication process leading to the formation of 8C nuclei in AMF-colonised cells (Tahiri-Alaoui et al. 2002).

Lignin and anthocyanin biosynthesis

Two proteins that, to our knowledge, have not yet been reported as differentially accumulated in arbuscular my corrhizal symbiosis were noticed as down-accumulated in SO4 roots upon their AMF colonization. Thus, a cinnamyl alcohol dehydrogenase (spot 2) involved in the lignin biosynthesis pathway showed a reduced amount in response to both AMF, as did an anthocyanidin dioxygenase (spot 4) that catalyses conversion of flavan-3,4-diols to 3-hydroxyanthocyanidins. Apart from their widely reported roles in roots under osmotic or toxin stress (Chalker-Scott 1999), modulation of anthocyanin accumulation is a characteristic response of plants to long-term P_i deficiency (Misson et al. 2005).

Ethylene biosynthesis

A protein also recorded as commonly down-accumulated was spot 10, identified as a 1-aminocyclopropane-1-carboxylic acid oxidase (ACC oxidase) involved in ethylene biosynthesis. In *Solanum tuberosum* roots grown under low phosphate supply, lower activities of ACC oxidase were reported to be concomitant to higher AM colonization, and an increase of P supply reversed ACC oxidase activity and intensity of colonisation. The reduced ACC oxidase activity, leading to decrease amounts of root ethylene, was interpreted as a mechanism by which plants under the P-deprived stress allow the AM fungus to colonise roots by repressing defence mechanisms (McArthur and Knowles 1992). Indeed, in many host–microbe interactions, ethylene is usually associated with or induces diverse biochemical pathways considered integral to a plant defence response.

Proteins involved in miscellaneous metabolisms

Four proteins involved in several metabolic pathways were recorded as differentially accumulated in response to the

AMF colonisation. For example, spot 1 was, comparatively to control roots, up-accumulated in *G. irregulare*-colonised SO4 roots. This spot matched to an aspartate carbamyl aminotransferase involved in the synthesis of pyrimidine nucleotides. On the contrary, three other spots (spots 9, 12 and 31) whose amounts were significantly reduced with both AMF were noticed. Spot 9 was identified as a putative phosphoesterase, belonging to a large family of proteins, which includes phosphatases (EC. 3.1.3.2). Acid phosphatases (APases) play a key role in phosphate acquisition by plants, but except for a few enzymes performing specific metabolic functions, it is difficult to ascribe a precise role to most of them (Duff et al. 2006). Hydrolysis of phosphate esters is a critical process in the energy metabolism, and metabolic regulations of plant cell intracellular APases are undoubtedly involved in the routine utilization of P_i reserves or other P_i-containing compounds. Thus, in mycorrhizal plants grown under low P availability for which the AMF fungus will drive up phosphorous from soil to the roots, a reduced amount in plant phosphoesterase in response to AMF colonisation is expected. Besides, two enzymes involved in nucleotide metabolism were identified: Spot 12 corresponded a putative dehydrogenase with dihydroorotate oxidase activity that catalyses the formation of orotate from dihydroorotate within the pyrimidine biosynthetic pathway, spot 31 matched to an adenosine kinase also named ATP:adenosine 5′-phosphotransferase which participates to purine metabolism, namely within the salvage purine degradation pathway. Nucleotides are crucial cellular components for plant growth, development and metabolism. Besides their roles as building blocks of DNA in the nucleus or DNA-synthesizing organelles and as components of transcripts, they also play a unique role in transferring phosphate into macromolecules like nucleic acids and phospholipids (Zrenner et al. 2006). Notably, one of the early changes in response to P_i deprivation is a decrease in the levels of nucleotides (Raghothama 1999). The decreased amounts reported here for spot 12 and 31, corresponding to pyrimidine and purine salvage enzymes, could be interpreted as linked to a reorganisation of phosphate fluxes within cell roots upon AMF colonisation. Indeed, beside the induction of specific P_i transporters in mycorrhizal roots (Javot et al. 2007; Branscheid et al. 2010 and references therein), a parallel down-regulation of

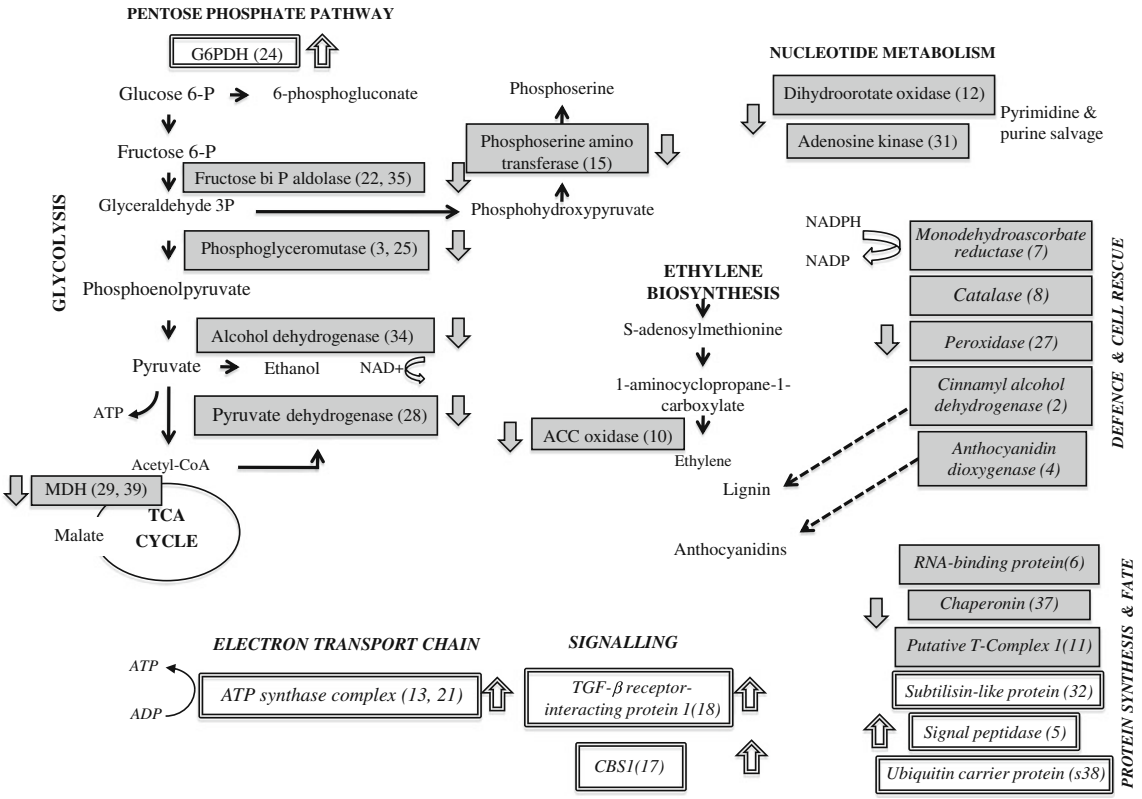

Fig. 5 Schematic representation of root proteins differentially accumulated upon AMF colonisation of SO4 rootstock cuttings (numbers in parentheses refer to numbering in Fig. 4 and identification in Table 1). Proteins whose abundance was increased in mycorrhizal roots compared to non-mycorrhizal ones (Nm) are indicated by *double-lined boxes* and *arrows*, while those which abundance was decreased are featured by *grey boxes* and *arrows*. Metabolic pathways are indicated in *capital letters* while defence and cell rescue ad protein synthesis and fate categories are indicated by *italics*. *G6PDH* glyceraldehyde-6-phosphate dehydrogenase, *TCA* tricarboxylic acid, *ACC oxidase* 1-aminocyclopropane-1-carboxylic acid oxidase, *MDH* malate dehydrogenase

conventional P_i-uptake systems leading to a reorganisation of P_i uptake does exist (Bucher 2007).

In summary, in this study, a total of 24 proteins were co-identified in SO4 rootstock upon colonisation with *G. irregulare* and *G. mosseae* (Fig. 4). This means that 61.5% of the AM-related protein modifications were conserved upon root colonisation with the two AMF, highlighting proteins that may be essential to symbiosis. This is in line with a recent report in *M. truncatula* roots colonised by the same strains of AMF (Recorbet et al. 2010). Thus, our data brought evidence for the first time in a perennial plant species, of a conserved proteomic response to different AMF species. However, contrasting to the publication of Recorbet et al. (2010) in which 11 proteins turned out to be of fungal origin, all the co-identified proteins of our study were of plant origin (Table 1). The failure to detect any fungal proteins in the symbiotic phase is likely to account for the mass spectrometry process used in the current work, as MALDI-TOF MS is known to be less efficient for organisms for which information at the nucleic acid level is restricted (Thelen 2007). Unexpectedly compared to previous AM symbiotic proteomes described so far (Bestel-Corre et al. 2002, 2004; Valot et al. 2005; Aloui et al. 2009; Recorbet et al. 2010, Schenkluhna et al. 2010), in AMF-colonised SO4 grapevine rootstock, much more co-identified proteins were recorded as down-accumulated (25) than up-accumulated (14). These data together with the limited overlap of proteins previously ascribed as mycorrhiza-related in herbaceous plants could indicate that AMF colonization of a ligneous plant species such as grapevine results in a more drastic reprogramming of host genes in order to accommodate the AMF, a conclusion that is schematically illustrated in Fig. 5, in which are presented the main proteins modulated in SO4 rootstock cuttings upon AMF colonization.

Remarkably, among the numerous proteins we recorded as down-accumulated in response to the AM symbiosis were several proteins known to be involved in plant adaptation to P deficiency. Plants acquire P as the inorganic phosphate ions (P_i) through P_i transporters in the roots (Marschner 1995). Moreover, plants have evolved sophisticated metabolic and developmental strategies to enhance P acquisition and remobilization in P_i-limiting conditions (Raghothama 1999). Recently, the molecular mechanisms were unravelled thanks to the identification of several genes involved in the regulation of P_i homeostasis (Abel et al. 2002; Poirier and Bucher 2002; Franco-Zorrilla et al. 2004; Yuan and Liu 2008; Lin et al. 2009). Interestingly, among the phosphate starvation-responding genes/proteins reported, there were proteins involved in carbon metabolism (some of them having potential function in signalling P deficiency), P remobilisation (such as phosphatases, pyrimidine and purine salvage enzymes etc.), stress and defence, development and root architecture and anthocyanin biosynthesis (Misson et al. 2005; Li et al. 2007, 2008, 2009; Valdes-Lopez and Hernandez 2008). It is therefore noteworthy that in the present work several proteins regarded as belonging to such functional categories were recorded as conversely accumulated upon AMF colonisation. One of the primary advantages delivered by AMF to their hosts is undoubtedly an improved mineral nutrition, particularly of P under P_i-limiting conditions (Smith and Read 2008), as was the case in this experiment. A crucial role of AMF in P acquisition, together with their capacity to store polyphosphates, relates to the development of a huge network of hyphae, which substantially extend the rhizospheric P_i depletion zone (Harrison 1999). Besides, AMF-colonised plants reprioritize their phosphate (P_i)-uptake strategies to take advantage of nutrient transfer via the fungus. The mechanisms underlying P_i transport are beginning to be understood, and recently, details of the regulation of plant and fungal P_i transporters in the AM symbiosis have been revealed (Javot et al. 2007; Branscheid et al. 2010 and references therein). Our proteomic study shed light on the molecular mechanisms that prevail during the AMF symbiosis of a perennial woody plant species, with notably a reverse effect of P deficiency affecting several categories of proteins. Further studies will compare the root proteome responses upon AMF colonisation and P supply. Additionally, time course proteomic studies in AMF-colonised SO4 grapevine rootstocks combined with either more sensitive 2D DIGE proteomic strategies or subcellular enrichment of a given cell root compartment will allow to further characterized the mycorrhiza responsive proteome of grapevine. Ongoing experiments are targeted to the microsome root compartment in order to reveal membrane proteins that could have essential functions in transport and signalling upon AMF colonization of a plant species with worldwide economic importance.

Acknowledgements This work was carried out in the frame of a CAPES/COFECUB project (SV553/07). GC Cangahuala-Inocente and MF Da Silva received 1-year postdoctoral grant from CAPES. Authors thank S. Gianinazzi and MC Lemoine for providing SO4 rootstock material and JL Rousselet for technical assistance in preparing the SO4 cuttings. They acknowledge G. Recorbet for critically reading the manuscript and helpful discussions.

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