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Review

Arbuscular mycorrhiza and heavy metal tolerance

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Abstract

Arbuscular mycorrhizal fungi (AMF) have repeatedly been demonstrated to alleviate heavy metal stress of plants. The current manuscript summarizes results obtained to date on the colonization of plants by AMF in heavy metal soils, the depositions of heavy metals in plant and fungal structures and the potential to use AMF-plant combinations in phytoremediation, with emphasis on pennycresses (*Thlaspi* ssp.). The focus of this manuscript is to describe and discuss studies on the expression of genes in plants and fungi under heavy metal stress. The summary is followed by data on differential gene expression in extraradical mycelia (ERM) of *in vitro* cultured *Glomus intraradices* Sy167 supplemented with different heavy metals (Cd, Cu or Zn). The expression of several genes encoding proteins potentially involved in heavy metal tolerance varied in their response to different heavy metals. Such proteins included a Zn transporter, a metallothionein, a 90 kD heat shock protein and a glutathione *S*-transferase (all assignments of protein function are putative). Studies on the expression of the selected genes were also performed with roots of *Medicago truncatula* grown in either a natural, Zn-rich heavy metal "Breinigerberg" soil or in a non-polluted soil supplemented with 100 μM $ZnSO_4$. The transcript levels of the genes analyzed were enhanced up to eight fold in roots grown in the heavy metal-containing soils. The data obtained demonstrate the heavy metal-dependent expression of different AMF genes in the intra- and extraradical mycelium. The distinct induction of genes coding for proteins possibly involved in the alleviation of damage caused by reactive oxygen species (a 90 kD heat shock protein and a glutathione *S*-transferase) might indicate that heavy metal-derived oxidative stress is the primary concern of the fungal partner in the symbiosis.

Keywords: *Glomus intraradices*; *Medicago truncatula*; *Thlaspi* ssp; Arbuscular mycorrhiza; Heavy metal tolerance; Phytoremediation; Oxidative stress alleviation

Contents

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1. Introduction: AMF status of plants on heavy metal-contaminated soils

Only few plants (the metallophytes) can cope with the adverse conditions on heavy metal soils. In Central Europe, typical examples are *Minuartia verna*, *Armeria maritima* ssp. *halleri*, *Cardaminopsis* (=*Arabidopsis*) *halleri*, *Thlaspi calaminare* and both forms of the zinc violets which have been reclassified as *Viola lutea* ssp. *calaminaria* or *V. lutea* ssp. *westfalica* (Hildebrandt et al., 2006a). *Viola lutea* ssp. *calaminaria* is endemic to heavy metal sites in the most westerly part of Germany and eastern Belgium whereas *V. lutea* ssp. *westfalica* occurs only at a heavy metal heap at Blankenrode, in the vicinity of Paderborn, Germany. Other metallophytes like the rare *Alyssum wulfenianum*, *Thlaspi cepaeifolium* or *Thlaspi goesingense*, occur on heavy metal sites in the South Eastern Alps. Some plant species, such as *Silene vulgaris* var. *humilis*, *Viola tricolor* or *Thlaspi praecox*, which occurs abundantly in Slovenia, have formed specific ecotypes adapted to heavy metal-polluted soils.

Metallophytes have developed various different physiological adaptations which enable them to compete successfully with the harsh conditions in heavy metal soils. In addition, protection by AMF that colonise plant roots and considerably reduce the uptake of heavy metals into plant cells may be one of the means that allow metallophytes to thrive on heavy metal-polluted sites (Weissenhorn et al., 1995; Leyval et al., 1997; Kaldorf et al., 1999; Berreck and Haselwandter, 2001; Ouziad et al., 2005; Vogel-Mikuš et al., 2006). For example, both zinc violets are strongly colonized by AMF (Hildebrandt et al., 1999) and leaves of *Viola lutea* ssp. *calaminaria* collected from a heavy metal site were earlier found to contain low amounts of heavy metals in ranges similar to those detected in non-metallophytes (Ernst, 1982). This correlation is not likely to be coincidental, since mycorrhizal colonization of the roots increases with increasing heavy metal content of the soil (Hildebrandt et al., 1999; Audet and Charest, 2006).

The diversity of AMF spores in heavy metal soils is frequently lower than in non-polluted sites (Pawlowska et al., 1996; Regvar et al., 2001). In line with this only few AMF species and a comparatively low number of AMF spores were found in the rhizosphere of the yellow zinc violet in its natural habitat (Ouziad, 1999; Tonin et al., 2001). However, a low number of spores does not necessarily reflect a limited AMF development (Whitfield et al., 2003; Regvar et al., 2006). In the course of our own study (Hildebrandt et al., 1999; Kaldorf et al., 1999), the AMF isolate *Glomus intraradices* Br1 was obtained, which consistently conferred heavy metal tolerance on a variety of plants in diverse heavy metal soils under optimum fertilization. In addition, the isolate Br1 was more effective in transferring heavy metal tolerance to tomato, maize or *Medicago truncatula* than *G. intraradices* Sy167, which was routinely used in our laboratory for the different studies on the AMF-plant interactions. Such results underline the importance of indigenous AM fungi, which are presumably more adapted to heavy metals. Nevertheless, *G. intraradices* Sy167 is also effective in alleviating heavy metal stress of plants (Hildebrandt et al., 1999; Kaldorf et al., 1999). Element localization studies by three different methods of microbeam analysis (Kaldorf et al., 1999) indicated that maize grown on two different heavy metal soils contained more essential elements such as K, P, and Mg but less heavy metals such as Ni, Fe, Zn or Cu when grown symbiotically with the AMF *G. intraradices* Br1 compared with non-AMF colonized roots (Kaldorf et al., 1999). Heavy metals (HM) that reached the interior of the roots were deposited mainly in the inner root parenchyma cells where most of the fungal structures (intraradical hyphae, arbuscules and vesicles) reside. The techniques employed, however, did not allow us to discriminate whether elements in the inner root parenchyma cells were deposited in the fungal, plant or both types of cells. Very recent results obtained from electron-dispersive X-ray spectrometry (EDXA) showed that Zn, Cu and Cd accumulated in the cell wall and in electron-dense granules in the cytoplasm of the fungi while their cytoplasm itself was essentially free of these elements (González-Guerrero et al., 2001). In addition, vesicles might serve as storage compartments for heavy metals (Turnau, 1998; Weiersbye et al., 1999). Recently glomalin, an insoluble glycoprotein produced by hyphae of AMF, was shown to bind potentially toxic elements including heavy metals (González-Chávez et al., 2004). In conclusion, evidence is now accumulating that AMF can filter out toxic heavy metals and thus keep them away from the plants.

2. The use of metallophytes in phytoremediation with a potential role of pennycresses (*Thlaspi* ssp.)

Throughout the world, soils exist that are almost vegetation-free due to their high content of toxic heavy metals. Phytoremediation comprises attempts to: (a) stabilize such heavy metal soils against erosion, lowering their contamination potential via the air (wind blow) by using metallophytes and (b) extract toxic elements from soils by these specifically adapted plants. Metallophytes are frequently characterized by their ability to hyperaccumulate heavy metals (Brooks, 2000; Prach and Pyšek, 2001; Wiegleb and Felinks, 2001; Regvar et al., 2006). The symbiotic combination with AMF could enhance their ability to grow on highly contaminated soils (Hall, 2002; Vogel-Mikuš et al., 2006). Most metal-hyperaccumulating plants, however, belong to the families of Brassicaceae and Caryophyllaceae that are widely accepted as non-mycorrhizal (Harley and Harley, 1987; DeMars and Boerner, 1996). Nevertheless, Brassicaceae from heavy metal sites such as *Biscutella laevigata* (Orlowska et al., 2002) or several *Thlaspi* species (Regvar et al., 2003; Vogel-Mikuš et al., 2005) were recently found to be mycorrhizal. *Thlaspi* species are colonized by *G. intraradices*, a common AM fungus, but this

could well be an AMF ecotype specifically adapted to heavy metal polluted soil types (Regvar et al., 2003). However, the low levels of colonization found in *Thlaspi* species, together with the difficulty to establish a mycorrhizal symbiosis under controlled environments, casts some doubts on the functionality of this symbiosis (Regvar et al., 2003; Vogel-Mikuš et al., 2005). Most recent reports suggest that AM development in hyperaccumulating species is favoured at elevated nutrient demands, e.g. during the reproductive period (Turnau and Mesjasz-Przybylowicz, 2003; Vogel-Mikuš et al., 2006). In mycorrhizal *Thlaspi praecox* the concentrations of phosphorus are higher and those of Cd and Zn are lower than in the non-colonized controls, indicating a selective advantage of even low levels of mycorrhizal colonization in metal enriched soils (Vogel-Mikuš et al., 2006). This may appear somewhat surprising; however, to-date, no correlation between the degree of colonization and fungal activity in the symbiosis has been demonstrated.

The product of biomass and metal content is the single most important quantity defining the suitability of metallophytes for phytoremediation (Bennett et al., 2000). Among these plants, the perennial *T. goesingense* and *T. montanum* are fairly productive and therefore appear to be suited for phytoremediation purposes. Colonization by AMF may indeed reduce the overall content of heavy metals in the plants, and the remainder may be mainly deposited in the vacuoles of the hyperaccumulating *Thlaspi* species (Vogel-Mikuš et al., 2006). However, in projects to stabilize heavy metal-contaminated soils against erosion, the application of a combination of AMF and *Thlaspi* species could possibly allow growth of plants even under extremely adverse soil conditions (Regvar et al., 2003). The effective colonization of the zinc violet by AMF has already been mentioned. This ornamental, though not very productive plant has been used to colonize abandoned heavy metal heaps near Katowice, Poland (Jędrzejczyk and Rostánski, 2001). The Ni hyperaccumulator *Berkheya coddii* (Asteraceae) from ultramafic soils of South Africa is heavily colonized by AMF in its natural habitats (Turnau and Mesjasz-Przybylowicz, 2003). However, all these plants and their potential symbioses with AMF have not yet been fully explored with respect to their phytoremediation potentials.

3. Expression of plant and fungal genes presumably involved in heavy metal tolerance

Colonization with AM-fungi has convincingly been shown to alleviate heavy metal-induced stress (Gildon and Tinker, 1981; Dehn and Schüepp, 1989; Diaz et al., 1996; Hall, 2002). Recently, Rivera-Becerril et al. (2002) and Rivera-Becerril et al. (2005) demonstrated a Cd-stress buffering effect of mycorrhizal colonization on pea plants. In line with this, a colonization with *G. intraradices* enables *M. truncatula* to grow almost as well in "Breinigerberg" HM soil as in non-polluted soil (Fig. 1). However, alleviating heavy metal toxicity by AMF colonization can vary to a large extent, depending on which heavy metal is involved, its concentration in the soil, the fungal symbiosis partner and the conditions of plant growth (Weissenhorn et al., 1995; Leyval et al., 1997; Hildebrandt et al., 1999; Turnau and Mesjasz-Przybylowicz, 2003). Buffering HM-stress had been assigned, at least partly, to selective immobilization of heavy metals within those root tissues that contain fungal structures (Kaldorf et al., 1999) or to the high metal sorption capacity of the extraradical mycelium of AMF (Joner et al., 2000).

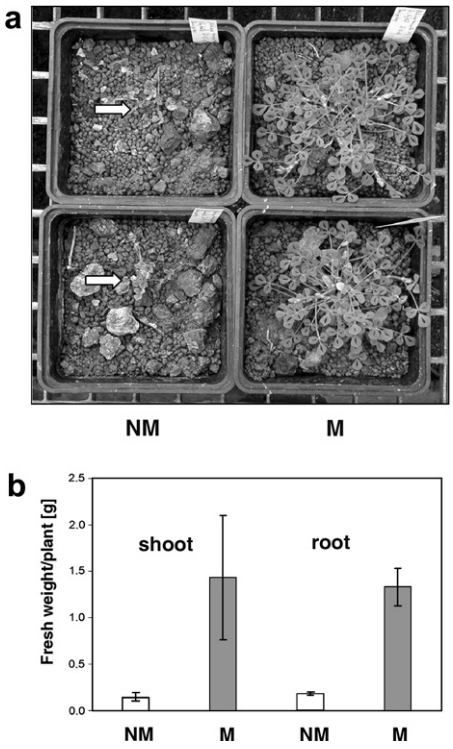

Fig. 1. (a) *Medicago truncatula* after growth for 12 weeks in the Breinigerberg heavy metal soil. M: steam sterilized soil inoculated with *Glomus intraradices*, NM: steam sterilized soil. White arrows indicate the position of the non-mycorrhizal plants in the respective pots. (b) *M. truncatula* root and shoot fresh weights of *G. intraradices* inoculated (M) and non-inoculated (NM) plants after twelve weeks of growth (standard deviations of mean values, $n = 6$).

AMF colonization of the roots has a significant impact on the expression of several plant genes coding for proteins presumably involved in heavy metal tolerance/detoxification (Repetto et al., 2003; Rivera-Becerril et al., 2005). Currently, rather limited information on the molecular basis of heavy metal tolerance mechanisms of AMF is available: the expression of a metallothionein gene of *Gigaspora margarita* (BEG 34) is up-regulated in symbiotic mycelia by Cu (Lanfranco et al., 2002) and increased transcript levels of a putative Zn transporter gene (*GintZnT1*) of the cation diffusion facilitator family (CDF) were observed in the mycelium of *G. intraradices* under short and long time exposure to Zn, indicating a possible function of this enzyme in protecting against Zn stress (González-Guerrero et al., 2005).

González-Guerrero et al. (2006) reported the Cd- and Cu-dependent up-regulation of a putative ABC transporter gene (*GintABC1*) in the extraradical mycelium of *G. intraradices*. *GintABC1* encodes a polypeptide with homology to the N-terminal region of the Multidrug-Resistance-Protein (MRP) subfamily of ABC transporters and may therefore be involved in Cd and Cu detoxification in the ERM of *G. intraradices*.

In a more comprehensive approach (Ouziad et al., 2005) studied the differential gene expressions in arbuscular mycorrhizal-colonized tomato grown under heavy metal stress. When the plants were grown either in the natural heavy metal soil "Breinigerberg", which is particularly rich in Zn, or in a non-polluted soil supplemented with Cd, growth of the AM colonized plants was distinctly better than that of the non-AMF inoculated controls. This material was used to analyze transcript formation of genes putatively involved in heavy metal tolerance. Northern blot analyses revealed that the mRNA of the plant genes *Lemt1*, *Lemt3* and *Lemt4* (coding for different metallothioneins), *LeNramp2* (for a broad range heavy metal transporter) or *LePCS1* (for phytochelatin synthase) showed no expression differences between heavy metal-grown and control plants, with or without AMF colonization. In contrast, *Lemt2* was strongly expressed in non-colonized control plants grown in the Breinigerberg soil or in soil supplemented with Cd, and colonization by AMF distinctly reduced the transcript levels of this gene. Similarly, *LeNramp1* was strongly expressed in tomato grown in Breinigerberg soil without AMF, whereas the transcription of this broad range metal transporter gene was down-regulated in AMF colonized plants. Transcript levels of another transporter, *LeNramp3* were reduced in roots, but not in leaves by colonization with AMF. These expression studies were largely confirmed by qRT-PCR based assays. RNA *in situ* hybridization experiments indicated that both *Lemt2* and *LeNramp1* were strongly expressed in all root tissues of non-AMF colonized plants grown in the Breinigerberg soil, whereas the expression of these genes was restricted to a few parenchyma cells in AMF colonized roots. Thus, among the genes with products putatively involved in alleviating heavy metal stress, some, but not all, are strongly expressed upon exposure to heavy metals, and these genes are down-regulated by symbiosis with the fungi. Colonization of roots might have lowered the concentration of heavy metals in the plant cells to an insufficient level to induce expression of these genes. In parallel with the down-regulation of these genes in the plant cells we expected an up-regulation of counterpart genes of the fungal symbiosis partner, due to the likely accumulation of heavy metals in the latter. This was, however, not the case. A suppression subtractive hybridization (SSH) library (Diatchenko et al., 1996) from hyphae of *G. intraradices* grown in the presence of high or low Zn concentrations (see review by Küster et al., 2006; Ouziad et al., 2005) provided no sequence at all with homologies to the plant genes mentioned above. However, the SSH library contained several EST-sequences putatively encoding enzymes involved in the detoxification of reactive oxygen species (glutathione *S*-transferase, superoxide dismutase, cytochrome P450, thioredoxin). Their differential expression was verified by reverse Northern analysis. It was therefore suggested that a primary function of the fungal cells in the symbiosis was to cope with the heavy metal-induced oxidative stress (Ouziad et al., 2005).

4. Gene expressions in arbuscular mycorrhiza under heavy metal stress in specific fungal structures

Further experiments have been have been performed to study the specific expression of selected fungal genes in the extraradical mycelium (ERM) of *G. intraradices* and in roots of mycorrhizal *M. truncatula*, grown either in the natural heavy metal-polluted Breinigerberg soil or under a defined Zn concentration in a Zn-enriched substrate (U. Hildebrandt, H. Bothe, unpublished). The four selected *G. intraradices* genes (Table 1) with a potential importance in heavy metal tolerance encode a glutathione *S*-transferase, a 90 kD heat-shock protein, both identified in the Zn-SSH library described by Ouziad et al. (2005), a metallothionein (BI451899) and a Zn transporter, with homology to members of the ZIP Zn-transporter family, obtained from another SSH-library (Hildebrandt et al., 2006; Küster et al., 2006).

To determine the transcript levels of the four selected genes in extraradical hyphae under HM-stress, *G. intraradices* Sy167 was grown for eight weeks in co-culture with Ri-T-DNA transformed carrot roots exactly as described in Hildebrandt et al. (2002) supplemented with either 1 μM $ZnSO_4$, 100 μM $ZnSO_4$, 2.5 μM $CdSO_4$ or 20 μM $CuSO_4$. The applied heavy metal stressors significantly reduced the ERM biomass by about 25% (data not shown).

Table 1
BlastX results of *Glomus intraradices* EST-sequences selected for qRT-PCR analysis

SSH library index (http://www.genetik.uni-bielefeld.de/MolMyk)/GenBank accession no.	Matching sequence from GenBank "nr" database (BlastX), Altschul et al. (1990)	Origin of matching sequence	*E*-value
__giaac240004b07/**EB741031**	Putative glutathione *S*-transferase **XP_470192**	*Oryza sativa*	7e−11
__giaac240004b09/**EB741032**	90-kDa heat-shock protein **AAP51221**	*Aphrocallistes vastus*	5e−31
__giaad240001_D7/**EB741033**	ZIP zinc transporter **BAE65501**	*Aspergillus oryzae*	8e−18
BI451899	Type 2 metallothionein **ABB05520**	*Arachis hypogaea*	1e−8

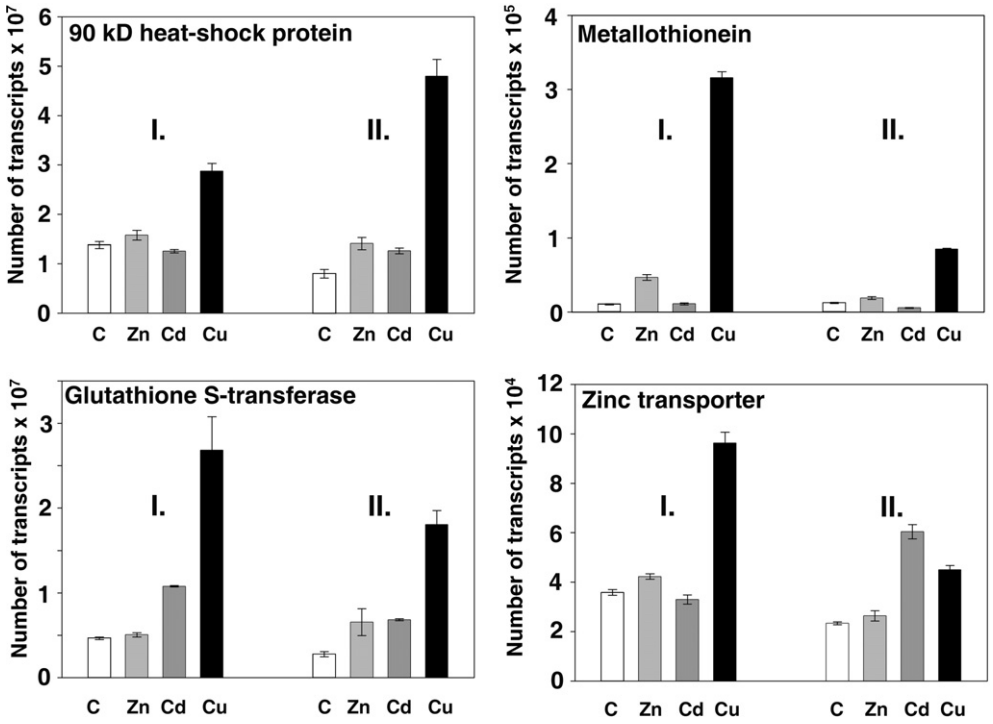

Fig. 2. Quantitative mRNA transcript analysis by real time RT-PCR of the indicated genes in the *in vitro* grown ERM of *Glomus intraradices* exposed to different heavy metals in two independent experiments (I and II). RNA isolation and subsequent real time RT-PCR analysis was performed as described by Ouziad et al. (2005). Standard curves for the selected genes were established by applying dilution series of recombinant plasmids (10^8–10^2 copies) and standardization was performed against the *G. intraradices* 28S-rRNA levels using the specific primers GiAM(F) and GiAM(Rev) (Alkan et al., 2004). Data are the means of three repetitions. Bars indicate ± standard deviations of mean values. C: control, 1 μM $ZnSO_4$; Zn: 100 μM $ZnSO_4$; Cd: 2.5 μM $CdSO_4$; Cu: 20 μM $CuSO_4$.

The expression levels of the four target genes measured by qRT-PCR varied considerably (Fig. 2). The *hsp90* and the GST-gene of *G. intraradices* were highly up-regulated by Cu-stress (6–7-fold) whereas under Zn-stress only slightly increased transcript levels were measured. A similar overall expression pattern was detected for the metallothionein gene, which exhibited the strongest up-regulation up to 30-fold in presence of 20 μM Cu, while growth with 2.5 μM Cd caused a slight decrease or almost no change for the metallothionein gene, and Zn-stress increased expression up to 4-fold. The Zn transporter gene transcripts of ERM grown with 100 μM Zn increased only marginally whereas Cu stress again caused at least a twofold increase of transcript levels. Mycelium from Cd-treated plates showed a small increase (ca. 1.5-fold) in the expression of the Zn transporter gene in one experiment.

The maximum transcript levels of the fungal heat-shock protein (HSP90) and glutathione *S*-transferase genes measured in mycorrhizal roots (Fig. 3) were lower by one order of magnitude, on average, after growth in Breinigerberg or Zn soil than in ERM obtained from the co-culture with carrot roots (Fig. 2). In contrast, the transcripts of the Zn transporter gene were in the same range in mycorrhizal roots (max. 10^5 transcripts) and in the *in vitro* ERM (3×10^4 transcripts). In comparison with non-heavy metal-treated controls, the *G. intraradices hsp90* and GST genes showed increased expression levels in mycorrhizal roots of *M. truncatula* when exposed to elevated amounts of Zn added to unpolluted substrate or in the natural heavy metal-polluted Breinigerberg soil at all sampling dates (Fig. 3). The Zn transporter gene responded to elevated stress in both heavy metal treatments with a 2–4 fold higher transcript accumulation, compared with a non-Zn-treated control. In the three RT-PCR analyses measured per experiment, transcript numbers of the metallothionein gene were too low to reveal statistically significant data.

HSP90, an abundant, evolutionarily conserved and highly stress-inducible molecular chaperone, regulates folding, transport, maturation and degradation of a variety of proteins (Cowen and Lindquist, 2005; Sangster and Queitsch, 2005). In line with this, the *G. intraradices* 90 kD heat shock protein gene (*hsp90*) was up-regulated in the ERM grown under HM-stress. The gene product could contribute to stabilization and refolding of denatured proteins damaged by heavy metal-induced reactive oxygen species (ROS). Oxidative stress might be a major cause of the toxicity of several heavy metals. Indeed, the highest levels of induction of *hsp90* in the *in vitro* grown ERM were obtained at high levels of Cu, which stimulate the Fenton reaction to increase the generation of highly damaging OH radicals from $O_2^{\cdot-}$ and H_2O_2, leading to oxidative stress (Avery, 2001).

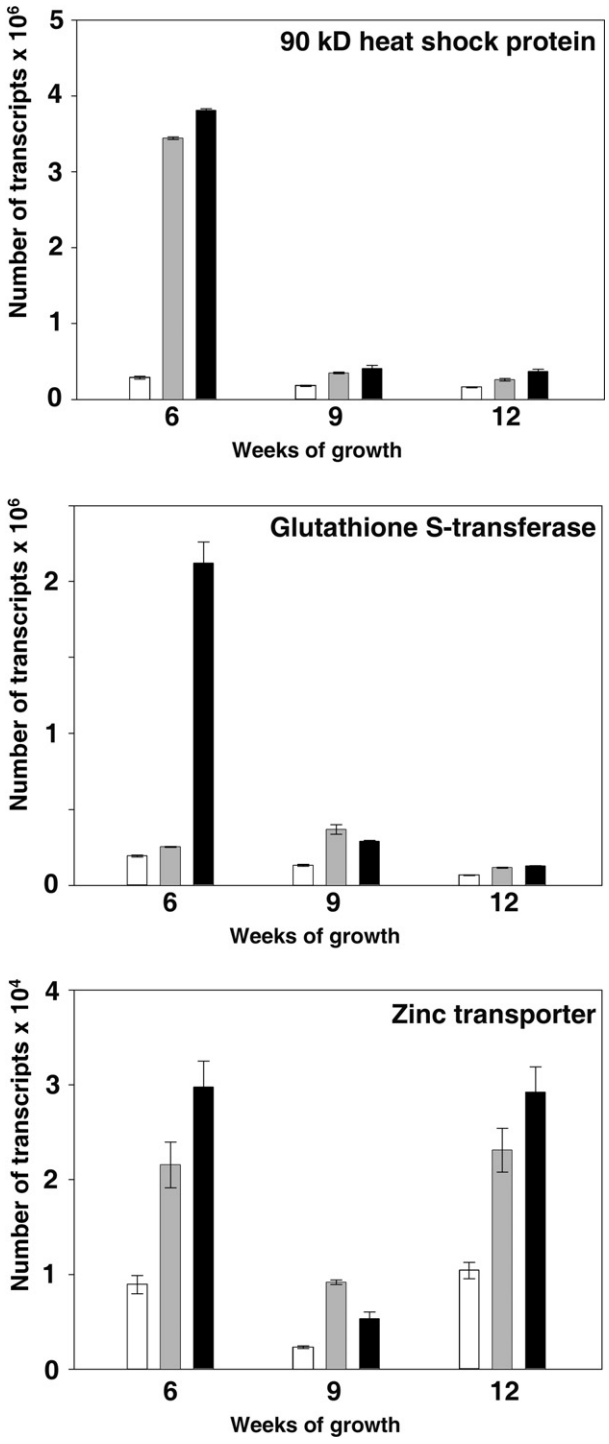

Fig. 3. Quantitative mRNA transcript analysis by real time RT-PCR of the indicated genes from total RNA isolated from *Medicago truncatula* roots colonized by *Glomus intraradices* Sy167, after 6, 9 and 12 weeks of growth in the greenhouse. For the pots with only Zn as the heavy metal contaminant, plants were watered with 100 ml of a 100 μM $ZnSO_4$ solution once a week starting 4 weeks after inoculation. Further details of the growth conditions were described earlier (Hildebrandt et al., 1999; Kaldorf et al., 1999; Ouziad et al., 2005). RT-PCR experimental conditions were the same as indicated in Fig. 2 and described in Ouziad et al. (2005). Data are the means of three repetitions. Bars indicate ± standard deviations of mean values. The fresh weights of the plants did not vary significantly among the different treatments. □ Control; ▭ irrigated with 100 μM $ZnSO_4$; ■ Breinigerberg soil.

Glutathione *S*-transferases (GSTs) catalyze the conjugation of glutathione with a variety of reactive electrophilic compounds and may provide protection against oxidative stress (Moons, 2003; Smith et al., 2004). In a SSH library obtained from *G. intraradices* grown under heavy metal stress, several ESTs had significant sequence homologies to GST-encoding genes from other organisms (Rhody, 2002). This finding, together with our observation on the transcriptional up-regulation of the glutathione *S*-transferase gene (4b07) by either Cd, Cu or Zn, could well indicate that GSTs participate in the alleviation of heavy metal toxicity in the symbiotic mycelium.

Metallothioneins (MTs) are metal binding proteins formed in a wide range of organisms upon their exposure to toxic concentrations of metals such as Cu, Zn or Cd. In non-AM-fungi, MTs are predominantly induced by Cu (Kumar et al., 2005). The distinct up-regulation of the metallothionein gene (BI451899) in ERM of *G. intraradices* by Cu-stress, to some extent by Zn but not by Cd, fully agrees with the proposed primary function of fungal MTs in the detoxification of Cu. This view is corroborated by the Cu-specific up-regulation of a MT from the AMF *G. margarita* (Lanfranco et al., 2002). However, transcript levels of the metallothionein gene (BI451899) were comparably low in ERM and mycorrhizal roots in quantitative RT-PCR experiments (Figs. 2 and 3).

Members of the ZIP family of Zn transporters are involved in the uptake of heavy metals from extracellular media or in the mobilization from intracellular stores (Gaither and Eide, 2001). Somewhat to our surprise, the *G. intraradices* Zn transporter gene with significant homologies to members of the ZIP family of zinc transporters (Table 1), was up-regulated in ERM by Cd and Cu treatment, but showed very little response to even 100 μM Zn in the medium. As this ZIP family Zn transporter gene did not show up in the Zn SSH library (Ouziad et al., 2005) and as it is apparently not regulated in response to 100 μM Zn, it might not be involved in Zn tolerance mechanism(s) of the ERM under the conditions employed. In contrast, in mycorrhizal roots of *M. truncatula* the transcript levels of the *hsp90*, the glutathione *S*-transferase and of the Zn transporter genes all increased in response to the natural HM-soil and to Zn-only exposure (100 μM) at all three sampling dates (Fig. 3). The high expression levels of the Zn transporter in roots suggests that this gene is preferentially expressed in the intraradical structures of *G. intraradices* and its gene product may play a more specific role at the plant fungal interface.

The AMF dependent down-regulation of plant genes potentially involved in HM-tolerance under HM-stress (Ouziad et al., 2005) and the concomitant up-regulation of stress related AMF genes indicate that effective fungal HM-tolerance mechanism(s) could provide a pivotal contribution to the increased heavy metal tolerance of mycorrhizal plants. This conclusion comes from data obtained from SSH libraries (Rhody, 2002; Ouziad et al., 2005; U. Hildebrandt, unpublished) and qRT-PCR experiments.

In a similar approach with AM colonized tomato grown under NaCl-stress (Ouziad et al., 2006), both plasmalemma and tonoplast aquaporin genes were down-regulated at the plant side. However, the expression of a Na^+/H^+ transporter gene presumably involved in salinity tolerance (Sottosanto et al., 2004) remained unaffected. Altogether, the results show the usefulness of SSH clone libraries to study plant and fungal gene expression under stress conditions. In conclusion, the HM dependent induction of genes encoding a heat-shock protein and a glutathione *S*-transferase in the mycelium of the AMF *G. intraradices* Sy167, suggests that alleviating the HM-induced oxidative stress might be of primary concern for AMF exposed to elevated HM. Other strategies possibly contributing to HM-tolerance appear to be involved as well, which is indicated by the significantly enhanced expression of the metallothionein and the Zn transporter gene, particularly under Cu-stress. The products of such HM responsive genes may act in a rather localized manner, potentially restricted to fungal structures like the arbuscules, which remains to be studied further.

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