



REVIEW ARTICLE

ROLE OF ARBUSCULAR MYCORRHIZA IN HEAVY METAL TOLERANCE IN PLANTS: PROSPECTS FOR PHYTOREMEDIATION

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SUMMARY

Arbuscular Mycorrhiza (AM) is an obligate biotroph, which mainly improves phosphorus nutrition, ability to withstand water stress and offers a natural potential for biological control of root pathogen. They are also reported to be present on the roots of plants growing on metal-contaminated soils and play an important role in metal tolerance and accumulation. Isolation of the heavy metal tolerant AM fungi can be a potential biotechnological tool for inoculation of plants for successful phytoremediation. AM fungi provide an attractive system to advance plant-based environmental clean-up. During symbiotic interaction the hyphal network functionally extends the root system of their hosts. Thus, plants in symbiosis with AM fungi have the potential to take up heavy metal (HM) from an enlarged soil volume. This review throws light on the potential and the prospects of AM fungi in heavy metal tolerance of plant and its utilization for enhancing phytoremediation of heavy metal contaminated soils.

Key words: Arbuscular Mycorrhiza (AM), Heavy metal, Phytoremediation

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1. Introduction

Heavy metals are among the most toxic inorganic substances which have contaminated large area of land due to use of sludge, pesticides, fertilizers, and emissions from municipal waste incinerators, car exhausts, residues from metalliferous mines, and smelting industries. Although metal ions such as copper (Cu), zinc (Zn), iron (Fe), manganese (Mn) and nickel (Ni) etc., are taking part in redox reactions, electron transfers, a multitude of enzyme catalyzed reactions in various cellular metabolism at optimum concentration, the toxic concentration of the same essential metals or the nonessential toxic ions of Cd, Hg, Pb, Ag, As, Al, etc. are strongly poisonous to metalsensitive enzymes, resulting in growth inhibition and death of the organism. How do plants regulate their metabolism in response to heavy metals and protect themselves against metal poisoning? The

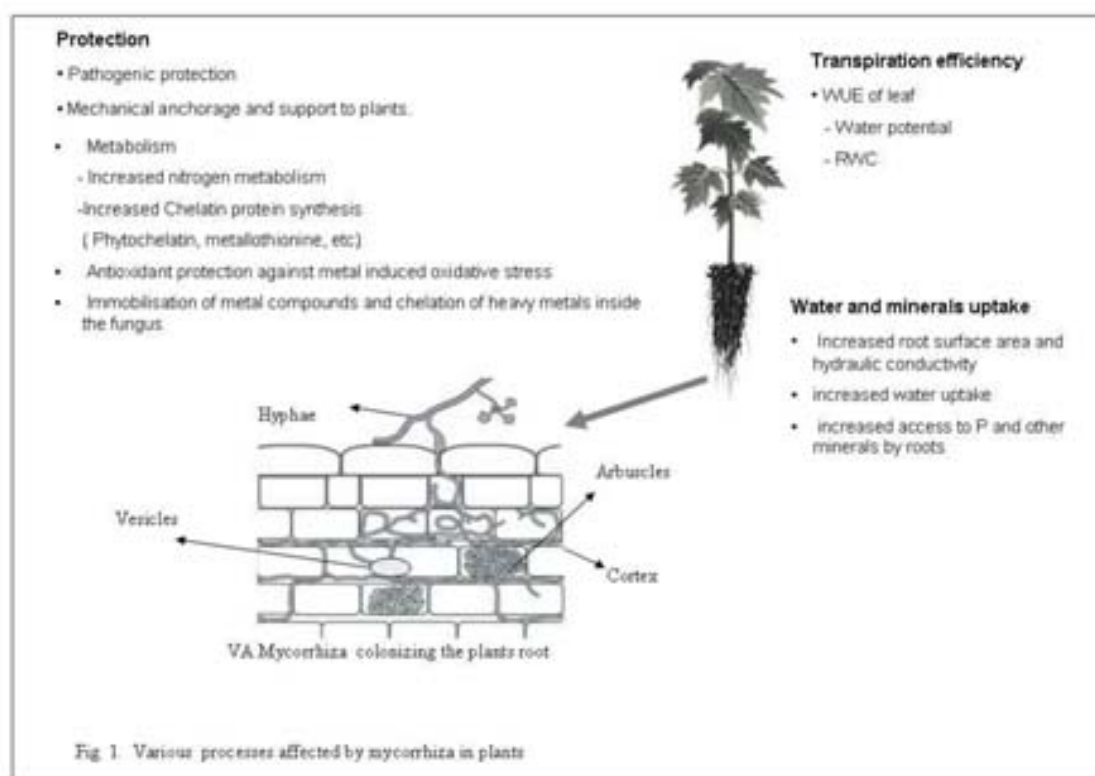
answer lies in understanding the mechanism of detoxification and tolerance of plants to heavy metal stress.

Metal toxicity and tolerance in plants is a subject that has been broadly reviewed (Hall, 2002; Clemens et al, 2002; Benavides et al., 2005; Misra and Dubey, 2006; Panda, 2008, Pal and Rai 2009). The presence of both essential and non essential heavy metals in the atmosphere, soil and water, in excessive amounts, can cause serious problems to all organisms. Knowledge of metal-plant interactions is not only important for the safety of the environment, but also for reducing the risks associated with the introduction of trace metals into the food chain. Heavy metals are also included in the main category of environmental pollutants as they can remain in the environment for long periods; their accumulation is potentially hazardous to humans, animals and plants

(Benavides et al., 2005; Gratão et al., 2005; Panda, 2008). We have witnessed in the last few decades a dramatic increase in contamination of the environment, including soil, air and water. It is well known that any pollutant, heavy metals in particular, has the potential to cause damage to plants, which, in turn, respond to the stress to survive. Heavy metal-induced oxidative stress in plants disturbs the balance between oxidants and antioxidants in the cell. The sensitivity of plants to heavy metals depends on an interrelated network of physiological and molecular mechanisms that includes uptake

and accumulation of metals through binding to extracellular exudates and cell wall, complexation of ions inside the cell by various substances, for example, organic acids, amino acids, ferritins, phytochelatins, and metallothioneins; general biochemical stress defense responses such as the induction of antioxidative enzymes and activation or modification of plant metabolism to allow adequate functioning of metabolic pathways and rapid repair of damaged cell structures (Hall, 2002; Azevedo and Azevedo, 2006; Tiwari et al., 2008; Panda, 2008) as depicted in Fig.1.

Fig. 1: Various processes affected by mycorrhiza in plants



Mycorrhiza is the mutualistic symbiotic association (non-pathogenic) of a specific group of soil-borne fungi with the roots of higher plants (Sieverding, 1991). Plant receives support from AM fungi, with the help of its symbiotic association, in the aspect of uptake of phosphorus and other nutrients, enhancement of growth hormones, increase of protein content, increase of lipid, sugars, amino acid levels, increase of tolerance to

heavy metals, increase of salinity tolerance, and resistance to root-borne pathogens.

The non-nutritional effects of AM would be due to increased survival ratio of transplanted seedlings, control of root diseases, increased tolerance to saline condition and increased soil aggregation by the external hyphal network (Marx, 1969). The carbohydrates are translocated from their source location (usually leaves) to the root tissues and then to the fungal partners.

In return, the plant gains the use of the mycelium's very large surface area to absorb water and mineral nutrients from the soil, thus improving the mineral absorption capabilities of the plant roots. About 80% of all terrestrial plant species form this type of symbiosis (Smith and Read, 1997) and 95% of the world's present species of vascular plants belong to families that are characteristically mycorrhizal (Quilambo, 2000). AM fungi affect various processes in plants as depicted in Fig .1. The mechanisms used by AM include increased root hydraulic conductivity, improved stomatal regulation, osmotic adjustment in the host, enabling extraction of water from smaller pores through improved contact with soil particles as a result of the hyphae binding effect (Joachim et al., 2009), increased evaporative leaf surface. AM fungi are known to assimilate and transport both NH_4^+ ions and some organic-nitrogen compounds to their host plants, particularly under conditions of low N availability and low pH

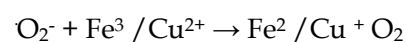
Phytoremediation has been successfully used to remove heavy metals from soils (Salt et al., 1995). Mycorrhizae have also been reported in plants growing on HM - contaminated sites indicating that these fungi have evolved a HM-tolerance and that they may play a role in the phytoremediation of the site. Joner and Leyval (1997) found that cadmium-tolerant *Glomus mosseae* isolates were responsible for uptake, transport and immobilization of cadmium. Copper (Cu) was absorbed and accumulated in the extraradical mycelium of three AMF isolates, as observed in a study with *Glomus spp.* Mycorrhizae were found to ameliorate the toxicity of trace metals in polluted soils growing in soybean and lentil plants (Jamal et al., 2002). As mycorrhizae may enhance the ability of the plant to cope with water stress situations associated to nutrient deficiency and drought (Schreiner et al., 1997), mycorrhizal inoculation with suitable fungi has been proposed as a promising tool for improving phytoremediation of metal contaminated soil. In the present review, the potential and the prospects of AM fungi in heavy metal tolerance of plant and its utilization for enhancing phytoremediation

of heavy metal contaminated soils have been highlighted.

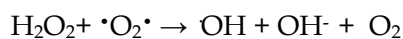
2. Heavy metal induced oxidative stress in plants

Reactive oxygen species (ROS) such as O_2^- , H_2O_2 , and $\cdot\text{OH}$ are commonly generated under stress conditions and bear strong oxidizing activities that can attack all types of biomolecules. In fact, these oxygen species represent intermediates emerging during the successive reduction of O_2^- to H_2O_2 (Asada and Takahashi, 1987; Dietz et al., 1999; Halliwell, 1987; Mittler, 2002; Panda 2008). Plants exposure to certain heavy metal ions shifts the balance of free radical metabolism towards an accumulation of H_2O_2 . In the presence of redox active transition metals such as Cu and Fe^{2+} , H_2O_2 can be converted to the highly reactive $\cdot\text{OH}$ molecule in a metal-catalyzed reaction via the Fenton reaction. The oxidized metal ions undergo a re-reduction in a subsequent reaction with superoxide radicals (O_2^-). An alternative mechanism of $\cdot\text{OH}$ formation directly from H_2O_2 and O_2^- , is the metal-independent Haber Weiss reaction

Fenton reaction;



Haber Weiss reaction;



The $\cdot\text{OH}$ molecule is one of the most reactive species known. Because of its ability to initiate radical chain reactions, it is very likely responsible for irreversible chemical modifications of various cellular components. Another ROS that might be involved mainly in lipid peroxidation is the protonated form of O_2^- , the hydroperoxyl radical ($\text{O}_2^- \text{H}$). These species exist in equilibrium. Possibly metal ions inhibit the activities of antioxidative enzymes especially of glutathione reductase, and also raise a transient depletion of GSH. Thus, a natural

accumulation of ROS would be the consequence. Heavy metals (HM) like Cu, Zn, Cd, Cr, Pb, Hg, As, Fe...etc under toxic concentration inactivates enzymic antioxidant defense system in plants resulting into increased ROS signaling generally leading to death of a plants. At high concentrations, HM interfere with essential enzymatic activities by modifying protein structure or by replacing a vital element resulting in deficiency symptoms (Halliwell, 1987; Mittler, 2002; Tiwari et al., 2008). The plasma membrane is particularly vulnerable to HM toxicity since membrane permeability and thus functionality can be affected by alterations of important membrane intrinsic proteins such as H⁺-ATPases (Hall, 2002). Also the production of reactive oxygen species leading to oxidative damage of plant tissue occurs in response to elevated HM levels (Schutzendubel and Polle 2002). As a consequence toxicity symptoms such as chlorosis, growth retardation, browning of roots, effects on both photosystems, cell cycle arrest and others can be observed. Plants and AM fungi have evolved several mechanisms to maintain ion homeostasis under elevated HM concentrations (Clemens 2001; Hall 2002). The basic principles of detoxification mechanisms include the extracellular HM-chelation by root exudates and/or binding of HM to the rhizodermal cell walls uptake of HM avoiding. Active plant efflux systems control cytosolic concentrations of HM. Intracellularly the plant cell produces chelating agents such as phytochelatins and metallothioneins, which have high-affinity HM binding properties. The resulting complex can finally be exported from the cytoplasm across the tonoplast and become sequestered inside the vacuole (Hall 2002, Fig. 2). Understanding how plants accumulate and store metal ions is relevant to two important applications: metal nutrition – for humans and in agriculture; and metal detoxification – using plants as biological detoxification systems for the

‘phytoremediation’ of metal contamination in the environment. Knowing how plant regulate essential metal ion metabolism will contribute to improvements in essential metal ion nutrition of plant and utilizing them as potential source of phytoremediation. Similarly, knowledge of the mechanisms by which metals, both essential and nonessential, can be sequestered, stored and detoxified in various organisms may contribute to the optimisation of phytoremediation processes (Clemens et al., 2002; Gohre and Paszkowski, 2006). The molecular genetics of model organisms can lend much to our understanding of the basic components of essential metal metabolism in plants. It has been well studied that some biochemical compounds involved in the physiological mechanisms of plants exposed to heavy metals are proteins capable of making linkages with metals, thereby forming complex biochemical compounds called metal-proteins, metallothionein and peptides like phytochelatins. Besides these, organic acids and amino acids takes active part in detoxification of heavy metals in plants (Fig.2.) (Hall, 2002; Mishra and Dubey, 2006; Pal and Rai, 2009). Conventional soil remediation practices in the past have relied mainly on the excavation of the contaminated soil. However, physical displacement, transport and storage or alternatively soil washing are expensive procedures and leave a site behind devoid of any soil microflora. Furthermore translocation of HM polluted soil instead of solving the problem rather shifts it to upcoming generations. In contrast plants offer an inexpensive and sustainable on-site approach (Gohre and Paszkowski, 2006), which relates to the above-described mechanisms for HM detoxification (Hall 2002) as shown in Fig. 2. There are two main strategies that use plants either to bind HM in the soil (phytostabilization) or to import and store HM in the plant’s above-ground tissues (phytoextraction)

Fig. 2: Cellular mechanism of heavy metal responses in VA mycorrhizal and non mycorrhizal plants

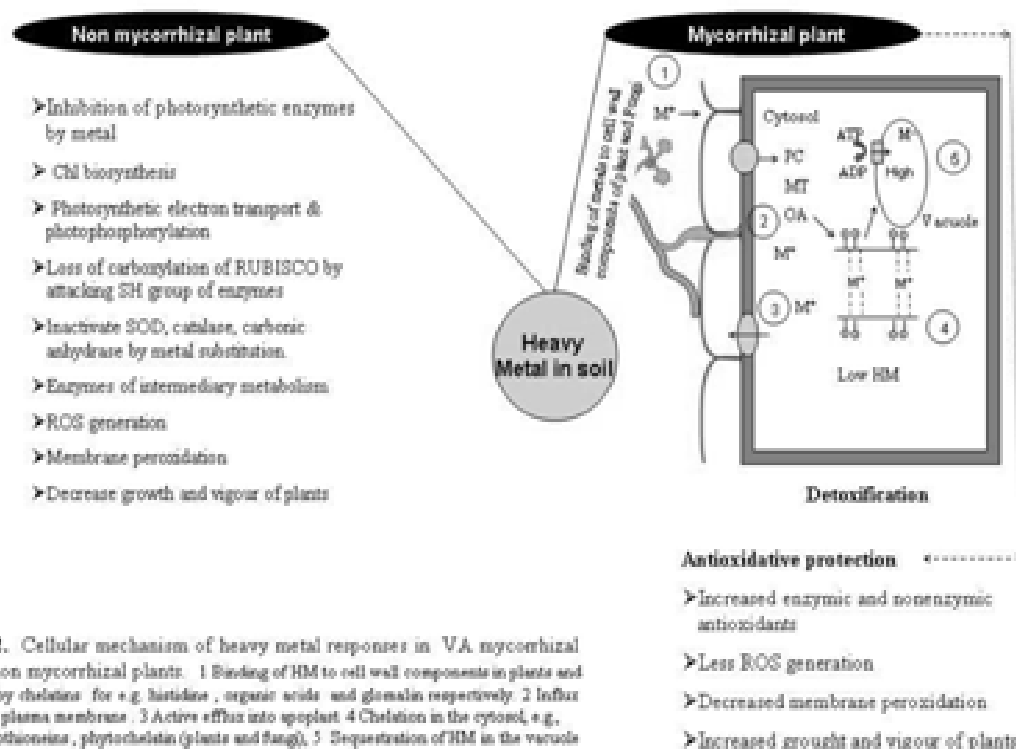


Fig. 2. Cellular mechanism of heavy metal responses in VA mycorrhizal and non mycorrhizal plants: 1 Binding of HM to cell wall components in plants and fungi by chelates for e.g. histidine, organic acids and glucanin respectively. 2 Influx across plasma membrane. 3 Active efflux into apoplast. 4 Chelation in the cytosol, e.g., metallothioneins, phytochelatins (plants and fungi). 5 Sequestration of HM in the vacuole of plant and fungal cells.

3. Phytochelatins and Metallothioneins

Phytochelatins (PCs) were first discovered as Cd-binding "Cadystins A and B" in a fission yeast and then in many plants as the major components of Cd-binding complexes. Phytochelatins (PCs) are enzymatically synthesized peptides known to involve in heavy metal detoxification and accumulation, which have been measured in plants grown at high heavy metal concentrations, but few studies have examined the response of plants even at lower environmentally relevant metal concentrations (Grill et al., 1985; Clemens, 2001; Cobbett, 2000; Goldsbrough, 2000; Pal and Rai, 2009). Genes encoding the enzyme PC synthase have been identified in plants and other species enabling molecular biological studies to untangle the mechanisms underlying PC synthesis and its regulation. Several published reports revealed advances in structure of PCs, their biosynthetic regulation, roles in heavy metal

detoxification and/or accumulation, and PC synthase gene expression for better understanding of mechanism involved in detoxification of heavy metals in plants (Mishra and Dubey 2006; Pal and Rai, 2009).

PCs have the general structure of (γ -glutamyl-cysteinyl)n-glycine (n=2-11) and the variants with the repeated γ -glutamyl-cysteinyl units are formed in some plants and yeast. They are capable of binding to various metals including Cd, Cu, Zn or As via the sulfhydryl and carboxyl residues, but their biosyntheses are controlled preferentially by the metal. PCs are synthesized from glutathione (γ -glutamyl-cysteinyl-glycine) in steps mediated by PC synthase. Inhibition studies of PC biosynthesis via glutathione have demonstrated their fundamental roles in the metal detoxification in yeast and fungi, green algae and some aquatic plants, and also in the suspension-cultured cells and intact tissues in higher plants. Over-expression of PC synthase genes increases the metal

tolerance in yeast and bacteria efficiently but not always in higher plant tissues especially in metal-accumulating species. "Hyperaccumulators" of Cd, Zn, Ni or As in terrestrial plants have a common features where massive metal transport to shoots prevails, besides the ability of their roots to form PCs. Hence PC based metal detoxification might be the primary defense mechanism established in lower plants and the additional PC-independent mechanism via vascular transport system became established later in higher plants. Existence of the PC-dependent and independent mechanisms at the metal-binding sites in the symplast and apoplast of shoots might improve metal detoxification and the tolerance characteristics of higher plants under heavy metal stress (Fig 2).

Metallothioneins (MTs) are small, metal-ion binding proteins (typically between 45 and 85 amino acids) which appear to be ubiquitous in eukaryotes and cyanobacteria. Several highly divergent MTs have been identified in cyanobacteria, algae, and higher plants. These MTs differ dramatically from mammalian MTs in amino acid sequences, charge, total cysteine content, cysteine spacing patterns, and length of linker domains. In particular, higher plants have moderately large, complex MT gene families, in contrast to the small, much less complex families found in mammals. The plant and algal MTs exhibit beneficial metal binding and induction properties that may protect these organisms from elevated levels of both toxic cadmium (Cd), mercury (Hg) and arsenic (As) and affect the homeostasis of nutrient metals like copper (Cu) and zinc (Zn). For example, in the marine brown algae *Fucus vesiculosus*, a 67-amino acid MT with 16 cysteine residues was found to be copper-inducible and bound both Cd(II) and Cu(II) in vitro. A pea class-2 MT increased copper tolerance and accumulation, when overexpressed in *Escherichia coli* and transgenic *Arabidopsis*, but did not affect levels of Zn and Cd (Evans et al., 1992). Despite animal MTs being among the first genes expressed in transgenic plants, little is known about the functions of the various

plant MT sequences themselves or why there is so much sequence diversity

Introduction of mammalian MT gene into *B. compsisir*, *B. napus* and *N. tabacum* confers Cd tolerance by increasing complexation of metal with MT and minimizing translocation of Cd to the shoots (Corbett, 2002; Hall, 2002; Pal and Rai, 2009). A number of genes in plants have been found to encode MT-like proteins having sequence similarity to animal MT proteins. A number of MT-encoding genes were identified in *Arabidopsis*. MTs have highest complexing affinity for Cu and are induced by exposure to it (Murphy et al., 1997). On Cu treatment, strong induction in MT2 mRNA level has been observed in *Arabidopsis*. When genes for MT 1 and MT2 from *Arabidopsis* were expressed in an MT-deficient yeast mutant, both genes complemented the mutation and provided a high level of resistance to Cu (Goldsbrough, 2000). Similarly, in Cu-sensitive mutants of *Arabidopsis* it was shown that MT genes could be induced by Cu. There appears to be similarity between MTs and PCS for complexation with metals as observed in plants such as datura and maize (Rival et al., 1990). A decrease in MT levels could result in increased level of free transition metal ions (Hall, 2002; Corbett, 2000; Pal and Rai, 2009). Both MTs and PCS helps in chelation and sequestering of toxic heavy metal enhancing the metal detoxification in plants.

4. An insight into heavy metal detoxification in plant: Role of AM fungi

Symbiosis with mycorrhizal fungi has been proposed as one of the mechanisms of plant HM-tolerance (Hall, 2002; Hildebrandt et al., 2007; Joachim et al., 2009). AM have been reported to evolve strategies which can alleviate heavy metal threats in mixed culture systems and, thus, from the food chains (Hall, 2002; Kramer, 2005; Pike and Rennenberg, 2005; Joachim et al., 2009). Amongst the strategies used include immobilisation of metal compounds, precipitation of polyphosphate granules in the soil, adsorption to chitin in the fungal cell walls and chelation of heavy metals inside

the fungus as shown in Fig 2 (Joachim et al., 2009). For example, AM can bind heavy metals beyond the plant rhizosphere by releasing an insoluble glycoprotein commonly known as glomalin (Gonzalez-Chavez et al., 2004; Gohre and Paszkowski, 2006). These authors reported that 1 g of glomalin could extract up to 4.3 mg Cu, 0.08 mg Cd and 1.12 mg Pb from polluted soils (Gonzalez-Chavez et al., 2004). In addition, hyphae binding in the soil are an important sink for heavy metals due to their large surface area. For instance, passive adsorption to the hyphae leads to binding of up to 0.5 mg Cd per g dry biomass (Joner et al., 2000). In other studies, the immobilisation and uptake of Pb was found to be greater in roots of mycorrhizal relative to the non-mycorrhizal plants (Chen et al., 2005). There is also scientific evidence suggesting that fungal vesicles (see fig. 1) may provide additional detoxification mechanisms by storing toxic compounds (Gohre and Paszkowski, 2006). In maize for example, heavy metals are selectively retained in the inner parenchyma cells coinciding with fungal structures (Kaldorf et al., 1999). Similarly, in cropping systems involving legumes such as pea (Joachim et al., 2009) and clover (Medina et al., 2005), AM can provide mycorrhizal buffer to stress imposed by Cd (Rivera-Becerril et al., 2002) or Cd tolerance by changing its polyamine metabolism, thus, stabilising Cd in the root system of colonised plants (Paradi et al., 2003). AM fungi colonise roots of dominant plant species even in highly contaminated soils (e.g., Pawlowska et al., 1996; Leung et al., 2007). These soil microorganisms are generally acknowledged as plant growth promoters, mainly due to the participation of their extensive extraradical mycelium (ERM) in plant nutrient acquisition, phosphorus in particular (Smith and Read, 1997). Although considerable variability in plant responses to AM inoculation has been observed in contaminated soils, the potential of AM fungi to buffer HM-stress has been demonstrated in a number of studies (e.g., Hildebrandt et al., 1999; Janouskova' et al., 2005; Chen et al., 2007). This alleviated influence can be attributed not only to AM-mediated

nutritional effects, but also to the impact of AM fungi on metal distribution at the soil-fungus-plant interface. Immobilisation of metals on both extra- and intraradical fungal tissues has been shown (Kaldorf et al., 1999; Joner et al., 2000), thus providing a plausible explanation for repeatedly reported enhancement of the barrier for metal translocation from the roots to the shoots of inoculated plants (e.g., Joner and Leyval, 1997; Janouskova' et al., 2005; Chen et al., 2007). Similar to plants, AM fungi from contaminated soils have been reported to cope better with HM-toxicity than those not exposed to such long-term selection pressure (Gildon and Tinker, 1981; Weissenhorn et al., 1993; Malcova' et al., 2003a). In spite of increasing knowledge on AM-plant interaction under HM-stress, little is known about whether there is a synergism between plant and fungal HM-tolerance. It can be hypothesized that tolerant AM fungi may confer additional HM tolerance on its host, thus leading to their higher survival rate and reproductive success on contaminated sites. Alternatively, carbon investment into maintenance of AM symbiosis (4-20% of total plant photosynthates) may represent luxurious costs for the tolerant plants that can cope well with HM contamination without being mycorrhizal, but not for the HM sensitive ones.

5. Phytoremediation of heavy metal: Prospect of AM Fungi

High concentrations of heavy metals (HM) in the soil have detrimental effects on ecosystems and are a risk to human health as they can enter the food chain via agricultural products or contaminated drinking water. Phytoremediation, a sustainable and inexpensive technology based on the removal of pollutants including HM from the environment by plants, is burning issue in plant research. However, as phytoremediation is a slow process, improvement of efficiency and thus increased stabilization or removal of HMs from soils is an important goal. It has been suggested that heavy metal-tolerant AM fungi could protect plants against harmful effect of heavy metals. During symbiotic

interaction the hyphal network functionally extends the root system of their hosts. Thus, plants in symbiosis with AM fungi have the potential to take up HM from an enlarged soil volume. Colonization with AM-fungi has convincingly been shown to alleviate heavy metal-induced stress. 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.

The prospect of AM fungi present in heavy metal-contaminated soils has significant implications for phytoremediation. Since heavy metal uptake and tolerance depend on both plant and soil factors,

including soil microbes, interactions between plant root and their symbionts such as AM fungi can play an important role in conferring plant tolerance to metal stress. Mycorrhizal associations increase the absorptive surface area of the plant due to extrametrical fungal hyphae exploring rhizospheres beyond the root-hair zone, which in turn enhances water use efficiency and mineral uptake. AM fungi can also act as a filtration barrier against transfer of heavy metals to plant shoots. The protection and enhanced capability of uptake of minerals result in greater biomass production, which is an important criteria for successful remediation. Indigenous AM isolates existing naturally in heavy metal-polluted soils are more tolerant than isolates from non-polluted soils, and are reported to efficiently colonize plant roots in heavy metal-stressed environments. Thus, it is important to screen indigenous and heavy metal-tolerant isolates in order to guarantee the effectiveness of AM symbiosis in clean up of metal contaminated soils. It is further suggested that the potential of phytoremediation of heavy metal polluted soil can be enhanced by inoculating hyper-accumulator plants with mycorrhizal fungi most appropriate for the polluted site. It is therefore of great importance that we combine selected plants with specific AM fungal isolates adapted to high concentrations of heavy metal in future research for phytoremediation programmes. However, there is need to develop new methods and optimize the conditions to grow in large quantities and characterize, develop and screen large number of AM fungi for tolerance to metals. The lack of correlation between colonization rates and a beneficial or detrimental host response perhaps suggests the need to dissect the molecular mechanism underlying interaction between AM fungi and plant roots resulting into altered rhizosphere properties. Identifying and culturing the most effective isolates could then be undertaken to select or develop genetically improved strains customized for a particular set of conditions or host plants.

AM fungi are asexual organisms and refractory to transformation. Therefore,

transgenic approaches cannot be undertaken to improve fungal phytoremediation properties. Thus it is essential to understand physio biochemical and molecular events during interaction host and AM symbionts to effectively employ them for phytoremediation or to define fungal genes attractive for introduction into plant backgrounds. For example, during analysis of differentially expressed genes in the presymbiotic versus the symbiotic stage of *Gigaspora margarita*, Lanfranco et al. (2002) identified a fungal metallothionin GmarMT1. Metallothionins are ubiquitous proteins that are involved in HM sequestration in plants. In heterologous complementation assays, the *G. margarita* protein conferred resistance to Cd and Cu, suggesting a similar function in AM fungi. GmarMT1 expression occurs throughout the whole life cycle of the fungus but is higher in presymbiotic than in symbiotic fungal structures. Copper induced expression of GmarMT1 in symbiotic mycelia, but no effect of Cd treatment and was suggested that the protein similar to plant metallothionins was involved in the HM resistance. Lanfranco et al. 2005 reported a gene encoding a functional Cu/Zn superoxide dismutase (SOD) (GmarCuZnSOD) act as a primary defense during oxidative stress by protecting cell membranes from damage caused by reactive oxygen species. A fungal Zn-transporter has been identified in *G. intraradices* (GintZnT1) (Gonzalez-Guerrero et al. 2005) that gene is upregulated in the extraradical hyphae by Zn. The authors suggested that GintZnT1 is involved in Zn efflux and, thus, in protection of *G. intraradices* against Zn stress. The identification of fungal genes with beneficial properties for soil remediation routines when expressed in phytoremediation crops represents an important goal. Since AM fungi can compensate for inefficient plant nutrient and HM uptake, they should be integrated into the design of future soil clean-up strategies with, for example, poplar. Studies of contaminated sites provide fungal isolates highly suitable for phytoremediation. It is, challenging to try to draw general conclusions about the usefulness of AM fungi for soil remediation. However,

enhanced understanding of mycorrhizal biology and of the HM tolerance of plants and fungi has defined valuable parameters for improving phytoremediation. One example is the use of adapted indigenous fungal strains that are more suitable for phytostabilization and extraction purposes than laboratory strains. It will be important in future to include the AM symbiosis in the design of both research plans and applications, with the ultimate goal of increasing the efficiency of phytoremediation

6. Conclusion and future perspectives

Phytoremediation is an emerging biobased alternative technology in the clean up of metal contaminated soil. The prospect of symbiont existing in heavy metal contaminated soil has important implication for phytoremediation. Mycorrhizal associations increased the absorptive surface area of the plant due to extra-matrical fungal hyphae exploring rhizosphere beyond the root hair zone, which in turn enhanced water and mineral uptake. The protection and increased mineral uptake results in greater biomass production which is important for successful remediation. The potentials of phytoremediation of metal polluted soil can be enhanced by inoculating hyper accumulator plants with mycorrhizal fungi most appropriate for polluted sites. The studies relating the dynamics of AM symbiosis in heavy metal phytoremediation revealed the existence of compromise between plant growth and heavy metal tolerance indicating the importance of metal binding process in buffering the soil environment. It is hence important to understand the contribution of AM symbionts to soil productivity and enhanced metal uptake at molecular level. Hence a comprehensive molecular physiological understanding of mycorrhizosphere dissecting the role of plant and mycorrhizal gene interaction in the process would be valuable to decipher plant tolerance mechanism under heavy metal stress specially with respect to phytoremediation.

References

- Asada, K and M. Takahashi, 1987. Production and Scavenging of Active Oxygen in Photosynthesis. In: Photoinhibition: Topics in Photosynthesis, Kyle, D.J., C.J. Osmond and C.J. Artzen (Eds.). Elsevier, Amsterdam, pp: 227-287.
- Azevedo, J.A and Azevedo, R.A, 2006. Heavy metals and oxidative stress: where do we go from here? Comm Biometry Crop Sci., 1 (2): 135-138.
- Benavides, M.P., Gallego, S.M and Tomaro, M.L, 2005. Cadmium toxicity in plants. Braz. J Plant Physiol., 17: 21-34.
- Chen, Z., Tang, J., Zhi, G and Hu, S, 2005. Arbuscular mycorrhizal colonization and phosphorus acquisition of plants: effects of coexisting plant species. Appl. Soil Ecol., 28: 259-269.
- Chen, B.D., Thu, Y.G., Duan, J., Xiao, X.Y and Smith, S.E, 2007. Effects of the arbuscular mycorrhizal fungus *Glomus mosseae* on growth and metal uptake by four plant species in copper mine tailings. Environ. Pollut., 147: 374-380.
- Clemens, S, 2001. Molecular mechanism of plant metal tolerance and homeostasis . Planta, 212: 475 - 486.
- Clemens, S., Bloss, T., Vess, C., Neumann, D., Nies, D.H and zur Nieden, U, 2002. A transporter in the endoplasmic reticulum of *Schizosaccharomyces pombe* cells mediates zinc storage and differentially affects transition metal tolerance. J Biol Chem., 277:18215- 18221.
- Cobbett, C.S, 2000. Phytochelatins and their role in heavy metal detoxification. Plant Physiol., 123: 825 - 883.
- Dietz, K.J., U. Kramer and M. Baier, 1999. Free Radicals and Reactive Oxygen Species as Mediators of Heavy Metal Toxicity. In: Heavy Metal Stress in Plants: From Molecules to Ecosystems, Prasad, M.N.V. and J. Hagemeyer (Eds.). Springer-Verlag, Berlin, pp: 73-97.
- Gildon, A and Tinker, P.B, 1981. A heavy metal tolerant strain of mycorrhizal fungus. Trans. Br. Mycol. Soc., 77, 648-649
- Gohre, V and Paszkowski, U, 2006. Contribution of arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. Planta, 223:1115-1122.
- Goldsbrough, P, 2000. Metal tolerance in plants: the role of phytochelatins and metallothiones - In: Terry, N., Banuelos, G. (ed) Phytoremediation of contaminated soil and water. Pp. 221 - 233, CRC Press, LLC.
- Gonzalez-Chavez, M.C., Carrillo-Gonzalez, R., Wright, S.F and Nichols, K.A, 2004. The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. Environ Pollut, 130:317-323
- Gonzalez-Guerrero, M., Azcon-Aguilar, C., Mooney, M., Valderas, A., MacDiarmid, C.W., Eide, D.J and Ferrol, N. 2005. Characterization of a *Glomus intraradices* gene encoding a putative Zn transporter of the cation diffusion facilitator family. Fungal Genet Biol., 42:130-140.
- Gratão, P.L., Polle, A., Lea, P.J and Azevedo, R.A, 2005. Making the life of heavy metal-stressed plants a little easier. Funct Plant Biol., 32: 481-494.
- Grill, E., Winnacker, E. L and Zenk, M.H. 1985. Phytochelatins: the principal heavy-metal complexing peptides of higher plants. Science, 230: 674- 676
- Hall, J.L, 2002. Cellular mechanism of heavy metal detoxification and tolerance. J. Exp. Bot., 53(366): 1 - 11.
- Halliwell, B, 1987. Oxidative damage, lipid peroxidation and antioxidant protection in chloroplast. Chem. Phys. Lipids, 44: 327-340.
- Hildebrandt, U., Kaldorf, M and Bothe, H, 1999. The zinc violer and its colonization by arbuscular mycorrhizal fungi. J. Plant Physiol., 154: 709-717.
- Hildebrandt, U., Regvar, M and Bothe, H, 2007. Arbuscular mycorrhiza and heavy metal tolerance. Phytochemistry, 68: 139-146.
- Jamal, A., Ayub, N and Usman Mand Khan, A.G, 2002 Arbuscular mycorrhizal fungi enhance zinc and nickel uptake from contaminated soil by bean and lentil. Int. J. Phytorem. 4, 205-221
- Janouskova, M., Pavlikova, D., Macek, T and Vosatka, M, 2005. Arbuscular mycorrhiza decreases cadmium phytoextraction by

- transgenic tobacco with inserted metallothionein. *Plant Soil*, 272: 29-40.
- Joner, E.J and Leyval, C, 1997. Uptake of ¹⁰⁹Cd by roots and hyphae of a *Glomus mosseae*/*Trifolium subterraneum* mycorrhiza from soil amended with high and low concentrations of cadmium. *New Phytol.*,135:353-360.
- Joner, E.J., Briones, R and Leyval, C, 2000. Metal-binding capacity of arbuscular mycorrhizal mycelium. *Plant Soil*, 226:227-234.
- Kaldorf, M., Kuhn, A.J., Schroder, W.H., Hildebrandt, U and Bothe, H, 1999. Selective element deposits in maize colonized by a heavy metal tolerance conferring arbuscular mycorrhizal fungus. *J. Plant Physiol.*,154:718-728.
- Kramer, U., 2005. Phytoremediation: novel approaches to cleaning up polluted soils. *Curr. Opin. Biotechnol.*,16: 133-141.
- Lanfranco, L, Novero, M and Bonfante P, 2005. The mycorrhizal fungus *Gigaspora margarita* possesses a CuZn superoxide dismutase that is up-regulated during symbiosis with legume hosts. *Plant Physiol.*, 137:1319-1330.
- Leung, H.M., Ye, Z.H and Wong, M.H, 2007. Survival strategies of plants associated with arbuscular mycorrhizal fungi on toxic mine tailings. *Chemosphere*, 66: 905-915.
- Joschim H.J., Makoi, R and Ndakidemi, P.A, 2009. The agronomic potential of vesicular-arbuscular mycorrhiza (AM) in cereals- legume mixtures in Africa. *Afr. J. Microbiol Res.*, 3(11) : 664-675.
- Malcova, R., Vosatka, M., Gryndler, M, 2003. Effects of inoculation with *Glomus intraradices* on lead uptake by *Zea mays* L. and *Agrostis capillaris* L. *Appl. Soil Ecol.*, 23: 255 - 267.
- Marx, D. H, 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections- antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. *Phytopathology* , 59: 158-163.
- Medina, A., Vassilev, N., Barea, J.M and Azcon, R, 2005. Application of *Aspergillus niger* treated agrowaste residue and *Glomus mosseae* for improving growth and nutrition of *Trifolium repens* in a Cd contaminated soil. *J. Biotechnol.*, 116: 369-378.
- Mishra, S and Dubey, R.S, 2006. Heavy metal uptake and detoxification mechanisms in plants. *Int J.Agric. Res.*, 1(2):122-141.
- Mittler, R, 2002. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.*, 7: 405-410.
- Ouziad, F., Hildebrandt, U., Schmelzer, E and Bothe, H, 2005. Differential gene expressions in arbuscular mycorrhizal-colonized tomato grown under heavy metal stress. *J Plant Physiol*,162:634-649.
- Ouziad, F., Wilde, P., Schmelzer, E., Hildebrandt, U and Bothe, H, 2006. Analysis of expression of aquaporins and Na⁺/H⁺ transporters in tomato colonized by arbuscular mycorrhizal fungi and affected by salt stress. *Environ. Exp. Bot.*, 57: 177-186.
- Pal, R and Rai J. P. N, 2009. Phytochelatin: Peptides Involved in Heavy Metal Detoxification. *Appl Biochem Biotech.* DOI. 10.1007/s12010-009-8565-4.
- Panda, S. K, 2008. Impact of copper on reactive oxygen species, lipid peroxidation and antioxidants in *Lemna minor*. *Biol Plant.*, 52 (3): 561-564.
- Parádi, I., Bratek, Z and Láng, F, 2003. Influence of arbuscular mycorrhiza and phosphorus supply on polyamine content, growth and photosynthesis of *Plantago lanceolata*. *Biol. Plant.*, 46: 563-569.
- Pawlowska, T.E., Blaszkowski, J and Ruhling, A , 1996. The mycorrhizal status of plants colonizing a calamine spoil mound in southern Poland. *Mycorrhiza*, 6: 499-505.
- Peuke, A.D and Rennenberg, H, 2005. Phytoremediation. *EMBO Rep*, 6:497-501.
- Quilambo, O.A, 2000. Functioning of peanut (*Arachis hypogaea*L.) under nutrient deficiency and drought stress in relation to symbiotic associations. PhD thesis. University of Groningen, the Netherlands. Van Denderen B.V., Groningen. ISBN 903671284X.
- Rhody, D, 2002. Erste Schritte zur Etablierung und Verbesserung von

- Transformationssystemen für wurzelbesiedelnde Pflanzen. PhD thesis, The University of Marburg, Germany.
- Rivera-Becerril, F., Calantzis, C., Turnau, K., Caussanel, J.P., Belimov, A.A., Gianinazzi, S., Strasser, R.J and Gianinazzi-Pearson, V, 2002. Cadmium accumulation and buffering of cadmium-induced stress by arbuscular mycorrhiza in three *Pisum sativum* L. genotypes. *J. Exp. Bot.*, 53: 1177-1185.
- Salt, D.E., Blaylock, M., Kumar, P.B.N., Dushenkov, E., Ensley, B.B., Chet, I and Raskin, I, 1995. Phytoremediation: A noble strategy for the removal of toxic metals from the environment using plants. *Biotechnology* 13: 468 - 474.
- Schreiner, R.P., Mihara, K.L., McDaniel H and Bethlenfalvay, G.J, 1997. Mycorrhizal functioning influence plant and soil functions and interactions *Plant Soil* , 188: 199-209.
- Sieverding, E, 1991. Vesicular-arbuscular mycorrhiza management in tropical agrosystems. Technical Cooperation, Federal Republic of Germany Eschborn. ISBN 3-88085-462.
- Schützendübel, A and Polle, A, 2002. Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. *J. Exp. Bot.*, 53: 1-15.
- Smith, S.E and Read, D.J, 1997. Mycorrhizal Symbiosis. Academic Press, London.
- Sottosanto, J.B., Gelli, A and Blumwald, E, 2004. DNA array analyses of *Arabidopsis thaliana* lacking a vacuolar Na⁺/H⁺ antiporter: impact of AtNHX1 on gene expression. *Plant J.*, 40: 752-771.
- Tewari, R.K., Kumar, P and Sharma, P.N., 2008. Morphology and physiology of zinc stressed mulberry plants. *J. Plant Nutr. Soil Sci.*, 171: 286-294.
- Weissenhorn, I., Leyval, C and Berthelin, J, 1993. Cd-tolerant arbuscular mycorrhizal (AM) fungi from heavy-metal polluted soils. *Plant Soil*, 157: 247-256.