Chapter 14
Arbuscular Mycorrhiza: Approaches for Abiotic Stress Tolerance in Crop Plants for Sustainable Agriculture

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1 Introduction

Plant growth and productivity is severely affected by various environmental stresses, such as, drought, salinity, and heavy metals worldwide. Global effects on desertification, soil salinization, scarcity of water resources, soil heavy metal contamination, and effects of other unfavorable factors are predicted to cause dramatic changes in the climatic conditions of arable lands, and together represent the primary cause of crop loss by more than 50% (Alcázar et al. 2006). Improving plant tolerance and maintaining crop productivity against such abiotic stresses is a major challenge for sustainable agriculture. Besides the intrinsic capacity of plants to tolerate abiotic stresses, most plants in nature establish a symbiosis with arbuscular mycorrhizal fungi (AMF) by which plants increase their tolerance to several stressful conditions thus, maintain growth and productivity (Ruiz-Lozano et al. 2006).

Arbuscular mycorrhiza is the symbiotic association between plant roots and fungi of the phylum Glomeromycota. Both of them derive benefits from the interaction; mycorrhizal fungi improve the nutrient status, water absorption, growth and enhance the host plant's resistance to biotic and abiotic stresses, while the host plant is necessary for fungal growth and reproduction by providing carbon in the form of photosynthates (Smith and Gianinazzi 1988; Smith and Read 1997). This interaction plays very important functions in plant ecosystem because more than 80% plant species depend on it for mineral uptake and their normal functioning (Remy et al. 1994; Smith and Read 1997).

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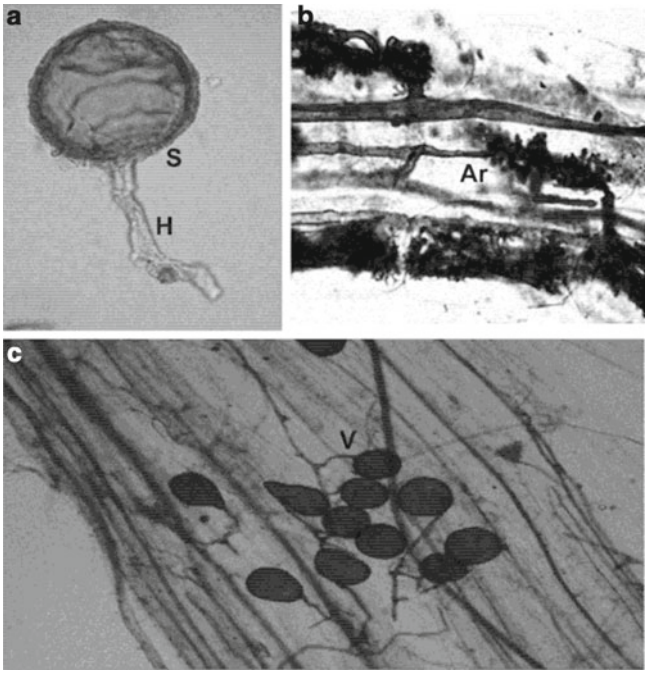

Fig. 14.1 AMF colonization in *Trigonella foenum-graecum.* The figure shows the different structures of AMF colonization: (**a**) a germinating spore (**b**) the arbuscules and (**c**) vesicles. The germinating spore colonizes the plant roots and forms arbuscules and vesicles in the cortical cell. The arbuscules are dichotomously branched tree like structures originating from branches of the intraradical hyphae after the branch hypha penetrates through the cortical cell wall. An arbuscule forms between the cell wall and plasma membrane and are the sites for exchange between the two symbiotic partners. Vesicles are thin-walled lipid-containing bodies produced terminally or intercalarily from hyphae in the root cortex and serve as the storage organ of the fungus

The three important components of mycorrhizal symbiosis are the root, the fungal intraradical structures in the root and an extraradical mycelium (ERM) in the soil. The intraradical structures formed by AMF in the host root cortical cells are intracellular hyphal coils and/or dichotomously branched structures—the arbuscules, hypertrophied hyphae—the vesicles (Fig. 14.1). Arbuscules are the sites of nutrient exchange between the symbionts and vesicles function as the fungal organs of storage (Smith and Read 1997). ERM surrounding the root is profusely branched absorbing hyphae, which form a network extending into the soil. ERM increases the total absorptive surface area of root and help in acquiring nutrients even beyond the depletion zone which develops around plant roots (George et al. 1992). The effect of AMF on phosphorus uptake is particularly large because of poor mobility of phosphate ions in soil (Hooker and Black 1995). AMF produces copious amounts of a stable hydrophobic glycoprotein called glomalin, which is deposited on the outer hyphal walls of the ERM and on adjacent soil particles and play an important role in soil structure stabilization (Wright et al. 1996; Wright and Upadhyaya 1998).

This involvement of fungal mycelium in soil conservation has a great relevance in sustainable systems (Barea and Jeffries 1995).

The development of a functional AM symbiosis is a multi-step process which is dependent on molecular cross talk between the two symbionts. It involves a sequence of recognition events leading to the morphological and physiological integration of these symbionts (Gianinazzi et al. 1995; Giovannetti and Sbrana 1998) (Fig. 14.1). The development of AM symbiosis involves induction of spore germination and hyphal growth (Becard and Piche 1990) contact between hyphae and root surfaces which includes recognition and formation of appresoria on the root epidermal cells (Giovannetti and Citernesi 1993; Bonfante and Bianciotto 1995); hyphal penetration into roots, which is characterized by localized production of wall-degrading hydrolytic enzymes by the fungus and by the exertion of hydrostatic pressure by the hyphal tip (Perotto et al. 1994; Giovannetti and Sbrana 1998); formation of arbuscules and establishment of a functional symbiosis (Samra et al. 1997).

Though, it is well established that AMF play very significant role in plant growth and productivity under abiotic stress conditions, however, due to their obligate nature the main obstacle in using AMF at large scale in agriculture is the lack of reliable and easily implementable methods for mass inoculum production. The most widely used standard and conventional method of maintaining and multiplying AMF is soil-based pot culture method. Besides, a few other methods such as inoculum rich soil pellets, aeroponic culture nutrient film technique, polymer-based inoculum, and root organ culture have also been introduced.

Under abiotic stress, colonization of plants with AMF assist plants to withstand these stresses. AMF enhance plant growth, productivity, and nutrient uptake under stress conditions. They also enhance osmolyte production, influence plant–water relation, and rate of photosynthesis, alter leaf water potential, ionic balance, antioxidant production and other physiological and biological parameters and thus improve plant's capacity to tolerate abiotic stresses. This chapter provides an overview of the mechanisms evolved by AMF to aid plants survive in these stressful conditions (drought, salinity, and heavy metals).

2 Salinity

Huge losses in arable land due to salinity are a major concern for sustainable agriculture. Salinity is a soil condition characterized by a high concentration of soluble salts. Soils are classified as saline when the electrical conductivity of the soil is 4 dS/m or more (Richards 1954), which is equivalent to approximately 40 mM NaCl and generates an osmotic pressure of approximately 0.2 MPa (Munns and Tester 2008). Salinity arises due to deposition of salts via two natural processes—weathering of rocks containing soluble salts of various types, mainly chlorides of sodium, calcium, and magnesium, and to a lesser extent, sulfates and carbonates; and deposition of oceanic salt (mainly NaCl) carried by inland wind and rain (Munns and Tester 2008). Thus, the major ions contributing to soil salinity include cations

(Na^+, Ca^{2+}, Mg^{2+}, and K^+), anions (Cl^-, SO_4^{2-}, HCO_3^-, CO_3^{2-}, and NO_3^-). Other constituents contributing to salinity in hyper saline soils and water include B, Sr^{2+}, SiO_2, Mo, Ba^{2+}, and Al^{3+} (Hu and Schmidhalter 2002). Besides, irrigation water and fertilizers used in agriculture, low precipitation, and over-exploitation of available water resources also contribute significantly to soil salinity (Canterll and Linderman 2001; Al-Karaki 2006). Of all the salts, sodium chloride is the most soluble and abundant salt released (Munns and Tester 2008). At present, cultivated land affected by salt amounts to 77 million hectares and constitutes 5% of 1.5 billion hectares cultivated land around the world. It is projected that increased salinization of arable land will result in to 50% land loss by the middle of the twenty-first century (Wang et al. 2003). The alarming rate of increase in soil salinity in agricultural land creates a distress to agriculture as most of the economically important crop species are very sensitive to soil salinity (Mahajan and Tuteja 2005) and have resulted in decreased crop production of more than 20% irrigated land worldwide (Porcel et al. 2012).

Excessive salts in soil, in particular, Na^+ ions alter the basic structure of the soil (Mahajan and Tuteja 2005). The presence of Na^+ ions in the cation exchange complex render the soil compact and subsequently reduce soil porosity and hamper soil aeration (Manchanda and Garg 2008). Low soil aeration due to high salt concentration has a direct relation with all major living processes, such as reduction in growth, photosynthesis, protein and lipid metabolism (due to salt-induced osmotic imbalance), nutritional disorder, and ion toxicity in plants (Evelin et al. 2012). Osmotic imbalance in a salt stressed plant is often manifested as retardation in growth of the plants with the leaves and stems appearing stunted (Singh and Charath 2001). This effect of salt is primarily due to (1) decrease in the plant's ability to take up water and nutrients as a result of osmotic or water-deficit (physiological drought) effect of salt (Evelin et al. 2009) and; (2) uptake of salt by plants from the soil through transpiration stream which injure cells in the transpiring leaves thereby inhibiting cell division and enlargement in plant's growing point (Manchanda and Garg 2008). Diversion of energy to counteract the accumulation of salts in the cells may also contribute to the stunted growth of the plants grown in saline soils (Evelin et al. 2009). Continued uptake of salt by plants and subsequent significant increase in the concentration of salts decreases the size of the leaves (Singh and Charath 2001) and induce leaf senescence by affecting the structure of chlorophyll molecules and photosynthesis. Specific effects of salt stress on leaf senescence have been related to accumulation of toxic ions (Na^+ and Cl^-) or to K^+ and Ca^{2+} depletion (Yeo et al. 1991). Salt-induced ionic imbalance and toxicity is perceptible in the form of disruption in the plant mineral relations. This may be explicated by the effects of salinity on nutrient availability, competitive uptake, transport or partitioning within the plant or may be caused by physiological inactivation of a given nutrient resulting in an increase in the plant's internal requirement for that essential element thereby causing ionic imbalance in the cell (Grattan and Grieve 1999). At the whole plant level, salinity frequently induces an increase in Na^+ and Cl^- ions as well as a decrease in K^+, Ca^{2+}, NO_3^-, and Pi concentrations (Shokri and Maadi 2009). Therefore, high concentrations of Na^+ and Cl^- ions in the soil solution may depress nutrient-ion activities and produce extreme ratios of Na^+: K^+, Na^+: Ca^{2+}, Ca^{2+}: Mg^{2+}, and Cl^-: NO_3^- (Evelin et al. 2012).

3 Arbuscular Mycorrhiza in Mitigation of Salt Stress

The role of AMF in mitigation of the effects of salt stress in plants is widely recognized. Arbuscular mycorrhizal fungi stimulated alleviation of salt stress effects and tolerance of host plants is brought about by combining nutritional (enhancing/selective uptake of nutrients and prevention of nutritional disorder), biochemical (accumulation of osmoregulators, control of reactive oxygen species (ROS) and enhanced activities of antioxidant enzymes and molecules), physiological (photosynthetic efficiency) and structural adaptations. Recent molecular studies have indicated that the regulation of AM-induced plant salt tolerance may involve mechanisms at the molecular level. Figure 14.2 illustrates the mechanisms of AM-mediated improved tolerance of host plants in salt and drought stress.

3.1 Enhance Growth and Biomass

Salinity-induced retardation of growth and fitness of plants has been shown to be alleviated by AM colonization. Under salt stress, plant growth and biomass, measured as indicators of plant fitness are higher in AM than the non-AM plants (Giri et al. 2007; Shokri and Maadi 2009; Evelin et al. 2012; Latef and Chaoxing 2011). The increased dependency of host plants on AMF under salt stress is an affirmation that AMF enhance plant growth (Borde et al. 2011; Kumar et al. 2011). Better growth of AM plants as compared to non-AM plants under salt stress is attributed to improved nutrient uptake, especially phosphorus (P), and other nutrients, mycorrhiza-mediated effects on water absorption and increased photosynthetic system (Giri et al. 2003; Garg and Manchanda 2009; Abdel-Fattah and Asrar 2011; Evelin et al. 2012; Kumar et al. 2011).

3.2 Prevention of Nutrient Deficiency and Ion Toxicity

Plants, for their growth and survival require 14 essential nutrients of which the macro-nutrients nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) are present in large amounts in tissues. The micronutrients (copper (Cu), iron (Fe), molybdenum (Mo), manganese (Mn), zinc (Zn)), though required in very small amount by the plants, are indispensable for plant growth and survival. Under salt stress, the plants are deprived of these nutrients due to the effects of salinity on availability, uptake, transport or physiological inactivation of a given nutrient (Grattan and Grieve 1999). Several studies have shown that the effects of salt-induced ion toxicity and nutrient deficiency can be ameliorated if the plants are colonized by AMF. The ERM of AMF has the ability to explore and exploit the growth medium thereby facilitating the uptake of nutrients.

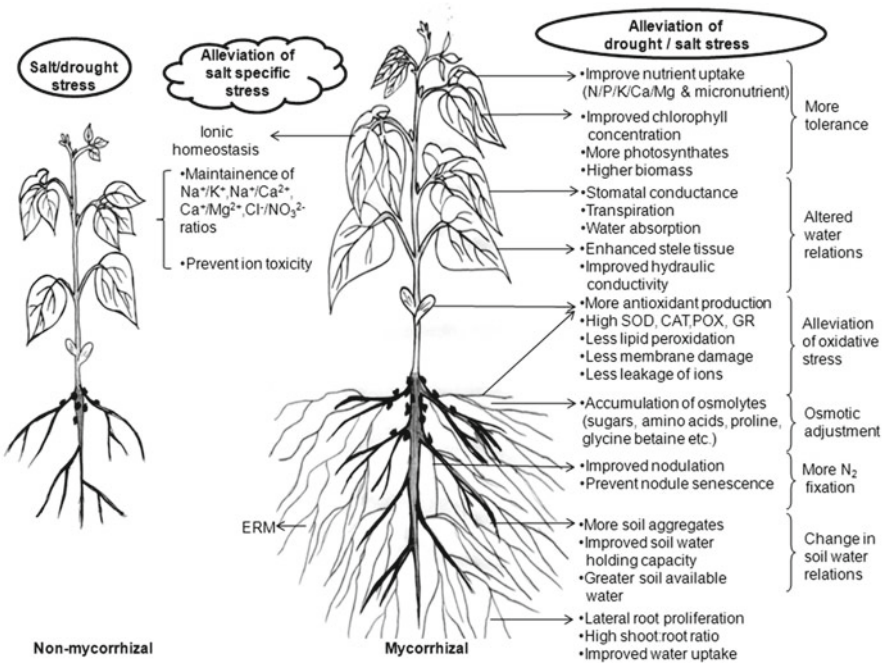

Fig. 14.2 Role of AMF colonization in alleviation of salt and drought stress. Under salt and drought stresses, the plants experience similar physiological effects. The plant on the *left* side depicts a salt/drought-stressed plant and shows lesser growth and biomass due to lower photosynthetic efficiency, greater degree of oxidative damage, lesser uptake water and nutrients and osmotic imbalance. On the *right* side is depicted an AMF-colonized plant under salt and drought stress. The presence of AMF in the plant roots and rhizosphere improves the quality and water holding capacity of the soil thereby altering soil-water relations. AMF colonization also induces lateral root proliferation and aids in uptake of water and nutrients. This is also facilitated by sturdier vascular system in AM plants which possess a higher rate of transpiration and stomatal conductance; thus improving water use efficiency. Better nutrient uptake in AM plants prevents nutrient deficiency. In plants under salt stress, better nutrient uptake also resulted in prevention of ion toxicity and maintenance favorable ionic ratios. There is higher accumulation of osmolytes in AM plants and to counteract the osmotic imbalance generated due to salt and drought stress. The oxidative damage under stress is also better prevented in AM plants by activation of antioxidant enzymes and molecules thereby preventing membrane and cellular damage preventing the leakage of ions. Lower damage in photosynthetic apparatus results in a higher rate of photosynthesis which leads to higher biomass, shoot: root ratio and leaf area, which in turn is responsible for more photosynthates. In legumes, AMF colonization improved nodulation and prevent nodule senescence thereby improving N_2 fixation

Furthermore, the ERM can also detect and show physiological plasticity in response to the nutrient status of their environment and host (Hodge et al. 2010). It is by virtue of this property of ERM that AMF pursue improved and/or selective uptake of nutrients as one key mechanism to prevent nutritional disorder and ionic imbalance in host plants in saline soils. The runner hypha radially extends the AMF colony and upon perception of signals from the nutrient ions, it produces branched

absorbing structures (BAS) or spores which absorb the nutrients and translocates them to the plants (Hodge et al. 2010). Mycorrhizal plants can therefore potentially access nutrients from a larger area than the non-mycorrhizal (NM) equivalents So, under salt stress, when the availability of the nutrients to plants are limited due to physiological drought, AM colonization offers huge benefit to host plants by improving the uptake of essential nutrients.

Arbuscular mycorrhizal fungi facilitate the uptake of N, P, K, Ca, Mg, Cu, Fe, Zn while successfully limiting the uptake of Na^+ and Cl^-. This buffering activity of AMF aids in preventing salt-induced ion toxicity while alleviating nutrient deficiency and other related cellular effects. For example, improved N and Mg uptake prevents degradation of chlorophyll and protein by Na^+. Higher P and Ca relieve the membrane system of the plant from the possible attack by ROS that cause peroxidation of the membrane lipids. While the maintenance of membrane integrity in the tonoplast imparts successful compartmentalization of excess Na^+ and Cl^- ions in the vacuole, the integrity of plasma membrane prevents leakage of cellular contents. In this way, ion toxicity is contained and cellular damage is prevented. The compartmentalization of Na^+ and Cl^- ensures higher K^+:Na^+, Ca^{2+}:Na^+, and NO_3^-:Cl^- ratios in the plant indicating smooth metabolic processes in the plant. Higher P-concentration in tissues also induces uptake and translocation of micro-nutrients by increasing the sink size in plants (Liu et al. 2000b). Understanding and elucidation of the above mechanism is a result of extensive investigation.

At the moment, the explanation of the molecular regulation of the mechanisms of AM-regulated uptake of nutrients is not complete. An encouraging point is that significant breakthroughs have been made in this aspect in the last few years. For instance, it has been proved that N absorbed through AMF constitutes a fifth of plant N (Leigh et al. 2009). Nitrogen is taken up by ERM as inorganic N from the soil in the form of nitrate and assimilated via nitrate reductase located in the arbuscule-containing cells (Kaldorf et al. 1998), and converted to arginine via the GS-GOGAT (glutamine synthetase-glutamine: 2-oxoglutarate amidotransferase) cycle. Arginine in the hyphae is broken down to urea and ultimately transferred to the plant as NH_4^+ with the resulting C skeletons from arginine breakdown re-incorporated in to the fungal C pools (Bago et al. 2002; Govindarajulu et al. 2005). The identification of ammonium transporter gene in ERM of *Glomus intraradices* (López-Pedrosa et al. 2006), a mycorrhiza-specific plant ammonium transporter in *Lotus japonicus* that is expressed in arbusculated cells (Guether et al. 2009), and up-regulation of an ammonium transporter in *Medicago truncatula* (Gomez et al. 2009) makes the mechanism clearer.

Similarly, in the case of P, high affinity phosphate transporters in ERM take up P and transport it within the fungus as polyphosphate, and once in the intraradical hyphae the long chains are hydrolyzed, facilitating transfer to the host plant via a phosphate transporter (Harrison and van Buuren 1995; Harrison 1999; Bago et al. 2002; Ohotomo and Saito 2005). Adding up, higher affinity for phosphate ions, lower threshold concentration for P, ability to store larger amounts of absorbed P than the plant roots also aids the continued movement of P by AM symbiosis into the hyphae (Bolan 1991).

AM-mediated maintenance of higher K^+:Na^+ ratio in host plants is accomplished by regulating the expression and activity of K^+ and Na^+ transporters and of H^+ pumps that generate the driving force for transport of ions (Parida and Das 2005). The Na^+/H^+ antiporter catalyze the transfer of Na^+ out of the cytoplasm into either vacuole or apoplasm (Ouziad et al. 2006). Though Ca^{2+}:Na^+ ratio has been shown to be influenced by AM colonization, the molecular mechanism is yet to be deciphered.

Compared to macro-nutrients, studies on the regulation of the mechanism of micro-nutrients uptake is rather rare. Till date, there is only a single report for a putative zinc transporter (González-Guerrero et al. 2005) in AMF. This lack of knowledge masks the AM-mediated uptake of micro-nutrients, therefore deserves extensive investigations.

3.3 Osmotic Adjustment

One of the mechanisms on how AMF impart tolerance to host plants is the adjustment of salt-induced osmotic imbalance. Osmotic adjustment is brought about by the accumulation of metabolites that act as compatible solutes. The compatible solutes, also called osmolytes, are so named because they do not interfere with normal biochemical reactions; rather they replace water in biochemical reactions. Frequently investigated osmolytes include proline, glycinebetaine, sugars, and polyols (Hasegawa et al. 2000; Parida and Das 2005).

The primary role of these osmolytes in plant cell is to adjust the osmotic potential of the soil with respect to the surroundings. However, these osmolytes are also bestowed with various other functions in plants. For instance, proline can also act as a signaling molecule and influence defense pathways, regulate complex metabolic and developmental processes, offers additional opportunities for plant improvement (Szabados and Savoure 2009).

Glycinebetaine, the non-toxic cellular osmolyte enhances tolerance to salt stress by various ways: (1) stabilize the structures and activities of enzymes and protein complexes; (2) maintain the integrity of membranes against the damaging effects of excessive salt; (3) protection of the photosynthetic machinery; (4) induction of specific genes whose products are involved in stress tolerance; (5) reductions in levels of ROS under stress; and (6) regulation of the activities of ion-channel proteins either directly or via protection of the plasma membrane (Gorham 1995; Chen and Murata 2008).

In AM plants, often the accumulation of proline, glycinebetaine, sugars, organic acids, and polyols is higher as compared to their non-AM equivalents under salt stress (Al-Garni 2006; Sannazzaro et al. 2007; Garg and Manchanda 2009; Sheng et al. 2011). Higher accumulation of osmolytes leads to better osmo-regulation. However, a few studies have reported lower proline accumulation in AM plants as compared to non-AM plants and may be explained due to stress avoidance (exclusion of salt). The variable effects of mycorrhizal colonization on proline levels of plants under salt stress may be related to the differences between plant species and effects of different composition of the inoculum.

The increase in total sugars in mycorrhizal plants under salt stress has also been related to improved osmotic adjustment. Sugars can prevent structural changes in soluble protein, maintain the osmotic equilibrium in plant cells, and protect membrane integrity (Abd-El Baki et al. 2000). The enhanced sugar accumulation in AM plants may be explained by the sink effect of fungus demanding sugars from the shoot tissues (Augé 2001), increased rates of photosynthesis and of carbon compounds to the root system, hydrolysis of starch, higher concentration of organic acid in AM plants (Sheng et al. 2011).

While modulation of polyamine pools may be projected as one of the mechanisms used by AMF to improve plant adaptation to saline soils (Sannazzaro et al. 2007), organic acids besides osmotic adjustement are credited with balancing cation excess and pH homeostasis (Hatzig et al. 2010). Moreover, high amount of organic acids, especially malic acid, can enhance sugar synthesis through facilitation of CO_2 delivery to the Calvin cycle (Chollet et al. 1996).

3.4 Maintenance of Reactive Oxygen Species Level

AM-mediated salt tolerance by host plants has also been attributed to its ability to detoxify ROS and maintain the delicate balance between ROS and antioxidants. ROS is an inevitable byproduct of plant metabolism produced essentially from photosynthesis, photorespiration and respiration. However, salt stress encourages the production of ROS in excess. While at low concentrations, ROS are required for signaling, growth and behavior, high concentrations of ROS is a threat to cells as it causes membrane lipid peroxidation, protein oxidation, enzyme inhibition and DNA and RNA damage (Miller et al. 2010; Singh et al. 2011). Enhanced generation of ROS under salt stress is accomplished in four ways: first, plants responds to salt stress by decreasing stomatal conductance to avoid excessive water loss. This in turn decreases the internal CO_2 concentrations (Ci) and slows the reduction of CO_2 by Calvin cycle. This response leads to depletion of oxidized $NADP^+$, which acts as the final acceptor of electrons in PSI, and alternatingly increases the leakage of electrons to O_2 forming O_2^- (Hsu and Kao 2003). Furthermore, Na^+ or Cl^- toxicity resulting from salt stress could disrupt the photosynthetic electron transport and provoke electron leakage to O_2 (Borsani et al. 2001; Slesak et al. 2002). Second: the decrease in C_i slows down the reactions of Calvin cycle and induces photorespiration particularly in C_3 plants, resulting in generation of more H_2O_2 in the peroxisome (Wingler et al. 2000; Ghannoum 2009). Third: the cell membrane-bound NADPH oxidase and the apoplastic diamine oxidase get activated during salt stress and therefore contribute to generation of ROS (Hernandez et al. 2001; Mazel et al. 2004; Tsai et al. 2005). Fourth: salt stress increases the rate of respiration resulting in higher respiratory electron leakage to O_2 (Fry et al. 1986; Moser et al. 1991; Jeanjean et al. 1993).

AM colonization restricts the excessive generation of ROS by enhancing the activities of antioxidant enzymes (superoxide dismutase (SOD), catalase, peroxidase

(POX), ascorbate peroxidase, glutathione reductase) and antioxidant molecules (carotenoids, ascorbic acid, glutathione, tocopherols). The benefits of AM symbiosis in containing the levels of ROS with concomitant enhanced activities of antioxidant enzymes and antioxidant molecules have been shown by many researchers (Wu et al. 2010b; Hajiboland et al. 2010; Borde et al. 2011; Singh et al. 2011). Mycorrhizal plants have lower concentration of malondialdehyde concentration while maintaining higher activities of SOD, catalase (CAT), POX, ascorbate peroxidase (APOX) activities than in non-mycorrhizal plants (Wu et al. 2010b; Latef and Chaoxing 2011). The increased SOD will help detoxify super oxide ($O_2^{\cdot -}$) to hydrogen peroxide (H_2O_2) (Smirnoff 1993). This H_2O_2 generated are scavenged by CAT and POD. APOX is also reported to involve in detoxification on hydrogen peroxide produced in the chloroplasts of the stressed host plants (Benavides et al. 2000; Lopez et al. 1996). The elevated levels of GR activity may serve to ensure the availability of $NADP^+$ to accept electrons derived from photosynthetic electron transport, thereby directing electrons away from oxygen and minimizing the production of $O_2^{\cdot -}$ (Gamble and Burke 1984; Menconi et al. 1995). Thus, AM-mediated rapid removal of excess ROS helps in maintaining the optimum concentration of ROS to perform its physiological role; at the same time, preventing the shift towards destructive mode.

3.5 *Prevention of Membrane Damage*

It is now becoming clearer that the presence of AMF in roots of plants help in maintaining the integrity and stability of plasma membrane under saline stress. Under NaCl stress, lower lipid peroxidation and concurrent lower electrolyte leakage were reported in mycorrhizal maize, pigeon pea, and fenugreek as compared to their non-AM counterparts (Feng et al. 2002; Garg and Manchanda 2009; Evelin et al. 2012). It is suggested that AM-conferred resistance to peroxidation of membrane lipids and hence leakage of electrolytes from the cell, mediated through improved nutrition, especially P and maintenance of higher Ca^{2+}:Na^+, are the key factors contributing to this beneficial effect of AM colonization on membrane integrity (Evelin et al. 2012). Other mechanisms include maintenance of higher antioxidant capacity and containment of ROS concentration in mycorrhizal plants as against non-AM plants.

3.6 *Higher Photosynthetic Efficiency*

Salinity-induced degradation of chlorophyll molecule and subsequent decrease in photosynthesis is major process driving the plants to die prematurely. On the positive side, these salt-induced toxic effects on photosynthetic machinery of the

plant may be restricted or prevented by inoculating plant with AMF. In this regard, many authors have demonstrated that AM plants had higher chlorophyll concentration and non-photochemical quenching capacity as compared to their non-AM equivalents (Sheng et al. 2008; Hajiboland et al. 2010; Kumar et al. 2011; Evelin et al. 2012). Arbuscular mycorrhiza-facilitated improved uptake of nutrients (higher K^+, Ca^{2+}, and Mg^{2+} ions) helps in avoiding the specific effects of salt stress on chlorophyll degradation and leaf senescence (Evelin et al. 2012). Furthermore, the ability of AM plants to regulate the energy bifurcation between photochemical and non-photochemical events also helps in maintaining photosynthesis (Sheng et al. 2008).

3.7 Improved Water Status

Arbuscular mycorrhizal colonization and the effective development of external mycelium is an important means for uptake of water in plants grown in saline soils. Under salt stress, mycorrhizal colonization have shown to provide better water status by maintaining higher relative water content over the non-AM plants (Aroca et al. 2006; Colla et al. 2008; Jahromi et al. 2008; Sheng et al. 2008). Improved water status due to AM colonization may also be attributed to its role in ensuring liquid continuity, high hydraulic conductivity of roots and hence water uptake (Smith et al. 2010). Since hydraulic conductivity is dependent on P-concentration (Carvajal et al. 1996), it is likely that water uptake would be more strongly expressed in P-sufficient AM plant than in P-deficient non-AM plants. Mycorrhizal plants are also shown to accumulate solutes and maintain the osmotic balance. Lower water saturation deficit and higher turgor potential in AM plants also improves the water status of the plant (Al-Garni 2006; Sheng et al. 2008).

Various studies have shown better water status in AM plants than their corresponding non-AM plants, however, it is still unclear of how water from the AM fungus is translocated to the plant system. The possibility of direct water transfer to plants via fungal hyphae have been put forward, however the idea remains controversial to be established (Smith et al. 2010). This gap in our knowledge is surprising, given the importance of AM-mediated uptake of water in plants under salt stress. However, a positive step towards bringing down this gap is the recent discovery of an aquaporin gene *GintAQP1* from *Glomus intraradices* (Aroca et al. 2009), the expression of *GintAQP1* is a compensatory alternative to plant aquaporins (Aroca et al. 2009). Aquaporins belong to the major intrinsic protein (MIP) family of transmembrane channels, which permit the selective membrane passage of water (and other few compounds) but not of H^+ and other ions (Chen et al. 2001; Hill et al. 2004) through the plasma lemma (by plasma lemma intrinsic proteins (PIPs)) and the tonoplast (by tonoplast intrinsic protein (TIPs)). Though a few studies have shown AMF influence on plant aquaporins (Ouziad et al. 2006; Aroca et al. 2007; Jahromi et al. 2008), these findings are not persuasive enough

to establish and characterize the role of aquaporins and point to the possibility that AMF differentially exert control on each PIP (plasma membrane intrinsic protein) gene and each *PIP* gene analysed may have a different function and regulation in AM-mediated alleviation of water stress.

3.8 Abscissic Acid Concentrations

Plant growth and response to a stress condition is largely under the control of hormones (Mahajan and Tuteja 2005). Under salt stress, the increase in transpiration results in increase in pH of leaf and accumulation of ABA. ABA, in turn promotes the efflux of K^+ ions from the guard cells, resulting in loss of turgor leading to stomatal closure. On the other hand, the analogous findings that AM colonization can alter the ABA levels in host plant, sponsor for an AM-induced regulatory mechanism for ABA accumulation (Duan et al. 1996; Ludwig-Müller 2000; Estrada-Luna and Davies 2003). AM-mediated higher ABA levels regulate free polyamine pools in the plant (Sannazzaro et al. 2007). However, in contrast to this report, Jahromi et al. (2008) reported lower ABA levels in *Glomus intraradices* colonized lettuce plants than the non-AM plants indicating that AM plants were less strained than non-AM plants by salinity stress imposed; hence, they accumulated less ABA.

3.9 Nodulation and Nitrogen Fixation

Plant inoculation with AMF has been shown to promote nodulation and prevent premature nodule senescence during salt stress (Garg and Manchanda 2009; Manchanda and Garg 2011; Evelin et al. 2012). Pre-mature nodule senescence during salt stress is induced due to acceleration of lytic activities, formation of green pigments from leghaemoglobin (Sarath et al. 1986) and loss of nitrogen fixation (Delgardo et al. 1994). In AM plants, higher leghaemoglobin concentration delays the change of colour in nodule from pink to brownish pink due to synthesis of green pigments from leghaemoglobin. Mycorrhizal plants also possess a higher nitrogenase activity. All these parameters contribute to a higher nitrogen fixing ability of AM plants. The increased in nitrogenase activity and nitrogen fixation in AM plants than non-AM plants has been attributed to relief from P-stress, which is beneficial for the functioning of nitrogenase enzyme of the bacterial symbionts and possibly due to uptake of some essential micro-nutrients which results both in improved growth of plants (Founoune et al. 2002) or vice versa (Rabie and Almadini 2005). Therefore it may be suggested that mycorrhizal and nodule symbioses often act synergistically on infection rate, mineral nutrition, and plant growth (Patreze and Cordeiro 2004), which supports the need for both N and P and increased tolerance of plants to salinity stress (Rabie and Almadini 2005).

3.10 Molecular Mechanism

The plant function is ultimately explained by operation of genes in cells and tissues to regulate its growth with a symbiont in coordination with the environmental stress. Notable absence of knowledge regarding the molecular basis for AM-induced tolerance to salt stress in plants indicates that study aimed at molecular levels is at nascent stage. Only a handful of reports in molecular studies (Harrison and van Buuren 1995; González-Guerrero et al. 2005; Ouziad et al. 2006; Aroca et al. 2007; Jahromi et al. 2008; Aroca et al. 2009; Guether et al. 2009), are a testimony to the declaration. Though molecular studies are racing at a fast pace in other plant biology/microbiology studies, the AM plant molecular studies are often impeded due to the obligate and heterokaryotic nature of AMF. So far, the scientists have concerted their efforts in unraveling the mechanisms for nutrient and water uptake in plants. In this regard, expression analyses of plant aquaporins have been studied in tomato (Ouziad et al. 2006), *Phaseolus vulgaris* (Aroca et al. 2007) and lettuce (Jahromi et al. 2008) (as described in the section on improved water status). Recently, an aquaporins gene (*GintAQP1*) from *Glomus intraradices* was reported (Aroca et al. 2009). This is a step nearer to elucidation of water uptake mechanism in AM symbioses; however, it deserves more research to reach a conclusion.

Molecular studies on AM plant mineral uptake have revealed a phosphate transporter (Harrison and van Buuren 1995), an ammonium transporter (López-Pedrosa et al. 2006; Guether et al. 2009), a putative zinc transporter (González-Guerrero et al. 2005) in AM fungus. The role of AMF in nutrient uptake at the molecular level is only beginning to be understood. Expression studies have been conducted for Na^+/H^+ antiporters—*LeNHX1* and *LeNHX2* in tomato (Ouziad et al. 2006). The authors reported that salt and mycorrhizal colonization had no significant effects on these two antiporters.

In another study, Jahromi et al. (2008) analysed the expression pattern of genes encoding Δ1-pyrroline-5-carboxylate synthetase (*LsP5CS*), late embryogenesis abundant protein *(LsLea*) and ABA (*Lsnced*) in mycorrhizal and non-mycorrhizal lettuce plants subjected to varied salt treatments (0–100 mM NaCl). The *PC5S* enzyme catalyzes the rate-limiting step in the biosynthesis of proline (Kishor et al. 1995), an osmoregulator in plants. Late embryogenesis abundant protein acts as stress markers. They also possess chaperone-like activity, thus having a protective role during osmotic stress. *Lsnced* encodes for 9-cis-epoxycarotenoid dioxygenase, a key enzyme for the biosynthesis of stress hormone ABA. ABA promotes stomatal closure to minimize transpirational water loss. It also mitigates stress damage through the activation of many stress-responsive genes, which collectively increase plant stress tolerance (Bray 2002). The authors reported a higher expression of genes *LsP5CS* and *Lsnced* in non-AM plants than AM plants at 50 mM NaCl, though at 100 mM, the levels were similar. *LsLea* gene was found to express under conditions of salt stress and the induction of this gene was found to be lower in AM plants

than non-AM plants. The lower expression of this gene suggests that AM plants suffer less stress than non-AM plants, which may be likely due to primary salt avoidance mechanism.

4 Drought

Available water resources for successful crop production have been decreasing in recent years rendering the lands more drought-prone. Furthermore, in view of various climate change models scientists suggest that in many regions of world, crop losses due to increasing water shortage will further aggravate its impacts (Anjum et al. 2011). Drought is the most important environmental stress and affects plant performance more than any other environmental factor. It severely impairs growth and development and limit plant production (Shao et al. 2009).

Plant experiences drought stress either when the water supply to root becomes deficient or transpiration rate becomes very high. Drought affects growth, yield, membrane integrity, pigment content, osmotic balance, water relations, and photosynthetic activity (Benjamin and Nielsen 2006; Praba et al. 2009). The susceptibility of plants to drought stress varies depending on stress degree, different accompanying stress factors, plant species, and their developmental stages (Demirevska et al. 2009). Acclimation of plants to water deficit is the result of different events, which lead to adaptive changes in plant growth and physio-biochemical processes, such as changes in plant structure, maintenance of growth rate, tissue osmotic potential, and antioxidant defenses (Duan et al. 2007).

5 Arbuscular Mycorrhiza in Mitigation of Drought Stress

AM symbiosis is known to complement plant's innate ability to tolerate drought stress and alleviate stress symptoms (Fig. 14.2). A number of studies have demonstrated that AM symbiosis can protect the host plants against the detrimental effects of drought stress (Rodriguez and Redman 2005; Cho et al. 2006; Subramanian et al. 2006; Augé et al. 2007; Wu et al. 2007; Aroca et al. 2008; Wu et al. 2008; Manoharan et al. 2010; Fan and Liu 2011).

5.1 Enhanced Growth and Nutrient Uptake

The most obvious explanation for AM-induced changes in plant water balance and drought resistance is indirect effects associated with changes in plant size and phenology that occur via increased acquisition of phosphorous and sometimes other nutrients (Augé et al. 2004). AM symbiosis generally leads to greater plant biomass and altered root-shoot; root length-leaf area ratios, enhanced plant growth, and nutrient uptake

ratios (Al-Karaki et al. 2004). The size of a plant can affect its water relations; larger plants with larger root system may have access to more extensive soil water reserves. The favorable contribution of AMF inoculation on plant growth may be attributed to the following facts (1) AMF inoculation leads to formation of AM, which might have enhanced water uptake from the soil in the host plant (Augé 2001). It has been well documented that AM formation results in an ecological niche for roots to be more accessible to water resources, as the fungal hyphae can penetrate soil pores inaccessible to the root hairs (Ruiz-Lozano 2003) (2) Enhanced plant growth might also result from soil-borne pathogen protection (St-Arnaud and Vujanovic 2007), improved soil structural development, aggregate stabilization (Rillig 2004; Wright 2005) (3) coupled with the water absorption, nutrient uptake in the host plants may be facilitated in a better manner than in the non-mycorrhizal one (Ruiz-Lozano 2003).

5.2 Prevention of Nutrient Deficiency

AM symbiosis has been shown to improve the acquisition of nutrients including phosphate, nitrogen, sulfur or even more trace elements like copper and zinc (Subramanian et al. 2006; Cavagnaro et al. 2010; Tian et al. 2010; Latef and Chaoxing 2011). Since nutrient mobility is limited under drought conditions, AMF may have a larger impact on overall plant growth and development in dry relative to well-watered conditions (Sánchez-Díaz and Honrubia 1994). AM hyphae have the potential to access nutrients from drier areas. For instance, phosphorous becomes less mobile in arid soils, and an enhanced P acquisition by AM would hence become more important in improving water relations of host plants (Augé et al. 2004; Allen 2006, 2007). Soil P is usually in the form of orthophosphate that may be directly absorbed at soil–root interface through root epidermis and hairs, and indirectly at the fungal-root interface through external AM hyphae (Garg et al. 2006; Requena et al. 2007). In addition, AM colonization has been found to be related with the increase in activities of certain enzymes that help in hydrolysis and mobilization of nutrients. Often, P forms complexes with Ca and Mg rendering P unavailable for uptake. Higher acid phosphatase in mycorrhizosphere as compared to rhizosphere of non-AM plants enables the hydrolysis and mobilization of P_i releasing P for uptake. Higher soil acid phosphatase activities in mycorrhizosphere were also found to be positively correlated to soil water content (Chethan Kumar et al. 2008; Sardans et al. 2008). Thus, increase of soil acid phosphatase mediated by AMF partially alleviates plant drought stress (Wu et al. 2011).

During periods of drought, N availability is reduced resulting in decreased N uptake and lower rates of N assimilation. In plants N assimilation begins with the reduction of (nitrate) NO_3^- to (nitrite) NO_2^- by nitrate reductase (NR). This step often serves as the rate-limiting step in the assimilation process and is drastically slowed by water stress (Ruiz-Lozano 2003). Colonization by AMF improves both the nutritional status and N-assimilation rate of drought-stressed plant (Subramanian and

Charest 1998; Boomsma and Vyn 2008). Increase in N assimilation may result from the direct uptake of NO_3^- or NH_4^+ by hyphae (Cardoso and Kuyper 2006). This may contribute to greater protein concentration in AM plants over non-AM plants under drought stress. Stress also affects the rate of catalysis of downstream NH_4^+-assimilation enzyme glutamine synthetase (GS) and glutamine synthase (GOGAT). Drought-stressed AM shoots and roots of maize cultivars exhibited higher (NR), GS and GOGAT activities than water-stressed non-AM shoots and roots (Subramanian and Charest 1998). More robust uptake of water and nutrients might provide sufficient substance necessary to maintain a well defined growth in AM plants.

5.3 Osmotic Adjustment

One of the significant responses to drought is the accumulation of compatible solutes, also known as osmolytes, which function for osmotic adjustment, so as to maintain favorable gradient for water flow from soil into roots (Ruiz-Lozano 2003). Osmotic adjustment in plants specifically involves the active accumulation of various ions, amino acids, and sugars. It is primarily necessary for the maintenance of turgor, cellular expansion and growth, stomatal opening, photosynthesis, and water influx during water stress (Chaves et al. 2003; Ruiz-Lozano 2003). Changes in amino acid concentrations in response to drought in AM colonized plants are variable with some studies reporting increased levels (Ogawa and Yamauchi 2006b) and others reporting the opposite (Augé 2001). The greater concentration of amino acids in AM roots and shoots indicates a greater capability of osmotic adjustment through amino acid accumulation in these plants. Proline has been regarded as a key osmolytes participating in osmotic adjustment (Molinari et al. 2007; Hassine et al. 2008). In plants exposed to water stress, proline often serves as an osmoprotectant, as a solute for the protection of proteins and enzymes from denaturation, as a hydroxyl radical scavenger, as an alleviator of cell acidity and as a sink for energy to control redox potential (Chaves et al. 2003; Ruiz-Lozano 2003). AM plants have been reported to accumulate significantly higher level of proline upon exposure to drought stress when compared with non-mycorrhizal plants. Through osmotic adjustment via sugar accumulation, plant typically maintains turgor better in roots than in leaves in response to water deficits. Higher root turgor helps maintain root growth, elongation, and nutrient water uptake (Ogawa and Yamauchi 2006a; Studer et al. 2007).

The enhanced sugar content in AM roots under well-watered conditions may be due to the sink effect of the mycorrhizal fungus demanding sugars from shoot tissues. Under drought, Porcel and Ruiz-Lozano (2004) observed that the sugar content in roots was similar in both AM and non-AM treatments, suggesting that osmotic adjustment occurred. In contrast, in shoots the sugar content of droughted AM plants was considerably lower than in non-AM plants. The authors proposed two explanations for lower hexose accumulation in leaves of mycorrhizal plants. One, lower availability of photosynthates for storage in these tissues; and another explanation, that AM shoots are less strained by drought than non-AM ones. The lower accumulation of

compatible solutes may indicate that the AM plants more successfully avoided drought stress (Augé 2001). In fact proline and other osmoregulator also accumulated less in shoots of AM plants than in non-AM plants suggest drought avoidances.

5.4 Maintenance of ROS Level

In plants, metabolism of ROS such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^·$) is kept in dynamic balance. Under water stress condition, this balance is broken leading to enhanced production of ROS, which is deleterious to cells and cause oxidative damage. AM symbiosis provokes a more powerful ROS scavenging system in host plants and reduces destruction of biomolecules at the cellular level. AM plants subjected to drought shows lower lipid peroxidation than non-mycorrhizal plants (Porcel and Ruiz-Lozano 2004). SOD and POX are two important enzymes involved in elimination of superoxide anion and H_2O_2 respectively. Ruiz-Lozano et al. (1996) and Porcel et al. (2003) proposed that AMF protects host plants against oxidative damage by increments of enzymatic and non enzymatic antioxidants. Higher activities of SOD and POX in AM plants have been reported by many authors indicating that the activity of antioxidant enzymes is induced by AMF inoculation (Wu and Xia 2006; Bressano et al. 2010; Latef and Chaoxing 2011; Fan and Liu 2011). In addition, Fan and Liu (2011) observed that mRNA levels of four genes in the AM plants were apparently higher than in non-mycorrhizal plants under drought stress. Genes were annotated as homologues to *CSD1* (Copper/zinc SOD), *MIOX1* (myco-inositol oxygenase), *GLX1* (glyoxalase) and TTC5 (Transparent Testa 5) gene of *Arabidopsis*. These genes encode enzymes responsible for elimination of ROS, alleviating oxidative stress and detoxification of cytotoxic compounds. All these studies demonstrate that AMF inoculation results in a well-established defense mechanism against the drought, in which mitigation of oxidative stress might be a crucial part (Fan and Liu 2011).

5.5 Higher Photosynthetic Efficiency

AM plants often display higher rate of photosynthesis than their non-mycorrhizal counter parts do, which is consistent with the AMF effects on stomatal conductance. Most of the studies suggest that AM symbiosis increases the units of photosynthesis (Ruiz-Sánchez et al. 2010) so as to increase the rates of photosynthetic storage and export at the same time (Augé 2001). It has been shown that concentration of chlorophyll in AM plants was higher than their control non-mycorrhizal plants (Fan and Liu 2011). Higher concentration of chlorophyll is associated with higher photosynthetic rate (Davies et al. 1993). The higher photosynthetic rates associated with mycorrhization can result in higher concentration of soluble sugars and

photosynthetic byproducts in the leaf symplasm, which can manifest itself as an increased cytoplast osmolarity in AM plants as against non-AM plants (Porcel and Ruiz-Lozano 2004).

5.6 Improved Water Status

AMF have the ability to affect plant water relation in both water-limiting and well-watered conditions. Augé (2001) has provided an extensive review on the effects of AM symbiosis on plant water relations in numerous host species colonized by various fungal symbionts, with a particular emphasis on these effects under drought conditions. Early studies examining the effects of AM symbiosis on plant water relations generally concluded that improved drought tolerance results from enhanced P nutrition. Further studies revealed that existence of other mechanisms either only partially correlated with or unrelated to plant nutrition or size. One proposed mechanism primarily focuses upon the impact of AM colonization on water absorption rates, which further involves alleviation of plant gas exchange parameters and subsequently, overall leaf hydration (Boomsma and Vyn 2008). Other mechanisms involve changes in plant hydraulic conductance (e.g. enhanced stele tissue size), soil water relations (e.g. increased aggregate stability, greater soil available water), soil–root water potential gradient (e.g. enhanced soil drying), plant water potential components (stomatal conductance-leaf water potential relationship alterations), and non-hydraulic root signals (differing cytokinin and auxin (AA) concentrations) (Boomsma and Vyn 2008). As suggested by Sánchez-Díaz and Honrubia (1994), AM-induced changes in water relations may involve complex interactions among multiple mechanisms. Primary impact of AM symbiosis involves changes in stomatal conductance (g_s) and transpiration (T), with T typically higher and g_s frequently unaffected or greater during drought stress in AM relative to non-AM plants. At times, AMF also postpone reductions in leaf water potential during periods of drought and hasten returns to control levels upon the alleviation of water-limiting conditions (Augé 2001).

5.7 Molecular Mechanism

The beneficial effect of AM symbiosis under drought stress conditions has been studied largely at the physiological level including the regulation of transpiration rate or increasing root water absorption (Augé et al. 2004). In last decade, it has also been noted that, under drought conditions, AM and non-AM plants differently regulate the expression of several stress genes in root tissue (Ruiz-Lozano et al. 2006). Among the genes regulated by the AM symbiosis during drought, aquaporins genes have been described (Porcel et al. 2006; Ruiz-Lozano et al. 2006; Aroca et al. 2007, 2008). Aquaporins are membrane intrinsic proteins that facilitate water and small

neutral solutes flow, always, following an osmotic gradient. Modulation of aquaporin gene in AM symbiosis under osmotic stress is discussed under salt stress.

Another important mechanism involved in adaptation to water deficit is the induction of specific genes encoding an important component of endoplasmic reticulum—the luminal binding protein (BiP). The protein BiP is a molecule present in all kingdoms. The role of BiP in the ER is to transiently bind to unfolded proteins and to prevent intramolecular and intermolecular interactions that can result in permanent misfolding or aggregation, with the subsequent loss of their function (Gething and Sambrook 1992). A BiP encoding gene from *Glomus intraradices* has been identified after differential hybridization of cDNA library constructed from the fungus growing in vitro and subjected to drought stress (Porcel et al. 2007). Its expression was up-regulated by drought stress not only during in vitro conditions (AM monoxenic cultures) but also ex vitro, when forming natural symbiosis with plants. The contribution of AMF to the enhanced drought tolerance of the host plant can be mediated by proteins with chaperone-like activity, such as that of BiP (Porcel et al. 2007).

5.8 Contributions of Extraradical AM Mycelia (Drought Avoidance)

Arbuscular mycorrhizal fungi also colonize soils, changing chemical and physical soil properties (Jastrow et al. 1998). These properties can affect plant response to drought (Augé 2001). Therefore, in addition to influencing plants directly by colonizing plant tissue, AM symbiosis has the potential to affect drought response by changing the soils in which plant is growing. In fact, merely growing plants in a soil that had previously been mycorrhizal resulted in higher stomatal conductance of non-AM bean plants, under drought conditions (Augé et al. 2004). The colonization of soil by hyphae may have a greater influence on host behavior during drought than colonization of roots. The ability to survive to lower soil hydration was associated with more soil hyphae implies that soil hyphae may somehow aid root systems in more thoroughly extracting water from drying soils. Others have suggested that, at similar bulk soil water potential or bulk water content in AM and non-AM soils, soil water potential might actually be slightly higher in the rhizosphere of AM plants, if mycorrhizae more effectively ramify and dry out a particular volume of soil than do non-AM roots (Hardie and Leyton 1981; Gupta 1991; Duan et al. 1996). On several occasions, AM plants have been observed to deplete soil water more thoroughly than non-AM plants before achieving a similar shoot response. AM plants developed lower soil water potential before wilting (Hardie and Leyton 1981) or at the permanent wilting point (Bethlenfalvay et al. 1988a, b), relative to non-AM plants. Soil of AM cowpeas had to lose more water than soils of similarly sized non-AM plants, before evoking similar stomatal conductance, shoot water potential, transpiration and ABA in xylem near stomatal closure (Duan et al. 1996). AM sorghum was also able to maintain leaf water potential to lower soil water potential than

similarly sized non-AM plants (Osonubi 1994). Dakessian et al. (1986), Bethlenfalvay et al. (1988a) and Franson et al. (1991) have provided evidence that AM plants apparently have access to soil water below the permanent wilting water potential of non-AM plants.

The extraradical AM mycelia increase the efficacy of root water absorption in dry soil (e.g. Reid 1979; Fitter 1985; Davies et al. 1992), soil hyphae may increase soil-to-root contact in drying soils. Perirhizal resistance—resistance to water flow across the soil–root interface—results from draw-down resistance, diurnally imposed by the rapid loss of water from the soil immediately adjacent to the root, and from contact resistance, which increases as the surface of the root has less contact with rhizosphere water (Tinker 1976; Klepper 1990). Contact resistance increases as water retreats from large pores into smaller and smaller capillary areas in the soil and decreases the amount of root length actually wetted (Herkelrath et al. 1977). Root and soil shrinkage creates gaps between the root and the soil, which can decrease water absorption (e.g. Nobel and Cui 1992). Root hairs can help prevent air gaps at the soil–root interface, as they grow into very small pores and effectively "glue" themselves to soil particles with exuded mucilage (Klepper 1990). AMF soil hyphae might serve this same function, perhaps even more effectively than root hairs, because most hyphae can enter finer pores than can root hairs (Tisdall 1991). Further, extraradical hyphal development and soil aggregation by AM plants have been greater under drought conditions (Davies et al. 1992).

Soil structure refers to pore space as well as to aggregates. Soil aggregate stability is a crucial soil property affecting soil sustainability, crop production, biological activity, soil carbon storage, and the movement and storage of water (Amezketa 1999). AMF and roots counteract as factors that affect soil aggregate stability, although the mechanism is still not known (Piotrowski et al. 2004). Glomalin, a glycoprotein produced by AMF and first reported by Wright and Upadhyaya (1996), is long-lived in soils (Rillig et al. 2001) and is tightly correlated with soil aggregate stability (Wright and Upadhyay 1998). Because soil aggregates regulate soil water flow (Prove et al. 1990), it seems logical to suspect that AMF colonization may improve the water relations of plants. Improved soil structure generally has positive impacts on soil moisture retention properties (Hamblin 1985). Colonization of soil by AMF has been shown to change soil moisture retention properties, in concert with changes in soil hyphal density and associated soil characters (Bearden 2001). Glomalin could influence soil carbon storage indirectly by stabilizing soil aggregates. Mycorrhizal soils maintain better soil structure, especially soil water-stable aggregates and Bradford reactive soil protein, which are important for (1) maintaining soil porosity; (2) increasing stability against wind and water erosion and (3) storing C by protecting organic matter from microbial decomposition (Augé et al. 2004). Mycorrhizal soils have more water-stable aggregates and consequently higher soil moisture (Augé et al. 2001). AMF colonization enhances plant growth under drought stress indirectly through affecting soil moisture retention via glomalin's effect on soil water-stable aggregates (Wu et al. 2008).

6 Heavy Metal

Heavy metals occur naturally in the environment and constitute a potential hazard for water, soils, plants, and sediments. Agro-ecosystem receive inputs of heavy metals from the increased use of agro chemicals, the application of metal containing wastes such as sewage sludge, coal, and wood ashes to soils and from atmospheric deposition (Mhatre and Pankhurst 1997). Heavy metals are grouped into one category of elements with specific weight higher than 5 g cm^{-3} (Göhre and Paszkowski 2006). Some of these metals are essential plant micro-nutrients such as Cu, Fe, Mn, Ni, and Zn and are required for beneficial plant growth and development, while others have no known biological function such as Cd, Pd, and Hg. High contents of heavy metals in soils are generally considered a matter of concern as they may adversely affect the quality of soil water and compromise sustainable food production.

At high concentration, heavy metal influence the structure of enzymes and hence their functionality by affecting the protein structure or substituting a necessary element. As the structure of plasma membrane, protein such as H^+-ATPase is sensitive to alteration by heavy metals; the toxic effects of heavy metals can influence the permeability and function of plasma membrane. In addition, metals cause oxidative stress (production of ROS) adversely affecting cellular components and hence plant tissues (Sajedi et al. 2010).

Remediation of metal compounds presents a different set of problems when compared to organics. Organic compounds can be degraded while metals normally need to be physically removed or immobilized (Kroopnick 1994). Contaminated soil can be remediated by chemical, physical, or biological techniques. The most common remediation technique is off-site management; the metal contaminated soil is taken for burial at landfill sites. This method of remediation merely shifts the contamination problem elsewhere (Vidali 2001). Moreover, physico-chemical technologies used for soil remediation render the land useless as a medium for plant growth, as they remove all the biological activities. Therefore, sustainable on-site techniques for remediation of heavy metal contaminated sites need to be developed. In recent years, attention has been paid to the remediation of polluted soil, by use of plants (phytoremediation) and microbes (micro-remediation). These two approaches are preferred to chemical or physical remediation, because of their cost effectiveness, environment friendliness, and fewer side effects (Karimi et al. 2011).

In plants, the root is typically the organ, which is in continuous contact with metal ions in soil. Therefore, interaction between the microorganism in the rhizosphere and the plant activity related to soil remediation is inevitable (Compant et al. 2010). Combination of a hyper accumulating plant with beneficial rhizo- and/or endospheric microorganisms holds great promise for low cost cleaning of contaminated sites (Karimi et al. 2011).

7 Arbuscular Mycorrhiza in Mitigation of Heavy Metal Stress

Arbuscular mycorrhizal fungi have been observed in soils containing heavy metals (Wulf et al. 2003; Göhre and Paszkowski 2006; Hildebrandt et al. 2007). AMF can both positively and adversely affect the uptake of heavy metals by plants. Although considerable variability in plant responses to AMF inoculation has been observed in contaminated soils, the potential of AMF to buffer heavy metal stress has been demonstrated in a number of studies (e.g. Hildebrandt et al. 1999; Janoušková et al. 2006; Chen et al. 2007). Similar to stresses such as soil compaction and salinity, the alleviating effects of AMF on plant growth may intensify with increasing heavy metal concentration (Hildebrandt et al. 1999; Audet and Charest 2006), indicating a significant interaction between AM and stress level. The role of AMF in enhancing plant tolerance to heavy metals is very much dependent on AMF species, plant genotype and type of element in the soil (Sudová et al. 2008). For example, according to Khan et al. (2000), Zn is absorbed and crystallized in AMF hyphae and cortical cells of mycorrhizal root, Zn transfer to shoot decreases. Although AMF enable enhanced uptake of Fe and Mn in plants (Miransari et al. 2006), at high concentrations, AM are able to decrease the translocation of Mn in shoots and retain Fe in roots (Leyval et al. 2002).

Similar to plants, AMF from contaminated soils have been reported to cope better with heavy metal toxicity than those not exposed to such long-term selection pressure (Weissenhorn et al. 1993; Malcova et al. 2003). In spite of increasing knowledge in AMF-plant interaction under heavy metal stress, little is known about whether there is a synergism between plant and fungal heavy metal tolerance. It can be hypothesized that tolerant AMF may confer additional heavy metal tolerance on its host thus leading to their higher survival rate and reproductive success on contaminated sites. Alternatively, carbon invested into maintenance of AM symbiosis (4–20% of total plant photosynthates) may represent high costs for the tolerant plants that can cope well with heavy metal contamination without being mycorrhizal, but not for the heavy metal sensitive ones.

7.1 Heavy Metal Sequestration in Root/Soil

Heavy metals in soil are associated with a number of soil components which determine their behavior in the soil and influence their bio availability (Boruvka and Drabek 2004).

7.2 Fungal Cell Wall

The AM-mediated alleviation of the effects of heavy metal stress cannot be only attributed to nutrition effects, but also to the impact of AMF on metal distribution at

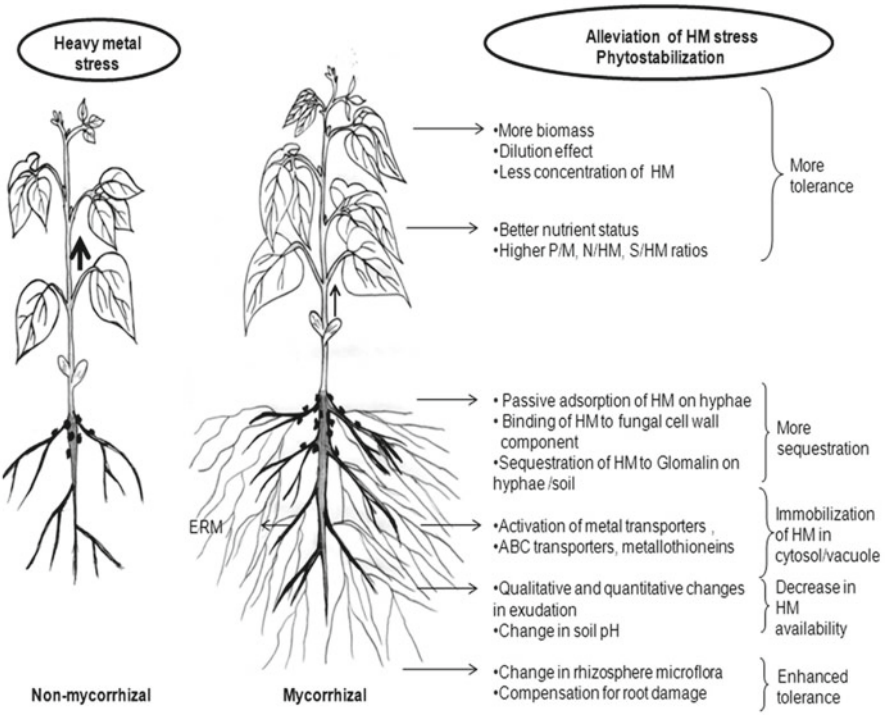

Fig. 14.3 Contribution of arbuscular mycorrhiza fungi to phytostabilization of heavy metals (HM). *Left*: Non-mycorrhizal plant in HM polluted soil showing enhanced uptake and transfer of HM to shoot. The plants are smaller as compared to the mycorrhizal plants on the *right* due to the effects of HM. *Right*: Mycorrhizal plant in HM polluted soil showing higher biomass and more tolerance to HM stress. In the rhizosphere, AMF colonization induces change in pH and microflora thereby decreasing HM availability. AMF in the roots also immobilize HM on its hyphae and sequester HM inside the cell; thereby lessening its transfer to shoot. On the other hand, AMF colonization also compensates for damaged root and enhances uptake of nutrients and water thereby maintaining better nutrient: HM ratios. These mechanisms facilitates for increased growth and higher biomass, which also complements to tolerance mechanism by diluting the effect of HM. Thickness of *arrows* indicates uptake level of HM

the soil-fungus-plant interfaces. AMF play an important ecological role in phytostabilization of toxic trace elements in soil by sequestration and, in turn help mycorrhizal plants survive in polluted soils (Fig. 14.3). The fungal cell wall components such as free amino, hydroxyl and carboxyl can bind to potentially toxic elements such as Cu, Pb and Cd (Kapoor and Viraraghavan 1995). Binding of heavy metal to chitin in the fungal cell wall reduces its local concentration in the soil. Joner et al. (2000) noted binding of up to 0.5 mg Cd per mg dry biomass, as a consequence of passive adsorption to the hyphae. Large surface area of hyphae in the soil is an important sink for heavy metals. Moreover, heavy metal tolerant fungi show greater affinity for heavy metals than roots (2–4 times more) and are thus suitable for immobilizing heavy metal in the soil (Joner et al. 2000).

Immobilization of metals on both extra and intraradical fungal tissue has been shown (Kaldorf et al. 1999; Joner et al. 2000), thus providing a plausible explanation of the barrier for metal translocation from the roots to the shoots of inoculated plants. Reduced transfer, as indicated by enhanced root:shoot Cd ratios in AM plants has been suggested (Tullio et al. 2003; Joner et al. 2000). This may be due to intracellular precipitation of metallic cations with phosphates.

7.3 Glomalin

Recently, glomalin, a glycoprotein produced by AMF has been suggested to have a metal chelating function influencing the metal availability for plants (Wright and Upadhyay 1998; Wright et al. 1998; Gonzalez-Chavez et al. 2004). One gram of glomalin could extract up to 4.3 mg Cu, 0.08 mg Cd and 1.12 mg Pb from the polluted sites (Gonzalez-Chavez et al. 2004). This protein may be one of the first cellular components in fungi coming in contact with ions from the surrounding environment; however, the exact mechanism of heavy metal binding by glomalin remains unclear (Volesky 1990). The glomalin stabilizes, reduces availability and decreases toxicity risk of heavy metals to other soil microorganisms and plants growing in these sites. The copious production and the recalcitrant nature of this molecule in the soil further enhance the potential usefulness of this compound in the soil (Rillig et al. 2001; Gonzalez-Chavez et al. 2004).

7.4 Change in Rhizosphere pH and Microflora

The rhizosphere of mycorrhizal plants (mycorrhizosphere) has lower heavy metal concentrations in soil solution as compared to that of non-mycorrhizal plants (Shen et al. 2006; Redon et al. 2008). This is because AMF reduces the availability of heavy metals to the host plant (Audet and Charest 2007). The lower soil solution concentration of heavy metals in the mycorrhizosphere has been often associated with high pH (Li and Christie 2001; Bi et al. 2003; Shen et al. 2006). In this regard, significant contributions from plants through AMF that is systemic effect of AM may also be responsible for modifications of soil pH and bacterial communities in the mycorrhizosphere or for the exudation of specific compounds (Marschner and Baumann 2003; Vierheilig et al. 2003). Root exudation is also an important factor influencing heavy metal mobility and bioavailability in soil (Hinsinger 2001). Thus, it may be suggested that AM-induced reduction in heavy metal availability in mycorrhizosphere is a combined endeavor of both plant-AMF. However, Janoušková and Pavliková (2010) showed cadmium immobilization in the rhizosphere of AM-*Nicotiana tobaccum* by the fungal ERM-induced alkalinization of substrate, while no indication was found for a significant involvement of plant-mediated effects of AM.

7.5 Enhance Growth and Biomass

Mycorrhizal associations increase the absorptive surface area of plant due to extra matrical fungal hyphae exploring rhizosphere beyond the root-hair zone, which in turn enhances water use efficiency and mineral uptake. The enhanced capability of uptake of minerals results in greater biomass production, a prerequisite for successful remediation. The stimulatory effect of AMF inoculation on the development of metal-treated plants was observed for maize, soybean, pea, and sunflower plants (Rivera-Becerril et al. 2002; Andrade et al. 2004, 2008; Jurkiewicz et al. 2004). Another possible mechanism of metal tolerance includes dilution of metal concentrations in plant tissues due to promotion of plant growth by AMF. For example Arsenate, As (V) is an analog of Pi and mycorrhizas would enhance uptake of both (Smith et al. 2010). Chen et al. (2007) pointed out that though total As (V) content increased concentration of As are frequently lower in AM plants, possibly reflecting tissue dilutions of As in the larger plants, rather than reduction in uptake per plant. AMF confers As tolerance in plants as the result of compensation by the fungal pathway for poor root growth. However, recent evidence indicate that this is an over simplified explanation and is not adequate (Smith et al. 2010).

8 Prevention of Nutrient Deficiency and Heavy Metal Toxicity

Excessive heavy metal concentrations (such as Cd) can induce deficiencies and imbalance of plant mineral nutrients (Greger and Lindberg 1987). AMF inoculation alleviates this effect and mycorrhizal plants show higher N, P, Ca, Mg, and S concentrations in shoot than non-mycorrhizal plants. Mycorrhizal maize plants showed higher P/Cd, N/Cd, and S/Cd ratios in both shoots and roots than non-mycorrhizal plants (Andrade et al. 2008). Higher ratios of P/metal in mycorrhizal plant species have been observed for several plant species (Andrade et al. 2004), suggesting that the higher P status of these plants may alleviate metal stress by phosphate complexation with metal ions inside the cells. The higher shoot N and S uptake in mycorrhizal plants lead to higher production of thiol rich proteins which, in addition to P complexation play an important role in heavy metal detoxification in vascular plants.

9 Phytoextraction

Phytoextraction is a rather recent technology and represents the most effective and attractive strategy on clean up contaminated soils (Kramer 2005). As phytoextraction relies on the capacity of plants to accumulate and tolerate heavy metal in their shoots, its efficiency depends both on the ability of metal to be translocated in aerial

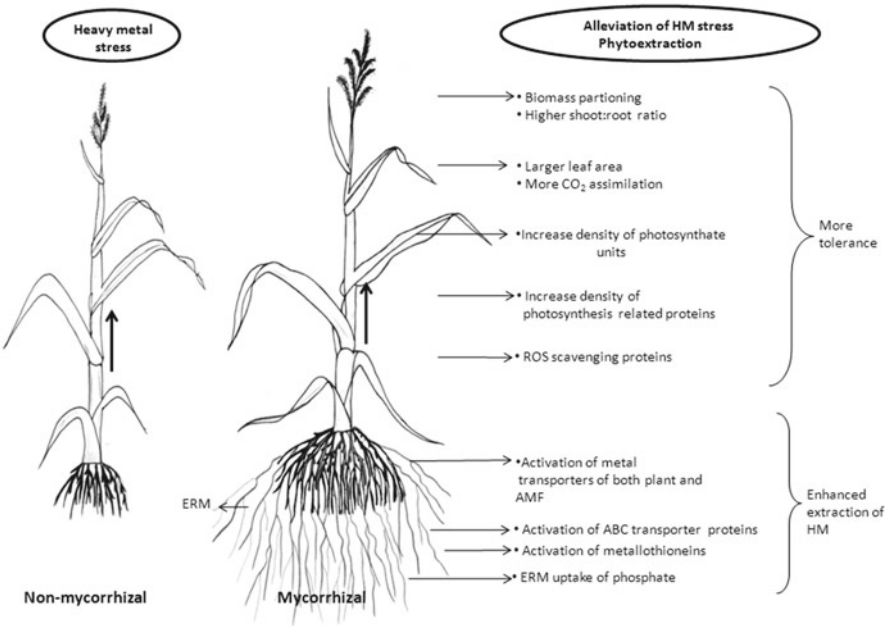

Fig. 14.4 Contribution of AMF to phytoextraction of HM. On the *left* is the non-mycorrhizal plant in HM polluted soil. The presence of HM in the rhizosphere reduced the growth of the plant; therefore the plant is smaller as compared to the mycorrhizal plants on the *right*. The mycorrhizal plant is able to tolerate HM stress due to the following reasons: (1) AMF colonization resulted in enhanced extraction of HM by activation of ABC transporter proteins and metallothioneins. At the same time, the extraradical mycelium (ERM) of extends the root system and enhances the uptake of P. (2) AMF also enhances the activities of ROS scavenging proteins and prevent the plants from oxidative stress. (3) AM plants maintain higher rate of photosynthesis thereby leading to higher biomass, shoot: root ratio and leaf area. Higher leaf area, in turn facilitates higher CO_2 assimilation. In this way, AMF colonization diminishes the phytotoxic effects of HM and imparts tolerance to the plant

plant organs and on shoot biomass production (Salt et al. 1998; Shi and Cai 2009; Wu et al. 2010a). Three main indicators have been used to measure plant effectiveness in extracting heavy metal from soil: the tolerance index expressed as the ratio of shoot growth parameters for plant grown in polluted soil to plants grown in metal-free soil (Shi and Cai 2009; Wu et al. 2010a); the transport factor calculated as the ratio of the heavy metal in shoot to that in roots (Wu et al. 2010a; Wang et al. 2007); and heavy metal partitioning that corresponds to the metal quantity present in plant organs (Redon et al. 2008). Figure 14.4 shows the mechanisms in mycorrhizal plants in alleviation of heavy metal stress through phytoextraction.

10 AM and Hyper Accumulators

There are only a limited number of plants (the metallophytes) that can grow under heavy metal stress (Miransari 2010; Tonin et al. 2001; Hildebrandt et al. 2006). In addition to the development of some special physiological processes, symbiosis with AM also enables metallophytes to grow under heavy metal stress by substantially reducing plant uptake of heavy metals (Berreck and Haselwandter 2001). Most metallophytes belong to the families Brassicaceae and Caryophyllaceae, which are non-mycorrhizal plants (de Mars and Boerner 1996). However, some species such as *Biscutella laerigata* and *Thlaspi* spp. are able to develop symbiosis with AM species such as *Glomus intraradices* (Hildebrandt et al. 2007).

It was discovered that addition of AMF further enhances the uptake and accumulation of As in *Pteris riltata* (Leung et al. 2006). At highest As concentration tested (100 mg/kg soil), non-mycorrhizal plants accumulated 60.4 mg As/kg soil while AM plants accumulated 88.1 mg As/kg soil. This was accompanied by enhanced growth, possibly due to improved phosphate (Pi) nutrition. Both effects combined allow for higher recovery of heavy metal. Similarly, *Berkheya coddii* belonging to Asteraceae family, used for phytomining Ni accumulated 30% more Ni on AMF inoculation by "adapted" AMF than in non-mycorrhizal controls (Turnau and Mesjasz-Przybylowicz 2003).

11 AM and Non-hyper Accumulators

Non-hyper accumulators can also be used for phytoextraction if they are sufficiently tolerant to heavy metal and produce high biomass. Research regarding shoot tolerance mechanism upon heavy metal phytoextraction has been essentially conducted in hyperacccumulator plant species; however, there is little evidence regarding processes by which mycorrhiza allow plant shoots to cope with metal stress (Davies et al. 2001). This is probably because roots are considered as the main site of metal toxicity exposure; therefore the cellular and molecular basis of heavy metal tolerance of mycorrhizal plants have been essentially grasped at the below ground level (Joner et al. 2000; Ouziad et al. 2005; Janoušková et al. 2006; Hildebrandt et al. 2007; Aloui et al. 2009; González-Guerrero et al. 2010).

12 Allocation Plasticity

Recently, Aloui et al. (2011) proposed that enhanced metal extracting capacity of mycorrhizal plant is not related to increase in root/shoot translocation rate, but to a high level of allocation plasticity. They observed that *Medicago truncatula* plants inoculated with *Glomus irregulare* displayed a significant increase in shoot tolerance to Cd relative

to those non-mycorrhizal. In spite of reduced root to shoot translocation rate of Cd, shoots of mycorrhizal plants contained the highest metal quantity relative to shoots of non-mycorrhizal plants (Kapoor and Bhatnagar 2007). From these observations they concluded that shoots of mycorrhizal *M. truncatula* have a capacity for extracting Cd, which is not related to an increased root to shoot transport factor. It has been proposed that a significant shift in root to shoot biomass partitioning permitted some plants to reduce the incidence of metal-induced stress in photosynthetic organs, a process referred to as allocation plasticity (Audet and Charest 2008).

The allocation plasticity of heavy metals in mycorrhizal plants may also be related to improved photosynthesis. AMF promotes photosynthesis by increasing the plant's ability to use light energy, maximize the area available for CO_2 assimilation per unit of carbon invested, facilitate the electron transport, prevent inhibition of aminolevulenic acid synthesis and protochlorophyllide photoreduction and by increasing the density of photosynthetic units (Stobart et al. 1985; Wright et al. 1998; Schoefs 2005; Aloui et al. 2011). A more tolerant photosynthetic system would allow plants maintaining high transpiration efficiency thus creating a water flux that can drive metals from the roots into the stem and leaves where the metal can be compartmentalized (Visioli et al. 2010). Taken together, both biomass partitioning and photosynthesis-related indicators support the idea that mycorrhizal plants extract and tolerate heavy metal by displaying a high level of allocation plasticity (Aloui et al. 2011) and do not invest in an intrinsic tolerance mechanism typical of *Arabidopsis thaliana*, which involves for example phytochelatin production (Audet and Charest 2007).

Although, several studies have demonstrated that mycorrhizal legumes can accumulate and tolerate heavy metal in their above-ground organs (Rivera-Becerril et al. 2002; Göhre and Paszkowski 2006; Aloui et al. 2009) information regarding molecular mechanisms by which shoots of mycorrhizal plants can escape heavy metal toxicity is lacking. In a pioneering work, Aloui et al. (2011) gave a first picture of shoot proteome modifications upon AM symbiosis and/or heavy metal stress in a legume plant. On performing 2-DE/MALDI/TOF-based comparative proteomic analysis of *Medicago truncatula* shoot responses upon mycorrhization and Cd exposure, they observed that in non-mycorrhizal plants, metal-responsive shoot proteins impaired CO_2 assimilation; the mycorrhiza responsive shoot proteome was characterized by an increase in photosynthesis-related proteins coupled to a reduction in gluconeogenesis/glycolysis and antioxidant processes. By contrast, Cd was found to trigger the opposite response coupled with the up-accumulation of molecular chaperones in shoot of mycorrhizal plants relative to those metal-free.

13 Molecular Mechanism

Although macroscopic symptoms and physiological effects of heavy metal stress are well documented in higher plants, especially those of agricultural importance like legumes and cereals (Das et al. 1997; Sanità di Toppi and Gabbrielli 1999) there

is lack of information concerning the molecular basis of such responses as well as on how they may be modulated by AM symbiosis. In this regard, targeted approaches have been developed within the last decade to monitor changes in plant gene expression and protein accumulation for understanding the molecular mechanism of heavy metal tolerance in mycorrhizal plants (Repetto et al. 2003; Rivera-Becerril et al. 2005).

When plant is subjected to high levels of heavy metal, these are translocated and accumulated in the parenchyma cells of the inner root, which is the place of colonization with different fungal structures including arbuscules, vesicles and hyphae (Kaldorf et al. 1999). The mechanisms directing heavy metal movement to plant roots by AMF have been proposed. These are (1) heavy metal may be deposited in the cellular wall or in the fungal vacuoles; (2) sequestration of heavy metals by siderophores may deposit heavy metal in root apoplasm or in soil; (3) metallothioneins or phytochelatins may result in the deposition of heavy metal in fungal or plant cell; and (4) the allocation of heavy metal from cytoplasm is performed by metal transporters located at the plasmalemma or tonoplast of both symbionts (Miransari 2011). Heavy metal content in roots of mycorrhizal plants is highly altered indicating that the related genes are expressed at transcriptional and translational levels by AMF (Ouziad et al. 2005).

At the genetic level, very few genes involved in heavy metal homeostasis have been analysed in detail in AMF: the Zn transporter *GintZnT1* from *Glomus intraradices* involved in Zn compartmentalization (González-Guerrero et al. 2005) and metallothionein gene *GmarMT1* and *GintMT1* from *Gigaspora margarita* and *Glomus intraradices* respectively that may provide protection against Cu (Lanfranco et al. 2002; González-Guerrero et al. 2007). Metallothioneins (MTs) constitute an extensive and diverse family of small cysteine-rich protein that bind metals via the thiol groups of their cysteine residues and may play a role in the intracellular sequestration of heavy metal. Although, the most widely accepted role for MTs is metal detoxification, several studies have indicated that MTs play a role in the protection against the effect of ROS. In fact, the MT protein itself acts as an antioxidant as it is a potent scavenger of hydroxyl radicals (Andrews and Geiser 1999). González-Guerrero et al. (2007) suggested that *GintMT1* might play a key role in the regulation of the redox status of the extraradical mycelia of *G. intraradices* through either its metal chelation activity or its thiol groups which might contribute to the pool of cytosolic thiols that regulate fungal redox status. In their other experiment, González-Guerrero et al. (2010) indicated that Cd and Cu result in expression of the related transporters *Gint ABC1* might play a key role in Cd and Cu detoxification. Given that Cu is an active redox metal that induces oxidative stress in *G. intraradices* (Benabdellah et al. 2009) and that transcription of *Gint ABC1* is induced by oxidative stress, up-regulation of *Gint ABC1* expression in the presence of Cu might be also due to the oxidative response elicited by Cu (González-Guerrero et al. 2010).

On the basis of 2D-based proteomic approach used to compare the proteomes of *Medicago truncatula* roots either colonized or not with the AM fungus *Glomus intraradices* in Cd-free and Cd-contaminated substrates, Aloui et al. (2009) reported

on the protective effect conferred by AM symbiosis. Their results indicated that at the proteome level, nine out of the 15 Cd-induced changes in non-mycorrhizal roots were absent or reversed in those Cd-treated and colonized by *G. intraradices*—including the *G. intraradices*—dependent down accumulation of Cd stress-responsive proteins. Out of the 26 mycorrhizal-related proteins that were identified only six displayed changes in abundance upon Cd exposure, suggesting that part of the symbiotic program, which displays low sensitivity to Cd, may be recruited to counteract Cd toxicity through the mycorrhiza-dependent synthesis of proteins having functions putatively involved in alleviating oxidative damages, including a cyclophilin, a guanine nucleotide-binding protein, an ubiquitin carboxy terminal hydrolase, a thiazole biosynthetic enzyme, an annexin, a glutathione S-transferase (GST) like protein and a S-adenosylmethionine (SAM) synthase.

14 Research Concerns

Although AMF usually can enhance plant potential to grow in heavy metal polluted soil and in case of hyperaccumulators may improve their metal uptake, the selection of appropriate mycobiont for the right plant is very important. This would result in more efficient bioaugmentation strategy, which would bioremediate the soil more efficiently (Miransari 2011). As mentioned previously, selection of AM species from contaminated areas can be more beneficial, because such species are adapted to high concentrations of heavy metals The colonization by AMF can lead to increased uptake and subsequent accumulation of heavy metal in above-ground tissues of plants—while this trait is required in remediation of land by phytoextraction, it is not desirable in agriculture crops grown for food. On the other hand, in several cases mycorrhizal colonization leads to accumulation of heavy metal in roots as described earlier within this review. Although this scenario could be desirable for enhanced plant heavy metal tolerance, it may interfere with efficient phytoextraction (Göhre and Paszkowski 2006).

15 Future Directions

Present-day industrial agricultural practices place several constraints on the use of services provided by mycorrhiza; however in order to manipulate AMF and to achieve their efficient use for long-term agricultural stability and productivity, we have to increase our knowledge on the impact of different production strategies on both the diversity of AM fungal communities and its relation to production quantity and quality (Gianinazzi et al. 2010).

The effects of AM in amelioration of abiotic stress on a variety of crop plants have typically been studied using pot-cultures in greenhouse or growth chamber environments in which interaction between the two symbionts were studied in a

controlled manner (Wright 2005). However, in field setting AM symbiosis is affected by factors not present in controlled greenhouse or laboratory conditions. In order to fully understand AMF effects on abiotic stress tolerance, future experiments should be conducted in field. These studies could be conducted at varying levels of stress and nutrient availability and at multiple locations so that the stress x soil fertility interaction could be interrogated across a number of environments (Boomsma and Vyn 2008). Cropping practices that mimic those used by growers should also be improved in future field research, including the use of various nutrient amendment, tillage systems, plant densities, crop rotations, and pesticide applications appropriate for optimum yield in a given environment. The findings of these studies could potentially serve as guides for examining the effects of cropping practices on the AM-mediated alleviation of abiotic stress.

The proportion of modern genotypes of crop plants capable of forming an effective symbiosis in a abiotic stress environment is generally unknown. A thorough analysis of the variation in colonization and responsiveness of susceptible and tolerant genotypes is necessary. Although future research should focus on identifying crop varieties capable of forming an effective symbiosis, it should also determine fungal isolates, species and communities that demonstrate the greatest enhancement of crop productivity in stress environment. Morphologically identical isolates of a particular fungal species can have varying effects on host growth, with the effect of a single isolate varying by such environmental factors as soil temperature, pH, moisture, nutrient status, and salinity (Picone 2003). Although an AM isolate or species can benefit a particular genotype it may not do so for another (Liu et al. 2000a, b). These conditional phenomena suggest that beneficial AM species may need to be determined both for specific genotypes and local agro-ecosystem environments. Such species need to be capable of surviving and spreading among native AM communities (when introduced) and in typical environmental and agronomic conditions.

Further understanding of plant metabolic and physiological responses to AM infection during a particular stress could be enhanced through the use of molecular and genomic techniques. There are a far and few studies on AM symbiosis under abiotic stress investigated on a molecular level (Porcel et al. 2005; Ruiz-Lozano 2003). Further studies could involve nontargeted screening of cDNA libraries from both AMF and AM colonized plants. Such techniques could enable the identification of stress-regulated genes that permit enhanced stress tolerance in AM colonized hosts. Using microarrays, stress-tolerance mechanisms (morpho-physiological and molecular processes) associated with AM symbiosis could be either identified or more fully understood through the comparison of AM and non-AM plants of same genotype.

In some cases, improving plant productivity through the use of AM inoculums instead of indigenous AM populations may be preferable or even necessary (e.g. in areas that received extensive use of fungicide or on severely degraded land) (Douds et al., 2005). Unfortunately, due to the obligate biotrophic nature of AM, the cost of inoculums production is presently quiet high. Thus, advances in technologies are imperative towards formulation of large scale, uniform, economically viable inoculums.

From the large number of studies extensively examined in this chapter, it is evident that AM symbiosis can potentially improve abiotic stress tolerance.

However, more research is required, particularly in field settings across multiple environments. Further endeavors seeking to elucidate, validate, and implement improvements in stress tolerance through AM symbiosis will likely require extensive collaboration among crop physiologists, plant breeders, genomicists, molecular and soil biologists.

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