Annals of Botany **104**: 1263–1280, 2009
doi:10.1093/aob/mcp251, available online at www.aob.oxfordjournals.org

ANNALS OF BOTANY
Founded 1887

INVITED REVIEW

Arbuscular mycorrhizal fungi in alleviation of salt stress: a review

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Received: 11 May 2009 Returned for revision: 27 July 2009 Accepted: 1 September 2009 Published electronically: 8 October 2009

• *Background* Salt stress has become a major threat to plant growth and productivity. Arbuscular mycorrhizal fungi colonize plant root systems and modulate plant growth in various ways.
• *Scope* This review addresses the significance of arbuscular mycorrhiza in alleviation of salt stress and their beneficial effects on plant growth and productivity. It also focuses on recent progress in unravelling biochemical, physiological and molecular mechanisms in mycorrhizal plants to alleviate salt stress.
• *Conclusions* The role of arbuscular mycorrhizal fungi in alleviating salt stress is well documented. This paper reviews the mechanisms arbuscular mycorrhizal fungi employ to enhance the salt tolerance of host plants such as enhanced nutrient acquisition (P, N, Mg and Ca), maintenance of the $K^+ : Na^+$ ratio, biochemical changes (accumulation of proline, betaines, polyamines, carbohydrates and antioxidants), physiological changes (photosynthetic efficiency, relative permeability, water status, abscissic acid accumulation, nodulation and nitrogen fixation), molecular changes (the expression of genes: *PIP*, Na^+/H^+ antiporters, *Lsnced*, *Lslea* and *LsP5CS*) and ultra-structural changes. Theis review identifies certain lesser explored areas such as molecular and ultra-structural changes where further research is needed for better understanding of symbiosis with reference to salt stress for optimum usage of this technology in the field on a large scale. This review paper gives useful benchmark information for the development and prioritization of future research programmes.

Key words: Arbuscular mycorrhizal fungi, salt stress, *PIP*, Na^+/H^+ antiporters, nutrient uptake, soil salinity.

INTRODUCTION

Salinization of soil is a serious problem and is increasing steadily in many parts of the world, in particular in arid and semi-arid areas (Giri *et al.*, 2003; Al-Karaki, 2006). Saline soils occupy 7 % of the earth's land surface (Ruiz-Lozano *et al.*, 2001) and increased salinization of arable land will result in to 50 % land loss by the middle of the 21st century (Wang *et al.*, 2003). At present, out of 1·5 billion hectares of cultivated land around the world, about 77 million hectares (5 %) is affected by excess salt content (Sheng *et al.*, 2008). High levels of salinity (>4 dS m^{-1} or $>0{\cdot}1$ % soil content; Richards,1954; Juniper and Abbott, 1993) in soils is mainly due to the soluble salts in irrigation water and fertilizers used in agriculture (Abrol, 1986; Copeman *et al.*, 1996; Al-Karaki, 2000), low precipitation and high temperature in these regions and over-exploitation of available water resources (e.g. ground water) (Cantrell and Linderman, 2001; Al-Karaki, 2006; Mouk and Ishii, 2006).

The significance of soil salinity for agricultural yield is enormous (Tester and Davenport, 2003) as it affects the establishment, growth and development of plants leading to huge losses in productivity (Giri *et al.*, 2003; Mathur *et al.*, 2007). The direct effects of salt on plant growth may involve: (*a*) reduction in the osmotic potential of the soil solution that reduces the amount of water available to the plant causing physiological drought – to counteract this problem plants must maintain lower internal osmotic potentials in order to prevent water movement from roots into the plant soil (Feng *et al.*, 2002; Jahromi *et al.*, 2008); (*b*) toxicity of excessive Na^+ and Cl^- ions towards the cell – the toxic effects include disruption to the structure of enzymes and other macromolecules, damage to cell organelles and plasma membrane, disruption of photosynthesis, respiration and protein synthesis (Juniper and Abbott, 1993; Feng *et al.*, 2002); and (*c*) nutrient imbalance in the plant caused by nutrient uptake and/or transport to the shoot leading to ion deficiencies (Marschner, 1995; Adiku *et al.*, 2001; Fig. 1).

To deal with saline soils and minimize crop loss, scientists have searched for new salt-tolerant crop plants (Gallagher, 1985; Glenn and O'Leary, 1985), and developed salt-tolerant crops through breeding (Cuartero and Fernandez-Munoz, 1999). To tackle the detrimental effects of salinity, scientists are also in the process of engineering plants genetically using different genes (Zhang and Blumwald, 2001; Sanan-Mishra *et al.*, 2005; Tang *et al.*, 2005; Wu *et al.*, 2005; Wei-Feng *et al.*, 2008). Wu *et al.* (2005) obtained a salt-tolerant perennial rye-grass (*Lolium perenne*) by transformaton with a rice vacuolar membrane Na^+/H^+ antiporter gene (*OsNHX1*) via an *Agrobacterium*-mediated method: the salt tolerance of perennial rye grass was improved by overexpression of the *OsNHX1* gene. Sanan-Mishra *et al.* (2005) reported that PDH45 (pea DNA helicase 45) mRNA is induced in pea seedlings in response to high salt, and its overexpression in tobacco plants, driven by a constitutive cauliflower mosaic virus 35S promoter, confers salinity tolerance. Recently, Wei-Feng *et al.* (2008) have reported that a transgenic *Arabidopsis thaliana* carrying a peroximal-type ascorbate peroxidase (pAPX) gene from barley was found to be more salt tolerant than the wild type. Leaching of excessive salts

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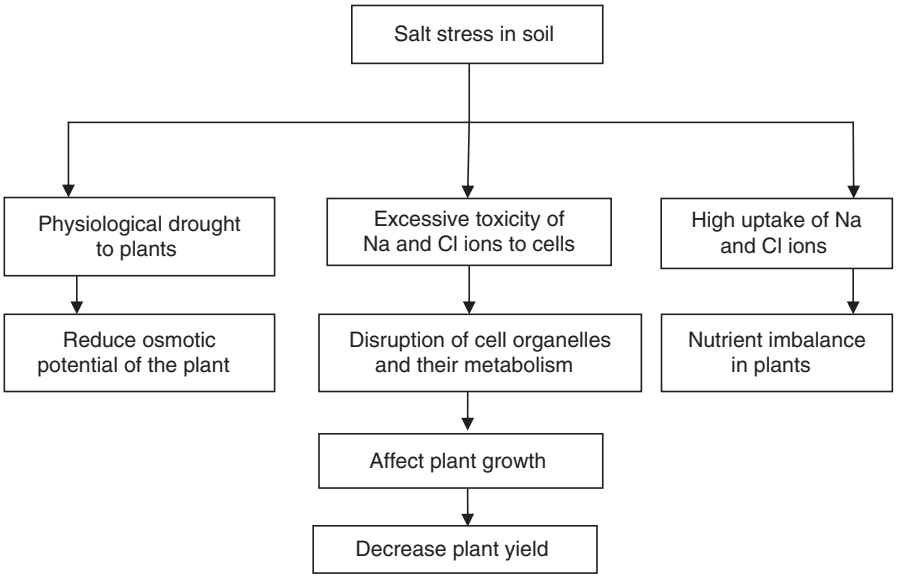

FIG. 1 Effect of salt stress on plants. Salt stress causes physiological drought to plants, imbalance in nutrient composition and excessive toxicity due to Na and Cl ions thereby leading to reduction in osmotic potential of plants, disruption of cell organelles and their metabolism. These ultimately affect plant growth and reduce the yield.

or desalinizing seawater for use in irrigation (Muralev *et al.*, 1997) are other methods employed to combat salt stress. Though successful, these approaches are costly and beyond the economic means of developing nations (Cantrell and Linderman, 2001).

Plants, in their natural environment are colonized both by external and internal microorganisms. Some microorganisms, particularly beneficial bacteria and fungi can improve plant performance under stress environments and, consequently, enhance yield (Brown, 1974; Levy *et al.*, 1983; Creus *et al.*, 1998). Arbuscular mycorrhizal fungi (AMF) are associated with the roots of over 80 % terrestrial plant species (Smith and Read, 1997; Hejiden *et al.*, 1998) including halophytes, hydrophytes and xerophytes. In this respect, biological processes such as mycorrhizal application to alleviate salt stress would be a better option. AMF have been shown to promote plant growth and salinity tolerance by many researchers. They promote salinity tolerance by employing various mechanisms, such as enhancing nutrient acquisition (Al-Karaki and Al-Raddad, 1997), producing plant growth hormones, improving rhizospheric and soil conditions (Lindermann, 1994), altering the physiological and biochemical properties of the host (Smith and Read, 1995) and defending roots against soil-borne pathogens (Dehne, 1982). In addition, AMF can improve host physiological processes like water absorption capacity of plants by increasing root hydraulic conductivity and favourably adjusting the osmotic balance and composition of carbohydrates (Rosendahl and Rosendahl, 1991; Al-Karaki and Clark, 1998; Ruiz-Lozano and Azcón, 2000; Ruiz-Lozano, 2003). This may lead to increased plant growth and subsequent dilution of toxic ion effect (Juniper and Abbott, 1993). These benefits of AMF have prompted it to be a suitable candidate for bio-amelioration of saline soils.

This review will cover the occurrence of AMF in saline soils and the effect of salinity on the arbuscular mycorrhizal (AM) fungus: colonization, hyphal length and sporulation both *in vivo* and *in vitro*. It will also cover literature relating to the alleviation of salt stress by AMF and its beneficial effects on growth, changes in biochemical, physiological and molecular mechanisms used by host plants to alleviate salt stress. The article has identified certain areas where more investigations are required in order to gain a whole understanding of the different mechanisms by which AMF symbiosis protects the plants against salt stress.

AMF IN SALINE SOILS

AMF have been known to occur naturally in saline environments (Khan, 1974; Allen and Cunningam, 1983; Pond *et al.*, 1984; Rozema *et al.*, 1986; Sengupta and Chaudhuri, 1990; Carvalho *et al.*, 2001; Hilderbrandt *et al.*, 2001; Harisnaut *et al.*, 2003; Yamato *et al.*, 2008), despite the low mycorrhizal affinity of the halophytes (Brundrett, 1991). The average density of spores in saline areas is reported to be low by some researchers (Barrow *et al.*, 1997; Carvalho *et al.*, 2001) but not others (Khan, 1974; Bhaskaran and Selvaraj, 1997; Aliasgharzadeh *et al.*, 2001; Landwehr *et al.*, 2002). Aliasgharzadeh *et al.* (2001) observed that the most predominant species of AMF in the severely saline soils of the Tabriz plains [with an electrical conductivity (EC_e) of 162 dS m^{-1}] were *Glomus intraradices*, *G. versiform* and *G. etunicatum.* The authors also found that the number of AMF spores did not significantly decrease with soil salinity and reported a relatively high spore number (mean of 100 per 10 g soil). The higher fungal spore density in saline soils may be due to the fact that sporulation is stimulated under salt stress (Tressner and Hayes, 1971) which means that AMF may produce spores at low root-colonization levels in severe saline conditions (Aliasgharzadeh *et al.*, 2001). This is in contrast to other reports on saline soils where low or even zero spore population was found in soils with EC_e approx. 45 dS m^{-1} (Hirrel *et al.*, 1978; Kim and Weber,

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1985; Barrow *et al.*, 1997). McMillen *et al.* (1998) reported that the spore germination and hyphal growth of AMF were inhibited by salt (150 mM NaCl). This may again cause the accumulation of spores in saline soil (Aliasgharzadeh *et al.*, 2001). F.Y. Wang *et al.* (2004), while investigating the relationship between the distribution of AMF in the rhizosphere of different wild plants in the Yellow River Delta (EC_e approx. 40·2 dS m^{-1}), observed a total of 33 species representing three genera of AMF, including two species of *Archaeospora*, seven in *Acaulospora* and 24 in *Glomus*. They also found most spores at a depth of 0–40 cm. There were significant differences between different depths. The number of AMF spores decreased with increasing soil depth in the rhizosphere. Ho (1987), had reported the same while studying AMF of halophytic grasses in the Alvord Desert of Oregon.

The occurrence of AMF in salt-marsh plants has also been reported by many authors (Khan, 1974; Rozema *et al.*, 1986; Sengupta and Chaudhuri, 1990; Carvalho *et al.*, 2001; Hilderbrandt *et al.*, 2001). Findings by Landwehr *et al.* (2002) reported abundant occurrence of AMF spores in extremely alkaline soils of pH values up to 11, independently of the soil type and irrespective of NaCl, Na_2CO_3, Na_2SO_4 or $CaSO_4$ salt types, though the degree of colonization varied from one individual to the next. Recently, Wilde *et al.* (2009) evaluated the distribution of AMF spores in two salt-marshes – Terschelling, The Netherlands (an almost natural site on the Atlantic Coast, with an EC_e of 6·2–19·0 mS cm^{-1}) and at Schreyahn, Germany (of anthropogenic origin due to potash mining, EC_e 6·3–20·1 mS cm^{-1}) – and reported that the distribution of AMF spores was unlikely to follow the salt gradient at both sites. They also reported that morphological analyses of spores from soil samples at both sites showed a higher AMF biodiversity, while the overall biodiversity of the AMF based on sequence analysis was comparably low in roots at both sites. There is correlation between AMF spore population and different edaphoclimatic factors. A positive correlation between spore density and soil pH and organic carbon has been reported by some authors (Ho, 1987; Mohammad *et al.*, 2003; Mathur *et al.*, 2007). A negative correlation was observed with available soil P and Na. Aliasgharzadeh *et al.* (2001) had reported negative correlation between spore density and available soil Mg, Ca, Cl, clay, electrical conductivity, SO_4 and the sodium absorption ratio and a positive correlation with sandy soil. Saint-Etienne *et al.* (2006) reported significantly negative correlations between salt levels and mycorrhizal soil infectivity (measured in most-probable number values), i.e. when the salinity of soil increased from 5 to 22 %, the infectivity level decreased from 301 to 20 most probable number per 100 g of soil.

AMF are also known to colonize halophytes and such a finding was reported as early as 1928 (Mason, 1928) and later by various authors (Khan, 1974; Hoefnagels *et al.*, 1993; Brown and Bledsoe, 1996). Halophytes, the salt-tolerant, salt-loving or saltwater plants can increase total plant growth within a range of 5–20 dS m^{-1} EC_e followed by a decrease in growth (O'Leary, 1995; Marcum, 2002). The extent of benefits derived by AM halophytes has been addressed by some authors (Allen and Cunningham, 1983; Rozema *et al.*, 1986; Baker *et al.*, 1995). Allen and Cunningham (1983) observed that, in saline soils, AM plants of *Distichlis spicata* had similar or lower biomass than non-AM plants. Further, under salt-stress conditions, a beneficial effect of AMF symbiosis was observed on the water status, accumulation of osmolytes and growth of *Phragmites australis* plants (Al-Garni, 2006). An improved water relationship was also reported in mycorrhizal *Aster tripolium* plants in saline soil (Rozema *et al.*, 1986). Johnson-Green *et al.* (2001) proposed that AMF could tolerate 50 mg total salts ml^{-1} soil water. They suggested that the mycorrhizal benefit to halophytes might occur primarily through improved mineral content, rather than through increased biomass.

In most of the studies mentioned above, identification of the AMF spores was carried out. In the earlier studies, identification was based mainly on the morphological criteria. Complementary to morphology based identification methods, use of molecular techniques such as polymerase chain reaction and restriction fragment length polymorphism for identification of AMF has been on the rise. Many authors have employed molecular techniques for identification of AMF spores (Landwehr *et al.*, 2002; Regvar *et al.*, 2003; Wilde *et al.*, 2009). The molecular identification techniques can overcome some of the pitfalls in morphology based identification; however, it is not advisable to rely solely on molecular identification techniques as the gene content of the different nuclei within an AMF individual is to some extent variable (Lloyd-MacGilip *et al.*, 1996; Sanders *et al.*, 1996). Pringle *et al.* (2000) reported that the sequences of the ITS region within a single spore can be more variable than between spores of a single isolate. According to Antoniolli *et al.* (2000), the divergence of the ITS region within a single spore can be between 2·4 % and 5·7 %. Therefore, to define a species, any characterization of a microbe by sequencing of ribosomal genes needs to be complemented by morphological and/or physiological characterization (Wilde *et al.*, 2009).

The AMF most commonly observed in saline soils are *Glomus* spp. (Allen and Cunningam, 1983; Ho, 1987; F. Y. Wang *et al.*, 2004). Studies employing molecular biological techniques revealed that 80 %, on average, of these spores belonged to one single species, *Glomus geosporum* (Wilde *et al.*, 2009). However, this finding does not necessarily indicate that *Glomus geosporum* confers salt tolerance on plants and enables them to grow under salt-stress conditions. Fuzy *et al.* (2008) reported that an isolate of *G. geosporum* from the inland salt-marsh at D-Jerxheim near Braunschweig consistently failed to give positive plant growth-promoting effects when diverse plants were grown with different NaCl concentrations under greenhouse conditions. Tian *et al.* (2004) observed that *G. mosseae* isolated from saline soil had a lower capacity to alleviate saline stress in cotton than the one isolated from non-saline soil. Recently, Porras-Soriano *et al.* (2009) tested the efficacy of three species of AMF – *Glomus mosseae*, *G. intraradices* and *G. claroideum* – to alleviate salt stress in olive trees under nursery conditions. The authors observed that *G. mosseae* was the most efficient fungus in terms of olive tree performance and particularly in the protection offered against the detrimental effects of salinity. These findings suggest that the capability of AMF in protecting plants from the detrimental effects of salt stress may depend on the behaviour of each species.

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Effect of salinity on colonization, spore germination and hyphal growth

Salinity, not only affects the host plant but also the AMF. It can hamper colonization capacity, spore germination and growth of hyphae of the fungus. Several researchers have reported the negative effects of salinity on the fungus (Hirrel, 1981; Estaun, 1989; McMillen *et al.*, 1998; Jahromi *et al.*, 2008). Colonization of plant roots by some AMF is reduced in the presence of NaCl (Hirrel and Gerdemann, 1980; Ojala *et al.*, 1983; Menconi *et al.*, 1995; Poss *et al.*, 1985; Rozema *et al.*, 1986; Duke *et al.*, 1986; Giri *et al.*, 2007; Juniper and Abbott, 2006; Sheng *et al.*, 2008) probably due to the direct effect of NaCl on the fungi (Juniper and Abbott, 2006) indicating that salinity suppresses the formation of arbuscular mycorrhiza (Tian *et al.*, 2004; Sheng *et al.*, 2008). Before primary colonization of a plant root by an AMF can commence, fungal propagules in the soil must become hydrated and activated and produce a germ tube. One or more hyphae then extend through the soil and encounter a receptive root. Hyphae elongate 20 times more slowly in the absence of host roots than in their presence (Becard and Piche, 1989). AMF respond to host exudates with extensive hyphal growth and branching (Giovannetti *et al.*, 1996). Despite the high mycorrhizal growth in the presence of roots, hyphae do not always appear to exhibit 'directional growth' toward the roots until they are very close to the host (Mosse and Hepper, 1975). Once, contact occurs, branching on the root surface takes place. However, hyphal branching does not occur on a non-host root, thereby suggesting that directional attraction may not be a general phenomenon, but may be more characteristic of the specific host tested (Vierheilig *et al.*, 1998). The relative timing of these stages is highly dependent on the characteristics of the individual fungus. The extent to which colonization by AMF is reduced in the presence of NaCl is dependent on the timing of the observation, such that there is more inhibition in the early than in the later stages of the symbiosis (McMillen *et al.*, 1998). It is, therefore, likely that the inhibition in these cases was due to an effect on primary rather than secondary infection (Wilson, 1984).

Besides, the AMF symbiosis with plant roots may also depend on various factors including the topographical or biochemical signals on the root surface (Gadkar *et al.*, 2001) and phenology of host plants (Wilson and Hartnett, 1998; Carvalho *et al.*, 2001). Carvalho *et al.* (2001) reported that the highest levels of AM colonization in *Aster tripolium* and *Inula crithmoides* corresponded, in both species, to the period of highest plant growth and the flowering period – summer and autumn, respectively. The varying levels of AM colonization may also be related to the different behaviour of each AM fungal species, even in similar ecosystems (Klironomos *et al.*, 1993) or to the influence of different environmental conditions (Carvalho *et al.*, 2001).

Contrary to the reports above, a few studies reported that AMF colonization is not reduced in the presence of NaCl (Levy *et al.*, 1983; Hartmond *et al.*, 1987). Increased AMF sporulation and colonization under salt-stress conditions has also been reported (Aliasgharzadeh *et al.*, 2001). Recently, Yamato *et al.* (2008) reported that colonization rates were not reduced in all AMF present in coastal vegetation on Okinawa Island, Japan even when treated with high salinity of 200 mM. This discrepancy in the results invites researchers to look out for salt-tolerant species of AMF.

In the presence of NaCl, germination of spores is delayed rather than prevented (Cantrell and Lindermann, 2001; Juniper and Abbott, 2006). It has been reported that preinoculation of a transplant with AMF bypasses the inhibitory effects that salt could have on spore germination (Cantrell and Lindermann, 2001; Al-Karaki, 2006).

The rate of germination and maximum germination of AMF spores may also depend on the salt type. According to Juniper and Abbott (1993), the different salts $NaNO_3$ and Na_2SO_4 with similar osmotic potentials ($-0{\cdot}48$ and $-0{\cdot}43$ MPa, respectively) impart differential effects on the rate and maximum germination of spores. They attributed the difference to a higher concentration of Na^+ in the latter. They also reported that NaCl allows a faster rate and maximum germination as compared with KCl with similar osmotic potentials. The percentage of AM colonization observed in a variety of host plants at varied levels of salinity is presented in Table 1.

The trend to decreasing hyphal length as soil salt concentration increases has been reported (Cantrell and Lindermann, 2001; Juniper and Abbott, 2006). These reports suggest that hyphal growth of the fungi can be taken as being more sensitive to NaCl than spore germination, which is delayed but not necessarily reduced.

The relative tolerance of different types of the same fungal genus can vary as is reported in the case of *Glomus* sp. Propagules of *Glomus* sp. within the colonized root pieces grow in 300 mM NaCl, but the spores of the same fungi extracted from the soil did not. This may indicate a difference in the energy status between them, or differences in the amount of water and energy required to initiate germination (Juniper and Abbott, 2006). Tommerup (1984) indicated that initial hyphal extensions have higher requirements for water than the intermediate stages for activation and germ-tube production. Therefore it may be possible that the germination rate of a prehydrated spore will have a greater percentage than the non-prehydrated spore in a saline environment (Juniper and Abbott, 2006). This contrasting result invites further studies in this aspect.

In a sole attempt, Jahromi *et al.* (2008) studied the effect of salinity on the AM fungus, *Glomus intraradices*, *in vitro*. They observed that there was no significant difference in hyphal length and branched absorbing structures (BAS) between control (no salt) and 50 mM NaCl, though there was a significant decrease in hyphal length and the number of BAS at 100 mM NaCl. It was also reported that salinity in the medium reduces the number of spores produced by *Glomus intraradices*. This decline suggests that if salinity persists, there can be a reduction in plant colonization by reducing the ability of inoculum (i.e. spores). An increase in salinity will reduce the hyphal length which will inhibit the colonization and symbiotic capability of AMF. BAS are believed to be associated with spore formation. Bago *et al.* (1998) had also shown that BAS can gradually form spores in their ramification. So, the reduction in the number of BAS can also reduce sporulation further.

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TABLE 1. *Studies on percentage of AMF colonization in root under salinity stress*

Range of salinity*	Plant	Fungus	% root colonization in AM plants	References
1·4–7·4 dS m^{-1}	*Lycopersicon esculentum*	*Glomus mosseae*	49·6–36	Al-Karaki (2000)
2 –12 dS m^{-1}	*Lactuca sativa*	Mixture of *Glomus*, *Acaulospora* and *Entrosphora* spp. procured from (*a*) saline playa or (*b*) non-saline vegetable farm	(*a*) 43·0–26·2 (*b*) 34·8–29·9	Cantrell and Linderman (2001)
2–12 dS m^{-1}	*Allium cepa*	Mixture of *Glomus*, *Acaulospora* and *Entrosphora* spp. procured from (*a*) saline playa or (*b*) non-saline vegetable farm	(*a*) 61·7–38·8 (*b*) 28·8–18·0	Cantrell and Linderman (2001)
0–13·19 dS m^{-1} (0–100 mM)	*Zea mays*	*Glomus mosseae*	70–80	Feng *et al.* (2002)
0–6·10 dS m^{-1} (0–3 g kg^{-1})	*Gossypium arboreum*	*Glomus mosseae*: (*a*) GM1 from non-saline soils; (*b*) GM2 from saline soils	(*a*) 38 ± 3 to 15 ± 2 (*b*) 46 ± 5 to 21 ± 3	Tian *et al.* (2004)
40·2 dS m^{-1}	*Tamarix chinensis*, *Phragmites communis*, *Suaeda glauca*, *Aeluropus littoralis* var. *sinensis* and *Cirsium setosum* (in Yellow River Delta, China)	Mixture of *Archaeospora*, *Acaulospora* and *Glomus*	0·2–9·5	F. Y. Wang *et al.* (2004)

* The range of salinity within brackets is the actual salt concentrations used by the authors.

ARBUSCULAR MYCORRHIZA AND SALT-STRESS AMELIORATION

Several studies investigating the role of AMF in protection against salt stress have demonstrated that the symbiosis often results in increased nutrient uptake, accumulation of an osmoregulator, an increase in photosynthetic rate and water-use efficiency, suggesting that salt-stress alleviation by AMF results from a combination of nutritional, biochemical and physiological effects (Fig. 2). Studies carried out so far have suggested several mechanisms by which AM symbiosis can alleviate salt stress in host plants. The various mechanisms employed are discussed in the following sections.

Plant growth and biomass

Under salt stress, plant growth and biomass suffered a setback. The reasons may be the non-availability of nutrients and the expenditure of energy to counteract the toxic effects of NaCl. However, mycorrhization was found to increase the fitness of the host plant by enhancing its growth and biomass. Several researchers have reported that AMF-inoculated plants grow better than non-inoculated plants under salt stress (Al-Karaki, 2000; Cantrell and Linderman, 2001; Giri *et al.*, 2003; Sannazzaro *et al.*, 2007; Zuccarini and Okurowska, 2008). It has been reported that mycorrhizal *Acacia nilotica* seedlings had higher root and shoot dry weight than the non-mycorrhizal seedlings (Giri *et al.*, 2007). Al-Karaki (2000) observed a higher shoot and root dry weight, fresh fruit yield, fruit weight and fruit number in a mycorrhizal tomato plant than in a non-mycorrhizal tomato plant. Colla *et al.* (2008) reported improved growth, yield, water status, nutrient content and quality of fruits of *Cucurbita pepo* plants colonized by *Glomus intraradices* when exposed to salinity stress. Enhanced growth of AM plants has been partly attributed to mycorrhizically mediated enhanced nutrient acquisition, especially better P nutrition (Plenchette and Duponnis, 2005; Sharifi *et al.*, 2007).

Nutrient uptake

AMF have been shown to have a positive influence on the composition of mineral nutrients (especially poor mobility nutrients such as phosphorus) of plants grown in salt-stress conditions (Al-Karaki and Clark, 1998) by enhancing and/or selective uptake of nutrients. This is primarily regulated by the supply of nutrients to the root system (Giri and Mukerji, 2004) and increased transport (absorption and/or translocation) by AMF (Al-Karaki, 2000; Sharifi *et al.*, 2007). Mycorrhizal dependency increases with increasing salt concentrations (Giri and Mukerji, 2004). It is found to vary with the isolates of fungus and species of plant (Tian *et al.*, 2004). A number of studies have shown the effect of salinity on the nutrient uptake of mycorrhizal plants (Table 2). The impact of mycorrhizal fungi on different mineral nutrients is given below.

Phosphorus. Soil salinity significantly reduces the absorption of mineral nutrients, especially phosphorus (P), because phosphate ions precipitate with Ca^{2+}, Mg^{2+} and Zn^{2+} ions in salt-stressed soils and become unavailable to plants

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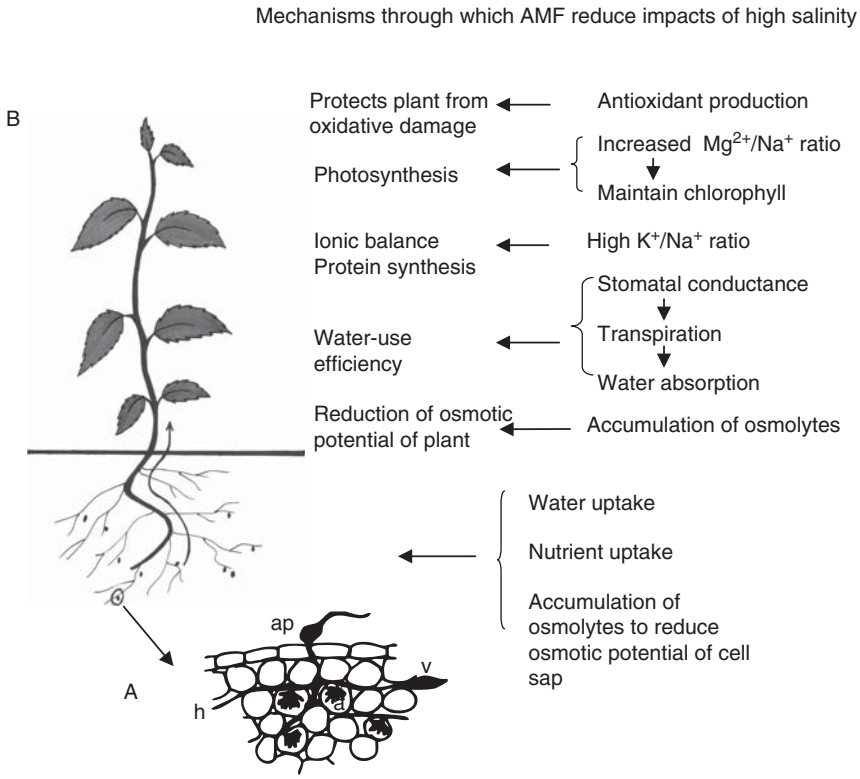

FIG. 2 The intricate functioning of arbuscular mycorrhizal (AM) fungi in ameliorating salt stress in plants. In AM symbiosis, the fungus forms an appressorium (ap) on the root surface and enters the root cortex by extending its hyphae (h). The hyphae form arbuscules (a) and vesicles (v) in the cortex. Salinity deprives plants of the basic requirements of water and nutrients, causing physiological drought and a decrease in osmotic potential accompanied by nutrient deficiency, rendering plants weak and unproductive. Arbuscular mycorrhiza help plants in salt stress by improving water and nutrient uptake: a decrease in osmotic potential is countered by increasing accumulation of osmolytes, and water-use efficiency, photosynthesis and antioxidant production (to scavenge ROS) is more efficient in salt-stressed plants in the presence of AMF (see text).

(Azcón-Aguilar *et al.*, 1979). Therefore, P solubilization or fertilization is necessary for plant growth which may be helpful in mitigating salt stress by overcoming P-binding capacity of the soil (Cantrell and Lindermann, 2001). Mycorrhizal inoculation can increase P concentration in plants by enhancing its uptake facilitated by the extensive hyphae of the fungus which allows them to explore more soil volume than the non-mycorrhizal plants (Ruiz-Lozano and Azcón, 2000). It is estimated that external hyphae deliver up to 80 % of a plant's P requirements (Matamoros *et al.*, 1999). Studies have shown higher P content (1. 2, 1·2, 0·9 and 0·6 %) in mycorrhizal than non-mycorrhizal *Acacia nilotica* plants (0·6, 0·5, 0·2 and 0·1 %) in saline soils at varied levels of soil salinity (1·2, 4, 6·5 and 9·5 dS m^{-1}), respectively (Giri *et al.*, 2007). Recently, Shokri and Maadi (2009) recorded that phosphorus concentration in *Trifolium alexandrium* plants was reduced with increasing levels of salinity. They observed that as salinity of soil increased (2·2, 5 and 10 dS m^{-1}), the concentrations of P decreased (0·41, 0·36 and 0·28 mg g^{-1}, respectively) in shoots of non-mycorrhizal *Trifolium alexandrium* plants while the P concentrations were relatively higher (0·68, 0·62 and 0·47 mg g^{-1}, respectively) in their mycorrhizal counterparts. The higher P concentrations in mycorrhizal plants at all salinity levels suggest that AMF increased P uptake in plants under saline conditions. Improved P nutrition in AM-inoculated plants may improve their growth rate, increase antioxidant production and enhanced nodulation and nitrogen fixation in legumes (Feng *et al.*, 2002; Alguacil *et al.*, 2003; Garg and Manchanda, 2008). Enhanced uptake of P by AMF in plants grown under saline conditions may reduce the negative effects of Na^{+} and Cl^{-} ions by maintaining vacuolar membrane integrity, which facilitates compartmentalization within vacuoles and selective ion intake (Rinaldelli and Mancuso, 1996), thereby preventing ions from interfering in metabolic pathways of growth (Cantrell and Lindermann, 2001).

Nitrogen. Salinity interferes with nitrogen (N) acquisition and utilization by influencing different stages of N metabolism, such as, NO_3^- uptake and reduction and protein synthesis (Aslam *et al.*, 1984, Frechill *et al.*, 2001). Application of AMF can help in better assimilation of nitrogen in the host plant. Giri and Mukerji (2004) recorded higher accumulation of N in shoots of mycorrhizal *Sesbania grandiflora* and *S. aegyptiaca* than non-mycorrhizal control plants. The extra-radical mycelia take up inorganic nitrogen from the soil in the form of nitrate and assimilated it via nitrate reductase, located in the arbuscule-containing cells (Kaldorf *et al.*, 1998) and the GS-GOGAT cycle leading to the formation of arginine. Arginine, so formed, is transported from the extra-radical to the intra-radical mycelia where it is catabolized again producing amongst other substances ammonia, which equilibrates

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TABLE 2. *Some examples of increased/decreased nutrient uptake in AM plants under salinity stress*

Nutrient	Range of salinity*	Plant	Fungus	Effect	References
Phosphorus	0–24·6 dS m^{-1} (0–200 mM)	*Glycine max*	*Glomus etunicatum*	Increase	Sharifi *et al.* (2007)
	1·2–9·5 dS m^{-1}	*Acacia nilotica*	*Glomus fasciculatum*	Increase	Giri *et al.* (2007)
	0–19·12 dS m^{-1} (0–150 mM)	*Citrus karma*	Mixed inoculum of *Glomus* sp. and *Gigaspora* sp.	Increase	Murkute *et al.* (2006)
	0–6·10 dS m^{-1} (0–3 g kg^{-1})	*Gossypium arboreum*	*Glomus mosseae*	Increase	Tian *et al.* (2004)
	3–10 dS m^{-1} (0·3–1·0 S m^{-1})	*Acacia auriculiformis*	*Glomus macrocarpum* and *Glomus fasciculatum*	Increase	Giri *et al.* (2003)
	0–13·19 dS m^{-1} (0–100 mM)	*Zea mays*	*Glomus mosseae*	Increase	Feng *et al.* (2002)
	1·4–7·4 dS m^{-1}	*Lycopersicon esculentum*	*Glomus mosseae*	Increase	Al-Karaki (2000)
Nitrogen	4–8 dS m^{-1}	*Cajanus cajan*	*Glomus mosseae*	Increase	Garg and Manchanda (2008)
	0–19·12 dS m^{-1} (0–150 mM)	*Citrus karma*	Mixed inoculum of *Glomus* sp. and *Gigaspora* sp.	Increase	Murkute *et al.* (2006)
	15·8 dS m^{-1} (1·58 S m^{-1})	*Sesbania aegyptiaca*	*Glomus macrocarpum*	Increase	Giri and Mukerji (2004)
Potassium	0–7·56 dS m^{-1} (0–3 g L^{-1})	*Ocimum basilicum*	*Glomus intraradices*	Increase	Zuccarini and Okurowska (2008)
	0–24·6 dS m^{-1} (0–200 mM)	*Glycine max*	*Glomus etunicatum*	Increase	Sharifi *et al.* (2007)
Calcium	0–24·6 dS m^{-1} (0–200 mM)	*Glycine max*	*Glomus etunicatum*	Increase	Sharifi *et al.* (2007)
	0·72–7·39 dS m^{-1}	*Musa* sp.	*Glomus clarum*	Increase	Yano-Melo *et al.* (2003)
Magnesium	15·8 dS m^{-1} (1·58 S m^{-1})	*Sesbania aegyptiaca*	*Glomus macrocarpum*	Increase	Giri and Mukerji (2004)
Sodium	1·2–9·5 dS m^{-1}	*Acacia nilotica*	*Glomus fasciculatum*	Increase	Giri *et al.* (2007)
	0–6·10 dS m^{-1} (0–3 g kg^{-1})	*Gossypium arboreum*	*Glomus mosseae*	Increase	Tian *et al.* (2004)
	0·12 S m^{-1}	*Acacia auriculiformis*	*Glomus macrocarpum* and *Glomus fasciculatum*	Increase	Giri *et al.* (2003)
	0–7·56 dS m^{-1} (0–3 g L^{-1})	*Ocimum basilicum*	*Glomus intraradices*	Decrease	Zuccarini and Okurowska (2008)
	0–24·6 dS m^{-1} (0–200 mM)	*Glycine max*	*Glomus etunicatum*	Decrease	Sharifi *et al.* (2007)
	1·4–7·4 dS m^{-1}	*Lycopersicon esculentum*	*Glomus mosseae*	Decrease	Al-Karaki (2000)
Chloride	0–6·10 dS m^{-1} (0–3 g kg^{-1})	*Gossypium arboreum*	*Glomus mosseae*	Increase	Tian *et al.* (2004)
	0–7·56 dS m^{-1} (0– g L^{-1})	*Ocimum basilicum*	*Glomus intraradices*	Decrease	Zuccarini and Okurowska (2008)
Copper	1·2–9·5 dS m^{-1}	*Acacia nilotica*	*Glomus fasciculatum*	Increase	Giri *et al.* (2007)
	1·4–7·4 dS m^{-1}	*Lycopersicon esculentum*	*Glomus mosseae*	Decrease	Al-Karaki (2000)
Zinc	0–24·6 dS m^{-1} (0–200 mM)	*Glycine max*	*Glomus etunicatum*	Increase	Sharifi *et al.* (2007)
	1·4–7·4 dS m^{-1}	*Lycopersicon esculentum*	*Glomus mosseae*	Decrease	Al-Karaki (2000)

* The range of salinity within brackets is the actual salt concentrations used by the authors.

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with ammonium according to the pH. These processes are consistent with increased expression of enzymes involved in primary nitrogen fixation in the extra-radical mycelia, whereas enzymes involved in arginine catabolism are up-regulated in the intra-radical mycelia. However, little is known about the transfer of nitrogen from the fungus to the plant and the authors have proposed the involvement of ammonium transporters (Govindarajulu *et al.*, 2005). Cliquet and Stewart (1993) observed increased N uptake in an AM plant due to a change in N metabolism brought about by changes in the enzymes associated with N metabolism (Mathur and Vyas, 1996). Studies have reported that improved N nutrition may help to reduce the toxic effects of Na ions by reducing its uptake and this may indirectly help in maintaining the chlorophyll content of the plant. The form of available N (NO_3^- or NH_4^+) strongly influences Na^+ accumulation (Giri and Mukerji, 2004). However, the exact mechanisms used by AMF to uptake N under salt-stress conditions are not clearly understood.

$K^+ : Na^+$ ratio. When Na^+ or salt concentration in the soil is high, plants tend to take up more Na^+ resulting in decreased K^+ uptake. Na^+ ions compete with K^+ for binding sites essential for various cellular functions. Potassium plays a key role in plant metabolism. It activates a range of enzymes, and plays an important role in stomatal movements and protein synthesis. High concentrations of K^+ are required in protein synthesis as K^+ is used in the binding of tRNA to the ribosomes (Blaha *et al.*, 2000). These functions cannot be replaced by Na^+ ions (Giri *et al.*, 2007); a higher $Na^+ : K^+$ ratio generated due to salinity disrupts the ionic balance in the cytoplasm, consequently disrupting various metabolic pathways (Giri *et al.*, 2007). Mycorrhizal colonization of a plant with AMF can reverse the effect of salinity on K^+ and Na^+ nutrition. Mycorrhizal colonization can enhance K^+ absorption under saline conditions (Alguacil *et al.*, 2003; Giri *et al.*, 2007; Sharifi *et al.*, 2007; Zuccarini and Okurowska, 2008) while preventing Na^+ translocation to shoot tissues. Na^+ uptake may also be influenced by the synthesis and storage of polyphosphate (Olrovich and Ashford, 1993) as well as by other cations, particularly K (Giri *et al.*, 2003). The uptake of K^+ increased in shoot tissues of mycorrhizal plants even at a high salinity level (9·5 dS m^{-1}). This increases the $K^+ : Na^+$ ratio in roots and shoots of AM plants (Giri *et al.*, 2007). It is accomplished by regulating the expression and activity of K^+ and Na^+ transporters and of H^+ pumps that generate the driving force to transport ions (Parida and Das, 2005). The Na^+/H^+ antiporter catalyses the transfer of Na^+ out of the cytoplasm into either vacuoles or apoplasm (Ouziad *et al.*, 2006). The higher $K^+ : Na^+$ ratio helps to prevent the disruption of various K-mediated enzymatic processes and inhibition of protein synthesis. High $K^+ : Na^+$ ratios are also beneficial in influencing the ionic balance of the cytoplasm or Na^+ efflux from plants (Allen and Cunningham, 1983; Founoune *et al.*, 2002; Colla *et al.*, 2008). Lower Na^+ in the mycorrhizal plants than non-AM plants may also be explained by the dilution effect due to growth enhancement (Al-Karaki, 2000, 2006).

There are contrasting reports that AMF sometimes enhance Na^+ uptake (Allen and Cunningham, 1983), while others suggest that AMF-colonized plants have lower levels of Na^+ (Dixon *et al.*, 1993; Sharifi *et al.*, 2007; Zuccarini and Okurowska, 2008). The concentration of Na^+ increased in AM plants with increasing salinity levels up to a certain level, and subsequently decreased at higher salinity. This suggests that AMF induce a buffering effect on the uptake of Na^+ when the content of Na^+ is within the permissible limit (Allen and Cunningham, 1983). This also indicates the possibility of a regulatory mechanism operating in the plant to contain Na^+ ions. P uptake by mycorrhizal plants also decreased in an excessive salt concentration (9·5 dS m^{-1}) due to the toxic effect of Na^+ on fungal development (Giri *et al.*, 2007), suggesting that mycorrhizal responses were only significant up to a certain level of salinity (4·7 dS m^{-1}; Al-Karaki, 2000).

Chloride ions. Root cells take up Cl^- from the soil solution through H^+/Cl^- symporters at low $[Cl^-]_{ext,}$ and also through anion channels under saline conditions. To reach the xylem and then the shoot, Cl^-traverses the root by a symplastic pathway and is released from cells within the stele through specific anion channels. At high salinity, Cl^- accumulation increases greatly, though it remains constant in the roots (White and Broadley, 2002). The high tissue Cl^- concentrations can be toxic to crop plants and may restrict agriculture in saline regions (Xu *et al.*, 2000). This problem can be tackled to some extent by the application of arbuscular mycorrhiza, which can reduce the uptake of Cl^- ions (Zuccarini and Okurowska, 2008). The Cl^- ions can be compartmentalized in vacuolar membranes, thereby preventing them from interfering with the metabolic pathways in the plant (Cantrell and Lindermann, 2001). However, there are reports of increased Cl^- accumulation due to mycorrhizal colonization, the reason of which may be due to the carbon drain imposed by mycorrhizal hyphae on plants, which enhances the translocation of highly mobile anions like Cl^- from the soil (Bulwada *et al.*, 1983; Graham and Syversten, 1984).

Calcium. Calcium acts as a second messenger and during salt stress the Ca^{2+} concentration is increased to transduce signals. Studies revealed that mycorrhization strongly affects Ca^{2+} in the plant. Cantrell and Linderman (2001) reported increased Ca^{2+} uptake in mycorrhizal lettuce. A higher Ca^{2+} concentration in mycorrhizal than in non-mycorrhizal banana plants was reported by Yano-Melo *et al.* (2003). High Ca^{2+} has a beneficial effect on toxic effects of NaCl by facilitating higher K^+/Na^+ selectivity leading to salt adaptation (Cramer *et al.*, 1985; Rabie and Almadini, 2005). Moreover, high Ca^{2+} was also found to enhance colonization and sporulation of AMF (Jarstfer *et al.*, 1998). However, in contrast to the reports above, Giri *et al.* (2003) reported that Ca^{2+} concentration remains unchanged in shoot tissues of mycorrhizal and non-mycorrhizal *Acacia auriculiformis* plants. This suggests that AMF may not be so important to the nutrients moving to plant roots by mass flow as compared with nutrients moving by diffusion (Tinker, 1975). Rhodes and Gerdemann (1978) indicated that Ca^{2+} is not translocated to onion roots through mycorrhizal hyphae as readily and efficiently as P. Moreover, AM inoculation depressed the Ca : P ratio by increased production of oxalate in the mycorrhizosphere, which is able to scavenge Ca^{2+} from the solution (Azcón and Barea, 1992).

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Magnesium. Biosynthesis of chlorophyll is impeded by salt stress, which prevents light harvesting and causes impairment of photosynthesis. Mycorrhiza, by improving Mg^{2+} can support a higher chlorophyll concentration (Giri *et al.*, 2003). This suggests that salt interfers less with chlorophyll synthesis in mycorrhizal than non-mycorrhizal plants (Giri and Mukerji, 2004). Effective Mg^{2+}-uptake helps by increasing the chlorophyll concentration and hence improving photosynthetic efficiency and plant growth. Some examples of increased or reduced nutrient uptake in AM plants under salinity are enlisted in Table 2.

Biochemical changes

As soil dries out and soil water potential becomes more negative, plants must decrease their water potential to maintain a favourable gradient for water flow from soil into roots. To achieve such an effect, plants develop a plethora of mechanisms, the most important being osmotic adjustment or osmoregulation, which may require a reduction in the plant osmotic potential which is mitigated by active accumulation of organic ions or solutes (Morgan, 1984; Hoekstra *et al.*, 2001). A number of nitrogen-containing compounds accumulate in plants exposed to saline stress. The most commmon of these include amino acids, amide and proteins; also quaternary ammonium compounds (betaines) and polyamines (Rabie and Almadini, 2005). These compounds are generally present in low concentrations when the plant is not under salt stress (Feng *et al.*, 2002). The specific nitrogen-containing compounds that accumulate in saline environments vary with plant species (Rabie and Almadini, 2005). Osmoregulation allows cells to maintain turgor and turgor-dependent processes including cellular expansion and growth, stomatal opening and photosynthesis, while keeping a gradient of water potential favourable to water entering the plant. Table 3 lists the effects of salinity on various parameters on AM plants.

Proline. Accumulation of amino acid proline is one of the most frequently reported modifications induced by water and salt stress in plants. Under saline conditions, many plants accumulate proline as a non-toxic and protective osmolyte to maintain osmotic balance under low water potentials (Stewart and Lee, 1974; Jain *et al.*, 2001; Parida *et al.*, 2002; Ashraf and Foolad, 2007; Sannazzaro *et al.*, 2007). It also acts as a reservoir of energy and nitrogen for utilization during salt stress (Goas *et al.*, 1982). Proline accumulation has been found to increase when the plant is colonized by AMF. Mycorrhizal mung bean (*Vigna radiata*) plants were reported to have a higher proline content than non-mycorrhizal plants at 12·5 and 25 mM NaCl at 40 and 60 d after sowing (Jindal *et al.*, 1993). Sharifi *et al.* (2007) also reported a higher proline concentration in AM soybean than the non-AM plants at different salinity levels (0, 50, 100, 150 and 200 mM NaCl). They also observed that in AM plants, a higher level of proline concentration is found in roots than shoots. This may be due to the fact that the roots are the primary sites of water absorption and, therefore, must maintain osmotic balance between water-absorbing root cells and the external media. However, in contrast to the reports above, Rabie and Almadini (2005) reported that non-AM *Vicia faba* plants accumulated more proline than

TABLE 3. *Some examples of increased/decreased content of the indicated compounds in AM plants compared with non-AM plants under saline conditions*

Compound	Range of salinity*	Plant	Fungus	Effect	References
Proline	0–24·6 dS m^{-1} (0–200 mM)	*Glycine max*	*Glomus etunicatum*	Increase	Sharifi *et al.* (2007)
	0–6 dS m^{-1}	*Vicia faba*	*Glomus clarum*	Decrease	Rabie and Almadini (2005)
Polyamines	0–24·6 dS m^{-1} (0–200 mM)	*Lotus glaber*	*Glomus intraradices*	Increase	Sannazzaro *et al.* (2007)
Betaines	0–34·5 dS m^{-1} (0–300 mM)	*Phragmites australis*	*Glomus fasciculatum*	Increase	Al-Garni (2006)
Carbohydrates	0–34·5 dS m^{-1} (0–300 mM)	*Phragmites australis*	*Glomus fasciculatum*	Increase	Al-Garni (2006)
	0–24·6 dS m^{-1} (0–200 mM)	*Glycine max*	*Glomus etunicatum*	Decrease	Sharifi *et al.* (2007)
Antioxidants	0–13·19 dS m^{-1} (0–100 mM)	*Glycine max*	*Glomus etunicatum*	Increase	Ghorbanli *et al.* (2004)
	4–8 dS m^{-1}	*Cajanus cajan*	*Glomus mosseae*	Increase	Garg and Manchanda (2002)
Abscissic acid	0–24·6 dS m^{-1} (0–200 mM)	*Lotus glaber*	*Glomus intraradices*	Increase	Sannazzaro *et al.* (2007)
	0–13·19 dS m^{-1} (0–100 mM)	*Lactuca sativa*	*Glomus intraradices*	Decrease	Jahromi *et al.* (2008)
Chlorophyll	15·8 dS m^{-1} (1·58 S m^{-1})	*Sesbania aegyptiaca* and *Sesbania grandiflora*	*Glomus macrocarpum*	Increase	Giri and Mukerji (2004)
	0–4·24 dS m^{-1} (0–2 g kg^{-1})	*Zea mays*	*Glomus mosseae*	Increase	Sheng *et al.* (2008)
	0·84–5·8 dS m^{-1}	*Lactuca sativa*	Mixture of *Glomus mosseae*, *Glomus intraradices* and *Glomus coronatum*	Increase	Zuccarini (2007)
Chlorophyll fluorescence	0–7·56 dS m^{-1} (0–3 g L^{-1})	*Ocimum basilicum*	*Glomus intraradices*	Increase	Zuccarini and Okurowska (2008)

* The range of salinity within brackets is the actual salt concentrations used by the authors.

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AM plants at various salinity levels (0–6 dS m^{-1}). S. Wang *et al.* (2004) suggested that proline accumulation in plants may be a symptom of stress in less-salt-tolerant species and its contribution to osmotic adjustment was apparently negligible as compared with potassium ions. The accumulation of proline may also be due to salinity and not necessarily by mycorrhizal colonization as reported by Sannazzaro *et al.* (2006). High levels of proline are known to accumulate in *Lotus glaber* in response to salinity (Maiale *et al.*, 2004) but, so far there are no reports regarding the influence of AMF on proline accumulation in this plant. The inconsistency in these reports needs to be elucidated to determine the salt-tolerance mechanisms operating in different plant systems.

Betaines. Accumulation of betaines in plants under salt stress is a common occurrence. Betaines are quaternary ammonium compounds which are *N*-methylated derivatives of amino acids. Once formed, they are seldom metabolized (Grattan and Grieve, 1985; Duke *et al.*, 1986). This can therefore be used as an effective indicator of salt stress (Duke *et al.*, 1986). Betaines are not merely non-toxic cellular osmolytes but they can also stabilize the structures and activities of enzymes and protein complexes and maintain the integrity of membranes against the damaging effects of excessive salt (Gorham, 1995). Accumulation of betaines under salt stress is found to increase when the plant is colonized by AMF. It was found that at higher salinity levels the glycine betaine content of AM plants was about 2-fold greater than that of non-AM plants (Al-Garni, 2006).

Polyamines. Free polyamines are small organic cations that are necessary for eukaryotic cell growth. There are three main polyamines in plants: putrescine (Put), spermidine (Spd) and spermine (Spm). Spd and Spm are synthesized from Put by successive addition of aminopropyl groups and Put is synthesized directly from ornithine via ornithine decarboxylase or indirectly following decarboxylation of arginine by arginine decarboxylase. These cations are thought to play an important role in plant responses to a wide array of environmental stressors such as salinity (Delauney and Verma, 1993; Krishnamurthy and Bhagwat, 1989), high osmolarity (Besford *et al.*, 1993) and anti-oxidative stress (Langebartels *et al.*, 1991; Kurepa *et al.*, 1998). They have been proposed as candidates for regulation of root development under saline situations (Couée *et al.*, 2004). Under saline conditions, free polyamine pools are reduced. However, inoculation of host plants with AMF increases free polyamine concentrations. Sannazzaro *et al.* (2007) reported an increase in total free polyamine pools in *Lotus glaber* plants colonized by *Glomus intraradices*. They observed variations in individual polyamines in response to salinity and mycorrhization depending on the plant genotype and organ (root/shoot) considered. Mycorrhizal, salt-tolerant genotypes of *Lotus glaber* plants showed higher levels of root Spm than non-AM plants. Mycorrhizal salt-sensitive *Lotus glaber* plants under salinity showed higher root Spm, lower shoot and root Put and lower Spd levels than the corresponding non-AM plants. It may be inferred that modulation of polyamine pools can be one of the mechanisms used by AMF to improve plant adaptation to saline soils (Sannazzaro *et al.*, 2007).

Carbohydrates. Several studies report the accumulation of soluble sugars to adjust the osmotic potential of plants during salt stress – a means of lowering the osmotic potential of the plant which constitutes an important plant protection mechanism against stress (Thanna and Nawar, 1994). The soluble sugar content of *Phragmites australis* was significantly increased by increasing concentrations of NaCl (Al-Garni, 2006). The increase in total carbohydrates is found to be positively correlated with mycorrhization of the host plant as reported by Thomson *et al.* (1990). Al-Garni (2006) reported that *Phragmites australis* plants colonized by *Glomus fasciculatum* had higher levels of soluble sugars than those non-mycorrhizal plants. Porcel and Ruiz-Lozano (2004) also reported increased sugar concentrations in soybean roots colonized by *Glomus intraradices*. The positive correlation between sugar content and mycorrhization is due to the sink effect of the fungus demanding sugars from the shoot tissues (Augé, 2000). The processes involved in the development of mycorrhiza frequently lead to increased rates of photosynthesis and of carbon compounds to the root systems of host plants (Finlay and Söderström, 1992). The increased sugar accumulation may also be due to hydrolysis of starch to sugars in the seedlings inoculated with mycorrhiza (Nemec, 1981). Feng *et al.* (2002) studied the prevalence of correlation between P concentration and sugar accumulation in host plants under saline conditions. They reported that in spite of similar P concentrations, the soluble sugar concentration in roots of mycorrhizal plants was higher than that of the non-mycorrhizal maize plants. This suggests that the higher soluble sugar concentration in mycorrhizal roots is due to AMF colonization and not due to an improvement in the P status of the plants. Trehalose, a non-reducing disaccharide, is the main storage carbohydrate in AMF and has been found to play an important role as an abiotic stress protectant stabilizing dehydrated enzymes and membranes and protecting biological structures from desiccation damage. It is present in the extra-radical mycelium as well as in spores of AMF (Becard *et al.*, 1991). In higher vascular plants, trehalose is a rare sugar, but it gets induced on AMF colonization of plant roots (Hoekstra *et al.*, 1992; Schubert *et al.*, 1992) and thus may help in protecting plants against salt stress. Recently, Ocon *et al.* (2007) made an attempt to decipher the effect of salt stress on trehalose content and metabolism in the extra-radical hyphae of *Glomus intraradices* and its possible role in protecting plants against abiotic stresses. They did not observe any change in trehalose content in *Glomus intraradices* when treated with 0·5 M NaCl. However, moderate transient activations of trehalose-6-phosphate phosphatase (required for the conversion of trehalose-6-phosphate to trehalose and orthophosphate) and neutral trehalase (required for the breakdown of trehalose into glucose) activities not associated with any transcriptional change were observed. Studying the accumulation of trehalose in extra-radical hyphae and mycorrhizal roots is of interest and it might provide a powerful insight into the response of AMF to stress conditions and trehalose being exploited as a stress-protective agent. Trehalose metabolism has been found to play a significant role in adaptation to hyperosmotic conditions of symbiotic bacteria (Lopez *et al.*, 2008). This strengthens the hypothesis that there is a role for this molecule

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in imparting tolerance against salt stress. Therefore, investigations are required to evaluate the potential of trehalose in protecting the cell from salt stress.

Conversely, some authors reported negative correlations between AMF colonization and sugar accumulation in host plants. Pearson and Schweiger (1993) reported a reduction in carbohydrate concentration with an increase in the percentage of root colonization. Sharifi *et al.* (2007) observed no role of soluble carbohydrates in responses of AM (colonized by *Glomus etunicatum*) soybean plants to salinity.

Antioxidants. Activated oxygen species such as singlet oxygen, superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide and hydroxyl radical ($^{\bullet}OH$) are inevitable by-products of the interaction between oxygen and electrons leaked from the electron transport chains in chloroplast and mitochondria during normal aerobic metabolism (Scandalios, 1993; Asada, 1999; Møller, 2001). All activated oxygen species can react with DNA, proteins and lipids (Fridovich, 1986) and in the absence of the protective mechanism; they can damage cell structure and function (Alguacil *et al.*, 2003). Thus plants have protective mechanisms to escape from oxidative damage (Jiang and Zhang, 2002; Núñez *et al.*, 2003; Yamane *et al.*, 2004) involving antioxidant molecules and enzymes (Jiang and Zhang, 2002; Núñez *et al.*, 2003). A correlation between antioxidant capacity and NaCl tolerance has been demonstrated in several plant species (Gossett *et al.*, 1994; Benavides *et al.*, 2000; Núñez *et al.*, 2003). Plants with high concentrations of antioxidants have been reported to have greater resistance to oxidative damage (Spychalla and Desbough, 1990; Dionisio-Sese and Tobita, 1998; Jiang and Zhang, 2002). Antioxidants include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APOX), glutathione reductase (Alguacil *et al.*, 2003; Ghorbanli *et al.*, 2004; Yamane *et al.*, 2004; Wu *et al.*, 2006), dehydroascorbate reductase (Ghorbanli *et al.*, 2004, Wu *et al.*, 2006), monodehydroascorbate reductase (Ghorbanli *et al.*, 2004), guaiacol peroxidase, oxidized glutathione (Wu *et al.*, 2006), glutathione peroxidase, and the enzymes involved in the ascorbate–glutathione cycle (Alguacil *et al.*, 2003). The non-enzymatic compounds which scavenge activated oxygen species include carotenoids, glutathione, tocopherols and ascorbic acid (Alguacil *et al.*, 2003; Wu *et al.*, 2006).

Several studies suggested that AM symbiosis helps plants to alleviate salt stress by enhancing the activities of antioxidant enzymes (Alguacil *et al.*, 2003; Harisnaut *et al.*, 2003; Zhong Qun *et al.*, 2007). Several researchers have observed higher antioxidant enzyme activity in mycorrhizal plants than the non-mycorrhizal plants. Ghorbanli *et al.* (2004) have shown higher activities of SOD, peroxidase, APOX activities in mycorrhizal soybean plants than in non-mycorrhizal plants. However, the activities of CAT and polyphenol peroxidase remain unchanged in both the plants. The higher activities of peroxidase and polyphenol peroxidase (a component of the plant defence mechanism against pathogens) in mycorrhizal plants have been reported by Mathur and Vyas (1996). Alguacil *et al.* (2003) also reported enhanced activities of CAT, APOX and SOD in *Olea europaea* and *Retama sphaerocarpa*. The increased SOD will help detoxify superoxide to hydrogen peroxide (Smirnoff, 1993). The hydrogen peroxide generated will be detoxified by CAT and peroxidase. APOX is also reported to be involved in detoxification of hydrogen peroxide produced in the chloroplasts of stressed host plants (Lopez *et al.*, 1996; Benavides *et al.*, 2000). The elevated levels of glutathione reductase 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 $O_2^{\bullet -}$ (Gamble and Burke, 1984; Menconi *et al.*, 1995).

The plants possess higher antioxidant enzyme activities as a result of mycorrhizal colonization but the response of the individual enzymes varies with respect to the host plant and the fungal species. This variation may also depend on the micronutrients available to some of the enzymes, e.g. CAT, APOX and SOD are metalloenzymes (Alguacil *et al.*, 2003) whose activities depend on availability. The activity of these metalloenzymes can be determined by the availability of the metals they utilize. Both excess and deficiency of micronutrients can modulate the expression of these metalloenzymes. For example, the presence of iron can enhance the activities of CAT and APX in *Nicotiana plumbaginifolia* (Kamfenkel *et al.*, 1995). The increase in Fe, Cu, Zn and Mn in shoots of plants inoculated with mycorrhiza could be involved in the increase in total SOD activity observed in mycorrhizal plants. These data indicate that mycorrhiza-induced activities of several antioxidant enzymes may be related to enhanced growth and acquisition of P or N in the plants (Alguacil *et al.*, 2003). However, the role of AMF on the status of non-enzymatic antioxidants such as carotenoids, tocopherols and ascorbic acid of the host plant has not been reported. Therefore, this aspect requires an in-depth investigation.

Physiological changes

Salt stress can affect the plant by disrupting its physiological mechanisms such as decreasing photosynthetic efficiency, gas exchange, membrane disruption, water status, etc. There is evidence demonstrating that AM symbiosis can alleviate such effects by employing various mechanisms which are discussed below.

Chlorophyll content. Increasing salinity causes a reduction in chlorophyll content (Sheng *et al.*, 2008) due to suppression of specific enzymes that are responsible for the synthesis of photosynthetic pigments (Murkute *et al.*, 2006). A reduction in the uptake of minerals (e.g. Mg) needed for chlorophyll biosynthesis also reduces the chlorophyll concentration in the leaf (El-Desouky and Atawia, 1998). A higher chlorophyll content in leaves of mycorrhizal plants under saline conditions has been observed by various authors (Giri and Mukerji, 2004; Sannazzaro *et al.*, 2006; Zuccarini, 2007; Colla *et al.*, 2008; Sheng *et al.*, 2008). This suggests that salt interfers less with chlorophyll synthesis in mycorrhizal than in non-mycorrhizal plants (Giri and Mukerji, 2004). In the presence of mycorrhiza, the antagonistic effect of Na^+ on Mg^{2+} uptake is counterbalanced and suppressed (Giri *et al.*, 2003). Inoculated plants under salt stress reach levels of photosynthetic capacity (estimated by chlorophyll content) even superior to those of

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non-stressed plants, showing that in this respect, mycorrhization is capable of fully counterbalancing stress (Zuccarini, 2007).

Chlorophyll fluorescence. Chlorophyll fluorescence is a measure of photosynthetic efficiency. It is calculated as the ratio between variable and maximum fluorescence (F_v/F_m) (Sheng *et al.*, 2008; Zuccarini and Okurowska, 2008). The ratio $F_v : F_m$ measures the capacity of the primary photochemistry of PSII which itself is particularly sensitive to a variety of environmental stress-inducing factors (Figueroa *et al.*, 1997). Salt stress could destroy the PSII reaction centre and disrupt electron transport in the photosynthetic apparatus of the plants. This toxic influence of salinity on the PSII reaction centre could be mitigated by AM symbiosis (Sheng *et al.*, 2008). The ratio $F_v : F_m$ in the leaves of mycorrhizal plants was significantly higher than that in non-AM plants (Sheng *et al.*, 2008; Zuccarini and Okurowska, 2008). Mycorrhiza-inoculated plants also showed higher non-photochemical quenching than the uninoculated plants (Sheng *et al.*, 2008). An increase in non-photochemical quenching can occur as a result of processes that protect the leaf from light-induced damage (Maxwell and Johnson, 2000). AM symbiosis also triggers the regulation of energy bifurcation between photochemical and non-photochemical events (Sheng *et al.*, 2008). Studies that showed a higher or lower content of the various compounds in AM plants compared with non-AM plants are listed in Table 3.

Relative permeability. Arbuscular mycorrhizal fungal inoculation of host plants enables plants to maintain a higher electrolyte concentration than the non-mycorrhizal plants by maintaining improved integrity and stability of the membrane (Feng *et al.*, 2002; Garg and Manchanda, 2008; Kaya *et al.*, 2009). Consequently, electrical conductivity of mycorrhizal roots was found to be higher than the non-mycorrhizal roots (Garg and Manchanda, 2008). The mycorrhizal *Cajanus cajan* roots showed a higher relative permeability than the non-mycorrhizal plants at different levels of soil salinity (4, 6 and 8 dS m^{-1} EC_e; Garg and Manchanda, 2008). Kaya *et al.* (2009) reported that the electrolyte leakage in leaves of *Capsicum annum* treated with 50 mM and 100 mM concentrations of NaCl were 31·66 and 42·45, respectively while the AMF-inoculated plants had a relatively lower electrolyte leakage of 26·87 and 30·98, respectively, This suggests that mycorrhizal plants had a much lower root plasma membrane electrolyte permeability than the non-mycorrhizal plants. The increased membrane stability has been attributed to mycorrhiza-mediated enhanced P uptake and increased antioxidant production (Feng *et al.*, 2002).

Abscissic acid (ABA) content. It has been documented that mycorrhization can alter the ABA levels in the host plant (Duan *et al.*, 1996; Ludwig-Muller, 2000; Estrada-Luna and Davies, 2003). Sannazzaro *et al.* (2007) reported higher ABA levels in *Lotus glaber* plants colonized by *Glomus intraradices* than non-colonized plants. They suggested that ABA regulates free spermine pools in the shoots and the increased spermine content in the mycorrhizal plant may be due to increased ABA content. However, in contrast to this report, Jahromi *et al.* (2008) reported lower ABA levels in lettuce plants colonized by *Glomus intraradices* than in the non-AM plants, indicating that AM plants are less strained by imposed salinity stress than non-AM plants and, hence, accumulated less ABA. Comparing the above observations, it might be suggested that the effect of AMF species on ABA content varies with the host plants.

Water status. Plants in saline soils are subjected to physiological drought as Na^+ and Cl^- ions bind water that is needed to be mobilized by the plants (Fuzy *et al.*, 2008). Studies have indicated that colonization by AMF can help plants in such situations. Many authors have reported that plants inoculated with AMF maintain a relatively higher water content compared with uninoculated plants (Colla *et al.*, 2008; Jahromi *et al.*, 2008; Sheng *et al.*, 2008). This is facilitated by the improved hydraulic conductivity of the root at low water potential (Kapoor *et al.*, 2008). The improved root conductance is associated with a longer root and an altered root system morphology induced by AMF (Dehne, 1982, Kothari *et al.*, 1990). AM plants are found to exhibit a higher stomatal conductance thereby increasing the demand for transpiration (Duan *et al.*, 1996; Ruiz-Lozano *et al.*, 1996; Dell'Amico *et al.*, 2002; Jahromi *et al.*, 2008; Sheng *et al.*, 2008). Mycorrhizal plants are also shown to possess a lower osmotic potential which is maintained by fungal accumulating solutes, consequently resulting in improved plant osmotic adjustment. 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). All these improved parameters facilitated by mycorrhizal colonization enable host plants to use water more efficiently (Graham and Syversten, 1984) allowing them to maintain a lower intercellular carbon dioxide concentration. As a consequence, the gas exchange capacity increases in the mycorrhizal plants.

Nodulation and nitrogen fixation. Nodules, formed through symbiosis with nitrogen-fixing bacteria are considered a soft target for salt stress and their occurrence decreases due to salt stress (Harisnaut *et al.*, 2003; Rabie and Almadini, 2005; Garg and Manchanda, 2008). This is likely due to premature nodule senescence triggered by salt stress (Gogorcente *et al.*, 1997; Gonzalez *et al.*, 1998; Matamoros *et al.*, 1999) which causes an acceleration of lytic activities, formation of green pigments from leghaemoglobin (Sarath *et al.*, 1986) and loss of nitrogen fixation (Delgado *et al.*, 1994). Application of AMF can counteract the harmful effects of salinity on nodulation and nitrogen fixation in legumes. There are reports that AM symbiosis could alleviate drought stress-induced premature nodule senescence (Ruiz-Lozano *et al.*, 2001; Porcel *et al.*, 2003). Giri and Mukerji (2004) reported a strong effect of mycorrhizal inoculation on nodule formation under salt stress. Colonization of a legume by AMF can increase the number of nodules (Giri and Mukerji, 2004; Rabie and Almadini, 2005; Garg and Manchanda, 2008). This may indicate a positive influence of AMF on legume–nitrogen-fixing bacteria symbiosis. Higher leghaemoglobin content was observed in mycorrhizal plants. The leghaemoglobin content was determined by estimating the change of colour in the nodule from pink to brownish pink due to synthesis of green pigments from leghaemoglobin. This greening of nodule was observed much earlier in non-AM plants (8 weeks) than AM plants (10 weeks; Garg and Manchanda, 2008). Mycorrhizal

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plants also possess a higher nitrogenase activity. All these parameters contribute to the higher nitrogen-fixing ability of AM plants. This increased nitrogenase activity and nitrogen fixation in AM plants as opposed to non-AM plants has been attributed to relief from P stress, which is beneficial for the functioning of the nitrogenase enzyme of the bacterial symbionts and possibly due to uptake of some essential micronutrients which results in either 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; Rabie, 2005) which supports the need for both N and P and increased tolerance of plants to salinity stress (Rabie and Almadini, 2005).

Molecular changes

Studies describing the effects of AMF on molecular responses to saline conditions are very rare due to the complex nature of the trait. The fact that the factors conferring salt tolerance to plants are generally encoded by complex gene families hampers any study evaluating the impact of mycorrhizal colonization on expression of genes with products involved in salt tolerance with the need to track numerous variables (Ouziad *et al.*, 2006). The heterokaryotic and obligate nature of AMF also substantiates the difficulty. However, molecular studies on salt tolerance by AMF are gaining a fast momentum as scientists are trying to unravel the molecular mechanisms of AM symbiosis to gain a whole understanding of the alleviation of salt stress by AM symbiosis.

In the few molecular studies done so far, the focus has been on the expression studies of a few proteins, including Na^+/H^+ antiporters, Δ1-pyrroline-5-carboxylate synthetase (*LsP5CS*), late embryogenesis abundant protein (*LsLea*) and ABA (*Lsnced*).

Aquaporins belong to the major intrinsic protein (MIP) family of transmembrane channels, which permit the selective membrane passage of water (and a few other compounds) but not of H^+ and other ions (Weig *et al.*, 1997; Chen *et al.*, 2001; Hill *et al.*, 2004) through the plasma lemma (by PIPs) and the tonoplast (by TIPs). It has been reported that abiotic factors such as drought and salt stress influence aquaporin expression most probably via phytohormones like ABA and giberrellic acid (Mariaux *et al.*, 1998; Siefritz *et al.*, 2001). Expression analysis of aquaporin genes in salt-stressed AM plants revealed contrasting results. Data from Ouziad *et al.* (2006) showed that continuous salt treatment in AM *Lycopersicon esculentum* down-regulated the amount of LeTIP and LePIP1 transcripts by roughly 20 % but not of LePIP2 transcripts. They also observed that the effect on transcript formation of aquaporin was more drastic after AMF colonization than after salinity. In the communication, AMF significantly reduced the mRNA transcripts of LePIP1 and LeTIP but not of LePIP2 in non-treated controls and salt-stressed roots. In contrast to the report of Ouziad *et al.* (2006), Aroca *et al.* (2007) reported that salt treatment (0·12–3·06 dS m^{-1}) induces a higher expression of all *PvPIP* genes in AM *Phaseolus vulgaris* plants than non-AM plants except the *PvPIP1;2* gene which reduced its expression in AM root after salt treatment, while maintaining its expression in non-AM roots. They also reported an increase in PIP1 protein in AM plants. Likewise, Jahromi *et al.* (2008) reported that under salt-stress conditions (0–100 mM NaCl), mycorrhizal lettuce plants maintained the expression of the *LsPIP2* gene and up-regulation of *LsPIP1* gene mainly at 100 mM NaCl. The enhanced expression of the *PIP1* gene and abundance of its protein could contribute to regulating root water permeability and, consequently, to better tolerance of the osmotic stress generated by salinity (Aroca *et al.*, 2007; Jahromi *et al.*, 2008). These results point to the possibility that AMF differentially exerts control on the expression of these genes (Ouziad *et al.*, 2006) and that each *PIP* gene analysed may have a different function and regulation in AM symbiosis. How much this contrasting result reflects biological or technical differences remains to be evaluated.

Ouziad *et al.* (2006) also evaluated the expression of two Na^+/H^+ antiporters – *LeNHX1* and *LeNHX2* – in dependence on salt and mycorrhizal colonization and reported that no significant alterations are observed under these conditions.

The expression of genes encoding Δ1-pyrroline-5-carboxylate synthetase (*LsP5CS*), late embryogenesis abundant protein (*LsLea*) and ABA (*Lsnced*) were determined following varied salt treatments (0–100 mM NaCl) on *Lactuca sativa* plants colonized by *Glomus intraradices* (Jahromi *et al.*, 2008). The *PC5S* enzyme catalyses the rate-limiting step in the biosynthesis of proline (Kishor *et al.*, 1995), an osmoregulator in plants. Late embryogenesis abundant proteins act 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 in the biosynthesis of the 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). They 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. The *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 due to a primary salt avoidance mechanism such as Na^+ and Cl^- accumulation (Giri *et al.*, 2003; Al-Karaki, 2006).

Ultra-structural changes

Salinity is reported to bring about drastic ultra-structural changes in plants (Yamane *et al.*, 2004; Mahmoodzadeh, 2008). Salt stress caused an increase in membrane surface and quantity of vesicles in root cells of *Sorghum* (Koyro, 1997). Thickened cell wall, increased frequency of plasmodesmata and vacuolization of cytoplasm were reported in the shoot apical meristem of canola (Mahmoodzadeh, 2008) due to salt stress. Yamane *et al.* (2004) observed disruptions in thyllakoid and thyllakoid membrane, while Sun *et al.* (2004) reported induction of ovule abortion in *Arabidopsis thaliana* due to dissipation of mitochondrial membrane potential (Hauser *et al.*, 2006). Subsequently, cells in the gametophyte

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accumulate reactive oxygen species which gradually leads to programmed cell death (Hauser *et al.*, 2006). The aborting gametophytes develop concentric rings of endoplasmic reticulum (autophagic bodies) surrounding chloroplasts, mitochondria, micro-bodies and cytoplasm. The cytoplasmic contents and organelles were invaginated into the vacuole. However, the formation of autophagic bodies and vacuoles is not found in every aborting ovule suggesting that these events are not related to programmed cell death (Hauser *et al.*, 2006). Up till now; there have been no published reports on the effect of AM in plants under this aspect of salt stress. Since, AMF inoculation can increase antioxidant activities in plants, it may be suggested that AMF can be applied to counteract the activities of reactive oxygen species and alleviate salt stress. Unfortunately, the role of AMF in this aspect has not yet been deciphered. Therefore, this aspect seeks more attention from the researchers to unveil the mechanism of salt-stress alleviation by AMF.

FUTURE PERSPECTIVES

While research over many years has broadened our understanding of the multi-complex processes directing plant–mycorrhiza symbiosis in ameliorating salt stress in plants, yet, there are several aspects that need to be addressed. Investigation of these issues in the future will lead to a better understanding of the process.

Our knowledge of the molecular mechanisms governing the process of salt amelioration by AMF in plants is limited to partial understanding of only a few genes. The role of salt overly sensitive (*SOS*) genes with respect to mycorrhizal application needs to be uncovered. Identification of the genes involved in production of various antioxidants and enzymes controlling the synthesis of various osmoregulators will provide further insights into the molecular basis of the mechanism.

The transporter systems operating in the symbiosis need to be elucidated. Various techniques such as PIXE (proton-induced X-ray emission) may also be employed for studying the localization of various micro- and macro-nutrients in the plant as well as the fungus.

The ultrastructural aspects of AM plants under salinity have remained untouched till now. Demonstrating the role of AMF in this aspect may contribute significantly to our understanding of the mechanism.

In vitro studies of the fungus have just begun to explore morphology under salt stress. Such studies need to be directed towards biochemical, physiological and molecular mechanisms and signalling systems operating in the fungus to confer tolerance of salt stress in plants.

ACKNOWLEDGEMENTS

Heikham Evelin thanks the Council of Scientific and Industrial Research (CSIR), New Delhi, India for financial support. The authors thank Mr Ajay Bahadur Singh for his assistance during the preparation of the manuscript.

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