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REVIEW PAPER

Salinity stress alleviation using arbuscular mycorrhizal fungi. A review

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Abstract Salinity is one of the most severe environmental stress as it decreases crop production of more than 20% of irrigated land worldwide. Hence, it is important to develop salt-tolerant crops. Understanding the mechanisms that enable plant growth under saline conditions is therefore required. Acclimation of plants to salinized conditions depends upon activation of cascades of molecular networks involved in stress sensing, signal transduction, and the expression of specific stress-related genes and metabolites. The stress signal is first perceived at the membrane level by the receptors and then transduced in the cell to switch on the stress-responsive genes which mediate stress tolerance. In addition to stress-adaptative mechanisms developed by plants, arbuscular mycorrhizal fungi have been shown to improve plant tolerance to abiotic environmental factors such as salinity. In this review, we emphasize the significance of arbuscular mycorrhizal fungi alleviation of salt stress and their beneficial effects on plant growth and productivity. Although salinity can affect negatively arbuscular mycorrhizal fungi, many reports show improved growth and performance of mycorrhizal plants under salt stress conditions. These positive effects are explained by improved host plant nutrition, higher K^+/Na^+ ratios in plant tissues and a better osmotic adjustment by accumulation of compatible solutes such as proline, glycine betaine, or soluble sugars. Arbuscular mycorrhizal plants also improve photosynthetic- and water use efficiency under salt stress. Arbuscular mycorrhizal plants enhance the activity of antioxidant enzymes in order to cope with the reactive oxygen species generated by salinity. At the molecular level, arbuscular mycorrhizal symbiosis regulates the expression of plant genes involved in the biosynthesis of proline, of genes encoding aquaporins, and of genes encoding late embryogenesis abundant proteins, with chaperone activity. The regulation of these genes allows mycorrhizal plants to maintain a better water status in their tissues. Gene expression patterns suggest that mycorrhizal plants are less strained by salt stress than non-mycorrhizal plants. In contrast, scarce information is available on the possible regulation by the arbuscular mycorrhizal symbiosis of plant genes encoding Na^+/H^+ antiporters or cyclic nucleotide-gated channels. These genes encode proteins with a key role in the regulation of uptake, distribution and compartimentation of sodium and other ions within the plant, and are major determinants for the salt sensitiveness of a plant. Thus, we propose that investigating the participation of cation proton antiporters and cyclic nucleotide-gated channels on arbuscular mycorrhizal symbiosis under salinity is a promising field that should shed further light on new mechanisms involved in the enhanced tolerance of mycorrhizal plants to salt stress.

Keywords Abiotic stress · Aquaporin · Antioxidant · Arbuscular mycorrhizal fungi · Cation antiporter · Homeostasis · Salinity · Stress tolerance

Abbreviations

ABA	Abscisic acid
AMF	Arbuscular mycorrhizal fungus
BAS	Branched-absorbing structure
CNGC	Cyclic nucleotide-gated channel
Lo	Root hydraulic conductivity
NMP	Nucleotide monophosphate
NIP	Nodulin 26-like intrinsic protein

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PIP	Plasma membrane intrinsic protein
P5CS	Δ^1-pyrroline-5-carboxylate-synthetase
ROS	Reactive oxygen species
RWC	Relative water content
TIP	Tonoplast intrinsic protein
SIP	Small and basic intrinsic protein
Ψleaf	Leaf water potential

Contents

1 Introduction

Salinization of soil is a serious land degradation problem and is increasing steadily in many parts of the world, in particular in arid and semiarid areas (Giri et al. 2003; Al-Karaki 2006). Increased salinization of arable land is expected to have devastating global effects, resulting in 30% land loss within the next 25 years and up to 50% by the middle of twenty-first century (Wang et al. 2003). High soil salinity causes both hyperionic and hyperosmotic stress and can lead to plant demise. Salinity in a given land area depends upon various factors like amount of evaporation (leading to increase in salt concentration), or the amount of precipitation (leading to decrease in salt concentration) (Mahajan and Tuteja 2005). High salt concentration (Na^+) in particular which deposit in the soil can alter the basic texture of the soil resulting in decreased soil porosity and consequently reduced soil aeration and water conductance. High salt depositions in the soil generate a low water potential zone in the soil, making it increasingly difficult for the plant to acquire both water as well as nutrients. The basic physiology of high salt stress and drought stress overlaps with each other. Therefore, salt stress essentially results in a water-deficit condition in the plant and takes the form of a physiological drought (Mahajan and Tuteja 2005). In plants, both drought and salinization are manifested primarily as osmotic stress, resulting in the disruption of homeostasis and ion distribution in the cell (Serrano et al. 1999; Zhu 2001). Plants differ greatly in their tolerance to salinity, as reflected in their different growth responses (Fig. 1). Salt sensitive plants, also known as glycophytes include rice (*Oryza sativa*), maize (*Zea mays*), soybean (*Glycine max*) or beans (*Phaseolus vulgaris*), while some halophytes plants are saltbush (*Atriplex amnicola*), alfalfa (*Medicago sativa*), or pepercress (*Lepidium sp.*).

Plants, in their natural environment are colonized both by external and internal microorganisms. Arbuscular mycorrhizal fungi (AMF) are ubiquitous among a wide array of soil microorganisms inhabiting the rhizosphere. These fungi constitute an important integral component of the natural ecosystem and are known to exist in saline environments (Giri et al. 2003). The proportion of vascular plant species forming AM is commonly overestimated (Trappe 1987), probably as a result of the low proportion of species and environments surveyed (Brundett and Abbott 1991). Although AMF exist in saline soils, the growth and colonization of plants may be affected by the excess of salinity, which can inhibit the growth of microbes due to osmotic and/or toxic effects of salts (Juniper and Abbott 2006). AM symbiosis has been demonstrated to increase resistance to soil salinity in a variety of host plants such as maize, clover, tomato, cucumber, and lettuce (Rosendahl and Rosendahl 1991; Ruiz-Lozano and Azcón 1996; Al-Karaki et al. 2001; Feng et al. 2002).

Although it is clear that AM fungi mitigate growth reduction caused by salinity, the mechanism involved remains unresolved. So far, studies on salt stress tolerance in mycorrhizal plants have suggested that AM plants grow better due to improved mineral nutrition and physiological processes like photosynthesis or water use efficiency, the production of osmoregulators, higher K^+/Na^+ ratios and compartmentalization of sodium within some plant tissues (Ruiz-Lozano et al. 1996; Giri et al. 2003; Al-Karaki 2006). In this review, we present a comprehensive analysis of nutritional, biochemical, physiological, and molecular changes that occur in plants when colonized by AM and subjected to salt stress.

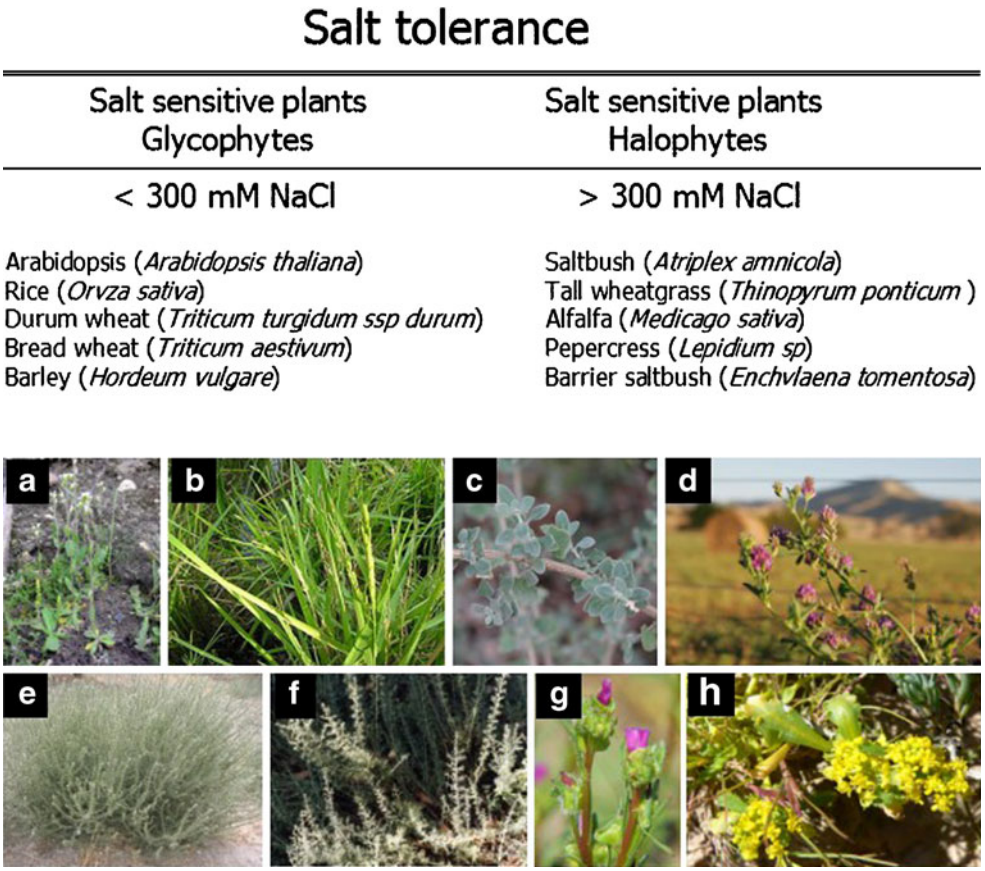

Fig. 1 Diversity in the salt tolerance of various cultivated and non-cultivated species. In the figure, species growing at less than 300 mM NaCl are considered glycophytes while those growing over 300 mM NaCl are considered halophytes. Pictures represent **a** arabidopsis (*Arabidopsis thaliana*), **b** rice (*Oryza sativa*), **c** saltbush (*Atriplex amnicola*), **d** alfalfa (*Medicago sativa*), **e** prickly saltwort (*Salsola kali*), **f** barrier saltbush (*Enchylaena tomentosa*), **g** fringed redmaid (*Calandrinia ciliata*), **h** pepercress (*Lepidium sp*.)

2 Morphological features and development of arbuscular mycorrhizal symbiosis

The arbuscular mycorrhizal fungi are the most complex group of mycorrhizas which forms intraradical and extraradical structures (Fig. 2): (1) intracellular hyphae forming coils, often found in the outer layers of cortical parenchyma, (2) the intercellular hyphae, (3) the intracellular hyphae with numerous ramifications, i.e., the arbuscules, (4) the inter or intracellular hypertrophied hyphae, i.e., the vesicles, (5) the extracellular ramified hyphae, i.e., branched-absorbing structures (BAS), and (6) resistance propagules, i.e., the spores.

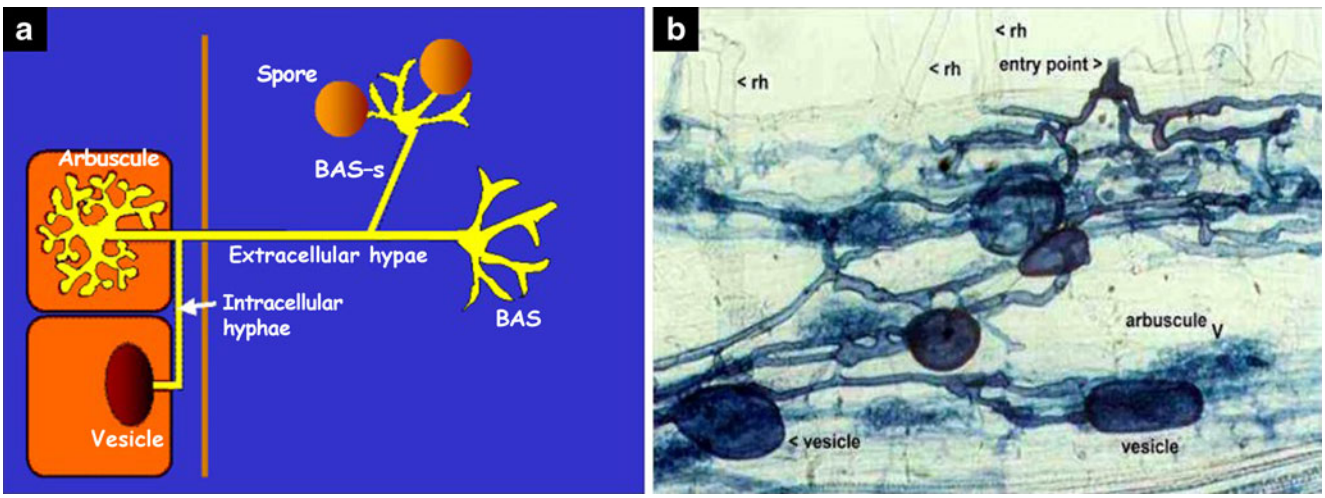

Fig. 2 **a** Schematic representation of arbuscular mycorrhizal symbiosis. Courtesy of Dr. A. Bago (CSIC, Spain). **b** Part of a clover root showing the distribution of fungal structures in the root. The main features shown are root hairs (rh) an entry point with a characteristic diamond-shaped swelling equivalent to an appressorium, large swollen vesicles, and "fuzzy" arbuscules. Courtesy of Dr. Jim Deacon (The University of Edinburgh)

Arbuscular mycorrhizal fungal spores are able to germinate in the absence of the host, but are unable to produce extensive mycelia and to complete their life cycle without establishing a functional symbiosis with a host plant. The lack of host-regulated spore germination did not represent a selective disadvantage, since AM fungi co-evolved with heir host plants for more than 360 and 400 million years (Remy et al. 1994; Redecker et al. 2000). Different efficient survival strategies have been inferred to be active in these ancestral organisms to compensate for the lack of host-regulated spore germination, e.g., a wide host range (Smith and Read 1997), the regulation of infection structure differentiation (Giovannetti et al. 1994), the ability of multiple germination (Koske 1981), an energy-saving mechanism operating when spores germinate in the absence of the host (Logi et al. 1998). In addition to these strategies, the ability to form wide hyphal networks by both pre-symbiotic and symbiotic mycelia may represent a fundamental mechanism for increasing the chances of AM symbionts to contact host roots (Giovannetti et al. 1999).

The establishment of the AM symbiosis begins with the colonization of a compatible root by the hyphae produced by AM fungal soil propagules, asexual spores or mycorrhizal roots. Even dead roots from annual plants might be a good source of inoculum because they protect the fungus from environmental hazards until the time when new hyphae can grow out of the roots and colonize other plants (Requena et al. 1996). After attachment of a hypha to the root surface by means of an appressorium, the fungus penetrates into the cortex and forms distinct morphologically specialized structures: Inter- and intracellular hyphae, coils, and arbuscules. Arbuscules are specialized hyphae similar to haustoria from plant pathogenic fungi. They are presumed to be the main site of nutrient exchange between the plant and the fungus (He and Nara 2007). After host colonization, the fungal mycelium grows out of the root exploring the soil in search of mineral nutrients, and it can also colonize other susceptible roots (Breuninger and Requena 2004). The fungal life cycle is completed after formation of asexual chlamydospores on the external mycelium (Fig. 3).

3 Ecological significance of arbuscular mycorrhizal symbiosis

Ecological consequences of the interactions between plants and the AMF for plant nutrition, growth, competition, stress tolerance and fitness, as well as for soil structuring have been often addressed. The key effects of AM symbiosis can be summarized as follows: (1) enhancing uptake of low mobile ions, (2) improving quality of soil structure, (3) enhancing plant community diversity, (4) improving rooting and plant establishment, (5) improving soil nutrient cycling, and (6) enhancing plant tolerance to (biotic and abiotic) stress (Smith and Read 2008).

The contribution of AMF to plant nutrient uptake is mainly through the acquisition of nutrients (especially phosphorous, which is extremely immobile element in soils) from the soil by the extraradical fungal hyphae,

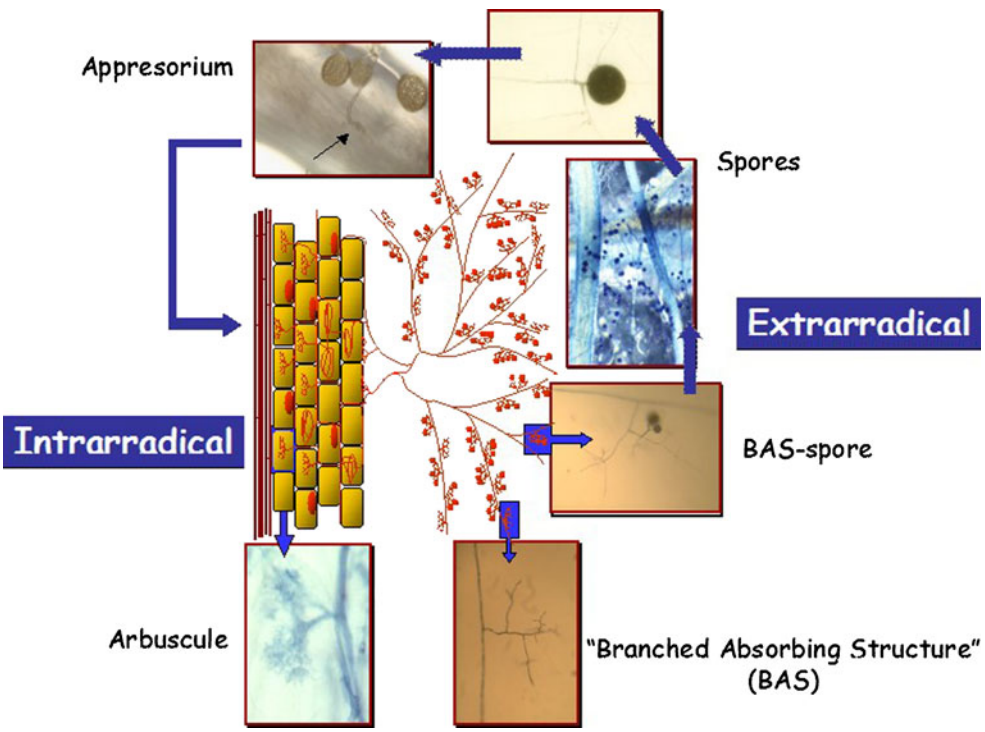

Fig. 3 Schematic representation of distinct morphological stages identified during the life cycle of arbuscular mycorrhizal fungi

especially from root-distant soil not depleted of nutrients by the root. Fungal hyphae are functionally analogous to fine root hairs, as both are nutrient uptake organs. Mycelium extends the plant's effective absorption surfaces beyond the nutrient depleted zone that develops around the root caused by direct root uptake processes (George 2000).

As a result of degradation/desertification processes, disturbance of natural plant communities is often accompanied, or preceded by lost of physicochemical and biological properties of the soil, such as soil structure, plant nutrient availability, organic matter content, and microbial activity. Therefore, in degraded and contaminated soils, that are often poor in nutrients and with low water-holding capacities, management of AM fungi is of great importance (Jeffries and Barea 2001). The effect of the AM fungi in cooperation with other microbes in the formation of water-stable soil aggregates is evident in different ecological situations (Andrade et al. 1995, 1998; Bethlenfalvay and Schüepp 1994; Bethlenfalvay et al. 1999; Requena et al. 2001), and the involvement of glomalin, a glycoprotein produced by the external hyphae of AM fungi, has been demonstrated (Wright and Upadhyaya 1998). Because of its glue-like hydrophobic nature, glomalin participates in the initiation and stabilization of soil aggregates (Miller and Jastrow 2000).

The influence of mycorrhizas on plant competition can be expected to lead to changes in plant coexistence and biodiversity. The microcosm experiment of Grime et al. (1987) provided one of the first clear demonstrations that AM fungi are potentially major determinants both of the structure and diversity of plant assemblages. Similar results have been obtained more recently by Van der Heijden et al. (1998) showing not only increased diversity, but also increased productivity, which was not observed in the experiments of Grime et al. (1987). The effect of arbuscular mycorrhizal in increasing diversity was the result of improved growth and survivorship of AM subordinates, associated with dominants that actually had their own competitive ability reduced by AM colonization.

The well-known activities of nitrogen-fixing bacteria and phosphate-solubilizing microorganisms improving the bioavailability of the major plant nutrients N and P, are very much enhanced in the rhizosphere of mycorrhizal plants where synergistic interactions of such microorganisms with mycorrhizal fungi have been demonstrated (Barea et al. 2002). AM mycelia have been shown to affect not only root morphology and functioning but also improve mycorrhizosphere soil properties.

AM symbiosis has been shown to increase tolerance to biotic and abiotic stresses. Regarding abiotic stress, several studies for years have demonstrated that AM symbiosis confers tolerance to drought (for reviews see Ruiz-Lozano 2003; Miransari 2010), heat (Compant et al. 2010), salinity (Evelin et al. 2009; Miransari 2010) or osmotic stress (Ruiz-Lozano 2003). On the other hand, early works on mycorrhizas and biotic stresses were mostly descriptive (for review, see Linderman 2000). A recent review by Pozo and Azcón (2007) summarizes the data on AM-induced protection against biotic stress and the possible mechanisms involved, with special emphasis on the role of plant defense responses. Generally, reports have focussed on beneficial effects of the symbiosis, aiming at using AM as potential biocontrol agents in integrated management programmes for disease control (Mukerji and Ciancio 2007). AM symbiosis has also been shown to occur in almost all habitats, including disturbed soils contaminated with heavy metals, and plays an important role in metal tolerance. A number of different mechanisms may be involved, including tissue dilution of the toxic element due to interactions with P nutrition and growth, sequestration of the toxic metal in the fungus and development of tolerance by the fungi (for reviews, see Hildebrandt et al. 2007; Gamalero et al. 2009). Thus, we can conclude that some plants are unable to endure habitat-imposed abiotic and biotic stresses in the absence of fungal endophytes.

4 Salinity effects on plants

Soil salinity affects the establishment, growth, and development of plants leading to huge losses in productivity (Evelin et al. 2009). Plants growing in saline soil are subjected to three distinct physiological stresses. First, the toxic effects of specific ions such as sodium and chloride, prevalent in saline soils, disrupt the structure of enzymes and other macromolecules, damage cell organelles, disrupt photosynthesis and respiration, inhibit protein synthesis, and induce ion deficiencies (Juniper and Abbott 1993). Second, plants exposed to the low osmotic potentials of saline soil are at risk of physiological drought because they must maintain lower internal osmotic potentials to prevent water moving from the roots into the soil. Finally, salinity also produces nutrient imbalance in the plant caused by decreased nutrient uptake and/or transport to the shoot (Marschner 1995; Adiku et al. 2001). As a consequence, salt stress affects all the major processes, such as growth, photosynthesis, protein synthesis, and energy and lipid metabolisms (Ramoliya et al. 2004).

A generic stress signal transduction pathway for the plant stress response is depicted in Fig. 4. The stress signal is first perceived at the membrane level by the receptor, which results in the generation of secondary signal molecules, such as Ca^{2+}, inositol phosphates, reactive oxygen species (ROS) and abscisic acid (ABA). The stress signal then transduces inside the nucleus to induce multiple

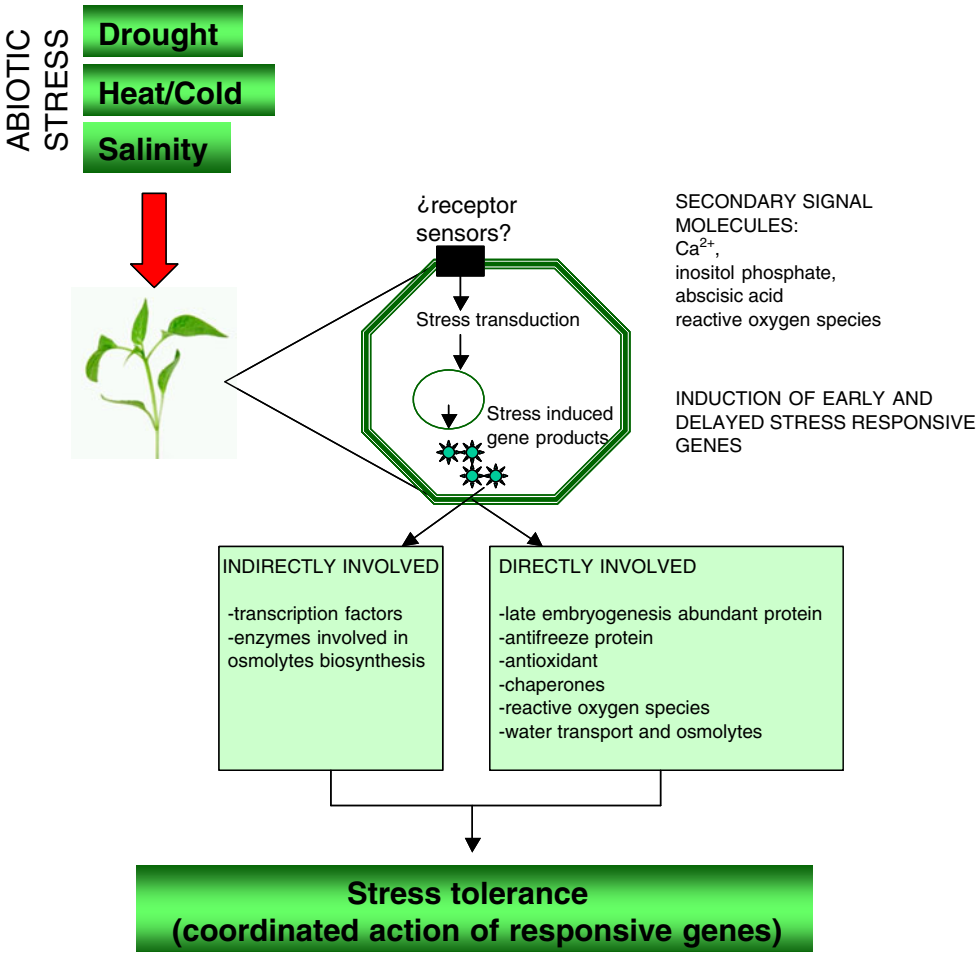

Fig. 4 Generic pathway for plant stress response. The extracellular stress signal is first perceived by the membrane receptor and then activates a large and complex signalling cascade intracellularly. The signal cascade results in the expression of multiple stress responsive genes, the products of which can provide the stress tolerance directly or indirectly. Overall, the stress response could be a coordinated action of many genes. Adapted from Tuteja (2007) with kind permission from Elsevier

stress responsive genes, the products of which ultimately lead to plant adaptation to stress tolerance. Early genes are induced within minutes of stress perception, and their products (e.g., various transcription factors) can activate the expression of delayed genes (e.g., RD [responsive to dehydration], KIN [cold induced], COR [cold responsive]). Overall, gene products are either involved directly in cellular protection against the stress (e.g., late embryogenesis abundant proteins, antifreeze proteins, antioxidants, chaperons, and detoxification enzymes) or involved indirectly in protection (e.g., transcription factor, enzymes of phosphatidylinositol metabolism; Tuteja 2007).

5 Salinity effects on arbuscular mycorrhizal fungi

Salinity, not only affects negatively the host plant but also the AMF. It can hamper colonization capacity, spore germination, and growth of fungal hyphae. Colonization of plant roots by some AMF is reduced in the presence of NaCl (Hirrel and Gerdemann 1980; Ojala et al. 1983; Poss et al. 1985; Duke et al. 1986; Rozema et al. 1986; Menconi et al. 1995; Juniper and Abbott 2006; Giri et al. 2007; Sheng et al. 2008) probably due to the direct effect of NaCl on the fungi (Juniper and Abbott 2006) indicating that salinity can suppress the formation of arbuscular mycorrhiza (Tian et al. 2004; Sheng et al. 2008). The varying levels of AM colonization under saline conditions may also be related to the different behaviour of each AM fungal species, even in similar ecosystems (Kilironomos et al. 1993) or to the influence of different environmental conditions (Carvalho et al. 2001).

In the presence of NaCl, germination of spores is delayed rather than prevented (Cantrell and Linderman 2001; Juniper and Abbott 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 impart differential effects on the rate and maximum germination of spores. They attributed the difference to a higher concentration of Na^+ in the latter.

Jahromi et al. (2008) studied in vitro the effects of salinity on the AM fungus, *Glomus intraradices*. They observed that there was no significant difference in hyphal length and 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 (Table 1).

Table 1 Total hyphal length and number of spores and branched absorbing structures (BAS) formed by *G. intraradices* grown in monoxenic culture and subjected to 0, 50, or 100 mM NaCl

	Salt level			
	0 mM	50 mM	100 mM	LSD
Hyphal length (sqrt mm cm^{-2})	48.4a	42.6a	30.7b	10.9
Number of spores (cm^{-2})	59.5a	14.0b	11.0b	29.4
Number of Bas	29.1a	21.7ab	13.2b	11.4

Data were subjected to analysis of variance (ANOVA) and followed by the Fisher's less significant Differences test (5% of significance). Table reproduced from Jahromi et al. (2008) with kind permission from Springer Science and Business Media

Means followed by different letters are significantly different ($P<0.05$)

LSD less significant difference, *sqrt* square root

Thus, all these results demonstrate that salinity affects directly the fungal development, reducing fungal mycelia formation and host root colonization.

Contrary to the reports above, 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 AMF species and to test if these AM isolates maintain colonization capacity and symbiosis efficiency under saline conditions.

6 Arbuscular mycorrhizal effects on plant biomass and nutrient uptake

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 osmoregulator compounds, and increase in photosynthetic rate and water use efficiency, suggesting that salt stress alleviation by AMF results from a combination of nutritional, biochemical, physiological, and molecular effects. However, this positive effect on plant development depends on the AMF species involved (Marulanda et al. 2003, 2007; Wu et al. 2007).

Hence, mycorrhization was found to increase the fitness of the host plant by enhancing its growth. Several researchers have reported that AMF-inoculated plants grow better than non-inoculated plans under salt stress (Al-Karaki 2000; Cantrell and Linderman 2001; Giri et al. 2007; Sannazzaro et al. 2007; Zuccarini and Okurowska 2008). For instance, Hajiboland et al. (2010) have recently reported that although high salinity reduced dry matter production by two tomato cultivars, in all treatments mycorrhizal plants grew better than non-mycorrhizal plants (Fig. 5).

The mycorrhizal association is well known to increase host nutrient acquisition, particularly P (Smith and Read 1997). The improved growth of mycorrhizal plants in saline conditions is primarily related to mycorrhiza-mediated enhancement of host plant P nutrition (Hanway and Heidel 1952; Hirrel and Gerdemann 1980; Ojala et al. 1983; Pond et al. 1984; Poss et al. 1985; Al-Karaki 2000).

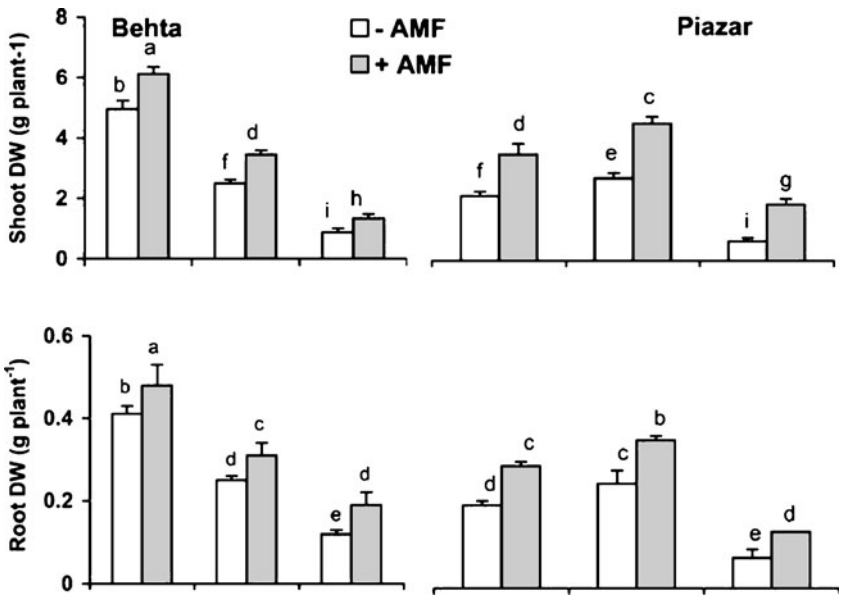

Fig. 5 Influence of different salinity levels on shoot and root dry weights (DW) of tomato cultivars Behta and Piazar colonized (+AMF) or not (−AMF) with an arbuscular mycorrhizal fungus (AMF). *Bars* of each parameter labeled by different letters indicate significant differences assessed by the Bonferroni test after performing three-way ANOVA ($P<0.05$). Adapted from Hajiboland et al. (2010) with kind permission from Springer Science and Business Media

Beneficial effect of Na^+ on growth of nonhalophytes was reported for some natrophilic crop species such as sugar beet (Marschner et al. 1981; Hajiboland et al. 2009) and was attributed mainly to an ability of plants for replacement of K^+ by Na^+ (Marschner et al. 1981; Hajiboland and Joudmand 2009). Elevated Na^+ in soil solution inhibits the uptake of other nutrients by disrupting the uptake of nutrients directly by interfering with various transporters in the root plasma membrane, such as K^+-selective ion channels, and inhibiting root growth by the osmotic effects of Na^+ on soil structure (Wild 1988). Thus, the uptake of water and essential mineral nutrients, such as P, K, Fe, Cu, and Zn, and the population of soil bacteria can be reduced (Barea et al. 2005). Certain ion ratios, such as K/Na, are accepted indicators for evaluation of salinity tolerance in tomato cultivars (Dasgan et al. 2002). Moreover, high Na^+/K^+ ratio disrupts various metabolic processes such as protein synthesis in the cytoplasm (Tester and Davenport 2003). Giri et al. (2007) showed that *Acacia nilotica* plants colonized by *G. fasciculatum* had a higher concentration of K^+ in root and shoot tissues at all salinity levels assayed. Similar increase in the concentration of K^+ has also been reported previously (Hanway and Heidel 1952; Ojala et al. 1983; Mohammad et al. 2003). It seems that higher K^+ accumulation by mycorrhizal plants under salt stress conditions may help in maintaining a high K/Na ratio, thus preventing the disruption of various enzymatic processes and inhibition of protein synthesis.

Apart from P and K, it has been demonstrated that arbuscular mycorrhizal fungi have a positive influence on the composition of mineral nutrients of plants grown under salt stress conditions (Al-Karaki and Clark 1998) by enhancing and/or selective uptake of nutrients. A number of studies have shown the effect of salinity on the nutrient uptake of mycorrhizal plants (Table 2).

7 Biochemical changes

The best characterized biochemical response of plant cells to osmotic stress is accumulation of some inorganic ions such as Na^+ and compatible organic solutes like proline, glycine betaine, and soluble sugars (Flowers and Colmer 2008). These compatible solutes can accumulate to high levels without disturbing intracellular biochemistry (Bohnert and Jensen 1996), protecting sub-cellular structures, mitigating oxidative damage caused by free radicals, and maintaining the enzyme activities under salt stress (Yokoi et al. 2002).

7.1 Proline and other osmolytes

One of the main consequences of NaCl stress is the loss of intracellular water. Plants accumulate many metabolites that are also known as "compatible (organic) solutes" in the cytoplasm to increase their tolerance against salt stress-induced water loss from the cells. Among these compounds, proline, betaines, sugars (chiefly fructose, sucrose and glucose) and complex sugars (trehalose, raffinose, and fructans) have been suggested to accomplish this function in halophytes.

Proline and glycine betaine (N, N, N-trimethylglycine betaine) are two major osmoprotectant osmolytes, which are synthesized by many plants (but not all) in response to stress, including salinity stress, and thereby help in maintaining the osmotic status of the cell to ameliorate the abiotic stress effect. Proline also plays roles in scavenging free radicals, stabilizing subcellular structures, and buffering cellular redox potential under stresses. The salinity stress responsive genes, whose promoters contain proline responsive elements (ACTCAT), are also known to be induced by proline (Chinnusamy et al. 2005). In higher plants, proline is synthesized from glutamic acid by the actions of two enzymes, pyrroline-5-carboxylate synthetase (P5CS) and pyrroline-5-carboxylate reductase (P5CR). Overexpression of the P5CS gene in transgenic tobacco resulted in increased production of proline and salinity/drought tolerance (Kishor et al. 1995).

Proline accumulation, in terms of amount, has been found to increase when the plant is colonized by AMF. Several authors have reported a higher proline concentration in AM plants than in non-AM plants at different salinity levels (Jindal et al. 1993; Sharifi et al. 2007). However, in contrast to the reports above, other authors reported that non-MA plants accumulated more proline that AM plants at various salinity levels (Wang et al. 2004; Rabie and Almadini 2005; Jahromi et al. 2008), suggesting that proline accumulation in plants may be a sympton of stress in less salt-tolerant species or that this accumulation may be also due to salinity and not necessarily to mycorrhizal colonization.

On the other hand, betaines 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 was found to increase when the plant is colonized by AMF (Al-Garni 2006).

The increase in sugar content is found to be positively correlated with mycorrhization of the host plant as reported by Thomson et al. (1990). Porcel and Ruiz-Lozano (2004) also reported increased sugar concentrations in soybean roots colonized by *G. intraradices* and subjected to drought stress. 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). Moreover, the increased sugar accumulation may also be due to hydrolysis of starch to sugars in the seedlings

Table 2 Some examples of increased/decreased nutrient uptake in AM plants under salinity stress

Nutrient	Range of salinity[a]	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 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)
	1–7·4 dS m^{-1}	*Lycoperssicon 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)
	2–24·6 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)

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[a] The range of salinity within brackets is the actual salt concentrations used by the authors

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

7.2 ABA content

ABA is a phytohormone that regulates plant growth and development and also plays an important role in the response of the plant to abiotic stress, including salinity stress. 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). Jahromi et al. (2008) reported lower ABA levels in lettuce plants colonized by *G. intraradices* than that in the non-AM plants (Fig. 6), indicating that AM plants are less strained by imposed salinity stress than non-AM plants and, hence, accumulated less ABA. It seems that the effects of AMF species on ABA content vary with the host plants (Evelin et al. 2009).

7.3 Antioxidant system

Many of the degenerative reactions associated with several biotic, abiotic, and xenobiotic stresses are mediated by

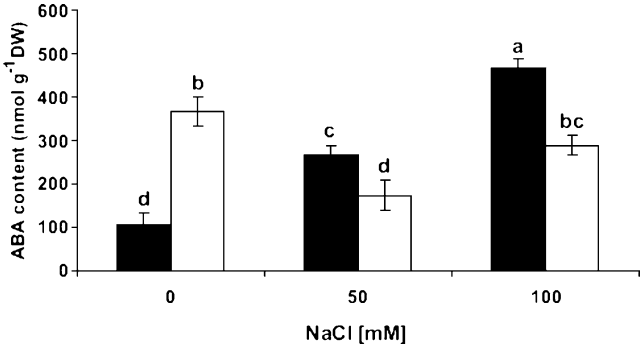

Fig. 6 Abscisic acid content (nmol g dry weight^{-1}) in lettuce roots. *Black columns* represent non-inoculated control plants and white columns represent plants inoculated with *G. intraradices*. Plants were subjected to 0. 50 and 100 mM NaCl. *Columns with different letters* are significantly different ($P<0.05$). *Columns* represents means±SE ($n=6$). *DW* dry weight. Data reproduced from Jahromi et al. (2008) with kind permission from Springer Science and Business Media

ROS. The term ROS is generic, embracing not only free radicals such as superoxide (O^{2-}) and hydroxyl radicals (OH^-), but also hydrogen peroxide (H_2O_2) and singlet oxygen ($^1\Delta_gO_2$). While it is generally assumed that the hydroxyl radical and singlet oxygen are so reactive that their production must be minimized (Jakob and Heber 1996), O^{2-} and H_2O_2 are synthesized at very high rates even under optimal conditions (Noctor and Foyer 1998). The chief toxicity of O^{2-} and H_2O_2 is thought to reside in their ability to initiate cascade reactions that result in the production of the hydroxyl radicals. These radicals (and their derivatives) are among the most reactive species known to chemistry, capable of reacting indiscriminately to cause oxidative damage to biomolecules such as lipid peroxidation, denaturation of proteins, and mutation of DNA (Gutteridge and Halliwell 1989; Bowler et al. 1992). Plant cells contain an array of protective and repair systems that minimize the occurrence of oxidative damage. According to Smirnoff (1993), these can be divided into two categories: systems that react with active forms of oxygen and keep them at a low level [i.e., superoxide dismutases, catalase, or peroxidases], and systems that regenerate oxidized antioxidants [glutathione (GSH), glutathione reductase (GR), ascorbate, and mono- and dehydroascorbate reductases]. The first group of enzymes are involved in the detoxification of O^{2-} radicals and H_2O_2, thereby preventing the formation of OH^- radicals. The GR, as well as the GSH, are important components of the ascorbate glutathione pathway (Fig. 7) responsible for the removal of H_2O_2 in different cellular compartments (Dalton 1995; Jiménez et al. 1997). Other non-enzymatic compounds which scavenge activated oxygen species include carotenoids, gluthatione, tocopherols and ascorbic acid (Alguacil et al. 2003; Wu and Xia 2006; Wu et al. 2006).

Like other abiotic stresses, salinity also induces oxidative stress in plants (Santos et al. 2001; Hajiboland and Joudmand 2009). 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). Several studies suggested that AM symbiosis helps plants to alleviate salt stress by enhancing the activities of antioxidant enzymes (Alguacil et al. 2003; Zhong et al. 2007).

8 Physiological changes

Salt stress inhibits photosynthetic ability and induces physiological drought in plants, which leads to a decrease in crop production (Pitman and Läuchli 2002). However, there have been very few attempts to study the influence of AMF inoculation on photosynthesis and particularly leaf photochemical properties under salt stress (Sheng et al. 2008). Salinity can affect several physiological mechanisms in the plant such as photosynthetic efficiency, membrane disruption, gas exchange, or water status. Some studies (Aroca et al. 2006; Porcel et al. 2006) have shown that colonization of plant roots by the AM fungus *G. intraradices* prevented leaf dehydration caused by salinity. 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).

AMF colonization induces an increase in root hydraulic conductivity of the host plants under osmotic stress conditions (Sánchez-Blanco et al. 2004; Aroca et al. 2007). AMF-colonized plants are able to fix more CO_2 than non-inoculated plants and hence their growth is improved (Querejeta et al. 2007). In addition, in some cases, also their water use efficiency is stimulated independent of changes in

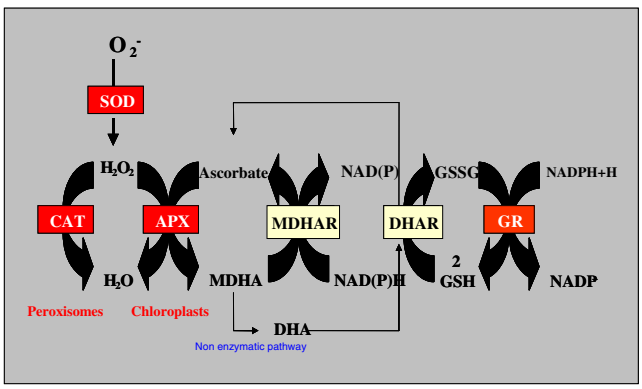

Fig. 7 Ascorbate-gluthation pathway. *SOD* superoxide dismutase, *CAT* catalase, *APX* ascorbate peroxidase, *MDHAR* monodehydroascorbate reductase, *DHAR* dehydroascorbate reductase, *GR* gluthation reductase, *MDHA* monodehydroascorbate, *GSSG* oxidized gluthation, *GSH* reduced gluthation

transpiration rate (Bolandnazar et al. 2007). These changes described in transpiration rate by AM symbiosis are correlated with changes in the ABA/cytokinins ratio (Goicoechea et al. 1997). At the same time, AMF-colonized plants, by the action of the fungal hyphae, are able to explore more soil and therefore to take up more water from it than noninoculated plants (Marulanda et al. 2003; Khalvati et al. 2005; Bolandnazar et al. 2007).

9 Molecular changes

The beneficial effects of the AM symbiosis on plant salinity tolerance have not been only assessed by measuring plant growth or plant water status. At the same time, these beneficial effects have been evidenced by measuring the expression of some plant genes related to salt stress responses.

9.1 P5CS

As mentioned previously, proline is probably the most widespread osmoprotectant in plants. Accumulation of proline is mainly due to de novo synthesis, although a reduced rate of catabolism has also been observed (Kishor et al. 1995). The first two steps of proline biosynthesis are catalyzed by P5CS by means of its γ-glutamil-kinase and glutamic-γ-semialdehyde-deshydrogenase activities (Fig. 8). Subsequently, the Δ^1-pyrroline-5-carboxylate formed is reduced by P5CR to proline (Hu et al. 1992). In *Arabidopsis*, the P5CS-encoding gene is induced by drought stress, salinity, and ABA, but P5CR is not (Yoshiba et al. 1995). The overexpression of the P5CS-encoding gene in transgenic tobacco plants has been shown to increase proline production and to confer tolerance of such plants to osmotic stress (Kishor et al. 1995). Hence, the P5CS-encoding gene is of key importance for the biosynthesis of proline in plants (Ábrahám et al. 2003).

Investigations carried out so far on osmoregulation in the AM symbiosis are scarce and somewhat contradictory. Moreover, information regarding P5CS gene expression in mycorrhizal plants subjected to salt stress is really limited. Jahromi et al. (2008) reported a higher expression of *Lactuca sativa* P5CS gene in non-MA plants that in AM

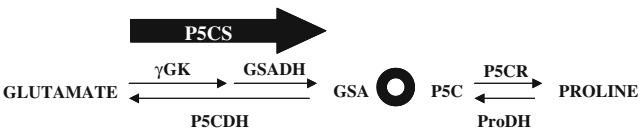

Fig. 8 Pathway of proline biosynthesis. *GSA* glutamyl-γ-semialdehyde, *P5C* Δ^1-pyrroline-5-carboxylate, *P5CS* Δ^1-pyrroline-5-carboxylate synthetase, *P5CR* P5C reductase, *γGK* γ-glutamyl kynase, *GSADH* glutamyl-γ-semialdehyde deshydrogenase, *P5CDH* P5C deshydrogenase, *ProDH* proline deshydrogenase

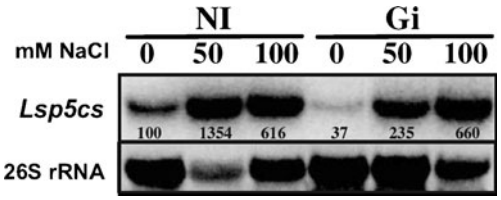

Fig. 9 Northern blot of total RNA (15 μg) from lettuce roots using *Lsp5cs* gene probe. Treatments are designed as NI, noninoculated control, or Gi, plants inoculated with *G. intraradices*. Plants were subjected to 0, 50, or 100 mM NaCl. The *lower panels* show the amount of 26 S rRNA loaded for each treatment. Numbers close to each Northern represent the relative gene expression (after normalization to 26 S rRNA) as a percentage of the value for control plants cultivated under nonsaline conditions. Data adapted from Jahromi et al. (2008) with kind permission from Springer Science and Business Media

plants at 50 mM NaCl, although at 100 mM, the levels were similar (Fig. 9).

9.2 Aquaporins

The negative water potential in drying or saline soils obliges plants to face with the problem of acquiring sufficient amount of water (Ouziad et al. 2006), a process in which aquaporins participate (Luu and Maurel 2005). Aquaporins are water channel proteins that facilitate and regulate the passive movement of water molecules down a water potential gradient (Kruse et al. 2006). Plants aquaporins are divided in four groups based on their sequence homology. These four groups are called plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), noduline like intrinsic proteins (NIPs), and small and basic intrinsic proteins (SIPs). The localization and function of SIPs are not clear at the moment (Luu and Maurel 2005), although the membrane of endoplasmic reticulum seems to contain SIPs (Ishikawa et al. 2005). Each subgroup is also subdivided, and for example there are PIP1 and PIP2 subgroups, having each subgroup several different proteins. In fact, in *Arabidopsis*, maize and rice, there are over 30 different aquaporins genes (Chaumont et al. 2001; Johanson et al. 2001; Sakurai et al. 2005). At the same time, each aquaporin group differs in their capacity of transporting water and other small and neutral solutes and in their subcellular localization. For recent reviews, see (Heinen et al. 2009; Maurel et al. 2009; Wudick et al. 2009).

Expression analysis of aquaporin genes in salt-stressed AM plants revealed contrasting results. Aroca et al. (2007) studied four aquaporin genes from *P. vulgaris* in mycorrhizal and nonmycorrhizal plants subjected to three different osmotic stresses: drought, cold, or salinity. Three of these PIP genes showed differential regulation by AM symbiosis under the specific conditions of each stress applied. Salt-treatment induced a higher expression of three *P. vulgaris* PIP genes in both groups of plants, especially in AM ones (Fig. 10).

On the other hand, Jahromi et al. (2008) reported that in the absence of salinity the expression of *L. sativa PIP1* and *PIP2* genes was inhibited by mycorrhization, while under saline conditionsmycorrhizal plants maintained the expression of *LsPIP2* gene, which was almost unaffected, whereas the expression of *LsPIP1* gene was upregulated, mainly at 100 mM NaCl. A differential effect of AM symbiosis on aquaporin isoforms under salinity has also been described by Ouziad et al. (2006). In this study, the amount of *Lycopersicon esculentum TIP* and *PIP1* transcripts was found to be downregulated in roots and upregulated in leaves of tomato plants subjected to salt stress (Fig. 11).

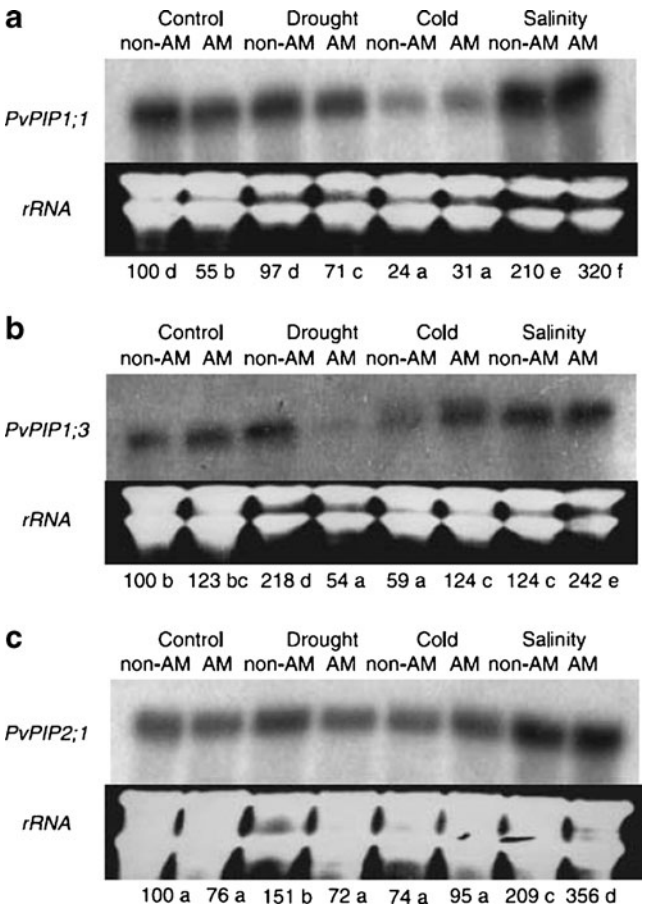

Fig. 10 Northern blot analyses using 3′ untranslated region as probes of *Phaseolus vulgaris PIP1;1* (**a**), *PIP1;3* (**b**), and *PIP2;1* (**c**) in total RNA of *P. vulgaris* roots not inoculated (nonAM) or inoculated (AM) with the arbuscular mycorrhizal fungus *Glomus intraradices*. Treatments: *control* plants kept at 23°C and watered at full capacity with tap water; *drought* plants kept at 23°C and not watered for 4 days; *cold* plants transferred to 4°C for 2 days and watered at full capacity with tap water; *salinity* plants kept at 23°C and watered every 2 days during a 6-day period with 10 ml of 0.5 M NaCl solution. Quantification of the gene expression was performed by dividing the intensity value of each band by the intensity of corresponding rRNA stained with ethidium bromide. Control value of nonAM roots was referred as 100. Treatments with different letters are significantly different ($P<0.05$) after analysis of variance and Fisher less significant differences test; $n=3$. Data reproduced from Aroca et al. (2007) with kind permission from John Wiley and Sons

Hence, results by Aroca et al. (2007), Jahromi et al. (2008), and Ouziad et al. (2006) clearly illustrate that each aquaporin gene may respond differently to AMF colonization and stress imposed. Such differences may be a consequence of the mode of the salt stress set, the differences between plant species tested and the complexity in expression pattern of different members of the large family of aquaporins (Sarda et al. 1999). This highlights the complex regulation of aquaporin genes in response to the AM symbiosis and to different abiotic stresses with an osmotic component (Table 3).

9.3 Late embryogenesis abundant proteins

These are a group of proteins that accumulate in plants seeds during their maturation phase, when tolerance to desiccation is required (Close 1996). It seems that during cellular dehydration, late embryogenesis abundant (LEA) proteins play an important role in maintenance of the structure of other proteins, vesicles, or endomembrane structures in the sequestration of ions such as calcium, in binding or replacement of water, and functioning as molecular chaperones (Close 1996; Koag et al. 2003). The overexpression of LEA proteins in plants and yeast confers tolerance to osmotic stresses (Imai et al. 1996; Xu et al. 1996; Babu et al. 2004). Dehydrins belong to LEA group 2 and represent the most conspicuous soluble proteins induced by a dehydration stress. They have been observed in over 100 independent studies of drought stress, cold acclimation, salinity stress, embryo development, and responses to ABA. However, the existence of multiple targets for dehydrins (euchromatin, cytosol, cytoskeleton) suggests that the direct consequences of dehydrin activity are biochemically diverse.

It is known that LEA gene expression increases under salt stress; however, there are not many studies relating LEA gene expression and MA symbiosis under salt stress conditions. Regarding drought stress, Porcel et al. (2005) cloned two dehydrin-encoding genes from *G. max* (*gmlea8* and *gmlea10*) and one from *L. sativa* (*lslea1*) and analyzed their contribution to the response against drought in mycorrhizal soybean and lettuce plants. Results demonstrate that the levels of *lea* transcript accumulation in soybean and lettuce plants colonized by either *G. mosseae* or *G. intraradices* were considerably lower than those of the corresponding nonmycorrhizal plants, suggesting that the accumulation of LEA proteins is not a mechanism by which the AM symbiosis protects their host plant. Moreover, results also suggested that mycorrhizal plants were less strained by drought due to primary drought-avoidance mechanisms. Regarding salinity, Jahromi et al. (2008) reported that *LsLea* was expressed under conditions of salt

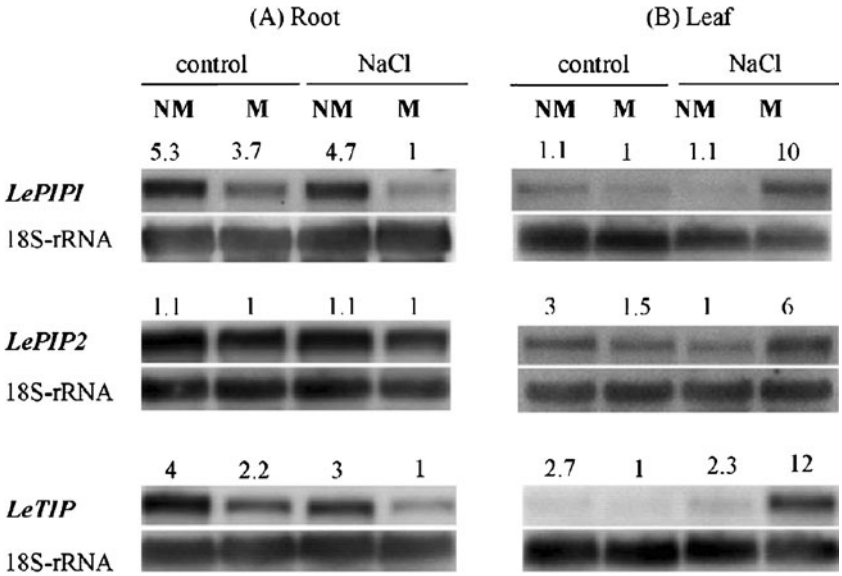

Fig. 11 Northern hybridizations using total mRNA and the different digoxigenin-labelled riboprobes for three *L. esculentum* aquaporins. Lowest signal intensity of one lane was arbitrary set to 1 to allow a comparison of the signal strengths within one blot (bloc of four lanes) for each gene expressed. Signal strengths were adjusted to the amount of 18 S rRNA blotted onto each lane. **a** Root and **b** leaf. *M* mycorrhizal plants, *NM* non-mycorrhizal plants, *control* plants not stressed with NaCl. Adapted from Ouziad et al. (2006) with kind permission from Elsevier

stress and the induction of this gene was also lower in AM plants than in non-MA plants (Fig. 12).

9.4 Cation channels and transporters

9.4.1 Na^+/H^+ antiporters

Sodium uptake and distribution within the plant is a major determinant for the salt sensitiveness of a plant. Prevention of Na^+ entry into the root, transport to, and allocation within the leaf, and sequestration into the vacuole are strategies by which plants cope with high salt environment. The Na^+/H^+ antiporters mediate the transfer of Na^+ out of the cytoplasm into either vacuole or apoplast. Transgenic plants with overexpressed Na^+/H^+ antiporters were reported to be more salt tolerant than the controls as shown for *Arabidopsis* (Gaxiola et al. 1999; Sottosanto et al. 2004) or rice (Fukuda et al. 1999). In *Arabidopsis*, transporters contributing

Table 3 Summary of the different effects of the mycorrhizal symbiosis on aquaporin gene expression under non stressed or under osmotic stress conditions

		OSMOTIC STRESS		
	NO STRESS	DROUGHT	COLD	SALINITY
	Effect Source	Effect Source	Effect Source	Effect Source
Mycorrhizal effects on aquaporin genes	↑ PcTIP Roussell et al. 1997 ↑ MtTIP Krajinski et al. 2000 ↑ PttPIP1.1 ↑ PttPIP2.3 Marjanovic et al. 2005 ↑ PttPIP2.5 ↑ MtPIP2.1 Uehlein et al. 2007 ↑ MtNIP1	↓ GmPIP1 ↓ GmPIP2 ↓ LsPIP1 Porcel et al. 2006 ↓ LsPIP2 ↓ PvPIP1.1 = PvPIP1.2 ↓ PvPIP1.3 Aroca et al. 2007 ↓ PvPIP2.1	↓ PvPIP1.1 = PvPIP1.2 ↑ PvPIP1.3 Aroca et al. 2007 = PvPIP2.1	↓ LePIP1 ↓ LeTIP Ouziad et al., 2006 = LePIP2 ↑ LsPIP1 = LsPIP2 Jahromi et al., 2008 ↑ PvPIP1.1 = PvPIP1.2 Aroca et al. 2007 ↑ PvPIP1.3 ↑ PvPIP2.1
Consequence	Plant water status not measured (Roussell et al., 1997; Krajinski et al., 2000; Uehlein et al., 2007) ↑ L_0 (Marjanovic et al., 2005)	↑ Ψ_{leaf} (Porcel et al., 2006) ↑ RWC (Porcel et al., 2006; Aroca et al., 2007) ↑ Sap flow rate (Aroca et al., 2007)	= RWC = L_0 (Aroca et al., 2007) = Sap flow rate	Plant water status not measured (Ouziad et al., 2006) ↑ RWC (Aroca et al., 2007; Jahromi et al., 2008) ↑ Sap flow rate (Aroca et al., 2007) ↑ L_0 (Aroca et al., 2007)
Proposed hypothesis	The enhanced aquaporin gene expression ameliorates the exchange of water and nutrients between both symbiotic partners	The down-regulation by the AM symbiosis of plant aquaporins allows conservation of water in plant tissues under drought	The arbuscular mycorrhizal fungi have little effect on plant water relations under cold stress	The up-regulation of aquaporin genes improves plant water flow and water status under salt stress

The consequences on plant water relations (when measured) and the proposed hypothesis are also included. Reproduced from Ruiz-Lozano and Aroca (2010) with kind permission from Springer Science and Business Media

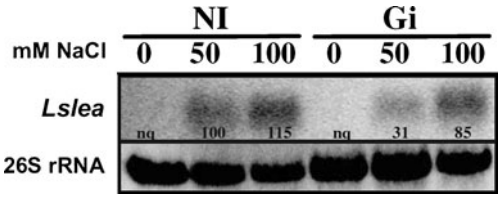

Fig. 12 Northern blot of total RNA (15 μg) from lettuce roots using *Lslea* gene probe. Treatments are designed as NI, noninoculated control, or Gi, plants inoculated with *G. intraradices*. Plants were subjected to 0, 50, or 100 mM NaCl. The *lower panels* show the amount of 26 S rRNA loaded for each treatment. Numbers close to each Northern represent the relative gene expression (after normalization to 26 S rRNA) as a percentage of the value for control plants, which were set at 100% in those plants subjected to 50 mM NaCl, *nq*, not quantifiable. Data adapted from Jahromi et al. (2008) with kind permission from Springer Science and Business Media

to Na^+ homeostasis include plasma membrane (SOS1) and vacuolar Na^+/H^+ antiporters (e.g., NHX1), and the plasma membrane uniporter HKT1 (Zhu 2003). Loss-of-function mutations in *AtHKT1* render plants Na^+ hypersensitive and disturb the distribution of Na^+ between roots and shoots.

Ouziad et al. (2006) analyzed the expression of two Na^+/H^+ antiporter genes in dependence on salt and mycorrhizal colonization (Fig. 13). They observed that under the conditions employed, no significant alterations by mycorrhization were detected in the expression of *LeNHX1* and *LeNHX2*, the latter of which had previously been shown to be also a K^+/H^+ antiporter (Venema et al. 2003).

9.4.2 Cyclic nucleotide-gated channels

Ion influx is central to signal transduction, and one of the potential pathways for the uptake of these ions is via cyclic nucleotide-gated ion channels (CNGCs) (Talke et al. 2003). Cyclic nucleotides monophosphate (NMP) have only recently been accepted as important secondary messengers in plants and they have been suggested to be involved in plant responses to both biotic and abiotic stresses. It is well known that salt and osmotic stress cause rapid increases in cGMP levels in *Arabidopsis thaliana* (Donaldson et al. 2004), and these studies are consistent with findings that cAMP and cGMP improve tolerance to salt stress (Maathuis and Sanders 2001). Interestingly, improved salt tolerance correlated with cNMP-dependent decrease of channel open probability and reduced influx of Na^+ (Maathuis and Sanders 2001; Rubio et al. 2003). Furthermore, cNMPs participate in various developmental processes in addition to photomorphogenesis.

Members of the CNGC family belong to the group of nonselective cation channels and enable the uptake of Na^+, K^+, and Ca^{2+} (Kaplan et al. 2007). They were first identified in vertebrate visual and olfactory signal transduction cascades (Zagotta and Siegelbaum 1996; Craven and Zagotta 2006), however, much less is known about them in plants. CNGCs are composed of six transmembrane domains and a pore region between the fifth and sixth domains (Fig. 14). The fourth transmembrane domain has similarities to the Shaker-type voltage sensor (Köhler et al. 1999; Rehmann et al. 2007). The N-terminal domain extends into the cytosol and is believed to bind calmodulin (CaM), while the C-terminal domain binds cNMPs. Plants, on the other hand, possess a slightly different structure where both the CaM and cyclic nucleotide binding domains occur in the cytosolic C-terminus in overlapping regions.

The *Arabidopsis* CNGC gene family comprises 20 members (Mäser et al. 2001). Studies on some *Arabidopsis* CNGCs have so far revealed their ability to transport cations that play a role in mediating various environmental stresses

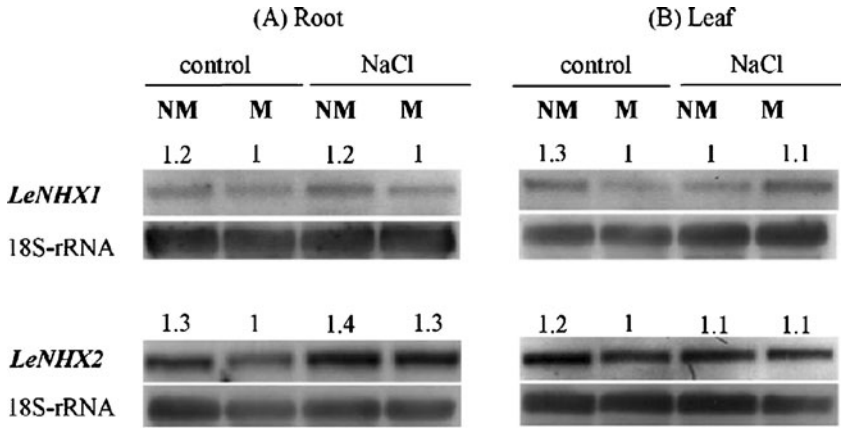

Fig. 13 Northern hybridizations using total mRNA and the different digoxigenin-labelled riboprobes for two *L. esculentum* Na^+/H^+ antiporters. Lowest signal intensity of one lane was arbitrary set to 1 to allow a comparison of the signal strengths within one blot (bloc of four lanes) for each gene expressed. Signal strengths were adjusted to the amount of 18 S rRNA blotted onto each lane. **a** Root and **b** leaf. *M* mycorrhizal plants, *NM* non-mycorrhizal plants, *control* plants not stressed with NaCl. Adapted from Ouziad et al. (2006) with kind permission from Elsevier

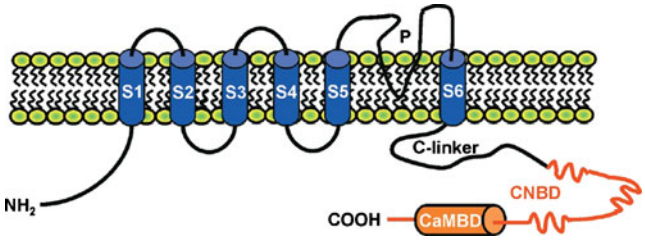

Fig. 14 Structure of plant cyclic-nucleotide-gated ion channels (CNGC). CNGCs are composed of six transmembrane domains (S) and a pore region (P) between the fifth and sixth domains. The cytosolic C-terminus has a C-linker followed by the cyclic nucleotide binding domain (CNBD) with an overlapping calmodulin bindig domain (CAMBD)

including salt stress, plant defense responses and development (Clough et al. 2000; Balagué et al. 2003; Chan et al. 2003; Gobert et al. 2006; Ma et al. 2006; Yoshioka et al. 2006; Borsics et al. 2007), summarized in Table 4.

Recently, Kugler et al. (2009), have reported that both *AtCNGC19* and *AtCNGC20* were upregulated in the shoot in response to elevated NaCl but not to mannitol concentrations. While in the root, *CNGC19* did not respond to changes in the salt concentration, in the shoot it was strongly upregulated. Salt induction of *CNGC20* was also observed in the shoot. No differences in K and Na contents of the shoots were measured in homozygous T-DNA insertion lines for *CNGC19* and *CNGC20*, respectively, which developed a growth phenotype in the presence of up to 75 mM NaCl similar to that of the wild type. All these results suggest that both channels are involved in the salinity response of different cell types in the shoot. Hence, *CNGC19* and *CNGC20* could assist the plant to cope with toxic effects caused by salt stress, probably by contributing to a reallocation of sodium within the plant.

In spite of the importance of CNGCs in plant cation homeostasis, there are no investigations about CNGCs in mycorrhizal plants. Thus, this is another key to understanding CNGC function in plants subjected to salt stress in future studies. Indeed, it seems apparent that CNGCs play important roles for plant survival and adaptation by mediating multiple stress responses and developmental pathways.

10 Perspectives for future studies

The complexion of the *Arabidopsis* genome-sequencing project revealed a large family of CNGCs composed of 20 members with overall sequence similarities ranging between 55% and 83% (Mäser et al. 2001). While mammalian genomes (in which they were discovered) have so far been found to contain six CNGC-encoding genes, the large number and diversity of their plant counterparts suggests important and perhaps very specialized physiological roles of CNGCs in plants. In the case of Na^+/H^+ antiporters, six fully sequenced members and at least 40 potential other candidates have been recognized (Xia et al. 2002). Although numerous studies have shown that overexpression of some Na^+/H^+ antiporters results in more salt tolerant plants (Gaxiola et al. 1999; Sottosanto et al. 2004) and it is known that some CNGCs such as *AtCNGC19* and

Table 4 Ion selectivity and physiological roles identified in plant cyclic-nucleotide-gated channels

Gene	Ion selectivity	Suggested physiological role
Arabidopsis thaliana		
AtCNGC1	K^+, Na^+, Ca^{2+}, Pb^{2+}	Cation uptake from soil
		Heavy metal uptake
AtCNGC2	K^+, Ca^{2+},Li^+ Cs^+, Rb^{2+}	Developmental cell death and senescence
		Growth and development
		Pathogen resistance
AtCNGC3	K^+, Na^+	Distribute and translocate ion from xylem
		Translocate Na^+ within embryo
AtCNGC4	K^+, Na^+	Pathogen resistance
AtCNGC10	K^+	Light modulated development
AtCNGC11	K^+, Ca^{2+}	Pathogen resistance
AtCNGC12	K^+, Ca^{2+}	Pathogen resistance
AtCNGC18	Ca^{2+}	Polarized pollen tube growth
Nicotiana tabacum		
NtCPB4	Pb^{2+}	Heavy metal uptake
Hordeum vulgare		
HvCBT1	Unknown	Ion transport in aleurona
NEC1	Unknown	Pathogen resistance

AtCNGC20 could assist the plant to cope with toxic effects caused by salt stress (Kugler et al. 2009), much remains unknown on this topic.

In relation to arbuscular mycorrhizal symbiosis, it is interesting to note that there are no studies regarding CNGCs and only a few investigations have been carried out with cation:proton antiporters (Ouziad et al. 2006). Thus, we propose that investigating the participation of cation proton antiporters and cyclic nucleotide-gated channels on arbuscular mycorrhizal symbiosis under salinity is a promising field that should shed further light on new mechanisms involved in the enhanced tolerance of AM plants to salt stress. Indeed, these studies would allow understanding if the AM symbiosis affects sodium uptake, distribution, and compartimentation within the plant cell. Overall, these investigations should open new research lines aimed at obtaining maximum benefit from the AM symbiosis under salinity or other osmotic stress conditions.

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