Chapter 15
Legumes–Microbes Interactions Under Stressed Environments

Hamdi H. Zahran

Abstract Legumes and their associated microbes are common and exist in different environments. Microbes have evolved many mechanisms, which enable them to cope with changing environment. Resilience to these changes is essential to their survival and depends on rapid and efficient control of genetic expression and metabolic responses. Legumes establish several mutual, antagonistic, and beneficial interactions with microbes, which are occasionally subject to unfavorable (stressed) environmental conditions. Stressed terrestrial environments include, deserts with arid climate (warm and dry), salt-affected soils, alkaline and acidic soils, soils contaminated with toxic metals, and nutrient deficiency. During the course of development, microbes inherit traits that enable them to survive under undesirable conditions. Legumes, however, are stress-sensitive plants, and only few of them can withstand stressed environments. Legume rhizospheres colonized by a consortium of microbes are influenced by nutrient-rich root exudates. Legumes and microbes exhibit mutual relationships such as, association, symbiosis, and parasitism and live together in one habitat for long periods. The associated microorganisms include plant-growth-promoting rhizobacteria (PGPR), which are either nitrogen-fixing or not, and many fungi. Symbiotic organisms include mycorrhiza and the root-nodule bacteria (rhizobia). Recent molecular and genetic tools have assisted in discovering new effective stress-tolerant microbes. This chapter broadens the scope of microbes interfering with growth of legumes – a relationship that has been misunderstood to be restricted to rhizobia. Therefore, future investigations have to consider a consortium of microbes in order to improve productivity of legume crops.

H.H. Zahran
Department of Botany, Faculty of Science, University of Beni-Suef, Beni-Suef 62511, Egypt
e-mail: hhz1952@hotmail.com

M.S. Khan et al. (eds.), *Microbes for Legume Improvement*,
DOI 10.1007/978-3-211-99753-6_15,

15.1 Introduction

Nitrogen (N) is one of the major liming nutrients for most crops and non-crop plant species. The acquisition and assimilation of biologically-fixed nitrogen is important but only second to photosynthesis for plant growth. Biological nitrogen fixation (BNF) involves the conversion of atmospheric N_2 to ammonium, a form of N that is easily utilized by plants. Many diverse biological associations contribute to BNF in both soil and aquatic systems. However, BNF is in the sole domain of certain bacteria (diazotrophs), which contain nitrogenase, the enzyme complex that catalyzes the conversion of gaseous N_2 to the combined form. The ability of a plant to supply all or part of its N requirements from BNF in its roots can be a great competitive advantage over non-N_2-fixing neighbors (Vessey et al. 2005). An essential element of agricultural sustainability is the effective management of N in the environment. This usually involves at least some use of biologically fixed N_2 because N from this source is used directly by the plant, and so is less susceptible to volatilization, denitrification, and leaching (Graham and Vance 2000). In most agricultural systems, the primary source of BNF (ca. 80%) occurs via the symbiotic interactions between legumes and rhizobia of the genera *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, and *Sinorhizobium.* The actinorhizal (*Frankia*) and *Anabaena–Azolla* types of interactions mainly contribute the other 20%. Legumes provide approximately 35% of worldwide protein intake and that ca. 250 million ha of legumes grown worldwide. There is great potential for all legumes to increase N derived from N_2 fixation as well as to enhance the total N_2 fixed through improved management and genetic modification of the plant. Legume N_2 fixation is a variable, but valuable process in agriculture, contributing almost 20% of the N needed for world grain and oilseed production. About 100 Tg N is required annually for the production of the world's grain and oilseed crops (Herridge and Rose 2000; Sadowsky 2005).

Legumes and their bacterial-nodules evolved about 60 and 58 million years ago, respectively (Sprent 2006, 2008). Nodulation is one of the interesting characteristic features of legumes, but non-nodulation remains common in Caesalpinioideae, but lesser in Mimosoideae and Papilionoideae. Legumes are within the order Fabales and represented by a single family, the Fabaceae (formerly the Leguminosae); however, most of the more than 650 genera in the family contain species that can form nodules (Vessey et al. 2005). Nodules are highly specialized organs formed by rhizobia on roots or stems of legume plants under N limited conditions. Within nodules, rhizobia are transformed into an endosymbiotic form - the bacteroids - in which N_2 is reduced to ammonia. The legume–rhizobia symbiosis is hence, of great ecological and agronomic importance.

Optimization of the symbiosis between legumes and their respective microsymbionts (the rhizobia or nonrhizobial bacteria) requires a competitive, infective, and highly efficient N_2-fixing rhizobial strains in sufficient numbers to maximize nodulation. Over 70 species of rhizobia varying in symbiotic and physiological characteristics are now identified (Vessey and Chemining'wa 2006) and relatively high degree of genetic diversity has been reported for rhizobia. The infection and nodulation process in rhizobia–legumes symbioses involves an intimate interaction of macro- and microsymbionts, mediated by bidirectional molecular communications between both symbionts. The rhizobia induce two types of nodules on legumes: determinate and indeterminate. The indeterminate nodules are formed most commonly on temperate legumes (e.g., pea, clover, alfalfa, etc.), inoculated with the fast-growing rhizobia, whereas determinate nodules are normally induced by bradyrhizobia on tropical legumes (e.g., soybean, common bean, etc.). Rhizobia infect host plants, and induce root- or stem-nodules, using three fundamentally different mechanisms: via root hairs, entry through wounds, cracks, or lesions, and via cavities located around primordia of adventitious roots.

Over the last two decades, advances in molecular biology and genetics have helped identify a large number of genes having symbiotic functions. In the fast-growing species, symbiosis-related genes are clustered on one or several relatively large plasmids, whereas in the bradyrhizobia, these genes are chromosomally-located (Debelle et al. 2001; Gualtieri and Bisseling 2000). Symbiotic N_2 fixation requires the coordinated interaction of two major classes of genes, the *nif* and *fix* genes. The *nif* genes encode the molybdenum-based enzyme system having structural and functional relatedness to the N_2-fixation genes of *Klebsiella pneumoniae*. In most rhizobia, *nif* genes are plasmid-borne, but located on the chromosome in the bradyrhizobia. Nitrogen fixation in symbiotic and free-living microbes is catalyzed by nitrogenase, an enzyme system encoded by the *nif*DK and *nif*H genes. Nitrogenase itself consists of a molybdenum-iron protein (MoFe), called component 1, and an iron-containing protein (Fe), called component 2. Environmentally, *nif*-gene expression is regulated by both O_2 and fixed N level. Moreover, several other genes in the rhizobia including those for exo-polysaccharide, hydrogen uptake, glutamine synthase, dicarboxylate transport, nodulation efficiency, B-1,2-glucans, and lipopolysaccharides, either directly or indirectly influence N_2-fixation (Sadowsky 2005). Legumes are genetically polymorphous for the balance between symbiotrophic and combined types of N nutrition. Wild-growing populations of legumes occasionally exceed crops in the activity of symbiotic N_2 fixation (Al-Sherif et al. 2004; Zahran 2006a). Legume species vary greatly in N_2 fixation ability and the amounts of fixed N under optimal conditions are several folds higher than the amount of N_2 usually fixed in the field. The major approaches for symbiotic N_2 fixation improvement are the selection and construction of effective rhizobial strains, and the breeding of the symbiotically active plants (Zahran 2006a, b, 2009). The amount of N_2 fixed by legumes–rhizobia symbioses may increase by 300% due to crop breeding and management practices (Vance 1998).

To advance analysis of the microbe–legume interactions, several model organisms, which provide either genomic or expressed sequence tags (EST), have been chosen – a prerequisite for large-scale protein identification by peptide fingerprinting. Two model legumes include *Medicago truncatula* and *Lotus japonicus*, which have EST databases with about 180,000 and 32,000 entries, respectively, and whose genome is being sequenced (Rolfe et al. 2003). Proteomic analysis has mainly focused on *M. truncatula*, for which a proteome reference map has been established

(Mathesius et al. 2001). On the other hand, the model symbiotic bacterium *Sinorhizobium meliloti*, able to infect both *M. truncatula* and its relative alfalfa (*Medicago sativa*), was chosen. *S. meliloti* genome consists of a 3.7 Mb chromosome and two megaplasmids of 1.4 and 1.7 Mb. The genome sequence contains 6,294 protein-coding frames, which provide a better understanding of the possible functions of *S. meliloti* (Galibert et al. 2001). However, the gene sequence alone often reveals little about the function of the gene products. Thus, functional proteomics is beginning to play a role in the identification and analysis of gene networks at the level of protein expression.

Among grain crops, pulses, or food legumes rank third after cereals and oilseeds in world production, and represent an important dietary constituent for humans and animals. Grain legumes are mainly cultivated in developing countries accounting for 61.3 million ha in 2002, compared to 8.5 million ha in developed countries (Graham and Vance 2003). Grain legumes play a crucial role in sustainability of agricultural systems and in food protein supply in developing countries (Zahran 2006b). In this chapter, various responses of legumes and their associated microbes to stressed environments is reviewed and discussed.

15.2 Arid and Saline Environments

Arid environments include desert areas characterized by water deficiency due to the atmosphere dryness and low rainfall, resulting in seriously degraded vegetation, and progressive reduction in biological diversity in the ecosystem. Vast tracts of arid and semiarid lands in the world are barren because the vegetation suffers due to water deficits. Under drying conditions, the soil water potential decreases and so does the soil hydraulic conductivity. It is more difficult for plants to extract water and, consequently, the plant water potential tends to decrease. This decrease may directly affect the physical aspects of some physiological processes. Such lands usually lack water supplies for supplemental irrigation, except ground water which is often very deep and saline and aquifers are low yielding. Despite these hostile living conditions being far from optimal, a considerable number of animal and plant species succeeded in adapting to these unhydrobiotic conditions, associated in some areas to high salinity. The rehabilitation of these degraded lands is limited to two possibilities (Tomar et al. 2003): first, the exploitation of plants native to arid environments and second, devising efficient systems for using limited saline water resources either by preventing its unproductive evaporation loss due to dry environment or drainage below rooting zone. Arid and semiarid regions offer optimal light and temperature conditions for most crops, but insufficient precipitation causes extensive reliance on irrigation. Plants developing in the Mediterranean climate (hot and dry summer), for example, are periodically subject to a combination of stresses including, not only the lack of water and high temperature coupled to high evaporative demand and high light intensity in summer but also limitation in the content of N, P, and other nutrients (Sánchez-Diaz 2001).

Irrigated lands are particularly prone to salinization, and salinity has profound effects on crop production. Reducing salinity and increasing salt tolerance of high yielding crops are becoming important global issues. Soil salinity is a wide spread problem representing the most serious forms of land degradation. It is the major cause of declining agricultural productivity and restricting plant growth and biomass production (Apse et al. 1999). In addition, excess salts in soil can bring drastic changes in some of the soil's physical and chemical properties resulting in the development of an environment unsuitable for cultivation. Soils having salts in the solution phase and/or sodium ions (Na^+) on the cation exchange sites exceeding the specified limits are called salt-affected soils. Major cations in salt-affected soils are Na^+, Ca^{2+}, Mg^{2+}, and to a lesser extent K^+. The major anions are Cl^-, SO_4^{2-}, HCO_3^-, CO_3^{2-}, and NO_3^-. These soils are generally divided into three broad categories: saline, sodic, and saline–sodic. A soil having electrical conductivity of saturated paste extract (EC_e) ≥ 4 dS/m and sodium adsorption ratio (SAR) <13 is called saline soil. Soils having $EC_e < 4$ dS/m and SAR ≥ 13 are designated as sodic soils. If a soil has $EC_e \geq 4$ dS/m and SAR > 13, it is categorized as a saline–sodic soil. Several means are used to ameliorate saline soils; cropping, in conjunction with leaching, is among those methods that are found to be the most successful and sustainable in ameliorating saline soils (Hamdy 1990; Qadir et al. 2000). About 23% of the 1.5×10^9 ha cultivated land considered saline and about half of all the existing irrigation systems of the world (3×10^8 ha) are influenced by secondary salinization, alkalization, and waterlogging. Further, about 10×10^6 ha of irrigated lands are abandoned each year because of the unfavorable effects of secondary salinization and alkalization (Dajic 2006). Approximately 400 million ha of agricultural lands throughout the world are affected by salinity (FAO 2005). The intensive irrigation of croplands under an arid climate is the main reason for secondary soil salinization in Egypt. Crops in Egypt are 100% irrigated, as precipitation is very scarce and evaporation is very high. According to government reports, almost 35% of the agricultural lands (ca. 1 M ha) in Egypt suffer from salinity, wherein the electrical conductivity of the extract from saturated soil is higher than 4 dS/m (Kotb et al. 2000). The major cause of soil salinization of the Nile Delta and Valley may include a high water table resulting from either over-irrigation or insufficient drainage system, irrigation with salty drainage and ground water, accumulation of surface runoffs in low-lying areas, and overuse of salt-generating agrochemicals (Kotb et al. 2000). Soil salinity problems usually relate to irrigation with low quality (saline) water occurring when salts accumulate in the crop-root zone and, consequently, the available water in soil for the crop is reduced. Such unfavorable soils of low fertility are generally unsuitable for agricultural production, causing unacceptable yield reduction, and in some cases, not being reasonably utilized. Because of the increased need for food production and increasing areas of salt-affected soils, research on plant responses to salinity has rapidly expanded in recent decades. The identification and use of plants adapted to saline environments is, therefore, of increasing importance if such areas are to remain productive. Recent investigations of plant tolerance to salt stress are focusing on improvement of breeding and modification of the genetic structure of existing crops, aiming at enhanced adaptation to salinity conditions.

Saline soil contains very little N and is thus not suitable for cultivation. An appropriate solution to this situation would be cultivation of salt-tolerant plants able to fix N through symbiotic systems (Zahran 1991, 1999, 2001). However, generally considered only marginally salt-tolerant, a number of legume trees have been used in the remediation of degraded land area, including salinized soils. Examples of these legume trees are *Albizzia lebbeck*, *Acacia auriculiformis*, *Acacia farnesiana*, *Acacia nilotica*, *Acacia tortilis*, *Cassia gluca*, *Cassia javanica*, *Cassia alata*, *Dalbergia sissoo*, *Glyricidia maculate*, *Prosopis juliflora,* and *Sesbania* spp. (Sharma et al. 2001, Zahran 2001; Giri et al. 2002; Tomar et al. 2003). However, legume trees usually exhibit considerable dependence on mycorrhiza for adequate supply of P, which enable them to thrive under salt stress conditions (Giri et al. 2003). Similarly, some herb legumes, such as *Medicago intertexta* and *Melilotus indicus,* are growing naturally in salt-affected soils (Al-Sherif et al. 2004; Zahran et al. 2007) or on seashores, e.g., the halophytic herb *Canavalia rosea* (Chen et al. 2000), are salt tolerant. Thus, rehabilitation of arid soils with salt-tolerant legume tree species will not only render these abandoned soils to be productive but will also ensure conservation and improvement of these lands.

15.3 Legume–Rhizobia Associations

15.3.1 The Rhizobial Bacteria

The Rhizobial bacteria exhibit several different lifestyles. It may colonize the soil environment as well as the root–soil interface (rhizosphere), and live within the root nodule. They can live in soils either as free-living saprophytic heterotrophs or as legume-host-specific N_2-fixing symbionts. These general features give rhizobia several distinct advantages with respect to survival and persistence over most other soil bacteria. A legume host may not be needed for persistence (saprophytic competence) of rhizobia and many of the rhizobia (bacteroids) released from nodules survive and persist in the soil indefinitely as free-living, heterotrophic saprophytes until they colonize the susceptible legume host (Lindström et al. 1990). Rhizobia are traditionally known to be highly stress resistant organisms compared to their compatible host legumes (Zahran et al. 2003; Vriezen et al. 2006, 2007) and some salt-tolerant rhizobia occasionally form functional symbiosis with their hosts. Salt-tolerant rhizobia may include *Sinorhizobium* sp. from the halophytic herb *Canavalia rosea*, grown at 3.5% NaCl (Chen et al. 2000), *Mesorhizobium* strain CCNWGX035 having high tolerance to NaCl, pH, and temperature (Wei et al. 2008), the halotolerant rhizobia from seedlings of *Acacia gummifera,* and *Acacia raddiana* grown at about 6% NaCl (Essendoubi et al. 2007). Similarly, *Bradyrhizobium* sp. from lupine grew at 5% NaCl and survived at acidic (pH 4–5) and alkaline (pH 9–10) conditions (Raza et al. 2001).

Abiotic stresses such as salt, osmosis, and heat may modify the synthesis pattern of some essential cellular components (e.g., proteins and lipopolysaccharides) of the salt-tolerant rhizobia (Zahran et al. 1994). For example, the salt-tolerant *Rhizobium etli* strain (EBRI 26) formed 49 differentially expressed proteins at 4% NaCl (Shamseldin et al. 2006), of which 14 were overexpressed and 35 downregulated. Proteins induced in response to stress may have an important role in homeostasis and maintenance of vital cellular functions (Wankhade et al. 1996). The mechanisms underlying salt tolerance have, however, not been completely elucidated in rhizobia and functional aspects of salt-stress proteins (SSPs). Like proteins, the synthesis pattern of phospholipids in *Bradyrhizobium* strain (SEMIA 6144) cells were modified under saline and temperature stresses and are suggested to be involved in the bacterial response to environmental stress (Medeot et al. 2007).

Rhizobia exposed to increased salinity can maintain osmotic equilibrium across the membrane by exclusion of salts and via intracellular accumulation of inorganic and/or organic solutes (Csonka 1991). For example, *Rhizobium meliloti* overcomes osmotic stress-induced growth inhibition by accumulating compatible solutes, such as K, glutamate, proline, glycine betaine, proline betaine, trehalose, and the dipeptide, *N*-acetylglutaminylglutamine amide (Boscari et al. 2002; Vriezen et al. 2007). Some compatible solutes are used as either N or C sources by rhizobia suggesting that their catabolism is regulated to prevent degradation during osmotic stress. However, the type of osmolytes and their concentrations depend on the level of osmotic stress, growth phase of the culture, C source, and the presence of osmolytes in the growth medium (Smith et al. 1994). Many bacteria are equipped with systems that facilitate the efficient transport of osmoprotectants under stressed conditions, and several of these osmoregulated systems have been identified (Wood et al. 2001). BetS, a system involved in the uptake of proline betaine (PB) in *S. meliloti*, is a Na^+-coupled secondary transporter with high affinity for glycine betaine and proline betaine (Boscari et al. 2004). This system is activated posttranslationally by osmotic stress and plays a crucial role in the rapid response to osmotic upshock. The salt tolerance of a salt-sensitive *Bradyrhizobium japonicum* strain was improved after transformation with *bets* gene of *S. meliloti*. An increased tolerance of transformant cells to a moderate NaCl concentration (80 mM) was detected in the presence of glycine betaine or proline betaine, whereas the growth of the wild-type strain was totally eliminated at 80 mM NaCl (Boscari et al. 2004).

Adaptation of rhizobia to salt is a complex multilevel regulatory process involving many genes (Nogales et al. 2000; Wei et al. 2004). As an example, Rüberg et al. (2003) determined that the prolonged exposure of *S. meliloti* 1021 to 380 mM NaCl activated genes related to polysaccharide biosynthesis and transport of small molecules (amino acids, amines, peptides, anions, and alcohols). In this bacterium, 137 identified genes showed significant changes in gene expression resulting from the osmotic upshift; 52 genes were induced and 85 were repressed. Similarly, sudden increase in external osmolarity of *S. meliloti* cultures, elicited by addition of either NaCl or sucrose stresses, induced large number of genes having unknown functions and in repression of many genes coding for proteins with known functions (Domínguez-Ferreras et al. 2006). Of the genes upregulated, 64% were located on plasmid (pSmbB) and 85% of the genes downregulated were chromosomal. This finding suggests the role of *S. meliloti* plasmid in osmoadaptation. Further, they reported that ribosomal genes and tricarboxylic acid cycle genes are repressed. Interestingly, 25% of all genes specifically downregulated by NaCl encode ribosomal proteins. Five salt-tolerance genes of *Sinorhizobium fredii* RT19 were identified by construction and screening of a Tn5-1063 library (Jiang et al. 2004). Na^+ intracellular content measurements established that *phaA2*, *phaD2*, *phaF2,* and *phaG2* are mainly involved in the Na^+ efflux in *S. fredii* RT19. Growth recovery of the *metH* mutants grown with different NaCl concentrations, obtained by addition of methionine, choline, and betaine, showed that the *metH* gene is probably involved in osmoregulation in *S. fredii* RT19 (Jiang et al. 2004).

Nodulation factors or Nod factors (lipochitooligosaccharides) of rhizobia are communication signals with leguminous plants and are major host-specificity determinants that trigger the nodulation program in a compatible legume host. Nod factor activities and cloning of genes required for their initiation, lead to an understanding of the first steps in signaling pathways and symbiotic interactions (Geurts et al. 2005; Mulder et al. 2005; Chen et al. 2006). Nod factors, which possess hormone-like properties, stimulate the plant to produce more *nod*-gene inducers to deform root hairs on their respective host plant, and initiate cell division in the root cortex. However, Nod factors from different *Rhizobium* species differ in the number of *N*-acetylglucose amine residues, the length and saturation of the acyl chain, and the nature of modifications on the basic backbone (e.g., sulphate, acetate, fucose, etc.). These differences define the host specificity observed in the symbiosis. The production of Nod factors and excretion of *nod* metabolites by *Rhizobium leguminosarum* bv. *trifolii* have been found to be disrupted by pH, temperature, and both P and N concentration (McKay and Djordjevic 1993). For instance, *Rhizobium tropici* strain CIAT899 grown under acid conditions formed 52 Nod factors, 37 of which differed from the 29 formed under neutral conditions (Morón et al. 2005). Under salt stress conditions, 46 different Nod factors were identified in a *R. tropici* CIAT899 culture, 14 different new Nod factor structures identified were not produced under neutral or acid conditions. High concentration of sodium enhanced *nod* gene expression (using a *nodP*::*lacZ* fusion) and Nod factor biosynthesis (Estevéz et al. 2009). Stimulation or suppression of Nod factors under stressed conditions might affect the rhizobia–legumes symbioses.

15.3.2 Legume–Rhizobia Symbioses

15.3.2.1 Effects of Water, Osmotic, and Desiccation Stresses

In nature, plants are frequently exposed to adverse environmental conditions that have a deleterious effect on their survival, development, and productivity. Drought and salinity are considered the most important abiotic factors limiting plant growth and yield in many areas of the world. Osmotic stress refers to a situation where insufficient water availability limits growth and development of plants (Zhu et al. 1997). Soil water content directly influence growth of rhizospheric microbes by decreasing water activity below critical tolerance limit and indirectly by altering plant growth, nutrient concentration, root architecture, and exudates.

Microbial cells are able to withstand lower water potentials than most higher-plant cells. Generally, the root-nodule bacteria (rhizobia) are more resistant to soil-water deficit (drought) than the plant itself and hence, the impact of drought stress conditions on N_2 fixation might be due to direct influence on the microsymbionts (Serraj et al. 1999; Hungria and Vargas 2000). Consequently, from the beginning of infection by rhizobia until the functioning of differentiated nodules, the most important factors limiting the fixation under water stress will probably depend on the host plant. The work done on different lucerne (*M. sativa*) cultivars suggests that those adapted to dry conditions are likely to show less water stress effects on N_2-fixation than those less adapted cultivars (Aguirreolea and Sánchez-Díaz 1989). Species of rhizobia, however, differ in their susceptibility to the detrimental effects of desiccation in natural soils. For example, slow-growing rhizobia is generally thought to survive desiccation better than fast-growing rhizobia (Zahran 2001). As far as the effect of water stress on symbiosis is concerned, it affects nodule establishment, C and N metabolism, nodule O_2 permeability, nitrogenase activity, and total plant N_2 fixation ability (Zahran and Sprent 1986; Aguirreolea and Sánchez-Díaz 1989; Sadowsky 2005). However, N_2-fixation is widespread in arid land legumes (e.g., *Acacia* and *Prosopis* species) and drought tolerant rhizobial strains have been reported for both tree and crop legume species (Nijiti and Galiana 1996). Differences exist between rhizobial species, with respect to drought or osmotic stress tolerance and the capacity to infect plants and fix atmospheric N as seen in *Acacia mangium* (Galiana et al. 1998), *Gliricidia sepium* (Melchior-Marroquin et al. 1999), *Sesbania* (Rehman and Nautiyal 2002), *Albizzia adianthifolia* (Swaine et al. 2007), and *Retama raetam* (Mahdhi and Mars 2006; Mahdhi et al. 2008).

Like bacterial partners, plants may also alleviate the impact of stress (e.g., osmotic), if grown with soil microorganisms like PGPR and AM-fungi (Valdenegro et al. 2001; Ruiz-Lozano 2003). The AM-fungi have improved ability for nutrient uptake and tolerance to biotic and abioic stresses. Tree legumes form an association with AM-fungi and rhizobia. This association could further be beneficial if they are used with PGPR. In this regard, *Medicago arborea*, a leguminous tree used for re-vegetation purposes under semi-arid conditions, was inoculated either singly or in combination with microorganisms [three *Glomus* species, two strains: wild type and genetically-modified *S. meliloti*, and PGPR (Valdenegro et al. 2001)]. Mycorrhizal fungi were effective in all cases, while PGPR inoculation was only effective when associated with specific mycorrhizal endophytes (*G. mosseae* plus wild type rhizobia and *Glomus deserticola* plus genetically-modified rhizobial strain). The effect of double inoculation with two species of AM-fungi (*G. deserticola and G. intraradices*) and two strains of *S. meliloti* (wild type and its genetic variant) was examined in three *M. sativa* (*Mimosa nolana*, *Mimosa rigidula,* and *Mimosa rotata*) plants. Nodulation and mycorrhizal dependency changed in each plant genotype in accordance with the *Sinorhizobium* strain and AM-fungi involved. Plants inoculated with both the AM-fungi and the genetically-modified *S. meliloti* were better adapted to drought stress (Vázquez et al. 2001).

AM-fungal symbiosis can also alleviate drought-induced reductions in nodule activity and senescence. The most remarkable observation was the substantial reduction in oxidative damage to lipids and proteins in nodules of mycorrhizal plants subject to drought as compared to the nodules of non-mycorrhizal plants. Mycorrhizal protection against the oxidative stress caused by drought is perhaps one of the most important mechanisms by which the AM symbiosis increases the tolerance of plants against drought (Ruiz-Lozano et al. 2001). The AM symbiosis considerably increased the glutathione reductase activity (an important component of the ascorbate glutathione cycle) both in roots and nodules of soybean plants subject to drought stress (Porcel et al. 2003). The AM-soybean plants respond to drought stress by down regulating the expression of two plasma-membrane intrinsic proteins (PIP) genes (Ruiz-Lozano et al. 2006). This is likely to be a mechanism to decrease membrane water permeability and to allow cellular water conservation. The role of AM-fungal symbiosis in the regulation of *Phaseolus vulgaris* root hydraulic properties and root plasma membrane aquaporins was evaluated under different stress (drought, salinity, and cold) conditions (Aroca et al. 2007). Hydraulic conductance and plasma-membrane intrinsic proteins (PIPs, proteins regulate the whole water transport through plant tissues) remained unchanged under various stress conditions in AM plants. The expression of each *PIP* gene responded differently to each stress and was dependent on the AM fungal presence. This finding indicates a specific function and regulation of each gene of AM symbiosis under the specific conditions of each stress tested.

The use of genetic engineering technology could lead to more effective gene-based approaches for improving crop tolerance to drought. Certain genes are expressed at elevated levels when a plant encounters stress–specific proteins such as water channel proteins, key enzymes for osmolyte biosynthesis, detoxification enzymes, and transport proteins (Vinocur and Altman 2005) are induced by abiotic stress. However, tolerance to complex stress like drought is very unlikely to be under the control of a single gene. Therefore, the successful strategy may be the use of genetic engineering to switch on a transcription factor regulating the expression of several genes related to abiotic stress (Bartels and Sunkar 2005; Chinnusamy et al. 2005). Transgenic plants over-express the *P5CS* (Δ^1-pyroline-5-carboxylate synthetase) gene from *Vigna aconitifolia*, accumulate high proline levels, and are more tolerant to osmotic stress (Kishor et al. 2005). Two *P5CS* genes have been isolated from the model legume *M. truncatula* (Armengaud et al. 2004): *MtP5CS1* (encode a developmental "housekeeping" enzyme) and *MtP5CS2* (shoot-specific osmoregulated isoform). *M. truncatula* transformed with the *P5CS* gene from *V. aconitifolia* (Verdoy et al. 2006). Over-expression of *P5CS* genes accumulates high levels of proline in tissues of *M. truncatula*, which display enhanced osmotolerance (Verdoy et al. 2006). Transgenic legume models allow analysis of some biochemical and molecular mechanisms that are activated in the nodule in response to high osmotic stress and ascertain the essential role of proline in the maintenance of nitrogen-fixing activity under these conditions. Recent molecular investigations thus indicate the active role of proline in alleviating the effects of osmotic stress on *Rhizoium*–legume symbiosis. A transcription factor DREB 1A from *Arabidopsis thaliana*, driven by the stress inducible promoter from the *rd29A* gene, was introduced in a drought-sensitive peanut cultivar JL24 through *Agrobacterium tumefaciens*-mediated gene transfer. All transgenic events were able to maintain a transpiration rate equivalent to the well-watered control in soils dry enough to reduce transpiration rate in the wild type (Bhatnagar-Mathur et al. 2007).

15.3.2.2 Effects of Salt Stress

Salinity is considered a significant factor affecting crop production and agricultural sustainability in arid and semiarid regions of the world. Soil infertility is often due to the presence of large quantities of salt and the introduction of plants capable of surviving under these conditions, is worth investigating(Soussi et al. 1998). Salt tolerance in plants is a complex phenomenon that involves morphological and developmental changes as well as physiological and biochemical processes. Salinity disrupts cell function, through the toxic effects of specific ions and by osmotic effects, or both (Munns 2005). Specific ion effect results from a reduction in metabolic activity, due to the presence of excessive concentrations within cells, and causes plant death when a critical salinity level exceeds. Osmotic effects, however, are manifest by water deficit due to reduction in cell turgor. The complexity of the plant response to salt stress is partially explained by the fact that salinity imposes salt toxicity in addition to osmotic stress (Hasegawa et al. 2000). Sodium is toxic to many organisms, except to halotolerant organisms such as halobacteria and halophytes, which possess specific mechanisms that keep intracellular sodium concentrations low. Sodium accumulation in the cytoplasm is prevented by restricting its uptake across the plasma membrane and by promoting its extrusion or sequestration in halophytes (Hasegawa et al. 2000). Therefore, a better understanding of physiological responses under salt conditions can be of value in programs conducted to breed salt-tolerant crop varieties. In the following section, plant responses to soil salinity are discussed with emphasis on molecular mechanisms of signal transduction and on the physiological consequences of altered gene expression. Understanding the mechanisms by which plants perceive and transduce stress signals to initiate adaptive responses is essential for engineering stress-tolerant crop plants (Xiong and Zhu 2001). Thus, in addition to the existing salt-tolerant crop genotypes, research is needed to develop genotypes with increased tolerance to salinity (Qadir et al. 2000). Genetic variability within a species offers a valuable tool for studying mechanisms of salt tolerance. One of these mechanisms depends on the capacity for osmotic adjustment. A general feature of many plants growing in a saline environment is that they decrease osmotic potential by accumulation of inorganic and/or compatible solutes in their cells.

High soil salinity can limit legume productivity by adversely affecting the growth of the host plant, the development of root-nodule bacteria, and finally the

N_2-fixation capacity (Zahran 1999). Furthermore, high salinity causes suppression of photosynthesis, reduces the yield of dry mass of stems, roots, and nodules, decreases the survival of root-nodule bacteria in soil and rhizosphere, increases generation time, and disrupts the cell ultrastructure (Novikova and Gordienko 1999). The identification of tolerant genotypes that may sustain a reasonable yield in salt-affected soils has thus been a strategy adopted by scientists to overcome salinity. On the contrary, numerous reports are available that explain the formation of the symbiosis between root-nodule bacteria and various legume species under salinized soils (Soussi et al. 1998, 1999; Zahran et al. 2003). The root-nodule bacteria grown under saline conditions may have specific traits, which enable them to establish a symbiotic interaction under salt stress (Zahran 2005). For example, 15 isolates of *S. meliloti* recovered from nodules of wild species of alfalfa, melilot, and trigonella, preferably formed symbiosis with a salt-tolerant legume grown in both salinized and nonsalinized soils (Ibragimova et al. 2006). It appears that the efficiency of symbiotic interaction under salinized conditions depends on the symbiotic efficiency of the isolates under standard conditions, but this did not correlate with the source of nodule bacteria (soil or nodule) or their salt tolerance.

Legume trees such as *Acacia*, *Prosopis*, *Sesbania,* and legume herbs such as *Melilotus* and *Medicago*, are salt-tolerant (Shamseldin and Werner 2005; Zahran et al. 2007). These legumes establish a symbiotic association with a wide range of rhizobia (*Rhizobium*, *Mesorhizobium*, and *Sinorhizobium*), welladapted to the drastic conditions of arid climates (Marcar et al. 1991; Räsänen and Lindström 2003; Nguyen et al. 2004). Grain legumes recognized as either sensitive or only moderately tolerant to salinity: *Cicer arietinum*, *Lens culinaris*, *P. vulgaris,* and *Pisum sativum* are sensitive to salt stress, while *Glycine max* and *Vicia faba* plants are particularly moderate salt-tolerant grain legumes (Zahran and Sprent 1986; Ashraf and Waheed 1990; Bouhmouch et al. 2005, Phang et al. 2008). The high sensitivity of the legume–*Rhizobium* symbiosis to salinity has been recognized, and the necessity to develop salt-tolerant symbioses has been emphasized (Sprent and Zahran 1988; Zahran 2005; Ibragimova et al. 2006; Zahran 2009). The limitation of N_2 fixation imposed by environmental factors could be resolved through the selection and breeding of improved legume cultivars. On the other hand, the unsuccessful symbiosis under salt stress may be due to a failure in the establishment of rhizobia populations in the rhizosphere, the failure of the infection process, and the inhibition of nodulation (Sprent and Zahran 1988; Bouhmouch et al. 2005). A best symbiotic N_2 fixation under salinity conditions is achieved if both symbiotic partners, as well as the different steps of their interaction (recognition, root colonization, infection, nodulation, and nitrogen fixation), are all tolerant to the imposed stress factor.

Annual pasture legumes and the naturally-growing annual herb legumes include salt-tolerant species, which usually adapt to increasing soil salinity. Al-Sherif et al. (2004) and Zahran et al. (2007) reported the existence of *M. indicus* and *M. intertexta* in salt-affected lands of Egypt. Some annual pasture legumes (e.g., *Melilotus siculus* and *Medicago polymorpha*) persist in saline soils ($EC_e > 8$ ds/m) of Australia (Boschma et al. 2008; Nichols et al. 2008). The ability to germinate and establish seedlings on saline lands is particularly important for annual pasture legumes; however, this point has received less attention compared to the work related to mature plants (Rogers et al. 2008). Seed germination of *T. subterraneum* and *Trifolium michelianum* was significantly reduced by about 50% at 110 mM NaCl (Rogers and Noble 1991), while *M. siculus* had no significant reduction in germination at 200 mM NaCl (Marañón et al. 1989). The mechanism of salinity tolerance and avoidance at germination of five self-regenerating annual pasture legumes of Mediterranean origin in Australia was studied (Nichols et al. 2009). The maximum NaCl concentrations, for which no reduction in germination percentage occurred, were 300 mM for *M. siculus*, 240 mM for *M. polymorpha*, and 120 mM for *T. subterraneum*, *Trifolium tomentosum*, and *Trifolium michelianum*. The results emphasized that *M. siculus and M. polymorpha* are among adapted annual pasture legumes for highly saline soils. The tropical pasture legume (*Stylosanthes humilis*) is a salt-sensitive legume, though significant differences in salt-tolerance were found between populations. The estimated concentrations that reduced shoot dry mass by 50% and 25% varied between populations from 84 to 108 and from 49 to 83 mM NaCl, respectively (Lovato et al. 1999). Populations from arid climate with saline soils show higher salt tolerance than those from nonsaline soils. In another study, salinity affected the germination, survival of seedlings, dry matter accumulation and yield of lentil, *L. culinaris* (Karterji et al. 2001). In soil with an EC_e of 2 dS/m and 3 dS/m (slightly saline soil), yield reduction in *L. culinaris* was about 20% and 90–100 %, respectively. Soybean is an important crop, and its productivity (growth, nodulation, and yield) was significantly hampered by salt stress. The final yield of soybean which is classified as a moderate salt-tolerant crop, reduced when salinity exceeded 5 dS/m (Ashraf 1994). Soybean production was inhibited by 52.5% and 61% when grown under moderate (14–15 dS/m) and high (18–20 dS/m) soil salinity, respectively (Chang et al. 1994). To cope with salt stress, soybean developed several tolerance mechanisms including maintenance of ion homeostasis, adjustment in response to osmotic stress, restoration of osmotic balance, and other metabolic and structural adaptations (Phang et al. 2008).

Under stress, plants maintain low concentration of Na^+ and high concentration of K^+ in the cytosol. However, Na^+ toxicity is not only due to toxic effects of Na^+ in the cytosol, but also because K^+ homeostasis is disrupted possibly due to the ability of Na^+ competing for K^+ binding sites. Plants possess a number of mechanisms to prevent accumulation of Na^+ in the cytoplasm that include minimizing Na^+ influx, intracellular compartmentation of Na^+, and maximizing Na^+ efflux as well as precirculation of Na^+ out of the shoot by the phloem (Ward et al. 2003; Bartels and Sunkar 2005). Salt tolerance correlates to an efficient Na^+ and Cl^- exclusion mechanism and to a better maintenance of leaf K^+ concentration at high levels of external NaCl (Sibole et al. 2003; Garthwaite et al. 2005). The tree legume, *A. nilotica*, however, exhibited different salt tolerance mechanism, which enabled the adjustment of osmotic potential by accumulation of Na^+, K^+, Cl^-, and proline under salt stress (Nabil and Coudret 1995). Salt-tolerant plants achieve the Na^+–K^+ balance in the cytosol by regulating the expression and activity of Na^+ and K^+ transporters and H^+ pumps that generate the driving force for transport (Zhu 2003).

Na^+ transporters, include the NHX and SOS families (salt overly sensitive) of Na^+/H^+ exchangers, HKT proteins, as well as components of the signaling pathway that regulate these transporters, such as SOS2 and SOS3 proteins (Horie and Schroeder 2004; Pardo et al. 2006). Proper regulation of ion flux is necessary for cells to maintain low concentrations of toxic ions and to accumulate essential ions. The vacuolar sodium sequestration is mediated by an Na^+/H^+ antiport at the tonoplast. Sequestration or compartmentalization of Na^+ into the vacuole through vacuolar Na^+/H^+ antiporters uses the proton motive force generated by the vacuolar H^+-translocating enzymes, H^+-adenosine triphosphate (ATPase), and H^+-inorganic pyrophosphatase (PPiase), to couple the downhill movement of H^+ with the uphill movement of Na^+ against the electrochemical potential (Blumwald and Gelli 1997). The presence of Na^+/H^+ antiporter activities has been physiologically characterized in tonoplast vesicles and is molecularly represented by six *Arabidopsis* genes *AtNHX1-6* (Blumwald et al. 2000; Yokoi et al. 2002). The first Na^+/H^+ exchanger identified was *AtNHX1*, a member of a family of six genes (*AtNHX1–AtNHX6*) that show sequence homology to mammalian and yeast NHE or NHX exchangers, respectively (Yokoi et al. 2002). Many reports have indicated the existence of Na^+/H^+ antiporters in plant vacuoles (Blumwald et al. 2000; Zörb et al. 2005). Several studies dealing with the occurrence, expression, and activity of Na^+/H^+ antiporters and NHX genes under salt stress were reviewed (Zahran et al. 2007). Overexpression of *AtNHX1* enhances salt tolerance in crop plants (e.g., tomato, rice, cotton, sugar beet, barley, sunflower, wheat, and maize) as well as in some halophytic plants (*Atriplex*, *Suaeda,* and *Thellungiella*) and this antiporter catalyzes both Na^+/H^+ and K^+/H^+ exchange. Among legumes, a vacuolar antiporter (*MsNHX1*) was cloned from alfalfa whose gene was induced by NaCl and ABA treatments (Yang et al. 2005). The involvement of Na^+/H^+ transporters in *M. intertexta* and *M. indicus*, growing in salt-affected cultivated soils of Egypt (Zahran 1998; Al-Sherif et al. 2004) have been investigated. NaCl induced gene expression of three genes in *M. intertexta* and one gene in *M. indicus*. *NHX* gene triggered in *M. intertexta* plants to cope with tissue Na^+ accumulation, while in *M. indicus*, the absence of Na^+ accumulation and the lack of induction of *NHX* genes in response to NaCl indicated that this species relied on different mechanisms to cope with salt stress.

Under stress conditions, proline, glycine betaine, sucrose, mannitol etc., are switched on to protect major processes such as cell respiration, photosynthetic activity, nutrient transport, and N and C metabolism (Zhu 2002). Trehalose (a nonreducing disaccharide found in a wide variety of organisms, including bacteria and plants) plays an important role as an abiotic stress protectant, stabilizing dehydrated enzymes and membranes as well as protecting biological structures from desiccation damage (Benaroudj et al. 2001; Sampedro and Uribe 2004). Accumulation of trehalose in crop plants improved their tolerance to drought and salinity (Romero et al. 1997). A significant increase in trehalose content was detected in nodules (bacteroids) of soybean (Müller et al. 1994), *M. truncatula,* and *L. japonicus* (López et al. 2008) in response to salt stress. These findings support the possible role of this disaccharide as an osmoprotectant against abiotic stress. Plant growth parameters and nitrogenase activity decreased in nodules of the model legumes (*M. truncatula* and *L. japonicus*) after treatment with 50 mM NaCl (López et al. 2008). Carbon metabolism in the *L. japonicus* nodule was less sensitive to salinity than in *M. truncatula*, as enzymatic activities responsible for C supply to the bacteroids to fuel nitrogen fixation such as sucrose synthase (SS), alkaline invertase (AI), malate dehydrogenase (MDH), and phosphoenolpyruvate carboxylase (PEPC), were less affected by salt than the corresponding activities in *M. truncatula*.

Cytokinins are a group of adenine derivatives that play a major role in many aspects of plant growth and development. Exogenous cytokinins induce cortical cell divisions in legume roots and the expression of several nodulin genes, thus enhancing legume–*Rhizobium* symbiosis (Jimenez-Zurdo et al. 2000; Mathesius et al. 2000; Gonzalez-Rizzo et al. 2006). Cytokinin levels decrease under adverse environmental conditions. However, application of exogenous cytokinin counteracts the negative physiological effects of salt stress (Hare et al. 1997). A new cytokinin receptor homolog (*MsHK1*) was induced in *M. sativa* seedlings by exogenous application of the cytokinin trans-zeatin (Coba De La Peña et al. 2008). *MsHK1* expressed in roots, leaves, and nodules of *M. sativa* under salt stress, and transcript accumulation in the vascular bundles pointed to a putative role in osmosensing for *MsHK1* receptor homolog (Coba De La Peña et al. 2008). Similarly, exogenous abscisic acid (ABA) pretreatment to plants subject to salinity improved growth parameters and ameliorated the effects of salt on nodule weight and nitrogenase activity of a salt-sensitive cultivar of the common bean (*P. vulgaris*) (Mills et al. 2001; Khadri et al. 2007). ABA treatment seems to limit sodium translocation to shoot resulting in the maintenance of high K^+/Na^+ ratio in salt-stressed plants. Therefore, ABA may function as a stress signal and play an important role in the tolerance of plants to salinity.

Molecular studies of rhizobia–legumes symbioses under stressed environments have sparked increasing interest in recent years. In this context, several kinds of genes or markers associated with stress tolerance have been identified in both bacteria and host legumes (Nguyen et al. 2004). For example, the *typA* gene (an orthologue of *typA*/*bipA* genes found in a wide range of bacteria and which is required for general housekeeping functions) of *S. meliloti* was described (Kiss et al. 2004). The *typA* gene is required for the establishment of nitrogen-fixing symbiosis with certain *M. truncatula* and *M. sativa* cultivars (Kiss et al. 2004). The *typA* gene is required for survival of *S. meliloti* under certain stress conditions such as growth at low temperature or low pH and in the presence of sodium dodecyl sulphate (SDS). In a recent study, Patankar and González (2009) explored the regulatory role of SMc04032 locus, named as nesR (one of the orphan LuxR-type response regulators): it causes the bacteria to cope with specific nutritional, environmental, and stress conditions. Through expression and phenotypic analysis, *nesR* was determined to affect the active methyl cycle and to influence nutritional and stress response activities in *S. meliloti*. These results suggest that *nesR* potentially contributes to the adaptability of *S. meliloti* when it encounters challenges such as high osmolarity, nutrient starvation, and/or competition for nodulation, thus increasing its chances for survival in the stressful rhizosphere. For plants, many genes encoding PR-5 proteins (proteins known to function as protein-based defensive system against abiotic and biotic stress) have been identified from a variety of plants, indicating that PR-5 is broadly distributed throughout higher plants. The involvement of PR-5 proteins in protection against abiotic stresses, such as osmotic imbalance has been suggested (Kononowics et al. 1992). Novel soybean genes, *GmOLPA* and *GmOLPB* (*G. max* osmotin-like protein), encoding an acidic homolog of PR-5 protein (Onishi et al. 2006) and neutral homolog PR-5 protein (Tachi et al. 2009), respectively, were highly induced in the leaves of soybean plants under conditions of high salt stress. An alfalfa cDNA library was induced by salt stress constructed by suppression subtraction hybridization (SSH) technology (Jin et al. 2009). 119 positive clones were identified by reverse Northern dot-blotting resulting in 82 uni-ESTs. Most of the annotated sequences were homologous to genes involved in abiotic or biotic stress in plants. In addition, several ESTs, similar to genes from other plant species, closely involved in salt stress were isolated from alfalfa, such as aquaporin protein and glutathione peroxidase.

The production of reactive oxygen species (ROS) is yet another major damaging factor, which disrupts normal metabolism through oxidative damage of lipids and proteins in plants exposed to different environmental stresses. Plants with high concentrations of antioxidants (e.g., ascorbate peroxidase APOX, catalase CAT, peroxidase POD, and superoxide dismutase SOD), have greater resistance to these oxidative damages (Jiang and Zhang 2002). Nodules are particularly rich in both quantity and diversity of antioxidant defenses that may protect the nodule structures from high rates of nodule respiration, as well as, conserve nitrogenase activity (Becana et al. 2000; Blokhina et al. 2003). Nitrogenase is O_2 sensitive; therefore, nodules have evolved mechanisms to downregulate their permeability to O_2 and maintain the infected cell O_2 concentration at approximately 5–50 nM compared to 250 μM for cells in equilibrium with air (Minchin 1997). Salinity induces the production of stress proteins or antioxidant enzymes in nodules to minimize damage caused by ROS such as, H_2O_2, O_2, and OH (Porcel et al. 2003). Salt stress (50 mM NaCl) or osmotic stress (50 mM mannitol) reduced plant growth, nitrogen fixation, and the activities of the antioxidant defense enzymes of common bean (*P. vulgaris*) nodules (Tejera et al. 2004; Jebara et al. 2005). The maintenance of sucrose synthase, together with isocitrate dehydrogenase, associated with a suitable antioxidant defense may be relevant for osmotic tolerance in *P. vulgaris* N_2 fixation (Sassi et al. 2008). The performance and responses to osmotic stress (50 mM mannitol) have been evaluated recently in chickpea-*Mesorhizobium* symbiosis (Mhadhbi et al. 2008). Nodular POX and APOX activities were significantly enhanced in chickpea plants under osmotic stress. The increase of POX and APOX inversely correlated with the inhibition of aerial biomass production and nitrogen-fixing capacity, suggesting a protective role for these enzymes in nodules. In a similar report, salinity (75 mM NaCl) significantly increased the nodule conductance in four genotypes of *S. meliloti* inoculated *M. truncatula* plants (Aydi et al. 2004). Thus, sensitivity to salinity appears to be associated with an increase in nodule conductance that supports the increased respiration of N_2-fixing nodules under salinity. In contrast, salinity did not change the nodule conductance and nodule permeability of the salt-tolerant variety of chickpea (L'taief et al. 2007). Salt tolerance of this variety appears to be associated with stability in nodule conductance and the capacity to form nodules under salt constraint. Nodule conductance to O_2 diffusion has been found to be a major factor in the inhibition of N_2 fixation by salinity that severely reduces the production of legumes.

15.3.2.3 Effects of pH and Temperature

Acid soils limit agriculture production, and as much as 25% of crops suffer from soil acidity (Munns 1986). Tolerance to acid conditions in rhizobia often correlates to the strain's ability to maintain internal pH approaching neutrality (Graham et al. 1994). Generally, bradyrhizobia are more acid-tolerant than rhizobia (Brockwell et al. 1991; Sadowsky and Graham 1998), although some strains of *R. tropici* are very acid-tolerant (Graham et al. 1994) due to the production of glutathione to grow in extreme acid stress conditions (Riccillo et al. 2000). Using Tn5 mutagenesis, acid-sensitive mutant of *S. meliloti* was isolated and some genes involved in acid tolerance have been characterized (Tiwari et al. 1996). Rrhizobia are sensitive to acidity (Hungria and Vargas 2000), but acidity also influences both the growth of the legume plant and the infection process (Munns 1986). This effect is, in part, most likely due to a disruption of signal exchange between macro- and microsymbionts (Hungria and Stacey 1997) and repression of nodulation genes and excretion of Nod factors in the rhizobia (Richardson et al. 1988). Stress parameters such as soil acidity affect rhizobial persistence, nodulation efficiency and N_2 fixation of some legumes (Graham and Vance 2000). Rhizobial strains nodulating *P. vulgaris* under arid conditions were analyzed for pH tolerance (Priefer et al. 2001). One strain (RP163) exhibiting high nodulation efficiency and broad pH tolerance was mutagenised by Tn5 and the resulting mutants unable to grow on extreme pH media were isolated. In these mutants, a suitable well-characterized promoter is now available to drive expression of rhizobial stress-tolerance genes. In a similar approach, promoters and genes inducible under extreme pH values were identified in *R. leguminosarum* bv. *viceae* VF39 (Priefer et al. 2001) - among them *gabT* encodes the GABA (γ-aminobutyrate) transaminase which is induced under acidic conditions.

Soil nutrient status has a tremendous influence on rhizobium–legume symbiosis. A nutrient stress is indirectly caused by changes in soil matric potential or acidity, which in turn limit nutrient bioavailability, rather than to the lack of the presence of nutrients per se (Sadowsky 2005). Stress conditions apparently increase requirements for essential elements, such as Ca^{2+}, P, and N, in both plants and microbes. The presence of Ca^{2+} may offset the deleterious influence of low pH on root growth while ion uptake increases *nod*-gene induction and expression, and concurrently affects the attachment of rhizobia to root hairs and nodule development (Richardson et al. 1988; Alva et al. 1990; Smit et al. 1992). Phosphorous (P) availability is another limiting factor for N_2-fixation and symbiotic interactions (Saxena and

Rewari 1991) and about 33% of the arable land in the world is P deficient, especially in low pH soil (Graham and Vance 2000). There are marked differences in rhizobial and plant requirements for P and the slow-growers are more tolerant to low P than the fast-growing rhizobia (Beck and Munns 1985).

High soil temperature has a marked influence on survival and persistence of rhizobial strains in temperate climate (Boumahdi et al. 2001). However, strains from naturally-growing legumes in tropical regions survive better at higher temperatures (Zahran et al. 1994). The influence of temperature on rhizobia appears to be strain dependent. For example, *Bradyrhizobium* sp. (lupine) was less susceptible than *R. leguminosarum* bv. *trifolii* to high soil temperature (Sadowsky 2005). However, rhizobial strains at elevated temperatures lose infectivity (Segovia et al. 1991). Moreover, excessive temperature shock cures plasmids in fast-growing strains, and some strains which were isolated from warm environments, had a Fix$^-$ phenotype (Moawad and Beck 1991; Hungria and Franco 1993). Soil temperature greatly influences competition for nodulation (Triplett and Sadowsky 1992). However, some high-temperature (up to 40°C)-tolerant rhizobia formed effective nitrogen-fixing nodules with *P. vulgaris* (Hungria et al. 1993; Michiels et al. 1994), *Prosopis* (Kulkarni and Nautiyal 1999), and *Acacia* (Zerhari et al. 2000). Each *Rhizobium*–legume combination has an optimum temperature relationship around 30–40°C; exposure of both symbiotic partners to temperature extremes much above or below these critical temperatures impairs infection, nodulation, nodule development, and general nodule functioning as well as plant growth and productivity (Michiels et al. 1994). Elevated temperatures directly influence the production or release of *nod*-gene inducers as reported for soybean and bean (Hungria and Stacey 1997) where it altered nodule functioning particularly leghemoglobin synthesis, nitrogenase activity, and H_2 evolution, and in addition, hastened nodule senescence (Hungria and Vargas 2000). Therefore, to obtain most competitive and effective bacterial strains, bacteria need to be isolated and screened from the pool of indigenous microbes that could adapt to a wide range of climatic conditions and hence increase growth and enhance nutrient uptake by plants in disturbed soils.

15.3.3 Effects of Metal Toxicity

Worldwide, contamination of soil and ground water by heavy metals is a severe problem. Soil contamination is a particularly serious environmental concern, as the majority of superfund sites are highly contaminated with heavy metals. To remediate such contaminated sites, conventional remediation methods such as, soil excavation followed by coagulation-filtration or ion exchange are applied. Such approaches are however, expensive and disruptive to the sites. On the contrary, in situ bioremediation is gaining momentum as it is a low-cost and effective method for restoration and remediation of polluted site (Khan et al. 2009a). In this context, the use of plants for rehabilitation of heavy-metal-contaminated soils is an emerging area of interest because it is an ecologically sound and safe method (Wu et al.

2006). During rhizoremediation, exudates released from plants can help stimulate the survival and action of bacteria, which subsequently results in a more efficient degradation of pollutants (Kuiper et al. 2004). The root system of plants helps spread bacteria through soil and facilitate penetration to otherwise impermeable soil layers. A suitable solution is to combine the advantages of microbe–plant associations in soil into an effective cleanup technology.

Soils contaminated with heavy metals present a major threat to sustainable agriculture, and legumes growing in these environments suffer heavily from metal toxicity. The effects of heavy metals, like, copper, cadmium, and chromium, used both separately or as mixtures, on growth of pea (*P. sativum*) inoculated with *Rhizobium* sp. was studied (Wani et al. 2008a). Copper was the most toxic of the three metals for pea plants and decreased seed yield by about 15%. Nevertheless, in another study (Wani et al. 2008b) some species of pea-nodulating *Rhizobium* proved to be tolerant to nickel and zinc. This study suggested that the intrinsic ability of N_2-fixation, growth promotion, and the ability to reduce toxicity of nickel and zinc of the tested strain could be of practical importance in augmenting the growth and yield of pea in polluted sites (Wani et al. 2008b). Furthermore, an expression of a metal-binding peptide (EC20) in *Pseudomonas putida* 06909 not only improved cadmium binding but also alleviated the cellular toxicity of cadmium (Wu et al. 2006). Arsenic (As) contamination of natural resources is a global environmental problem. Arsenic-contaminated ground water was reported in over 20 countries (Reichman 2007). Legumes have, however, been identified as naturally occurring pioneer species on arsenic-contaminated sites, and free-living rhizobia are commonly found in soils with high arsenic content (Macur et al. 2001; Carrasco et al. 2005). Legumes and their symbiotic rhizobia are often desirable species, during and after the remediation of arsenic-contaminated lands. For example, excess As reduced the formation of root-nodules and dry weight of roots and shoots of soybean plants (Reichman 2007). However, inoculation of soybean plants by *B. japonicum* had significantly larger dry weights than noninoculated soybean plants. It is hypothesized that *B. japonicum* stimulated the growth of soybean via the production of growth-promoting hormones at elevated concentrations of a heavy metal via mechanisms other than improved nitrogen nutrition. Therefore, the potential use of rhizobia as growth promoting bacteria for the remediation of heavy-metal contaminated sites is an exciting new area of research.

15.4 Legume–Bacteria Associations Under Stressed Environments

Rhizosphere microorganisms influence plant growth, development, productivity, and environmental adaptation. The inoculation with bacterial mixtures provides a more balanced nutrition and improves nutrient uptake by plants (Belimov et al. 1995; El-Komy 2005). The use of beneficial microbes in agriculture production systems was started about 60 years ago and there is now increasing evidence that it can enhance plant resistance to adverse environmental stresses (Sheng 2005). Among heterogeneously distributed microbes in soils, plant growth promoting rhizobacteria (PGPR) facilitate plant growth and development directly or indirectly (Khan et al. 2009b). Direct stimulation may include providing plants with nutrients through nitrate reductase activity and nonsymbiotic N_2-fixation, phytohormones (indole acetic acid, zeatine, gibberellic acid, and abscisic acid), iron sequestered by bacterial siderophores, and soluble P. Indirect stimulation of plant growth includes preventing phytopathogens, allelopathy, antibiotic production, and competition with deleterious agents (Egamberdiyeva and Islam 2008).

In the rhizosphere of legumes, there are abundant nonsymbiotic rhizobia, which are not able to infect plants but which play a significant role in the rhizosphere of plants. Strains of rhizobia within a single species can have three different genetically determined strategies (Denison and Kiers 2004): mutalistic rhizobia provide N to their legume hosts, parasitic rhizobia infect legumes, but fix little or no N, and nonsymbiotic strains unable to infect legumes at all. Successful growth of legumes at various environments is not dependent only on the symbiotic activities of rhizobia, but may be stimulated by other PGPR. Of the most significant PGPR is the genus *Azospirillum*, a free-living, surface colonizing (sometimes living as endophyte) diazotroph. *Azospirillum* bacteria are capable of increasing the yield of important crops growing in various soils and climatic regions and a significant increase (5–30%) in the yield has been reported (Castro-Sowiniski et al. 2007). *Azospirillum* inoculation improves root development and enhanced water and mineral uptake due to the secretion of indole-3-acetic acid (Spaepen et al. 2007). Many reports have focused on the ability of *Azospirillum* species to promote plant growth and increase agricultural productivity through certain mechanisms that act additively or synergistically with BNF to enhance the overall performance of plants. For example, *Azospirillum* significantly improved yield of legumes when coinoculated with other effective, N_2-fixing bacteria. It has been shown (Rodelas et al. 1996, 1999) that dual inoculation of *Rhizobium* with *Azospirillum* and other PGPR (e.g., *Azotobacter*) significantly increased nodulation and N_2-fixation of legumes (e.g., *V. faba*). Inoculation of chickpeas and faba bean with *Azospirillum brasilense* has shown to significantly reduce the negative effects on growth and nodulation caused by irrigation with saline water (Hamaoui et al. 2001). During interactions, rhizobia synthesize lipochitooligosaccharides (LCOs), also called Nod factors, consisting of approximately 2–60 different individual structures (D'Haeze and Holsters 2002). Nod factors allow rhizobia to enter the root and cortical cells, and induce nodulin gene expression and cell division, leading to nodule formation (Cooper 2007). In a follow up study, the effects of *A. brasilense* inoculation on plant growth, nodulation, and production of flavonoids and LCOs was reported for a *Rhizobuim–P. vulgaris* interaction under salt stress (Dardanelli et al. 2008). *A. brasilense* promoted root branching in seedlings of *P. vulgaris* and increased secretion of *nod* gene-inducing flavonoid species. The negative effects detected under salt stress on gene expression and on Nod factor production were relieved in coinoculated plants. Moreover, insoluble P compounds in the rhizosphere are converted into available P for plant uptake by bacteria. A range of bacterial genera, including *Bacillus, Mesorhizobium, Pseudomonas, Rhizobium,* and *Sinorhizobium,* are active acid producers and involved in P solubilization (Rodriguez and Fraga 1999; Zaidi et al. 2009). The effects of the P-solubilizing *P. putida* on the symbiosis between rhizobia and legumes (e.g., soybean and alfalfa), usually grown in arid climates, were investigated (Rosas et al. 2006). Modification of shoot and root system dry weights occurred in soybean but not in alfalfa in the presence of *Pseudomonas* strains. A greater number of nodules and dry weight were recorded for soybean when coinoculated with *P. putida* and *B. japonicum*. In addition to N_2 fixation and phytohormone biosynthesis, *A. brasilense* produces specific polyamines. Among polyamines, cadaverine (1,5-diaminobentane) has been identified in *A. brasilense* and some α-proteobacteria (Bohin et al. 2005; Perrig et al. 2007). Cadaverine correlates with root growth promotion and osmotic stress mitigation in some plant species, like *V. faba* (Liu et al. 2000), *Lactuca sativa* (Barassi et al. 2006), and *Oryza sativa* (Cassán et al. 2009).

Certain PGPR produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which regulate ethylene production by metabolizing ACC (an immediate precursor of ethylene biosynthesis in higher plants) into α-ketobutyrate and ammonia (Glick 2005). Bacterial strains containing ACC deaminase alleviates stress-induced ethylene-mediated negative impact on plants (Safronova et al. 2006). PGPR containing ACC deaminase activity sustains plant growth and development under stress conditions by reducing stress-induced by ethylene production (Saleem et al. 2007). Some rhizobacteria (e.g., *Bacillus* species) associated with plants in saline soils, grew and fixed N_2 at 5% NaCl (Zahran et al. 1995; Egamberdiyeva and Isalm 2008). Seed inoculation with the salt-tolerant bacteria *B. japonicum*, *Bacillus polymyxa*, *Bacillus amyloliquefaciens*, *Mycobacterium phlei*, and *Pseudomonas alcaligenes* significantly increased shoot growth, root length, uptake of N, P, and K, and yield of soybean, pea, and wheat as compared to the control (Egamberdiyeva and Hoflich 2003).

15.5 Legume–Fungal Associations under Stressed Environments

Besides N, phosphorus availability is very important for crop productivity. One of the benefits of the Arbuscular mycorrhizal (AM) fungi is the improvement of P uptake by the plant. AM-fungi effect on plant water status has also been associated with improved host nutrition, particularly P. Better understanding of the interactions between AM-fungi and other microorganisms is necessary for the development of sustainable management of soil fertility and crop production. The implication of these interactions on sustainable agriculture has been reviewed (Johansson et al. 2004). Nitrogen-fixing bacteria clearly have the potential to influence AM-fungi. *Rhizobium* species may act synergistically with AM-fungi on their plant hosts. Further intracellular interactions could be important because they allow rapid exchange of energy and nutrients between plant roots, mycorrhizal fungi, and associated bacteria (Johansson et al. 2004).

Arbuscular mycorrhizal fungi have been shown to promote plant growth and salinity tolerance mainly by enhancing nutrient acquisition, producing plant growth hormones, improving rhizospheric and soil conditions, altering host physiological and biochemical properties, and defending roots against soil-borne diseases (Ghorbanli et al. 2004; Rabie 2005). AM-fungi protect plants against salt stress via better access to nutritional status and plant physiology modification (Rabie and Almadini 2005) and are considered as bio-ameliorators of saline soils (Yano-Melo et al. 2003; Tain et al. 2004). In saline environments (e.g., saline–alkali soils), vesicular AM plant root colonization is host-dependent and significantly affected by various amendments (e.g., PGPR amendments) given to reclaim such soils (Raghuwanshi and Upadhyay 2004). Double inoculation with rhizobia and an endomycorrhizal complex increased tolerance of *Acacia cyanophyla* plants to salinity (Hatimi 1999). The leguminous plants possessing high levels of vesicular AM colonization (50–70%) in saline–alkali soil included *A. nilotica*, *A. lebbeck, and D. sissoo* (Raghuwanshi and Upadhyay 2004). Mycorrhhizal seedlings of two species of *Sesbania* (*Sesbania aegyptica and Sesbania grandiflora*) had significantly higher root and shoot dry biomass, chlorophyll content, nodule number, and increased concentrations of P, N, and Mg^{2+}, but lower Na^+ concentration, than nonmycorrhizal seedlings (Giri and Mukerji 2004). Mycorhizal fungus (*Glomus fasciculatum*) alleviated the deleterious effects on growth of *A. nilotica* plants grown in saline soils that might be related to improved P nutrition (Giri et al. 2007). The reduction of Na^+ uptake, together with concomitant increase in P, N, and Mg^{2+} absorption and high chlorophyll content in mycorrhizal plants, may be important salt-alleviating mechanisms for plants growing in saline soil. Under saline conditions (150 mM NaCl), the halotolernat legume (*Lotus glaber*) colonized by *Mesorhizobium loti* and *Glomus intraradices*, was more dichotomous and total biomass increased (Echeverria et al. 2008). The improved K^+/Na^+ ratios in root and shoot tissues of mycorrhizal *A. nilotica* plants may help in protecting disruption of K-mediated enzymatic processes under salt stress conditions. Exposure of pigeonpea (*Cajanus cajan*) plants to salinity stress (up to 8 dS/m) markedly decreased nodule mass, acetylene reduction activity (ARA), and leghemoglobin content (Garg and Manchanda 2008). However, AM-fungi inoculation significantly improved nodulation, nitrogenase activity, and leghemoglobin content of salt-stressed pigeonpea plants. Under salt stress, soybean plants inoculated with salt pretreated AM-fungi showed increased SOD and POD activity in shoots relative to those inoculated with the nonpretreated AM fungi (Ghorbanli et al. 2004). Further, activities of enzymes involved in the detoxification of O_2^- radicals and H_2O_2 (superoxide dismutase SOD, catalase CAT, and peroxidase POX), and enzymes of the ascorbate glutathione pathway responsible for the removal of H_2O_2 (glutathione reductase GR and ascorbate peroxidase APOX) increased markedly in AM-salt stressed plants (Garg and Manchanda 2008).

Drought resistance of mycorrhizal plants is independent of plant P concentration (Peña et al. 1988; Sánchez-Diaz et al. 1990). In *Medicago–Rhizobium–Glomus* symbiosis, subjected to drought stress, nodule activity in infected plants was significantly higher than in noninfected plants (Peña et al. 1988). AM-fungi may increase drought resistance of plants by several mechanisms including enhancing water uptake due to hyphal extraction of soil water and lowering leaf osmotic potential for greater turgor maintenance by regulating photosynthesis (Sánchez-Diaz et al. 1990; Ruiz-Lozano and Azcón 1995). However, this effect is independent of the P nutrition in plant tissues.

Mycorrhizal colonization is recognized further as the key to plant growth and fitness in stressed environments and in sustainable soil–plant systems. AM-fungi and bacteria, isolated from metal-contaminated sites, are often more resistant to metals than those collected from uncontaminated environments. In certain cases, the plant growth promoting effect of bacteria in the presence of nickel is attributed to the bacterial ability in reducing the detrimental Ni-induced stress on plants (Burd et al. 2000). Therefore, the combination of Ni-adapted AM-fungal isolates with those Ni-tolerant bacteria could increase phytoremediation potential. However, prolonged exposure of microorganisms to heavy metals may lead to reduced growth rate or to the loss of several beneficial properties, such as the N_2-fixing ability in the case of rhizobia (Zahran 1999; Biró et al. 2001). Thus, selected metal-tolerant saprophytic and symbiotic microorganisms may play an important role for plant establishment in metal-contaminated soils. For instance, the dual symbiosis between AM-fungi and N_2-fixing rhizobia showed a synergistic effect and significantly increased plant tolerance to heavy metals (Biró et al. 2000). Growth of clover (*Trifolium repens*)–*R. leguminosarum* bv. *trifolii*, and uptake of N, P, and Ni , was studied in Ni-contaminated soil (Vivas et al. 2006). Dual inoculation of clover with the Ni-tolerant bacteria (*Brevibacillus brevis*) and AM-fungus (*Glomus mosseae*) increased shoot and root plant biomass and nodule number that was highly depressed, and substantially reduced the specific absorption rate for Ni compared to plants grown in soils inoculated only with *G. mosseae*. These results suggest that selected bacterial inoculation improved the mycorrhizal benefit in nutrient uptake and in decreasing Ni toxicity, and inoculation of adapted beneficial microorganisms (e.g., *B. brevis* and *G. mosseae*), used as a tool to enhance plant performance in soil contaminated with nickel.

15.6 Conclusion

The legume–rhizobia symbiosis is of tremendous ecological and agronomic importance. Optimization of symbiosis between the legumes and their respective microsymbions requires the competitive, infective and highly efficient N_2-fixing rhizobial strains in sufficient numbers to maximize legume productivity. Advances in molecular biology and genetic tools have helped elucidate numerous genes having symbiotic functions. The major approaches employed for improving N_2-fixation include the selection and construction of effective rhizobial strains and breeding symbiotically-active plants. Proteomics is another approach used in the identification of proteins involved in *Rhizobium*–legume symbiosis. Soil salinity, a wide spread problem, is the major cause of declining agricultural productivity in different ecological niches. The identification and use of plants adapted to saline environments is of increasing importance for raising the productivity of crops in these areas. Recent investigations on plant tolerance to salt stress have focussed on improvement of breeding and modification of the genetic structure of existing crops aiming at enhanced adaptation to salinity conditions. In this regard, cultivation of legumes, especially the nitrogen-fixing trees, is recommended for the rehabilitation of arid saline soil. This solution is not only likely to make abandoned soils productive but will also ensure conservation and improvement of the environment. The legume trees such as, *Acacia*, *Prosopis*, and *Sesbania* and legume herbs such as, *Melilotus* and *Medicago*, have been found to be salt-tolerant. These legumes establish a symbiotic association with a wide range of rhizobia (*Rhizobium*, *Mesorhizobium*, and *Sinorhizobium*) and can adapt to the unfavorable arid climates. On the other hand, salt-tolerant rhizobia can also change the pattern of cellular constituents such as proteins, phospholipids, and polysaccharides, which are essential for nodulation and maintenance of physiological events. Under salt or acid stress, the rhizobia are reported to form different new Nod factors the stimulation or suppression of which under stressed conditions might affect the rhizobia–legumes symbioses.

Molecular studies on rhizobia–legumes symbioses for understanding how such interaction works under stressed environments are receiving increasing interest. Because of these studies, several kinds of genes or markers associated with stress tolerance have been identified both in bacteria and legumes. For example, the *typA* gene required for the establishment of nitrogen-fixing symbiosis with certain *M. truncatula* and *M. sativa* cultivars and for survival of *S. meliloti* under certain stress conditions is reported. A locus, named as nesR, potentially contributes to the adaptability of *S. meliloti* when it encounters challenges such as high osmolarity, nutrient starvation, and/or competition for nodulation, thus increasing its chances for survival in the stressful rhizosphere. In addition, rhizosphere microorganisms influence plant growth, development, productivity, and environmental adaptation. The composite inoculation of beneficial microbes therefore, provides more balanced nutrition and improves growth and yield of crops. The role of AM-fungi in improving growth and N_2-fixation of legumes is another alternative in sustainable agricultural production systems. The AM-fungi promote plant growth and salinity tolerance mainly by enhancing nutrient acquisition, producing plant growth hormones, altering host physiological and biochemical properties, and defending roots against soil-borne diseases. In combination with other PGPR, the AM-fungi have shown dramatic increase in yield of legumes by increasing salinity tolerance, P level, phosphatases, nodule numbers, N level, protein content, and nitrogenase activity, in comparison to the sole application of AM-fungi or PGPR. Leguminous species are often used in the remediation of contaminated sites because of their capacity to fix N and enhance soil fertility. The rhizobial bacteria facilitate growth of the host legume via the production of phytohormones in derelict soils besides improving N nutrition. Therefore, the potential use of rhizobia–legume associations as growth promoting organism for the remediation of stressed sites is an exciting new area of research, which, however, urgently requires further testing under field environments.

References

Aguirreolea J, Sánchez-Díaz M (1989) CO_2 evolution by nodulated roots in *Medicago sativa* L. under water stress. J Plant Physiol 134:598–602

Al-Sherif EA, Zahran HH, Atteya MA (2004) Nitrogen fixation and chemical composition of wild annual legumes at Beni-Suef Governorate, Egypt. Egypt J Biol 6:32–38

Alva AK, Assher CJ, Edwards DG (1990) Effect of solution pH, external calcium concentration, and aluminm activity on nodulation and early growth of cowpea. Aust J Agric Res 41:359–365

Apse MP, Dharon GS, Snedden WA, Bumerold E (1999) Salt tolerance conferred by overexpression of a vascular Na+/H+ antiport in Arabidopsis. Science 285:1256–1258

Aroca R, Porcel R, Ruiz-Lozano JM (2007) How does arbuscular mycorrhizal symbiosis regulate root hydraulic properties and plasma membrane aquaporins in *Phaseolus vulgaris* under drought, cold or salinity stresses? New Phytol 173:808–816

Ashraf M (1994) Breeding for salinity tolerance in plants. Crit Rev Plant Sci 13:17–42

Ashraf M, Waheed A (1990) Screening of local/exotic accessions of lentil (*Lens culinaris*) for salt tolerance at two growth stages. Plant Soil 128:167–176

Aydi S, Drevon J-J, Abdelly C (2004) Effect of salinity on root-nodule conductance to the oxygen diffusion in the *Medicago truncatula–Sinorhizobium meliloti* symbiosis. Plant Physiol Biochem 42:833–840

Barassi C, Ayrault G, Creus C, Sueldo R, Sobrero M (2006) Seed inoculation with Azospirillum mitigates NaCl effects on lettuce. Sci Hortic 109:8–14

Bartels D, Sunkar R (2005) Drought and salt tolerance in plants. CRC Crit Rev Plant Sci 24:23–58

Becana M, Dalton DA, Moran JF, Iturbe-Ormaetxe I, Matamoros MA, Rubio MC (2000) Reactive oxygen species and antioxidants in legume nodules. Physiol Plant 109:372–381

Beck DP, Munns DN (1985) Effect of calcium on the phosphorous nutrition of *Rhizobium meliloti*. Soil Sci Soc Am J 49:334–337

Belimov AA, Kojemiakov AP, Chuvarliyeva CV (1995) Interaction between barley and mixed cultures of nitrogen fixing and phosphate-solubilizing bacteria. Plant Soil 173:29–37

Benaroudj N, Lee DH, Al G (2001) Trehalose accumulation during cellular stress protects cells and cellular proteins from damage by oxygen radicals. J Biol Chem 276:24261–24267

Bhatnagar-Mathur P, Devi MJ, Reddy DS, Lavanya M, Vadez V, Serraj R, Yamaguchi-Shinozaki K, Sharma KK (2007) Stress-inducible expression of At DREB1A in transgenic peanut (*Arachis hypogaea* L.) increases transpiration efficiency under water-limiting conditions. Plant Cell Rep 26:2071–2082

Biró B, Köves-Péchy K, Vörös I, Takács T, Eggenberg P, Strasser RJ (2000) Interrelation between Azospirillum and Rhizobium nitrogen-fixers and arbuscular mycorrhizal fungi in the rhizosphere of alfalfa at sterile, AMF-free or normal soil conditions. Appl Soil Ecol 15:159–168

Biró B, Tiricz H, Morvai B (2001) Investigations on the vitality, resistance and diversity of metal-adapted and non-adapted Rhizobium strains. Acta Microbiol Immunol Hung 48:156–157

Blokhina O, Virolainen E, Fagerstedt KV (2003) Antioxidants, oxidative damage and oxygen deprivation stress; a review. Ann Bot 91:179–194

Blumwald E, Gelli A (1997) Secondary inorganic ion transport in plant vacuoles. Adv Bot Res 25:401–407

Blumwald E, Aharon GS, Apse MP (2000) Sodium transport in plant cells. Biochim Biophys Acta 1465:140–151

Bohin A, Bouchart F, Richet C, Kol O, Leroy V, Timmerman P, Huet G, Bohin J, Zane J (2005) GC/MS identification and quantification of constituents of bacterial lipids and glycoconjugates obtained after methanolysis as heptafuorobutyrate derivatives. Anal Biochem 340:231–244

Boscari A, Mandon K, Dupont L, Poggi M-C, Le Rudulier D (2002) BetS is a major glycine betaine/proline betaine transporter required for early osmotic adjustment in *Sinorhizobium meliloti*. J Bacteriol 184:2654–2663

Boscari A, Mandon K, Poggi M-C, Le Rudulier D (2004) Functional expression of *Sinorhizobium meliloti* BetS, a high-affinity betaine transporter, in *Bradyrhizobium japonicum* USDA110. Appl Environ Microbiol 70:5916–5922

Boschma SP, Lodge GM, Harden S (2008) Herbage mass and persistence of pasture legumes and grasses at two potentially different saline and waterlogged sites in northern New South Wales. Aust J Exp Agric 48:553–567

Bouhmouch I, Souad-Mouhsine B, Brhada F, Aurag J (2005) Influence of host cultivars and Rhizobium species on the growth and symbiotic performance of *Phaseolus vulgaris* under salt stress. J Plant Physiol 162:1103–1113

Boumahdi M, Mary P, Hornez JP (2001) Changes in fatty acid composition and degree of unsaturation of (brady) rhizobia as a response to phases of growth, reduced water activities and mild desiccation. Antonie Van Leeuwenhoek 79:73–79

Brockwell J, Pilka A, Holliday RA (1991) Soil pH is the major determinant of the number of naturally-occurring *Rhizobium meliloti* in non-cultivated soils in New South Wales. Aust J Exp Agric 31:211–219

Burd IG, Dixon DG, Glick BR (2000) Plant growth promoting bacteria that decrease heavy metal toxicity in plants. Can J Microbiol 46:237–245

Carrasco JA, Armario P, Pajuelo E, Burgos A, Caviedes MA, Lopez R, Chamber MA, Palomares AJ (2005) Isolation and characterization of symbiotically effective Rhizobium resistant to arsenic and heavy metals after the toxic spell at the Aznalocollar pyrite mine. Soil Biol Biochem 37:1131–1140

Cassán F, Maiale S, Masciarelli O, Vidal A (2009) Cadaverine production by *Azospirillum brasilense* and its possible role in plant growth promotion and osmotic stress mitigation. Eur J Soil Biol 45:12–19

Castro-Sowiniski S, Herschkovitz Y, Okon Y, Jurkevitch E (2007) Effects of inoculation with plant growth-promoting rhizobacteria on resident rhizosphere microorganisms. FEMS Microbiol Lett 276:1–11

Chang RZ, Chen YW, Shao GH, Wan CW (1994) Effect of salt stress on agronomic characters and chemical quality of seeds in soybean. Soybean Sci 13:101–105

Chen W-M, Lee TM, Lan C-C, Cheng C-P (2000) Characterization of halotolerant rhizobia isolated from root nodules of *Canavalia rosea* from seaside areas. FEMS Microbiol Ecol 34:9–16

Chen J-L, Lin S, Lin L-P (2006) Rhizobial surface biopolymers and their interaction with lectin measured by atomic force microscopy. World J Microbiol Biotechnol 22:565–570

Chinnusamy V, Jagendorf A, Zhu JK (2005) Understanding and improving salt tolerance in plants. Crop Sci 45:437–448

Coba De La Peña T, Cárcamo CB, Almonacid L, Zaballos A, Lucas MM, Balomenos D, Pueyo JJ (2008) A salt stress-responsive cytokinin receptor homologue isolated from *Medicago sativa* nodules. Planta 227:769–779

Cooper JE (2007) Early interactions between legumes and rhizobia: disclosing complexity in a molecular dialogue. J Appl Microbiol 103:1355–1365

Csonka LN (1991) Prokaryotic osmoregulation: genetics and physiology. Annu Rev Microbiol 45:569–606

Dajic Z (2006) Salt stress. In: Madhava Rao VK, Raghavendra AS, Janardhan Reddy K (eds) Physiology and molecular biology of stress tolerance in plants. Springer, Netherlands, pp 41–99

Dardanelli MS, Fernández De Córdoba FJ, Espuny MR, Rodríguez Carvajal MA, Soria Díaz ME, Gil Serrano AM (2008) Effect of *Azospirillum brasilense* coinoculated with Rhizobium on

Phaseolus vulgaris flavonoids and nod factor production under salt stress. Soil Biol Biochem 40:2713–2721

Debelle F, Moulin L, Mangin B, Denarie J, Boivin C (2001) Nod genes and nod signals and the evolution of the Rhizobium–legume symbiosis. Acta Biochim Pol 48:359–365

Denison RF, Kiers ET (2004) lifestyle alternatives of rhizobia: mutualism, parasitism, and forgoing symbiosis. FEMS Microbiol Lett 237:187–193

D'Haeze W, Holsters M (2002) Nod factor structures, responses, and perception during initiation of nodule development. Glycobiology 12:79–105

Domínguez-Ferreras A, Pérez-Arnedo R, Becker A, Olivares J, Soto MJ, Sanjuan J (2006) Transcriptome profiling reveals the importance of plasmid pSymB for osmoadaptation of *Sinorhizobium meliloti*. J Bacteriol 188:7617–7625

Echeverria M, Scambato AA, Sannazzaro AI, Maiale S, Ruiz OA, Menéndez AB (2008) Phenotypic plasticity with respect to salt stress response by *Lotus glaber*: the role of its AM fungal and rhizobial symbionts. Mycorrhiza 18:317–329

Egamberdiyeva D, Hoflich G (2003) Effect of plant growth promoting bacteria on growth and nutrient uptake of cotton and pea in a semi-arid region of Uzbekistan. J Arid Environ 56:293–301

Egamberdiyeva D, Isalm KR (2008) Salt-tolerant rhizobacteria: plant growth promoting traits and physiological characterization within ecologically stressed environments. In: Ahmad I, Pichtel J, Hayat S (eds) Plant-bacteria interactions: strategies and techniques to promote plant growth. WILEY-VCH Verlag Gmbh & Co. KGaA, Weinheim, pp 257–281

El-Komy HMA (2005) Coimmobilization of A. lipoferum and B. megaterium for plant nutrition. Food Technol Biotechnol 43:19–27

Essendoubi M, Brhada F, Eljamail JE, Filali-Maltouf A, Bonnassie S, Georgeault S, Blanco C, Jebbar M (2007) Osmoadaptive responses in the rhizobia nodulating Acacia isolated from south-eastern Moroccan Sahara. Environ Microbiol 9:603–611

Estevéz J, Soria-Díaz ME, De Córdoba FF, Morón B, Manyani H, Gil A, Thomas-Oates J, Van Brussel AAN, Dardanelli MS, Sousa C, Megías M (2009) Different and new Nod factors produced by *Rhizobium tropici* CIAT899 following Na+ stress. FEMS Microbiol Lett 293:220–231

FAO (2005) Global network on integrated soil management for sustainable use of salt-affected soils. FAO Land and Plant Nutrition Management Service, Rome, Italy. http://www.fao.org.ag/agl/agll/spush

Galiana A, Gnahoua GM, Chaumont J, Lesueur D, Prin Y, Mallet B (1998) Improvement of nitrogen fixation in *Acacia mangium* through inoculation with Rhizobium. Agroforest Syst 40:297–307

Galibert F, Finan TM, Long SR et al (2001) The composite gnome of the legume symbiont *Sinorhizobium meliloti*. Science 293:668–672

Garg N, Manchanda G (2008) Effect of mycorrhizal inoculation on salt-induced nodule senescence in *Cajanus cajan* (pigeonpea). J Plant Growth Regul 17:115–124

Garthwaite AJ, Von Bothmer R, Colmer TD (2005) Salt tolerance in wild Hordeum species is associated with restricted entry of Na^+ and Cl^- into the shoots. J Exp Bot 56:2365–2378

Geurts R, Fedorova E, Bisseling T (2005) Nod factor signaling genes and their function in the early stages of Rhizobium infection. Curr Opin Plant Biol 8:346–352

Ghorbanli M, Ebrahimzadeh H, Sharifi M (2004) Effect of NaCl and mycorrhizal fungi on antioxidative enzymes in soybean. Biol Plant 48:575–581

Giri B, Mukerji KG (2004) Mycorrhizal inoculation alleviates salt stress in *Sesbania aegyptica* and *Sesbania grandiflora* under field conditions: evidence for reduced sodium and improved magnesium uptake. Mycorrhiza 14:307–312

Giri B, Kapoor R, Mukerji KG (2002) Va mycorrhizal techniques/VAM technology in establishment of plants under salinity stress conditions. In: Mukerji KG, Manorahari C, Singh J (eds) Techniques in mycrorrhizal studies. Kluwer, Dordrecht, The Netherland, pp 313–327

Giri B, Kapoor R, Mukerji KG (2003) Influence of arbuscular mycorrhizal fungi and salinity on growth, biomass, and mineral nutrition of *Acacia auriculiformis*. Biol Fertil Soils 38:170–175

Giri B, Kapoor R, Mukerji KG (2007) Improved tolerance of *Acacia nilotica* to salt stress by arbuscular mycorrhiza, *Glomus fasiculatum* may be partly related to elevated K/Na ratios in root and shoot tissues. Microb Ecol 54:753–760

Glick BR (2005) Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiol Lett 251:1–7

Gonzalez-Rizzo S, Crespi M, Frugier F (2006) The *Medicago truncatula* CRE1 cytokinin receptor regulates lateral root development and early symbiotic interaction with *Sinorhizobium meliloti*. Plant Cell 18:2680–2693

Graham PH, Vance CP (2000) Nitrogen fixation in perspective: an overview of research and extension needs. Field Crops Res 65:93–106

Graham PH, Vance CP (2003) Legumes: importance and constraints to greater use. Plant Physiol 131:872–877

Graham PH, Draeger KJ, Ferrey ML, Conroy MJ, Hammer BE, Martinez E, Arons SR, Quinto C (1994) Acid pH tolerance in strains of Rhizobium and Bradyrhizobium, and initial studies on the basis for pH tolerance of *Rhizobium tropici* UMR 1899. Can J Microbiol 40:198–207

Gualtieri G, Bisseling T (2000) The evolution of nodulation. Plant Mol Biol 42:181–194

Hamaoui B, Abbadi JM, Burdman S, Rashid A, Sarig A, Okon Y (2001) Effects of inoculation of *Azospirillum brasilense* on chickpeas (*Cicer arietinum*) and faba bean (*Vicia faba*) under different growth conditions. Agronomie 21:553–560

Hamdy A (1990) Management practices under saline water irrigation. Acta Hortic 278:745–754

Hare PD, Cress WA, Van Staden J (1997) The involvement of cytokinins in plant responses to environmental stress. J Plant Growth Regul 23:79–103

Hasegawa PM, Bressan R, Zhu J-K, Bohmert H-J (2000) Plant cellular and molecular responses to high salinity. Annu Rev Plant Physiol Plant Mol Biol 51:463–499

Hatimi A (1999) Effect of salinity on the association between root symbionts and *Acacia cyanophyla* Lind: growth and nutrition. Plant Soil 216:93–101

Herridge D, Rose I (2000) Breeding for enhanced nitrogen fixation in crop legumes. Field Crops Res 65:229–248

Horie T, Schroeder JI (2004) Sodium transporters in plants. Diverse gene and physiological functions. Plant Physiol 136:2457–2462

Hungria M, Franco AA (1993) Effects of high temperature on nodulation and nitrogen fixation by *Phaseolus vulgaris* (L.). Plant Soil 149:95–102

Hungria M, Stacey G (1997) Molecular signals exchanged between host plants and rhizobia, basic aspects and potential application in agriculture. Soil Biol Biochem 29:519–530

Hungria M, Vargas MAT (2000) Environmental factors affecting N2 fixation in grain legumes in the tropics, with emphasis on Brazil. Field Crops Res 65:151–164

Hungria M, Franco AA, Sprent JI (1993) New sources of high-temperature tolerant rhizobia for *Phaseolus vulgaris* (L.). Plant Soil 149:103–109

Ibragimova MV, Rumyantseva ML, Onishchuk OP, Belova VS, Kurchak ON, Andronov EE, Dzyubenko NI, Simarov BV (2006) Symbiosis between the root-nodule bacterium *Sinorhizobium meliloti* and alfalfa (*Medicago sativa*) under salinization conditions. Microbiology 75:77–81

Jebara S, Jebara M, Limam F, Aouani ME (2005) Changes in ascorbate peroxidase, catalase, guaiacol peroxidase and superoxide dismutase activities in common bean (*Phaseolus vulgaris*) nodules under salt stress. J Plant Physiol 162:929–936

Jiang M, Zhang J (2002) Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves. J Exp Bot 53:2401–2410

Jiang JQ, Wei W, Du BH, Li XH, Wang L, Yang SS (2004) Salt-tolerance genes involved in cation efflux and osmoregulation of *Sinorhizobium fredii* RT19 detected by isolation of Tn5 mutants. FEMS Microbiol Lett 239:139–146

Jimenez-Zurdo JI, Frugier F, Crespi MD, Kondorosi A (2000) Expression profiles of 22 novel molecular markers for organogenetic pathways acting in alfalfa nodule development. Mol Plant Microbe Interact 13:96–106

Jin H, Sun Y, Yang Q, Chao Y, Kang J, Jin H, Li Y, Margaret G (2009) Screening of genes induced by salt stress from Alfalfa. Mol Biol Rep. doi:10.1007/s11033-009-9590-7

Johansson JF, Paul LR, Finlay RD (2004) Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. FEMS Microbiol Ecol 48:1–13

Katerji N, Van Hoorn JW, Hamdi A, Mastrorilli M, Oweis T, Erskine W (2001) Response of two varieties of lentil to soil salinity. Agric Water Managem 47:179–190

Khadri M, Tejera NA, Lluch C (2007) Sodium chloride-ABA interaction in two common bean (*Phaseolus vulgaris*) cultivars differing in salinity tolerance. Environ Exp Bot 60: 211–218

Khan MS, Zaidi A, Wani PA, Oves M (2009a) Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. Environ Chem lett 7:1–19

Khan MS, Zaidi A, Wani PA, Ahemad M, Oves M (2009b) Functional diversity among plant growth-promoting rhizobacteria: current status. In: Khan MS, Zaidi A, Musarrat J (eds) Microbial strategies for crop improvement. Springer, Germany, pp 105–132

Kishor PBK, Sangam S, Amrutha RN, Laxmi PS, Naidu KR, Rao KRSS, Rao S, Reddy KJ, Theriappan P, Sreenivasulu N (2005) Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. Curr Sci 88:424–438

Kiss E, Huguet T, Poinsot V, Batut J (2004) The typA gene is required for stress adaptation as well as for symbiosis of *Sinorhizobium meliloti* 1021 with certain *Medicago truncatula* lines. Mol Plant Microbe Interact 17:235–244

Kononowics AK, Nelson DE, Singh NK, Hasegawa PM, Bressan RA (1992) Regulation of the osmotin gene promoter. Plant Cell 4(513):524

Kotb THS, Watanabe T, Ogino Y, Tanji KK (2000) Soil salinization in the Nile Delta and related policy issues in Egypt. Agric Water Manage 43:239–261

Kuiper I, Lagendijk EL, Bloemberg GV, Lugtenberg JJ (2004) Rhizo-remediation: a beneficial plant–microbe interaction. Mol Plant Microbe Interact 17:6–15

Kulkarni S, Nautiyal CS (1999) Characterization of high-tmperature tolerant rhizobia isolated from *Prosopis juliflora* grown in alkaline soil. J Gen Appl Microbiol 45:213–220

Lindström K, Lipsanen P, Kaijalainen S (1990) Stability of markers used for identification of two Rhizobium galegae inoculation strains after five years in the field. Appl Environ Microbiol 56:444–450

Liu K, Fu H, Bei K, Luan S (2000) Inward potassium channel in guard cells as a target for polyamine regulation of stomatal movements. Plant Physiol 124:1315–1325

López M, Herrera-Cervera JA, Iribarne C, Tejera NA, LLuch C (2008) Growth and nitrogen fixation in *Lotus japonicus* and *Medicago truncatula* under NaCl stress: nodule carbon metabolism. J Plant Physiol 165:641–650

Lovato MB, De Lemos Filho JP, Martins PS (1999) Growth responses of *Stylosanthes humilis* (Fabaceae) populations to saline stress. Environ Exp Bot 41:145–153

L'taief B, Sifi B, Zaman-Allah M, Drevon J-J, Lachaâl M (2007) Effect of salinity on root-nodule conductance to the oxygen diffusion in the *Cicer arietinum–Mesorhizobium ciceri* symbiosis. J Plant Physiol 164:1028–1036

Macur RE, Wheeler JT, McDermott TR, Inskeep WP (2001) Microbial populations associated with the reduction and enhanced mobilization of arsenic in mine tailings. Environ Sci Technol 35:3676–3682

Mahdhi M, Mars M (2006) Genotypic diversity of rhizobia isolated from *Retama raetam* in arid regions of Tunisia. Ann Microbiol 56:305–311

Mahdhi M, Nzoué A, De Lajudie P, Mars M (2008) Characterization of root-nodulating bacteria on *Retama raetam* in arid Tunisian soils. Prog Nat Sci 18:43–49

Marañón T, Garcia LV, Troncoso A (1989) Salinity and germination of annual Melilotus from the Gauadalquivir delta (SW Spain). Plant Soil 119:223–228

Marcar NE, Dart P, Sweeney C (1991) Effect of root zone salinity on growth and chemical composition of Acacia ampliceps B.R. Maslin, *A. auriculiformis* A. Cunn. Ex. Benth. and *A. mangium* Wild. at two nitrogen levels. New Phytol 119:567–573

Mathesius U, Charon C, Rolfe BG, Kondorosi A, Crespi M (2000) Temporal and spatial order of events during the induction of cortical cell divisions in white clover by *Rhizobium leguminosarum* bv. *trifolii* inoculation or localized cytokinin addition. Mol Plant Microbe Interact 13:617–628

Mathesius U, Keijzers G, Natera SHA et al (2001) Establishment of a root proteome reference map for the model legume *Medicago truncatula* using the expressed sequence tag database for peptide mass fingerprinting. Proteomics 1:1424–1440

McKay IA, Djordjevic MA (1993) Production and excretion of nod metaboloites by *Rhizobium leguminosarum* bv. *trifolii* are disrupted by the same environmental factors that reduce nodulation in the field. Appl Environ Microbiol 59:3385–3392

Medeot DB, Bueno MA, Dardanelli MS, De Lema MG (2007) Additional changes in lipids of Bradyrhizobium SEMIA 6114 nodulating peanut as a response to growth temperature and salinity. Curr Microbiol 54:31–35

Melchior-Marroquin JI, Vargas-Hernandez JJ, Herrera-Cerrato R, Krishnamurthy L (1999) Screening Rhizobium spp. strains associated with *Gliricidia sepium* along an altidudinal transect in Veracruz, Mexico. Agroforest Syst 46:25–38

Mhadhbi H, Jebara M, Zitoun A, Limam F, Aouani E (2008) Symbiotic effectiveness and response to mannitol-mediated osmotic stress of various chickpea–rhizobia associations. World J Microbiol Biotechnol 24:1027–1035

Michiels J, Verreth C, Vnderleyden J (1994) Effects of temperature on bean-nodulating Rhizobium strains. Appl Environ Microbiol 60:1206–1212

Mills D, Zhang G, Benzioni A (2001) Effect of different salt and of ABA on growth and mineral uptake in jojoba shoots grown in vitro. J Plant Physiol 158:1031–1039

Minchin FR (1997) Regulation of oxygen diffusion in legume nodules. Soil Biol Biochem 29:881–888

Moawad H, Beck D (1991) Some characteristics of *Rhizobium leguminosarum* isolates from uninoculated field-grown lentil. Soil Biol Biochem 23:917–925

Morón B, Soria-Díaz ME, Ault J et al (2005) Low pH changes the profile of nodulation factors produced by *Rhizobium tropici* CIAT899. Chem Biol 12:1029–1040

Mulder L, Hogg B, Bersoult A, Cullimore JV (2005) Integration of signalling pathways in the establishment of the legume-rhizobia symbiosis. Physiol Plant 123:207–218

Müller J, Xie ZP, Staehelin C, Mellor RB, Boller T, Wiemken A (1994) Trehalose and trehalase in root nodules from various legumes. Physiol Plant 90:86–89

Munns DN (1986) Acid soils tolerance in legumes and rhizobia. Adv Plant Nutr 2:63–91

Munns R (2005) Genes and salt tolerance: bringing them together. New Phytol 167:645–663

Nabil M, Coudret A (1995) Effects of sodium chloride on growth, tissue elasticity and solute adjustment in two *Acacia nilotica* subspecies. Physiol Plant 93:217–224

Nguyen NT, Moghaieb REA, Saneoka H, Fujita K (2004) RAPD markers associated with salt tolerance in *Acacia auriculiformis* and *Acacia mangium*. Plant Sci 167:797–805

Nichols PGH, Craig AD, Rogers ME, Albertsen TO, Miller S, McClements DR et al (2008) Production and persistence of annual legumes at five saline sites in southern Australia. Aust J Exp Agric 48:518–535

Nichols PGH, Malik AI, Stockdale M, Colmer TD (2009) Salt tolerance and avoidance mechanismss at germination of annual pasture legumes: importance for adaptation to saline environments. Plant Soil 315:241–255

Nijiti CF, Galiana A (1996) Symbiotic properties and Rhizobium requirements for effective nodulation of five tropical dry zone acacias. Agroforest Syst 34:265–275

Nogales J, Campos R, Ben Abdelkhalek H, Olivares J, LLuch C, Pandey A, Mann M (2000) Proteomics to study genes and genomes. Nature 405:837–846

Novikova TI, Gordienko NY (1999) Specific features of functioning of the symbiotic system Rhizobium–Glycyrrhiza uralensis under the conditions of chloride salinization. Sibirskii Ekolog Z 3:295–302

Onishi M, Tachi H, Kojima T, Shiraiwa M, Takahara H (2006) Molecular cloning and characterization of a novel salt-inducible gene encoding an acidic isoform of PR-5 protein in soybean. Plant Physiol Biochem 44:574–580

Pardo JM, Cubero B, Leidi EO, Quintero FJ (2006) Alkali cation exchangers: roles in cellular homeostasis and stress tolerance. J Exp Bot 57:1181–1199

Patankar AV, González JE (2009) An orphan LuxR homolog of *Sinorhizobium meliloti* affects stress adaptation and competition for nodulation. Appl Environ Microbiol 75:946–955

Peña JI, Sánchez-Diaz M, Aguirreolea J, Becana M (1988) Increased stress tolerance of nodule activity in the Medicago-Rhizobium Glomus symbiosis under drought. J Plant Physiol 133:79–83

Perrig D, Boiero L, Masciarelli O, Penna C, Ruíz O, Cassán F, Luna V (2007) Plant growth promoting compounds produced by two agronomically important strains of *Azospirillum brasilense*, and their implications for inoculant formulation. Appl Microbiol Biotechnol 75:1143–1150

Phang T-H, Shao G, Lam H-M (2008) Salt tolerance in soybean. J Integr Plant Biol 50:1196–1212

Porcel R, Barea JM, Ruiz-Lozano JM (2003) Antioxidant activities in mycorrhizal soybean plants under drought stress and their possible relationship to the process of nodule senescence. New Phytol 157:135–143

Priefer UB, Aurag J, Boesten B, Bouhmouch I, Defez R, Filali-Maltouf A, Miklis M, Moawad H, Mouhsine B, Prell J, Schlüter A, Senatore B (2001) Characterization of Phaseolus symbionts isolated from Mediterranean soils and analysis of genetic factors related to pH tolerance. J Biotechnol 91:223–236

Qadir M, Ghafoor A, Murtaza G (2000) Amelioration strategies for saline soils: a review. Land Degrad Dev 11:501–521

Rabie GH (2005) Influence of VA-mycorrhizal fungi and kinetin on the response of mungbean plants to irrigation with seawater. Mycorrhiza 15:225–230

Rabie GH, Almadini AM (2005) Role of bioinoculants in development of salt-tolerance of *Vicia faba* plants under salinity stress. Afr J Biotechnol 4:210–222

Raghuwanshi R, Upadhyay RS (2004) Performance of vesicular-arbuscular mycorrhizae in saline-alkali soil in relation to various amendments. World J Microbiol Biotechnol 20:1–5

Räsänen LA, Lindström K (2003) Effects of biotic and a biotic constraints on the symbiosis between rhizobia and the tropical leguminous trees Acacia and Prosopis. Indian J Exp Biol 41:1142–1159

Raza S, Jørnsgård B, Abou-Taleb H, Christiansen JL (2001) Tolerance of Bradyrhizobium sp. (Lupini) strains to salinity, pH, CaCO3 and antibiotics. Lett Appl Microbiol 32:379–383

Rehman A, Nautiyal CS (2002) Effect of drought on the growth and survival of the stress-tolerant bacterium Rhizobium sp. NBRI2505 sesbania and its drought sensitive trnsposon Tn5 mutant. Curr Microbiol 45:368–377

Reichman SM (2007) The potential use of the legume–rhizobium symbiosis for the remediation of arsenic contaminated sites. Soil Biol Biochem 39:2587–2593

Riccillo PM, Muglia CI, De Brujin FJ, Row AJ, Booth IR, Aguilar OM (2000) Glutathione is involved in environmental stress responses in *Rhizobium tropici*, including acid tolerance. J Bacteriol 182:1748–1753

Richardson AE, Simpson RJ, Djordjevic MA, Rolfe BG (1988) Expression of nodulation genes in *Rhizobium leguminosarum* bv. *trifolii* is affected by low pH and by Ca2+ and Al ions. Appl Environ Microbiol 54:2541–2548

Rodelas B, González-López J, Salmerón V, Pozo C, Martínez-Toledo MV (1996) Enhancement of nodulation, N2 fixation and growth of faba bean (*Vicia faba* L.) by combined inoculation with *Rhizobium leguminosarum* bv. *viceae* and *Azospirillm brasilense*. Symbiosis 21:175–186

Rodelas B, González-López J, Martínez-Toledo MV, Pozo C, Salmerón V (1999) Influence of Rhizobium/Azotobacter and Rhizobium/Azospirillum combined inoculation on mineral composition of faba bean (*Vicia faba* L.). Biol Fertil Soils 29:165–169

Rodriguez H, Fraga R (1999) Phosphate-solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339

Rogers ME, Noble CL (1991) The effect of NaCl on the establishment and growth of balansa clover (Trifolium michelianum Savi var. balansae Boiss.). Aust J Agric Res 42:847–857

Rogers ME, Colmer TD, Frost K, Henry D, Cornwall D, Hulm E et al (2008) Diversity in the genus Melilotus for tolerance to salinity and water logging. Plant Soil 304:89–101

Rolfe BG, Mathesius U, Djordjevic M, Weinman J, Hocart C, Weiller G, Bauer WD (2003) Proteomic analysis of legume–microbe interactions. Comp Funct Genomics 4:225–228

Romero C, Bellés JM, Vayá JL, Serrano R, Culiáñez-Macia FA (1997) Expression of the yeast trehalose-6-phosphate synthase in transgenic tobacco plants: pleiotropic phenotypes include drought tolerance. Planta 201:293–297

Rosas SB, Andrés JA, Rovera M, Correa NS (2006) Phosphate-solubilizing *Pseudomonas putida* can influence the rhizobia–legume symbiosis. Soil Biol Biochem 38:3502–3505

Rüberg S, Tian Z-X, Krol E, Linke B, Meyer F, Wang Y, Pühler A, Weidner S, Becker A (2003) Construction and validation of a *Sinorhizobium meliloti* whole genome DNA microarray: genome-wide profiling of osmoadaptive gene expression. J Biotechnol 106:255–268

Ruiz-Lozano JM (2003) Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress. New perspectives for molecular studies. Mycorrhiza 13:309–317

Ruiz-Lozano JM, Azcón R (1995) Hyphal contribution to water uptake in mycorrhizal plants as affected by the fungal species and water status. Physiol Plant 95:472–478

Ruiz-Lozano JM, Collados C, Barea JM, Azcón R (2001) Arbuscular mycorrhizal symbiosis can alleviate drought-induced nodule senescence in soybean plants. New Phytol 151:493–502

Ruiz-Lozano JM, Porcel R, Aroca R (2006) Does the enhanced tolerance of arbuscular mycorrhizal plants to water deficit involve modulation of drought-induced plant genes? New Phytol 171:693–698

Sadowsky MJ (2005) Soil stress factors influencing symbiotic nitrogen fixation. In: Werner D, Newton WE (eds) Nitrogen fixation in agriculture, forestry, ecology, and the environment. Springer, The Netherlands, pp 89–112

Sadowsky MJ, Graham PH (1998) Soil biology of the Rhizobiaceae. In: Spaink HP, Kondorosi A, Hooykaas PJJ (eds) The Rhizobiaceae. Kluwer Acadmic Publishers, Dordrecht, pp 155–172

Safronova VI, Stepanok VV, Engqvist GL, Yv A, Belimov AA (2006) Root-associated bacteria containing 1-aminocyclopropane-1-carboxylate deaminase improve growth and nutrient uptake by pea genotypes cultivated in cadmium supplemented soil. Biol Fertil Soils 42:267–272

Saleem M, Arshad M, Hussain S, Bhatti AS (2007) Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. J Ind Microbiol Biotechnol 34:635–648

Sampedro JG, Uribe S (2004) Trehalose–enzyme interactions result in structure stabilization and activity inhibition. The role of viscosity. Mol Cell Biochem 256–257:127–319

Sánchez-Diaz M (2001) Adaptation of legumes to multiple stresses in Mediterranean-type environments. Options Mediterr 45:145–151

Sánchez-Diaz M, Pardo M, Antolin MC, Peña J, Aguirreolea J (1990) Effect of water stress on photosynthetic activity in the Medicago-Rhizobium Glomus symbiosis. Plant Sci 71: 215–221

Sassi S, Gonzalez EM, Aydi S, Arrese-Igor C, Abdelly C (2008) Tolerance of common bean to long-term osmotic stress is related to nodule carbon flux and antioxidant defenses: evidence from two cultivars with contrasting tolerance. Plant Soil 312:39–48

Saxena AK, Rewari RB (1991) The influence of phosphate and zinc on growth, nodulation and mineral composition of chickpea (*Cicer arietinum* L.). World J Microbiol Biotechnol 7:202–205

Segovia L, Pinero D, Palacios R, Martinez-Romero E (1991) Genetic structure of a soil population of nonsymbiotic *Rhizobium leguminosarum*. Appl Environ Microbiol 57:426–430

Serraj R, Sinclair T, Purcell L (1999) Symbiotic N_2 fixation response to drought. J Exp Bot 50:143–155

Shamseldin A, Werner D (2005) High salt and high pH tolerance of new isolated *Rhizobium etli* strains from Egyptian soils. Curr Microbiol 50:11–16

Shamseldin A, Nyalwidhe J, Werner D (2006) A proteomic approach towards the analysis of salt tolerance in *Rhizobium etli* and *Sinorhizobium meliloti* strains. Curr Microbiol 52:333–339

Sharma MP, Bhatia NP, Adholeya A (2001) Mycorrhizal dependency and growth responses of *Acacia nilotica* and *Albizzia lebbeck* to inoculation by indigenous AM fungi as influenced by available soil P levels in a semi-arid Alfisol wasteland. New For 21:89–104

Sheng XF (2005) Growth promotion and increased potassium uptake of cotton and rape by a potassium releasing strain of *Bacillus edaphicus*. Soil Biol Biochem 37:1918–1922

Sibole JV, Cabot C, Poschenrieder C, Barceló J (2003) Efficient leaf ion partitioning, an overriding condition or absicisic acid-controlled stomatal and leaf growth responses to NaCl salinization in two legumes. J Exp Bot 54:2111–2119

Smit G, Swart S, Lugtenberg BJJ, Kijne JW (1992) Molecular mechanisms of attachment of Rhizobium bacteria to plant roots. Mol Microbiol 6:2897–2903

Smith LT, Smith GM, D'Souza MR, Pocard J-A, Le Rudulier D, Madkour MA (1994) Osmoregulation in *Rhizobium meliloti*: mechanism and control by other environmental signals. J Exp Zool 268:162–165

Soussi M, Ocaña A, Lluch C (1998) Effect of salt stress on growth, photosynthesis and nitrogen fixation in chick-pea (*Cicer arietinum* L.). J Exp Bot 49:1329–1337

Soussi M, Lluch C, Ocaña A (1999) Comparative study of nitrogen fixation and carbon metabolism in two-chick-pea (*Cicer arietinum* L.) cultivars under salt stress. J Exp Bot 50: 1701–1708

Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol Rev 31:425–448

Sprent JI (2006) Evolving ideas of legume evolution and diversity: a taxonomic perspective on the occurrence of nodulation. New Phytol 174:11–25

Sprent JI (2008) 60 Ma of legume nodulation: what's new? what's changing? J Exp Bot 59:1081–1084

Sprent JI, Zahran HH (1988) Infection, development and functioning of nodules under drought and salinity. In: Beck DP, Materon LA (eds) Mediterranean agriculture. Martinus Nijhoff/Dr. W. Junk, Dordrecht, The Netherlands, pp 145–151

Swaine EK, Swaine MD, Killham K (2007) Effect of drought on isolates of Bradyrhizobium elkanii cultured from *Albizzia adianthifolia* seedlings of different provenances. Agroforest Syst 69:135–145

Tachi H, Fukuda-Yamada K, Kojima T, Shiraiwa M, Takahara H (2009) Molecular characterization of a novel soybean gene encoding a neutral PR-5 protein induced by high-salt stress. Plant Physiol Biochem 47:73–79

Tain CY, Feng G, Li XL, Zhang PS (2004) Different effects of arbuscular mycorrhizal fungus isolates from saline or non-saline soil on salinity tolerance of plants. Appl Soil Ecol 26:143–148

Tejera NA, Campos R, Sanjuan J, Lluch C (2004) Nitrogenase and antioxidant enzyme activities in *Phaseolus vulgaris* nodules formed by *Rhizobium tropici* isogenic strains with varying tolerance to salt stress. J Plant Physiol 161:329–338

Tiwari RP, Reeve WG, Dilworth MJ, Glenn AR (1996) Acid tolerance in *Rhizobium meliloti* strains WSM419 involved a two-component sensor-regulator system. Microbiology 142:1693–1704

Tomar OS, Minhas PS, Sharma VK, Singh YP, Gupta RK (2003) Performance of 31 tree species and soil conditions in a plantation established with saline irrigation. For Ecol Manage 177:333–346

Triplett EW, Sadowsky MJ (1992) Genetics of competition for nodulation. Annu Rev Microbiol 46:399–428

Valdenegro M, Barea JM, Azcón R (2001) Influence of arbuscular-mycorrhizal fungi, *Rhizobium meliloti* strains and PGPR inoculation on the growth of *Medicago arborea* used as model legume for re-vegetation and biological reactivation in a semi-arid mediterranean area. Plant Growth Regul 34:233–240

Vance CP (1998) Legume symbiotic nitrogen fixation: agronomic aspects. In: Spaink HP, Kondorosi A, Hooykaas PJJ (eds) The Rhizobiaceae. Kluwer Acad. Pub, Dordrecht, pp 509–530

Vázquez MM, Azcón R, Barea JM (2001) Compatibility of a wild type and its genetically modified Sinorhizobium strain with two mycorrhizal fungi on Medicago species as affected by drought stress. Plant Sci 161:347–358

Verdoy D, De La Peña TC, Redondo FJ, Lucas MM, Pueyo JJ (2006) Transgenic *Medicago truncatula* plants that accumulate proline display nitrogen-fixing activity with enhanced tolerance to osmotic stress. Plant Cell Environ 29:1913–1923

Vessey JK, Chemining'wa GN (2006) The genetic diversity of *Rhizobium leguminosarum* bv. *viciae* in cultivated soils of the eastern Canadian prairie. Soil Biol Biochem 38:153–163

Vessey JK, Pawlowski K, Bergman B (2005) Root-based N2-fixing symbiosis: legumes, actinorhizal plants, Parasponia sp. and cycads. Plant Soil 274:51–78

Vinocur B, Altman A (2005) Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. Curr Opin Biotechnol 16:123–132

Vivas A, Biró B, Németh T, Barea JM, Azcón R (2006) Nickel-tolerant *Brevibacillus brevis* and arbuscular mycorrhizal fungus can reduce metal acquisition and nickel toxicity effects in plant growing in nickel supplemented soil. Soil Biol Biochem 38:2694–2704

Vriezen JAC, De Bruijn FJ, Nüsslein K (2006) Desiccation responses and survival of *Sinorhizobium meliloti* USDA 1021 in relation to growth phase, temperature, chloride and sulfate availability. Lett Appl Microbiol 42:172–178

Vriezen JAC, De Bruijn FJ, Nüsslein K (2007) Responses of rhizobia to desiccation in relation to osmotic stress, oxygen and temperature. Appl Environ Microbiol 73:3451–3459

Wani PA, Khan MS, Zaidi A (2008a) Effects of heavy metal toxicity on growth, symbiosis, seed yield and metal uptake in pea grown in metal amended soil. Bull Environ Contam Toxicol 81:152–158

Wani PA, Khan MS, Zaidi A (2008b) Effect of metal-tolerant plant growth-promoting Rhizobium on the performance of pea grown in metal-amended soil. Arch Environ Contam Toxicol 55:33–42

Wankhade S, Apte SK, Rao KK (1996) Salinity and osmotic stress regulated proteins in cowpea Rhizobium 4a (peanut isolate). Biochem Mol Biol Int 39:621–628

Ward JM, Hirschi KD, Sze H (2003) Plants pass the salt. Trends Plant Sci 8:200–201

Wei W, Jiang J, Li X, Wang L, Yang SS (2004) Isolation of salt-sensitive mutants from *Sinorhizobium meliloti* and characterization of genes involved in salt tolerance. Lett Appl Microbiol 39:278–283

Wei G-H, Yang X-Y, Zhang Z-X, Yang Y-Z, Lindström K (2008) Strain Mesorhizobium sp. CCNWGX035: a stress-tolerant isolate from *Glycyrrhiza glabra* displaying a wide host range of nodulation. Pedosphere 18:102–112

Wood JM, Bremer E, Csonka IN, Kramer R, Poolman B, Van Der Heide T, Smith IT (2001) Osmosensing and osmoregulatory compatible solute accumulation by bacteria. Comp Biochem Physiol A Mol Integr Physiol 130:437–460

Wu CH, Wood TK, Mulchandani A, Chen W (2006) Engineering plant–microbe symbiosis for rhizoremediation of heavy metals. Appl Environ Microbiol 72:1129–1134

Xiong L, Zhu J-K (2001) Abiotic stress signal transduction in plants: molecular and genetic perspectives. Physiol Plant 112:152–166

Yang Q, Wu M, Wang P, Kang J, Zhou X (2005) Cloning and expression analysis of a vacuolar Na+/H+ antiporter gene from alfalfa. DNA Seq 16:352–357

Yano-Melo AM, Saggin OJ, Maia LC (2003) Tolerance of mycorrhizal banana (Musa sp. cv. Pacovan) plantlets to saline stress. Agric Ecosyst Environ 95:343–348

Yokoi S, Quintero FJ, Cubero B, Ruiz MT, Bressan RA, Hasegawa PM, Pardo JM (2002) Differential expression and function of *Arabidopsis thaliana* NHX Na+/H+ antiporters in the salt stress response. Plant J 30:529–539

Zahran HH (1991) Conditions for successful Rhizobium–legume symbiosis in saline environments. Biol Fertil Soils 12:73–80

Zahran HH (1998) Structure of root nodules and nitrogen fixation in Egyptian wild herb legumes. Biol Plant 41:575–585

Zahran HH (1999) Rhizobium–legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol Mol Biol Rev 63:968–989

Zahran HH (2001) Rhizobia from wild legumes: diversity, taxonomy, ecology, nitrogen fixation and biotechnology. J Biotechnol 91:143–153

Zahran HH (2005) Rhizobial nitrogen fixation in agriculture: biotechnological perspectives. In: Ray RC (ed) Microbial biotechnology in agriculture and aquaculture, vol 1. Science Publishers, Inc., Enfield, USA, pp 71–100

Zahran HH (2006a) Wild legume rhizobia: biodiversity and potential as biofertilizers. In: Rai MK (ed) Handbook of microbial biofertilizers. Haworth Press Inc., New York, pp 203–222

Zahran HH (2006b) Nitrogen (N2) fixation in vegetable legumes: biotechnological perspectives. In: Ray RC, Ward OP (eds) Microbial biotechnology in horticulture, vol 1. Science Publishers, Inc., Enfield, USA, pp 49–82

Zahran HH (2009) Enhancement of rhizobia–legumes symbioses and nitrogen fixation for crops productivity improvement. In: Khan MS, Zaidi A, Musarrat J (eds) Microbial strategies for crop improvement. Springer, Germany, pp 227–254

Zahran HH, Sprent JI (1986) Effects of sodium chloride and polyethylene glycol on root-hair infection and nodulation of *Vicia faba* L. plants by *Rhizobium leguminosarum*. Planta 167:303–309

Zahran HH, Räsanen LA, Karsisto M, Lindström K (1994) Alteration of lipopolysaccharide and protein profiles in SDS-PAGE of rhizobia by osmotic and heat stress. World J Microbiol Biotechnol 10:100–105

Zahran HH, Ahmad MS, Afkar E (1995) Isolation and characterization of nitrogen-fixing moderate halophilic bacteria from saline soils of Egypt. J Basic Microbiol 35:269–275

Zahran HH, Abdel-Fattah M, Ahmad MS, Zaki AY (2003) Polyphasic taxonomy of symbiotic rhizobia from wild leguminous plants growing in Egypt. Folia Microbiol 48:510–520

Zahran HH, Marin-Mansano MC, Sánchez-Raya AJ, Bedmar EJ, Venema K, Rodríguez-Rosales MP (2007) Effect of salt stress on the expression of NHX-type ion transporters in *Medicago intertexta* and *Melilotus indicus*. Physiol Plant 131:122–130

Zaidi A, Khan MS, Ahemad M, Oves M (2009) Plant growth promotion by phosphate solubilizing bacteria. Acta Microbiol Immunol Hung 56:263–284

Zerhari K, Aurag J, Khbaya B, Kharchaf D, Filali-Matlouf A (2000) Phenotypic characteristics of rhizobia isolates nodulating Acacia species in the arid and Saharan regions of Morocco. Lett Appl Microbiol 30:351–357

Zhu J-K (2002) Salt and drought stress signal transduction in plants. Annu Rev Plant Biol 53:247–273

Zhu J-K (2003) Regulation of ion homeostasis under salt stress. Curr Opin Plant Biol 6:441–445

Zhu J-K, Hasegawa PM, Bressan R (1997) Molecular aspects of osmotic stress in plants. Crit Rev Plant Sci 16:253–277

Zörb C, Noll A, Karl S, Leib K, Yan F, Schubert S (2005) Molecular characterization of Na+/H+ antiporters (ZmNHX) of maize (Zea mays L.) and their expression under salt stress. J Plant Physiol 162:55–66