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Actinorhizal and Rhizobial-Legume Symbioses for Alleviation of Abiotic Stresses

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Abstract

Plant symbiotic interactions with nitrogen-fixing bacteria could be used in agricultural and forest soils under harsh conditions and for land reclamation. These associations include rhizobia interacting with legumes species and the actinobacterium *Frankia* interacting with actinorhizal plants. These plants are distributed worldwide and suffer in many areas – especially in arid and semiarid regions from harsh biotic and abiotic conditions. Abiotic stress conditions include climatic conditions, soil characteristics, water and nutrient availability, salinity, and soil contamination by heavy metals, xenobiotics, and toxins. Thus, the improvement of symbiotic nitrogen fixation under stress conditions may rely on strategies devised to select more tolerant plant-bacteria associations and to use successfully efficient synergistic systems such as those involving mycorrhizal fungi and rhizobacteria, particularly plant growth-promoting rhizobacteria (PGPR). This chapter focuses on main stress conditions affecting plant-bacteria symbioses with emphasis on mechanisms that underpin plant resistance to stress. Biological strategies involving microbial inoculants aiming to protect and improve plant symbioses against detrimental effects of abiotic stresses so as to enhance the crop production in such conditions are discussed. In addition, plant-bacteria symbioses can also be used to promote the establishment of perennial vegetation that might limit the heavy metal pollution of soils and consequently represent an effective management procedure in disturbed soils. This chapter also focuses on main techniques successfully used for the purpose of land reclamation by plant-bacteria symbioses.

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Introduction

Most subtle known plant-bacteria associations are dinitrogen-fixing symbioses. They occur in nature as associations involving certain gram-negative bacteria (rhizobia) and legumes (legume-rhizobia symbioses) or between the gram-positive bacteria of the genus *Frankia* (order *Frankiales*, class *Actinobacteria*) and woody dicotyledonous actinorhizal plants (Pawlowski and Sirrenberg 2003). Diverse bacteria, commonly named rhizobia, are known to infect and nodulate legumes, while only genus *Frankia* is associated with actinorhizal plants as nodulating microorganism. Currently, 98 species of legume-nodulating bacteria belonging to 13 bacterial genera and 13,000 legume species from 700 genera are identified (Rajwar et al. 2013). Among these plants, soybean (*Glycine max*) is the dominant crop legume, representing 50 % of the global legume crop area. Taxonomically, rhizobia belong to the family *Rhizobiaceae* (α-subclass of *Proteobacteria*) and include species of the genera *Rhizobium*, *Bradyrhizobium*, *Ensifer* (formerly named *Sinorhizobium*), *Mesorhizobium*, *Allorhizobium*, and *Azorhizobium*. Currently, an ongoing number of rhizobia are being discovered. Recent findings indicate that rhizobial bacteria are diverse and also include *Devosia*, *Methylobacterium*, *Ochrobactrum*, *Phyllobacterium*, and *Shinella*. Some legumes are nodulated by strains of *Burkholderia*, *Cupriavidus*, and *Herbaspirillum* species belonging to the β-subclass of *Proteobacteria* (Chen et al. 2003; Masson-Boivin et al. 2009).

Actinorhizal plants comprise 200 plant species belonging to 25 genera of woody plants from eight non-leguminous dicotyledonous angiosperm families *Casuarinaceae*, *Betulaceae*, *Rhamnaceae*, *Rosaceae*, *Myricaceae*, *Coriariaceae*, *Elaeagnaceae*, and *Datiscaceae* (Chaia et al. 2010). While most known legume plants produce seeds and fodder with high protein content and exert beneficial effects on crop productivity in diverse cropping ecosystems over the world (Zahran 1999; Terpolilli et al. 2012), actinorhizal plants are of great ecological importance. They occupy ecological niches where legumes do not thrive and are mainly used for reclamation of degraded lands and as a source of fuel and wood. Also, *Frankia* species are considered as pioneer microorganisms in soils and as early successional species following deglaciation or fires (Chapin et al. 1994; Roy et al. 2007). Among actinorhizal plants, *Casuarinaceae* and *Betulaceae* families are the most widely planted around the world. *Casuarina* species, which are Mediterranean trees that can reach a final height of 20–30 m, are among the fastest growing trees in the world (can grow up to 2–3 m per year). *Casuarina equisetifolia* is the most widespread and the best known species. Members of this genus are used as soil binder in landslide areas and planted extensively in coastal areas of tropical and subtropical regions as windbreaks against wind erosion (Diem and Dommergues 1990). Wood from these plants is used for carpentry, construction, and fuel production. Some plants produce edible fruits, and leaves are used as fodder. For example, in India, 5,000,000 ha is planted with *C. equisetifolia* and produces 10 million tons of pulpwood (Karthikeyan et al. 2009).

Biological nitrogen fixation (BNF) by N_2-fixing plants is a sustainable and environmental-friendly alternative to chemical fertilization. By supplying combined nitrogen to plants, microbes forming association contribute to plant health and productivity in agricultural and forest soils. It is generally accepted that more than 60 % of the N input to plants has a biological origin and more than 30 % of the biological N input to plants is due to the activity of symbiotic plant-bacteria systems (Barea et al. 2005). Annual levels of at least 35 million tons of nitrogen are attributed to rhizobia-legume symbiosis (Freire 1992) corresponding to a 25–30 % of global annual terrestrial N inputs. On the other hand, it is estimated that actinorhizal plants are responsible for as much as 15–25 % of global nitrogen fixation (Dawson 2008). In most agricultural systems, the symbiotic interactions of legumes-rhizobia provide 80 % of the primary source of biologically fixed N (Sadowsky and Graham 1998). Regarding crop and forestry sustainment, it is well known that many countries are using bacterial-plant symbioses as fertilizers in agriculture. Thus, many researches are aimed to select more efficient nitrogen-fixing plants, able to grow in agrosystems with low available nutrients. These biological strategies were encouraged by the major public concern for reducing agrochemical inputs as they constitute a threat to global environment. Indeed, these plants are nonpolluting and more cost-effective soil fertilizers compared to chemical fertilizers and sewage sludge. In addition, these associations could succeed in many areas where harsh abiotic conditions are inimical to the growth of many plants. Abiotic stress conditions in relatively harsh environments include harsh climatic conditions, soil characteristics, water and nutrient availability, salinity, and soil contamination by heavy metals, xenobiotics, and toxins. These symbioses deploy various resistance mechanisms to combat the abiotic stress conditions. These mechanisms underlie physiological and metabolic shifts that help plant to adapt to stress conditions. The most important responsive trait is the involvement of antioxidant enzymes such as peroxidase and catalase that have the ability to remove free radicals and help plant to mitigate oxidative damage and consequently to overcome stress conditions.

Plant bacterial symbioses are applied to alleviate abiotic stresses in many crop systems. It is well known that inoculation with indigenous N_2-fixing bacteria increases soil fertility and quality especially in poor soils and can contribute to the plant tolerance to abiotic environmental stresses (Richards et al. 2002; Mnasri et al. 2007; Bianco and Defez 2009; Tani and Sasakawa 2003; Oufdou et al. 2014). Efforts are made to search for new stress-tolerant crops or genetically engineered plants. However, as yet these strategies are costly and out of reach for developing countries. Easier and cheaper strategies are based on the application of microbial inoculants. Thus, efforts are made to select more tolerant rhizobia or *Frankia* strains and/or effective plant associations. For efficient applications of this strategy, it is suggested that bacterial strain–plant genotype combination should be considered for selecting the most adapted microbe-plant combinations to environmental stress conditions (Requena et al. 1997; Valdenegro et al. 2001; Remans et al. 2008). Apart from this, other soil microbiota are also able to interact with N_2-fixing plants. They involve plant growth-promoting bacteria (PGPB) and mycorrhiza (Barea and Azcón-Aguilar 1983; Probanza et al. 1996; Yamanaka et al. 2003; Han and Lee 2005; Aroca et al. 2007; Saravanakumar and Samiyappan 2007; Solans 2007; Egamberdiyeva and Islam 2008; Figueiredo et al. 2008; He and Critchley 2008; Marulanda et al. 2009). The simultaneous occurrence of these microorganisms together with the N_2-fixing bacteria generates a multipartite relationship able to significantly improve nitrogen fixation and the host plant growth. Bioaugmentation by stress-tolerant rhizobacteria particularly PGPR could be used as a stress alleviation mechanism. One interesting mechanism of action of PGPR is mediated by 1-aminocyclopropane-1-carboxylate (ACC) deaminase, an enzyme that cleaves ACC, the precursor molecule of ethylene. By this mechanism, the level of ethylene in a stressed plant is lowered. In addition, rhizobacterial species have the ability to elevate the levels of stress enzymes in the plant. Such mechanism and others confer to plant a tolerance commonly named “induced systemic tolerance” (IST) (Yang et al. 2009). Associations with mycorrhizae are commonly called tripartite associations. Dual inoculation of these microorganisms with rhizobia or *Frankia* strains resulted often in tolerance compared to those formed by the bacterial symbiont alone. Indeed, PGPR and mycorrhizae play important roles in the life of plants, not only ensuring more efficient nutrition but also improving plant health through increased protection against biotic and abiotic stresses. Increasing extension of salinized and contaminated/degraded lands is of global concern. N_2-fixing plants and their associations are also used for mitigating negative impact of toxicity in heavy metal-contaminated soils and for soil bioremediation.

There are many comparative features of the legume-*Rhizobium* symbiosis with those of the actinorhizal symbiosis regarding physiological resistance to stress conditions and their ability to interact with microbial inoculants able to enhance their tolerance (N_2-fixing symbiont bacteria and beneficial soil microbiota). Thus, this chapter focuses on main resistance mechanisms deployed by these two main plant-bacteria symbioses and their applications or their associations with beneficial telluric microorganisms (mainly PGPRs and mycorrhizae) under soil stressed systems. This chapter also focuses on main applications of plant-bacteria symbioses in contaminated soils and advances in soil bioremediations. Figure 14.1 summarizes mechanisms deployed by N_2-fixing bacteria, PGPRs, and AM fungi to alleviate abiotic stresses.

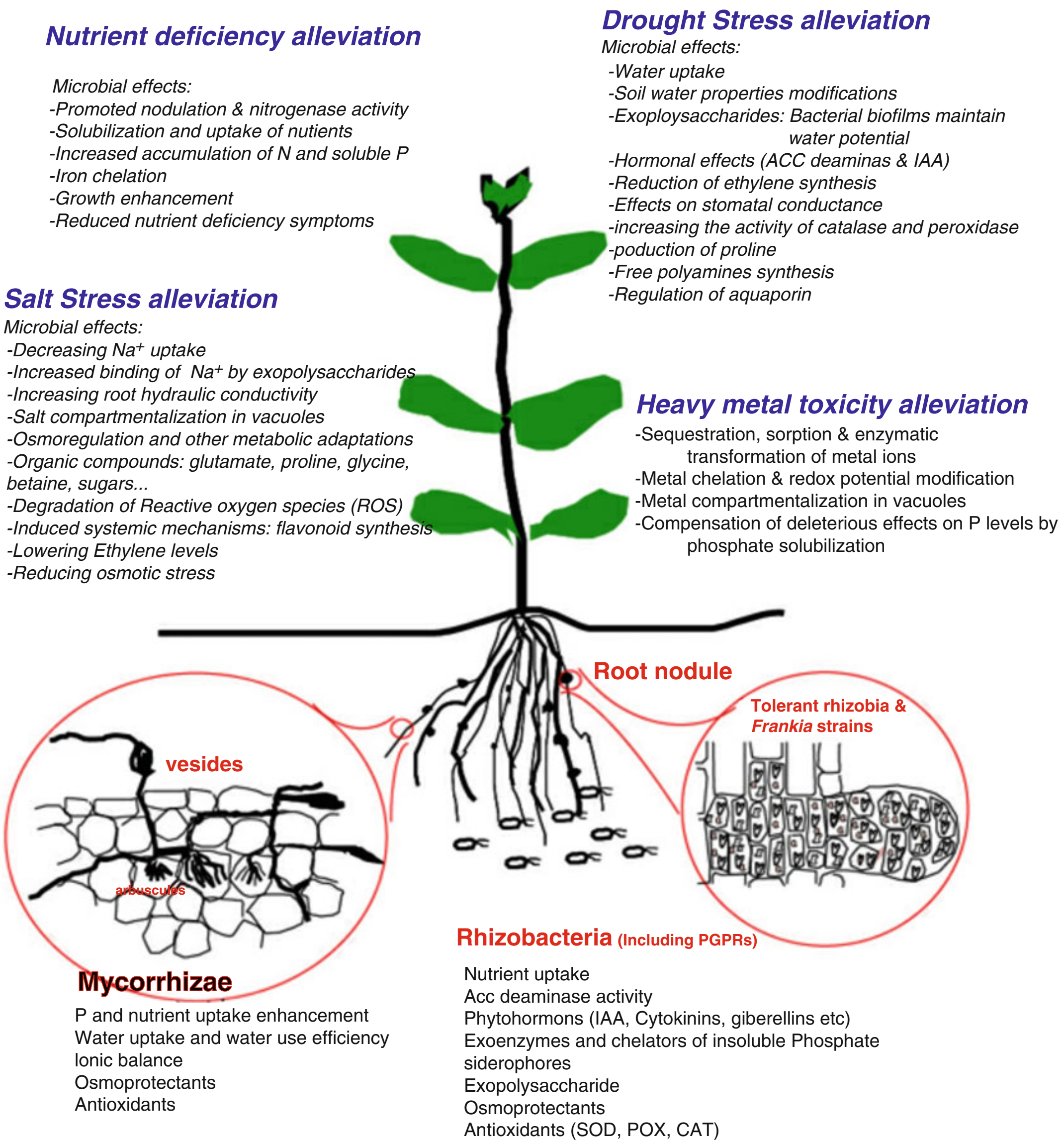

Fig. 14.1 Scheme summarizing the mechanisms deployed by N_2-fixing bacteria, PGPRs, and AM fungi to alleviate abiotic stresses. *CAT* catalase, *IAA* indoleacetic acid, *PGPR* plant growth-promoting rhizobacteria. *POX* peroxidase, *SOD* superoxide dismutase

Application of Plant-Bacteria Symbioses to Alleviate Abiotic Stresses

Tolerance and Growth Promotion Under Nutrient Deficiency

Soil nitrogen status is considered as one of the main factors affecting sustainability of plant growth in a crop or forest ecosystem. N status of soils under N-fixing plants including actinorhizals and legumes is often considered as high. They are very important in agriculture and forestry as they are responsible for a substantial part of the global flux of nitrogen from atmospheric N_2 to fixed mineral forms. Alders are, for example, of major interest for soil nitrogen status as their leaf litter is highly N rich and therefore mineralized much faster than that of other tree species (Claessens et al. 2010). However, in many nutrient-poor areas, colonization of primary successional substrates is largely limited by low levels of nutrients. In most soils where low levels of available nitrogen are a major limitation, N_2-fixing plants which are the primary source of fixed nitrogen (Zahran 1999; Barritt and Facelli 2001) could succeed. Nitrogen fixation and root nodule formation are substantially affected by soil nutrient availability of mineral N (Arnone et al. 1994; Bollman and Vessey 2006) and P (Reddell et al. 1986a; Sanginga et al. 1989; Yang 1995; Kouas et al. 2005; Sadowsky 2005). It is well known that nodulation is stimulated in poor N soils, whereas high levels of mineral forms of nitrogen (nitrate and ammonium) have a negative effect on nodulation and nitrogen fixation activity (Arnone et al. 1994; Baker and Parsons 1997; Wall et al. 2000; Tiffany and Williams 2005; Bollman and Vessey 2006; Shamseldin and Moawad 2010). Nodule development requires P consumption for DNA and plasma membrane synthesis, for cell division, and also for symbiotic N_2 fixation due to the need for large amount of ATP for nitrogenase activity (reduction of N_2). In general, the fixation of atmospheric N_2 in plant-microbe symbioses can negatively be impacted by phosphorus limitation (Reddell et al. 1986a; Tang et al. 2001). Indeed, it was demonstrated that nodulation and growth in some actinorhizal plants are frequently limited by P in early successional environments (Chapin et al. 1991; Uliassi et al. 2000). Also, *Frankia* infective abilities following deglaciation were only showed if P was supplemented (Chapin et al. 1994). Phosphorus has been shown to have a positive effect on nodulation in many actinorhizal and legume plants (Wall et al. 2000; Olivera et al. 2004; Gentili et al. 2006). This was also observed in some soils from Australia, where Reddell et al. (1986a) found that nodulation occurred only when P amendments were performed.

N_2-fixing plants able to grow under nitrogen deficiency are considered as important for revegetation in such soils as these can accumulate nitrogen. Different perennial legumes were studied for their potential use in revegetating Australian soils with phosphorus deficiency (Pang et al. 2010). In addition, efforts have been made to select efficient legume plants for N_2 fixation and biomass and seed yield, particularly under conditions of N and P deficiency (Elisondo Barron et al. 1999; Tang et al. 2004; Remans et al. 2008). The selection of N_2-fixing plants tolerant to N deficiency in combination with tolerant bacterial symbionts seems to be a valuable strategy. Consequently, screening and inoculation of efficient N_2-fixing bacteria in order to provide improvement in plant production equal to or better than that of nitrogen fertilization are aspects that have been investigated by numerous researchers (Sellstedt and Huss-Danell 1986; Romdhane et al. 2008).

Many researches are aimed to select and apply PGPRs for their beneficial effects on plant fitness and productivity. These rhizobacteria are well known to promote plant growth by various direct and indirect mechanisms including phytohormone synthesis and phosphate solubilization and are consequently used as phytostimulators and biofertilizers for agricultural crops. In addition, the use of microbial inoculants could represent an alternative strategy to the use of chemical fertilizers notably NP-based fertilizers. Rhizobia can also be considered as PGPR endophytes as they contribute to plant growth. However, researchers prefer to denote PGPR as free root-colonizing rhizobacteria. Most known PGPRs belong to the genera *Arthrobacter*, *Bacillus*, *Micromonospora*, *Pseudomonas*, and *Streptomyces*. Some of these bacteria were applied for growth stimulation and nodulation in both legume and actinorhizal plants (Solans 2007; Malik and Sindhu 2011). The most important traits of PGPRs in association with N_2-fixing plants are the improvement of nodulation (Knowlton and Dawson 1983; Zhang et al. 1996; Lucas-Garcia et al. 2004) and supply of soluble P by solubilization (Toro et al. 1998; Valverde et al. 2006). The application of PGPRs to N_2-fixing plants often resulted in enhanced legume or actinorhizal plants' growth. Hence, fitness was enhanced in various crops when PGPRs were co-inoculated with rhizobia or with *Frankia* (Knowlton and Dawson 1983; Probanza et al. 1996, 1997; Solans 2007; Wani et al. 2007a; Dardanelli et al. 2008; Egamberdieva et al. 2010; Malik and Sindhu 2011). An example of such application is the single, dual, or triple seed inoculations of common bean (*Phaseolus vulgaris*) with *Rhizobium leguminosarum* bv. *phaseoli*, N_2-fixing *Bacillus subtilis*, and P-solubilizing *Bacillus megaterium* in a highland region in Turkey as indicated by significantly increased nodulation, plant growth, nutrient uptake, and seed yield equal to or higher than nitrogen (N), phosphorus (P), and NP mineral fertilizer application. Moreover, triple inoculations were most efficient for enhancement of macro- and micronutrient uptake resulting in an increase in mineral concentration in the plant and seeds (Elkoca et al. 2010). Similar results were found in *Vicia faba* L. inoculated with four different *Rhizobium*/*Azospirillum* and *Rhizobium*/*Azotobacter* combinations which led to changes in mineral macro- and micronutrients, K, P, Ca, Mg, Fe, B, Mn, Zn, and Cu, compared with plants inoculated with *Rhizobium* alone (Rodelas et al. 1999). Recently, Solans et al. (2011) reported that three root-associated saprophytic actinomycetes (*Streptomyces* sp., *Actinoplanes* sp., and *Micromonospora* sp.) stimulated nodulation by *Frankia* in the actinorhizal plant *Ochetophila trinervis*. These root associated actinobacteria produced phytohormones (indole acetic acid (IAA), gibberellic acid, and zeatin) at levels higher than produced by the symbiotic *Frankia* strain alone. One interesting strain *Micromonospora* MM18 showed direct promotion effect on shoot growth of *Ochetophila trinervis* when inoculated as mycelium as well as cell-free growth medium (Solans 2007). However, in some cases, effects of dual inoculation with *Rhizobium* and PGPR depend on various parameters including legume species, PGPR species, and the nature and concentration of secondary metabolites released by PGPR. Co-inoculation with *Pseudomonas fluorescens* 2137 increased colonization of *Bradyrhizobium japonicum* on soybean roots. Nodule numbers and acetylene reduction increases were observed probably due to release of growth-promoting substances by *Pseudomonas* that stimulate *B. japonicum*, whereas co-inoculation with another strain *P. fluorescens* WCS365 had an opposite effect (Chebotar et al. 2001). Under field conditions, co-inoculation of PGPR, *Serratia* and *P. fluorescens* with *R. leguminosarum*, had positive effects on lentil but had no effect on pea (Chanway et al. 1989). These opposite effects are likely due to PGPR species and/or legume plant species. Elo et al. (2000) isolated a number of bacteria from the humus of spruce and tested their potential beneficial effects on alder (*Alnus incana*), red fescue (*Festuca rubra*), and birch (*Betula pendula*).

Under stress conditions of soil nutrient deficiency, the effectiveness of PGPRs as bioinoculants for N_2-fixing plants has been demonstrated in legume plants. Positive effects were reported in relation to iron, phosphorus, magnesium, calcium, and the nutrient uptake under iron-limiting conditions. Siderophores produced by certain bacteria could contribute to enhanced availability of iron. Inoculation of mung bean (*Vigna radiata*), with the siderophore-producing *Pseudomonas* strain GRP3, resulted in a reduced chlorotic symptoms and enhanced chlorophyll level (Sharma et al. 2003). In general, differences in the responsiveness to PGPR among cultivars of legumes were observed. Thus, PGPRs could be used efficiently for enhancement of tolerance to P deficiency in non-tolerant cultivars. In the

P. vulgaris genotype DOR364, a poor nitrogen fixer under P deficiency, Remans et al. (2008) succeeded to partly alleviate this constrain by using a combination of *Rhizobium etli* and *Azospirillum* inoculation. However, in another *Phaseolus* genotype, the beneficial effect of co-inoculated *R. etli* and *Azospirillum* was shown to be dependent on P nutrition (Remans et al. 2007). These authors showed that co-inoculation resulted in efficient growth enhancement only at high P conditions and combinations failed under low P conditions. It is also worth mentioning that in general, the interplay between *Rhizobium*, PGPR species, and plant host species plays a major role in the success of these combinations under P stress conditions. Under deficient P conditions, while combination of *Rhizobium* and *Azospirillum* was ineffective, the beneficial effect of co-inoculation of *Rhizobium* with *Pseudomonas putida* was strongly expressed (Remans et al. 2007).

In addition, it is well known that N_2-fixing plants can also harbor ecto- or endomycorrhizae forming either tripartite or tetrapartite associations. These kinds of associations were found in both legume plants and actinorhizae (Vance 2001; Yamanaka et al. 2003; Roy et al. 2007; He and Critchley 2008; Gherbi et al. 2008), and their importance for plant growth promotion in poor soils has been documented extensively. Their beneficial effects on plant growth are mainly due to the positive effects of the mycorrhizal fungi through P supply (Barea et al. 2005). In many cases, dual inoculation of actinorhizal plants with arbuscular mycorrhizal fungi (AMF) and *Frankia* resulted in a more enhanced growth than single inoculation with *Frankia* alone (Vasanthakrishna et al. 1994; Oliveira et al. 2005; Elumalai and Raaman 2009; Muthukumar and Udaiyan 2010; Orfanoudakis et al. 2010). In addition, the usefulness of AMF co-inoculation with N_2-fixing bacteria is well established in nutrient-poor soils. Inoculation of mycorrhiza fungi promotes the growth of actinorhizal plants by various mechanisms including increased uptake of phosphorus (Theodorou and Reddell 1991; He and Critchley 2008; Elumalai and Raaman 2009). It was demonstrated that *Elaeagnus* and *Shepherdia* inoculated with soil containing *Frankia* and mycorrhizal fungi under nutrient deficiency had a superior nodulation than those uninoculated (Visser et al. 1991). Theodorou and Reddell (1991) found that symbiotic mycorrhizae could contribute to a successful *Frankia* symbiosis in *Casuarina* plants by increasing N_2 fixation especially in P-deficient soils, in arid climates. However, inoculation experiments showed that mutual interactions and delays between inoculations of the two symbionts could play a major role in the efficiency of tripartite association (Gardner 1986; Theodorou and Reddell 1991). Theodorou and Reddell (1991) observed that *Amanita* sp. formed ectomycorrhizae on *Allocasuarina littoralis* and *Casuarina cunninghamiana* but not on *Casuarina equisetifolia*. It was also shown that the intensity of ectomycorrhizal infection with *Pisolithus tinctorius* and *Laccaria laccata* was higher in *C. equisetifolia* rather than *C. cunninghamiana* suggesting specificity between the plant and mycorrhizae. In addition, under limited supply of mineral N and P, Sempavalan et al. (1995) found a high degree of coordination between *Frankia* and *Glomus* for nodulation and mycorrhizal colonization of roots of *C. equisetifolia*, while Gardner (1986) reported a possible competition between *Frankia* and AMF microsymbionts for infection sites and/or nutrients in AMF-colonized *C. equisetifolia* resulting in negative effect on nodulation. It was thus suggested that efficiency of these tripartite associations in poor soils could be ensured if the moments of inoculations of both symbionts are skillfully monitored (Gardner 1986). All these observations emphasize the importance of knowledge of specific combinations involving mycorrhizae/*Frankia*/actinorhizal plant species.

Alleviation of Drought Stress

Arid or semiarid land areas are increasing worldwide due to climate change and global warming. This condition has deleterious effects on plants in these areas. Drought stress affects plant hormone balance by decreasing the endogenous cytokinin level and increasing the levels of abscisic acid

(ABA) content in the leaves. This effect leads to stomatal closure. Many N_2-fixing species undergo deleterious physiological effects of water deficiency and show a reduction in nitrogen fixation when subjected to drought (Zahran 1999; Sharma et al. 2010). In addition to its deleterious effects on nodules and nitrogenase activity (Sundström and Huss-Danell 1987; Devries et al. 1989; Porcel et al. 2003), drought could affect the survival of rhizobia and the size of the soil population of infective *Frankia* and their infectivity (Mahler and Wollum 1981; Sayed et al. 1997). Drought also alters the soil properties and renders it unsuitable for soil microbial activity and crop growth (Selvakumar et al. 2012).

Actinorhizae are more drought tolerant than other nitrogen-fixing microorganisms. Numerous actinorhizal plants mainly those belonging to the genus *Casuarina* were reported as drought tolerant (Diem and Dommergues 1990). Though they are high water demanders, alders (*Alnus*) are well adapted to water deficits due to their root systems (Claessens et al. 2010). The performance of tolerant *Casuarina* under conditions of drought could be due to their high rate of photosynthesis and decreased stomatal conductance. Thus, researches focused on propagation of *Casuarina* species tolerant to drought via in vitro tissue culture for seedling production or via cuttings of plant parts.

Numerous legume plants are adapted to drought stress conditions. Among these are arid legumes, such as cluster bean (*Cyamopsis tetragonoloba*), dew bean (*Vigna aconitifolia*), and cowpea (*Vigna unguiculata*) (Kumar 2005). It was observed that numerous drought-tolerant species (cultivars) are characterized by a deeper root profiles, a greater root biomass, and a greater root-to-shoot ratio. Those variabilities could be exploited in breeding programs for N_2 fixation drought tolerance. In addition, efforts are made to select more tolerant N_2-fixing plant species to drought by using conventional selection and breeding (El-Lakany 1983; Pimentel et al. 1999; Sinclair et al. 2001; Khan et al. 2010; Asfaw et al. 2012), and efforts are also made to screen more tolerant *Frankia* and rhizobia strains (Sayed et al. 1997; Romdhane et al. 2009; Zahran 2001; Sayed 2011). For instance, salt-tolerant rhizobia that were able to nodulate chickpea (*Cicer arietinum*) were selected for their efficiency in conditions of water deficiency (Romdhane et al. 2009). Mnasri et al. (2007) reported that when inoculated to *P. vulgaris*, a salt-tolerant *Ensifer meliloti* induced more tolerance to drought than a salt-sensitive bacterial strain *Rhizobium tropici. Frankia* may be absent in some arid soils, whereas non-leguminous N_2-fixing plants are often nodulated in well-hydrated areas. Thus, there is a need to inoculate host plants with a selected microsymbiont in arid and semiarid areas. Attempts to select more tolerant *Frankia* strains were performed by Sayed et al. (1997) who found that the infectivity of a soil inoculum decreased by storage under dry conditions and pointed out the importance of screening *Frankia* strains for drought tolerance during preparation of inoculum for use in hot climates. Thereafter, inoculation of tolerant bacterial symbionts was also used as a strategy to alleviate drought stress in many N_2-fixing plants. Bacterial aggregation as biofilms in the vicinity of roots was reported as a mechanism that could contribute to maintain convenient water potential (Khan et al. 2011). Bacteria producing extracellular materials were reported for their beneficial effects. Exopolysaccharides (EPS) act as adhering material that help bacteria to attach to soil particles and by binding soil particles together lead to soil aggregate formation. EPS contribute to hold water within soil aggregate and thus lead to ameliorate plant growth under drought conditions. Among EPS-producing bacteria, rhizobia were applied by Hartel and Alexander (1986). They observed a significant correlation between the levels of EPS produced by cowpea *Bradyrhizobium* strains and their drought tolerance.

On the other hand, rhizobacteria and mycorrhizae were also investigated for their ability to help plants to alleviate drought stress. PGPR bacteria could also contribute to drought alleviation in legume plants through hormonal effects that involve IAA and ACC deaminase. Marulanda et al. (2009) used three bacteria: *Pseudomonas sp.*, *P. putida*, and *B. megaterium* with IAA-producing abilities to alleviate drought stress in

Trifolium repens. Alleviation effect could also involve the regulation of ethylene synthesis by ACC-deaminase-producing *Pseudomonas* which showed to be effective in both pot and field trials (Arshad et al. 2008). Indeed, reduction of ethylene synthesis by such bioinoculants effect could result in better growth under drought stress (Zahir et al. 2008). ACC-deaminase-producing rhizobacteria could obviously reduce the negative effects of ethylene on nodulation and N_2 fixation. Dual inoculation involving PGPRs and rhizobia proved their effectiveness in mitigating water stress deficiency in N_2-fixing plants. In bean, Figueiredo et al. (2008) used dual inoculation of *Paenibacillus polymyxa* and *R. tropici* to alleviate drought stress. Plants co-inoculated with *Rhizobium* and *Paenibacillus* exhibited greater nodulation and nitrogen content compared to drought-stressed plants inoculated with only *Rhizobium*. In addition, PGPR induced systemic mechanisms to drought could represent a powerful tool to alleviate such alterations. IST by increasing the activity of catalase and peroxidase in water stress was found in *P. fluorescens* Pf1-treated green gram plants (*V. radiata* L.) (Saravanakumar et al. 2012).

Mycorrhizae could also be used as bioinoculants for drought stress alleviation (Ruiz-Lozano et al. 2001). Several studies have stressed their role in improving legume and actinorhizal plant tolerance to drought (Goicoechea et al. 1998; Ruiz-Lozano et al. 2001; Valdenegro et al. 2001; Zhang et al. 2010). Mycorrhizal inoculation significantly improved both nodulation and nitrogen fixation in these conditions (Azcón et al. 1988; Theodorou and Reddell 1991; Ruiz-Lozano et al. 2001). In soybean, mycorrhizae were reported to have antioxidant activities and to protect legume plants against premature nodule senescence induced by drought stress (Porcel et al. 2003). It was also established that they could reduce the production of proline, a marker of the water stress effect in plants (Goicoechea et al. 1998). In addition, they induce free polyamine synthesis (Goicoechea et al. 1998). This contributes to increased accumulation of nonstructural carbohydrates that could serve as osmoprotectants by lowering the osmotic potential under drought conditions. The regulation of root hydraulic properties by AM symbiosis under drought stress is also correlated with the regulation of aquaporins which are membrane intrinsic proteins that form a pore in cell membranes of living organisms (Aroca et al. 2007). Uehlein et al. (2007) found an AM-induced gene expression of specific aquaporin genes in mycorrhized *Medicago truncatula*. The application of mycorrhizae for drought tolerance in actinorhizal plants was also reported (Zhang et al. 2010). Zhang et al. (2010) found that AM associations improve the drought tolerance of *C. equisetifolia* seedlings by lowering the permeability of plasma membrane and malondialdehyde contents, enhancing concentrations of P nutrition, soluble sugars, soluble proteins, and peroxidase activities. However, efficient dual inoculation involving symbiotic mycorrhizae and rhizobia seems to be variable according to bacterial interactions in a given combination. Ruiz-Lozano et al. (2001) reported that dual inoculation of *Bradyrhizobium* and *Glomus intraradices* was less effective than dual inoculation with *G. mosseae*, in alleviating drought-induced nodule senescence in soybean plants, suggesting the existence of a competitive interaction between the very aggressive *G. intraradices* and *Bradyrhizobium*. The importance of efficient combinations was also discussed by Valdenegro et al. (2001) who used combinations of three mycorrhizae and one PGPR, *Enterobacter* sp., and found that the positive effects of AMF isolates on growth of *Medicago arborea* plants under drought conditions were stimulated by the co-inoculation. This effect was however found to be dependent on the inoculated rhizobial strain and on the AMF isolate suggesting the importance of efficient combinations of AMF, PGPR, and rhizobia in such conditions. Table 14.1 presents some selected examples of drought stress alleviations in legume plants by rhizobia and microbial inoculants (i.e., rhizobacteria or mycorrhizae).

Alleviation of Salt Stress

Salinity in soil is one of the main environmental stress parameters, especially in arid and semiarid

Table 14.1 Selected examples of drought stress alleviation in legume plants by rhizobia and microbial inoculants

Stress	Plant species	Symbiotic bacteria	Microbial inoculant	References
Drought	*Glycine max* (soybean)	*Bradyrhizobium japonicum*	*Glomus mosseae*	Ruiz-Lozano et al. (2001)
	Medicago arborea	*Rhizobium meliloti*	Arbuscular mycorrhizal fungi and *Enterobacter* sp.	Valdenegro et al. (2001)
	Medicago sativa L. cv. Arag6n (Alfalfa)	*R. meliloti*	*Glomus fasciculatum*	Goicoechea et al. (1998)
	Phaseolus vulgaris (common bean)	*Rhizobium tropici*	*Paenibacillus polymyxa*	Figueiredo et al. (2008)
	Vicia faba (faba bean)	*R. leguminosarum* bv. *viciae*	*Azotobacter chroococcum*	Dashadi et al. (2011)

regions, and can severely reduce crop production. Salinization is mainly due to human activities such as excessive tillage or irrigation with saline water. Over 800 million hectares of land throughout the world is salt affected (Rengasamy 2006) with over 70 million ha being farmlands (FAO 2005). The osmotic stress is currently one of the most serious environmental factors in agriculture (Manchanda and Garg 2008). In legume plants, salt toxicity results in reduction in survival and productivity (Shannon 1998; Yuan et al. 2007), whereas actinorhizae, particularly *Casuarina* species, are well known for their relatively high resistance to salt stress conditions (Luard and El-Lakany 1984; Graves and Gallagher 2003; Diagne et al. 2013). Many reports stressed that toxicity is mainly due to plant uptake of salts. Under elevated salt levels, movement of water in response to the water potential gradient results in a water stress. It was stressed that salinity above 50 mM NaCl has deleterious effects on legume plants. It was also reported that nodule formation in N_2-fixing plants is very sensitive to salt stress (Zahran and Sprent 1986; Young et al. 1992). Salinity also affects nitrogenase activity in legumes (Cordovilla et al. 1999) and actinorhizal plants (Sande and Young 1992; Oshone et al. 2013) and consequently reduces N_2 fixation. For instance, in the common bean, high Na^+ and Cl^- concentrations were found in nodules and leaves (Ashraf and Bashir 2003). Nodule formation is even more sensitive than the rhizobia themselves (Zahran 1991). Moreover, in the nodule formation process, it appears that the ability of the rhizobia to infect roots and nodule initiation are more sensitive to salt stress than nodule development (Zahran and Sprent 1986; Craig et al. 1991). Consequently, salinity affects plant performance resulting in reduction in shoot and root growth and legume crop production (Oufdou et al. 2014). In addition, salinity could aggravate this effect through increase in levels of ethylene, a stress hormone that significantly reduces plant growth. Salinity can also indirectly exert deleterious effects by restricting the carbon supply from the host plant as a result of photosynthesis inhibition (Soussi et al. 1999). Other deleterious effects are calcium deficiency, production of reactive oxygen species (ROS), tissue necrosis, and increase in ABA levels that lead to early senescence of older leaves.

Several works emphasized that salt tolerance depends on both plant and bacterial species. For example, most reports agreed that fast-growing rhizobial strains are more tolerant than slow-growing strains (Ghittoni and Bueno 1996). Members of the fast-growing genus *Rhizobium* are generally more tolerant to salts than members of the genus *Bradyrhizobium*. It is also well known that some legumes, e.g., *Sesbania aculeata*, *V. faba*, and *G. max*, are more salt tolerant than other legume species such as *Pisum sativum*. In a given species, tolerance could be variable according to cultivars (Soussi et al. 1999). Resistance to saline conditions is the result of adaptive processes and is mainly due to ability to exclude Na and Cl ions and to prevent the transport of these ions to shoots. They also involve control of ion transport and compartmentation and osmotic solute synthesis and accumulation, which lead to osmotic adjustment. Development of plant symbiont-tolerant systems for cultivation in salt-affected soils is currently considered as necessity. Indeed, several studies are aimed at identifying salt-tolerant genotypes belonging to legumes (Moreno et al. 2000; Sadiki and Rabih 2001) and actinorhizal plants (El-Lakany and Luard 1982; Luard and El-Lakany 1984; Nambiar-Veetil et al. 2011). It is thus well established that N_2-fixing plant symbiosis tolerance to salt stress conditions is variable among species or cultivars. Moreno et al. (2000) found a large genotypic variability in bean cultivars for salt tolerance at the seedling stage and identified some salt-tolerant cultivars with higher performances. Fast-growing salt-tolerant actinorhizal plants showing variable tolerance levels could also be used in salt-affected terrains. Actinorhizal trees such as alders (Graves and Gallagher 2003) and members of the *Casuarinaceae* (*C. equisetifolia* and *C. glauca*) are able to grow under saline conditions (Nambiar-Veetil et al. 2011), whereas *Myrica cerifera* are intolerant to high salinity (Young et al. 1992). Tani and Sasakawa (2006) found that in *C. equisetifolia* plants, which are highly tolerant to salt stress, proline is primarily synthesized as a major compatible solute to adjust the osmotic pressure when Na^+ accumulates in the cells, allowing to maintain cell homeostasis under salt stress conditions. Currently, several members of the *Casuarinaceae* are used in the reclamation of salt-affected lands, and more recent researches focused on tissue culture of salt-tolerant *C. equisetifolia* clones (Liu et al. 2003) and creation of transgenic trees that are more tolerant to salt in tropical regions (Nambiar-Veetil et al. 2011).

Greater performance of N_2-fixing plants under saline conditions seems to be determined mainly by the tolerance of the host plant and its interaction with the bacterial symbionts (Zahran 1991; Tani and Sasakawa 2003). Consequently, inoculation with more tolerant symbiotic bacterial strains with efficient nodulation and N_2 fixation under saline conditions may contribute to the compensation of the salinity-induced plant growth loss (Girgis et al. 1992; Hafeez et al. 1999; Zahran 2001; Tani and Sasakawa 2003). Therefore, the selection of osmotolerant strains of symbiotic bacteria able to efficiently nodulate plants under saline conditions is considered as a great challenge to improve the productivity of N_2-fixing plants. Several salt-tolerant *Rhizobium* and *Frankia* species have been reportedly isolated that could tolerate saline environments (Rai 1983; Hafeez et al. 1999; Zahran 1999; Tani and Sasakawa 2003; Srivastava et al. 2012; Oshone et al. 2013). Among them, some strains of *Rhizobium meliloti* and *R. fredii* (Kassem et al. 1985) and *Sinorhizobium* (*Ensifer*) *arboris* (isolated from *Acacia*) (Zahran et al. 1994) were able to grow at NaCl concentration of more than 300 mM. Similarly, Hafeez et al. (1999) described a *Frankia* strain CcO1 that could tolerate up to 500 mM NaCl (Ec_e of 47 dSm-1), and Tani and Sasakawa (2003) reported nodulation of *C. equisetifolia* with a selected strain of *Frankia* Ceq1 under saline conditions up to 300 mM (28 dSm^{-1}). Accumulation of various solutes was correlated to tolerance of *Rhizobium* (Zahran 1999). Among accumulated compatible solutes are K^+, glutamate, proline, glycine betaine, proline betaine, trehalose, and the dipeptide N-acetylglutaminylglutamine amide. Recently, the role of poly-β-hydroxyl butyrate (PHB) in cell protection of rhizobia during high saline conditions was reported (Arora et al. 2006). Glycine betaine was found to increase more in the salt-tolerant strains of *R. meliloti* than in sensitive strains (Smith et al. 1988). It has also been suggested that rhizobia synthesizing IAA may prevent the deleterious effects of salinity. Bianco and Defez (2009) reported an increased tolerance against salt stress by *M. truncatula* nodulated by a strain of an IAA-overproducing mutant of *Sinorhizobium meliloti*. This effect was accompanied by higher amount of proline and accumulation of antioxidant enzymes in mutant inoculated plants in comparison with plants inoculated with the wild strain. Such inoculation with symbiotic bacteria could help plants to tolerate salt conditions. For instance, in arid saline soils, it was reported that *B. japanicum* S2492 has significantly increased dry weight, plant height, and yield (>35 %) of soybeans (Egamberdiyeva et al. 2004).

The application of other beneficial microbes as inoculants have recently gained interest as beneficial microbes could help plants to mitigate

Table 14.2 Selected examples of salinity stress alleviations in legume plants by combinations of rhizobia and rhizobacteria

Salinity	*Galega officinalis* (goat's rue)	*Rhizobium galegae* sv. *officinalis*	*P. extremorientalis* or *P. trivialis*	Egamberdieva et al. (2013a)
	P. vulgaris (common bean)	*R. tropici* or *R. etli*	*Azospirillum brasilense*	Dardanelli et al. (2008)
	P. vulgaris (common bean)	*R. tropici*	*Chryseobacterium balustinum*	Estevez et al. (2009)
	P. vulgaris (common bean)	*Ensifer fredii*	*C. balustinum*	Estevez et al. (2009)
	Glycine max (soybean)	*B. japonicum*	*Pseudomonas putida*	Egamberdieva et al. (2013b)
	Cicer arietinum (chickpea)	*Rhizobium*	*Azospirillum brasilense*	Hamaoui et al. (2001)

stress conditions. Hamaoui et al. (2001) reported that inoculation with the PGPR *Azospirillum brasilense* significantly enhanced nodulation by native rhizobia in chickpea. This bacterial treatment was shown to significantly enhance growth parameters and to reduce the negative effects on plant growth caused by irrigation with saline water. Dual inoculation including rhizobia and bacterial inoculants could also be used to alleviate salinity stress conditions in N_2-fixing plants (Dardanelli et al. 2008; Estevez et al. 2009; Egamberdieva et al. 2013b). In soybean, Estevez et al. (2009) reported that co-inoculation of the PGPR *Chryseobacterium balustinum* and *Ensifer fredii* led to a significant increase in the number of nodules and root growth under moderate saline conditions. This symbiotic performance was more pronounced than in single inoculation with *E. fredii*. Similar results were found by Egamberdieva et al. (2013b) in soybean co-inoculated by *B. japonicum* and *P. putida*. PGPRs include bacteria with ACC-deaminase activity (Shaharoona et al. 2006; Saravanakumar and Samiyappan 2007; Egamberdieva et al. 2013a). Saravanakumar and Samiyappan (2007) reported that *P. fluorescens* with ACC-deaminase activity led to plant growth promotion and enhanced saline resistance in groundnut plants compared with that inoculated with *Pseudomonas* strains lacking ACC-deaminase activity. PGPR effects could also be mediated by "induced systemic mechanisms" to salt. Indeed, through mechanisms such as osmoregulation and other metabolic adaptations, PGPR could ameliorate the tolerance of plants to saline conditions (Egamberdiyeva and Islam 2008). Phenolic compounds are well known as resistance mechanisms, and their elicitation by PGPRs was reported in many research works as accompanying salinity alleviations in co-inoculated plants (Dardanelli et al. 2008, 2012). Enhanced synthesis of flavonoids (known as phenolic substances involved in *Rhizobium* attraction), following PGPR inoculation, was reported by Dardanelli et al. (2008). They showed that co-inoculation of *A. brasilense* and *R. tropici* strain CIAT899 or *R. etli* ISP42 resulted in enhanced nitrogen fixation concomitant to production of a variety of flavonoid species in *P. vulgaris* cv. *Negro jamapa* under salt stress. This effect is linked to an efficient activation of nod factor genes under such conditions. It was also reported that most of PGPRs could produce EPS able to bind Na^+. Han and Lee (2005) observed that inoculation of EPS-producing strain resulted in a decrease in Na^+ content of soybean grown under saline conditions. The production of osmoprotectants, a kind of organic compounds under inhibitory osmolarities, by either rhizobia or beneficial bacteria that are subsequently taken up by plants, is also reported by several authors. Table 14.2 presents selected examples of salinity stress alleviations in legume plants by combinations of rhizobia and rhizobacteria.

Tripartite association involving ectomycorrhizal and endomycorrhizal fungi was also reported as a powerful tool to alleviate saline stress. Colonization by AMF can help plants to cope with salinity stresses (Ruiz-Lozano 2003) by compensating for its negative effects on nodulation and N_2 fixation (Ruiz-Lozano and Azcón 1993). It was reported for instance that dual inoculation with AM and rhizobia decreased the deleterious effects of sulfate salinity on plant growth and on P and N accumulation in *Lathyrus sativus* (Jin et al. 2010). Zhong et al. (2010) demonstrated that the ectomycorrhizal fungus (EMF) *P. tinctorius* increased *Casuarina* tolerance to salt condition. One mechanism of osmotic stress tolerance induced by AMF colonization was shown by Aroca et al. (2007) in bean (*P. vulgaris*) for which an increase in root hydraulic conductivity of the host plants was observed. Reddell et al. (1986b) observed that dual inoculation of *Frankia* and the EMF *Suillus* excludes salt and toxic metals from metabolic pathways by compartmentalization in vacuoles and cell walls.

Alleviation of Heavy Metal Toxicity in Contaminated Soils

Several soil systems are contaminated by mineral compounds such as heavy metals or by organic compounds of various origins. Many of these compounds originate from industrial and intensive agriculture activities and discharges of untreated or partially treated wastewater. It is well known that heavy metals even in traces are toxic against organisms (Giller et al. 2009; Nonnoi et al. 2012) including humans and result in major health problems. The most common heavy metal contaminants are cadmium (Cd), chromium (Cr), cupper (Cu), mercury (Hg), lead (Pb), nickel (Ni), manganese (Mn), and zinc (Zn). The elevated levels of heavy metals in the environment are excessively absorbed by roots and translocated to shoots, leading to impaired metabolism and reduction in plant growth (Rodríguez-Llorente et al. 2010; Perez-Palacios et al. 2013). For instance, it was reported that in legume plants, Cd contamination could result in a decreased uptake of nutrients, inhibition of various enzyme activities, and induction of oxidative stress (Sandalio et al. 2001) or inhibition of chlorophyll synthesis and photosynthesis (Padmaja et al. 1990). For actinorhizal plants, inhibition of growth, nodulation, and nitrogenase activity by heavy metals such as Cd (Hensley and Carpenter 1987), Cu (Fessenden and Sutherland 1979), Ni (Wheeler et al. 2001), and Zn (Cusato et al. 2007) have been reported. It is currently accepted that the sensitivity to a given heavy metal is variable according to plant species. In *Alnus crispa*, Cu concentration of 60 mg kg^{-1} caused a reduction of nodule dry weight, while nodulation was totally inhibited at 100–150 mg kg^{-1}. However, Lorenc-Pluciñska et al. (2013) described two species of *Alnus*, *A. incana* and *A. glutinosa* tolerant to Cu concentrations higher than 1,500 mg kg^{-1} of soil. Unfortunately, heavy metals cannot be destroyed biologically. The physical-chemical methods to remediate heavy metal pollution are not suitable for practical applications. In addition to their high costs, they led to a large destruction of soil structure and fertility and soil properties. Thus, biological remediation presents the advantage to be of low-cost, effective, eco-friendly, and socially accepted strategy.

Nitrogen-fixing plants could play a key role in the detoxification of heavy metals (Lorenc-Pluciñska et al. 2013; Vivas et al. 2003a, b). Among strategies that should be adopted prior to introduction of N_2-fixing plants in contaminated sites is the selection of tolerant plant species and/or bacterial symbionts. Actinorhizal plants such as alders have been used for the reclamation of sites contaminated with toxic levels of trace elements (Lefrancois et al. 2010; Babu et al. 2013; Lorenc-Pluciñska et al. 2013). Lorenc-Pluciñska et al. (2013) reported that two actinorhizal species *A. incana* and *A. glutinosa* grow well in heavily polluted soil. In spite of accumulation of toxic metals in their roots and nodules and a decreased rate of N_2 fixation, concentrations of Cu, Zn, Cd, and Pb in leaves of both alder species were within normal ranges. In fact, heavy metals are accumulated in roots of these species, and only small proportions are transferred to aboveground parts, including leaves. These species of alders can be recommended for the revegetation of areas polluted with phytotoxic concentrations of trace metals. However, these results were obtained only from short-term pot trials and

Table 14.3 Selected examples of heavy metal bioremediation by legume and actinorhizal plants in association with microbial inoculants

Plant	Symbiont bacterium	Tolerant microbial inoculant species	Heavy metal contamination[a]	Reference
Alnus glutinosa (alder)	Soil as *Frankia* inoculum		Cu	Whitbread-Abrutat (1997)
A. glutinosa (alder)	*Frankia* (Ni tolerant)		Ni	Wheeler et al. (2001)
A. glutinosa and *Alnus incana* (tolerant plants)	*Frankia*		Cu and Pb	Lorenc-Pluciñska et al. (2013)
Alnus firma (alder)		*Bacillus thuringiensis* (endophyte)	Pb, Zn, As, Cd, Cu, and Ni	Babu et al. (2013)
Pisum sativum (pea)	*Rhizobium* (metal tolerant)		Ni, Zn	Wani et al. (2008)
Trifolium (clover)	*Rhizobium*	PGPRs and the mycorrhizal fungus *Glomus mosseae*	Zn	Vivas et al. (2006a)
Lupinus luteus (lupine)	*Bradyrhizobium*	*Pseudomonas* and *Ochrobactrum cytisi*	Cd, Cu, and Pb	Dary et al. (2010)
Pisum sativum (pea)		*Pseudomonas brassicacearum* and *P. marginalis*	Cd	Safronova et al. (2006)
Trifolium pratense (red clover)	*Rhizobium*	*Brevibacillus* sp.	Pb	Vivas et al. (2003a)
Trifolium repens (white clover)	*Rhizobium*	*G. mosseae* (mycorrhizae) and *Brevibacillus* sp.	Cd	Vivas et al. (2003b)
T. repens (white clover)	*Rhizobium trifolii*	*Brevibacillus brevis* and *Glomus mosseae* (AM fungus)	Ni	Vivas et al. (2006b)
Vigna radiata (mung bean)		*P. putida*	Cd, Pb	Tripathi et al. (2005)
V. radiata (mung bean)	*Bradyrhizobium* (metal tolerant)		Ni, Zn	Wani et al. (2007b)

[a] *Cd* cadmium, *Cu* copper, *Mn* manganese, *Ni* nickel, *Pb* lead, *Zn* zinc

require further field validation. *Frankia* and rhizobia strains were also consequently screened for their tolerance to heavy metals (Richards et al. 2002; Carrasco et al. 2005; Dary et al. 2010; Bélanger et al. 2011). Heavy metal-tolerant *Frankia* could allow actinorhizal plants to colonize contaminated terrains, and their spectra of tolerance were reported to vary depending on strains tested (Richards et al. 2002; Bélanger et al. 2011). Richards et al. (2002) investigated the tolerance of 12 *Frankia* strains to a wide variety of heavy metals. While all of the 12 strains were sensitive to low concentrations (<0.5 mM) of Ag^+, AsO_2^-, Cd_2^+, SbO_2^-, and Ni_2^+, most were less sensitive to Pb^{2+} (6–8 mM), CrO_4^{2-} (1.0–1.75 mM), AsO_4^{3-} (>50 mM), and SeO_2^{2-} (1.5–3.5 mM). These authors also found that four strains are resistant to elevated levels of Cu^{2+} (up to 20 mM for strain CN_3). Carrasco et al. (2005) and Dary et al. (2010) have isolated effective rhizobia resistant to heavy metals from polluted soils in Spain. These strains could be exploited for revegetation programs of many other polluted areas.

Other microbial inoculants such as PGPRs or mycorrhizae could act as helper microbia in heavy metal-contaminated areas. Table 14.3 presents selected examples of heavy metal bioremediation by legume and actinorhizal plants in combination with microbial inoculants. PGPRs as bioinoculants proved their utilities to alleviate heavy metal stress conditions and were used as a tool for rhizoremediation in contaminated soils. For instance, elevated levels of heavy metals interfere with P uptake by plants. By using P-solubilizing PGPRs, this effect could be compensated (Gupta et al. 2001). Rhizobacteria have been shown to possess several characteristics that can alter heavy metal bioavailability through acidification of the microenvironment, releasing of chelating substances, and modifying heavy metal redox potential (Abou-Shanab et al. 2003). One of the major traits of these bacteria is the sequestration and accumulation of heavy metals (Richards et al. 2002; Gupta et al. 2004; Lee et al. 2006). It was suggested to be the role of polysaccharides or proteins as potential binding sites for these heavy metals (Richards et al. 2002). Works by Vivas et al. (2003a, b, 2006a) showed the utility of bioinoculants for alleviation of heavy metals as a strategy to grow legumes in contaminated soils. In these studies, bacteria with the ability to survive and colonize the rhizosphere were used in Zn-, Pb-, or Cd-contaminated soils. Clover (*Trifolium*) growing in soil contaminated with Cd inoculated with a Cd-adapted autochthonous PGPR, *Brevibacillus*, resulted in growth-promoting effects and a reduction in Cd transfer from soil to plants (Vivas et al. 2003b). A significant enhancement of nitrogen and phosphorus accumulations and nodule formation concomitant to decrease the amount of Pb absorbed by plants was also reported (Vivas et al. 2003a). Dary et al. (2010) used *Lupinus luteus* and a consortium of metal-resistant PGPR co-inoculated (including *Bradyrhizobium*, *Pseudomonas* sp., and *Ochrobactrum cytisi*) for reclamation of multi-metal-contaminated soil. They observed root accumulation of heavy metals concomitant with increment in lupine growth promotion effect when compared to *Lupinus* inoculated with *Bradyrhizobium* alone. This mixture also succeeded to reduce plant toxicity symptoms and metal accumulation in both shoots and roots. The alleviating effect could also be due to more intimate bacteria-plant relationships such as those ensured by endophytic PGPRs. Babu et al. (2013) reported a significant enhancement of heavy metal phytoremediation by *Alnus firma* with an endophytic strain of *Bacillus thuringiensis*.

Tripartite associations involving mycorrhizae were also used as a tool to alleviate heavy metal stress and for bioremediation (Díaz et al. 1996; Roy et al. 2007; Karimi et al. 2011). Mycorrhizae can help contribute to phytoremediation by their ability to sequester heavy metals through the production of chelates or by absorption. Consequently, less heavy metals are translocated to plant shoots and leaves. AMF were used in association with *C. equisetifolia* to rehabilitate heavy metal-polluted soils (Karimi et al. 2011). Tripartite association established by *Alnus*, mycorrhizae-*Frankia*, was reviewed by Roy et al. (2007). In their work aiming to provide *Trifolium pretense* growth promotion and lead toxicity reduction by *Brevibacillus*, Vivas et al. (2003a) reported that association of bacteria and mycorrhizae could interact synergistically resulting in significant positive effects. They reported that mycorrhization of AM fungi was positively stimulated by the PGPR bacterium resulting in significant growth under Pb contamination. All these associations could provide a basis for selecting nodulating bacteria associated with other microbial inoculants that may have applications in formulating appropriate inocula, effective when used for phytoremediation and revegetation of contaminated soils, but also could be a tenable strategy for improving growth while avoiding high input of chemical fertilizers in such sites.

Conclusions

Enhancing application and management of biologically fixed N could result in very important environmental and economic benefits for the humanity. N_2-fixing plants have an exceptional ability to form symbiotic association with rhizobia and *Frankia*. Hence, they have been used on a broad scale so as to improve the nitrogen status of soils. Symbiosis could represent a key factor in abiotic stress conditions, and many researchers stressed the importance of both host specificity and environmental variations in determining patterns of symbiont efficiencies under such conditions. Therefore, the selection of more tolerant plant/symbionts has been one of the multiple approaches. A valuable selection approach could be performed by targeting multitolerant rhizobia. Recently, Elboutahiri et al. (2010) isolated rhizobia from drought and salt-affected regions of Morocco that were tolerant to salinity, water stress, high temperature, acidity, and heavy metals. However, bacterial strain–plant genotype combination should be considered for selecting the most adapted microbe-plant combinations to stress conditions. On the other hand, the application of microbial inoculants to N_2-fixing plants is one of the most interesting eco-friendly strategies for agriculture and forestry sustainability. Plant inoculation with more tolerant strains of these bacteria could contribute to their tolerance to stress conditions (Figueiredo et al. 2008). In comparison with legume plants, the use and exploitation of the beneficial effects of PGPRs to actinorhizal plants is still a rarely used approach by researchers (Knowlton and Dawson 1983; Probanza et al. 1996; Rojas et al. 2002). Very little information is available on the effectiveness of PGPRs as bioinoculants for actinorhizal plants under stress conditions. This topic needs to be further investigated. Unexplored fluorescent *Pseudomonas* and other PGPR species with beneficial properties (e.g., phytohormone or siderophore production) (Arshad et al. 2008; Figueiredo et al. 2008) could contribute to more tolerance to abiotic stress conditions. There is in fact a need to explore more number of effective combinations of PGPR and *Frankia*. Also, the application of helper bacteria or PGPRs in association with actinorhizal plants for bioremediation of eroded or degraded areas following industrial waste pollution is another aim to be focused on.

In addition, tripartite associations involving mycorrhizae seem to be promising for sustainable agriculture and forestry based on N_2-fixing plants. To help actinorhizal plants to withstand abiotic stresses, mostly used bioinoculants in tripartite associations are mycorrhizae. In researches focusing on tripartite associations, one of the most important technical traits is the selection of the appropriate microbial inoculants (mycorrhizae and PGPRs). In addition, the use of compatible multiple microbial consortia including bacterial symbionts and fungal symbionts acting synergistically, providing various beneficial effects, is also a powerful strategic tool (Requena et al. 1997; Muthukumar and Udaiyan 2010). In general, the use of tripartite and tetrapartite associations is largely studied and applied in both legumes and actinorhizal plants. However, the use of these associations should be approached cautiously in relation to efficient combinations, particularly those involving mutual interactions between the N_2-fixing bacterium and the bioinoculant (rhizobacterium or mycorrhizae) (Requena et al. 1997; Gardner 1986; Ruiz-Lozano et al. 2001; Valdenegro et al. 2001; Remans et al. 2007).

In addition to classical rhizobia and *Frankia* strains, another way is to study and evaluate other endophytic bacteria potentially found in nodules (nodule inhabitants) in experiments aiming to alleviate abiotic stress effects. Recently, an actinobacterium, *Micromonospora*, was found in actinorhizal and legume nodules as holding nitrogenase activity and nifH-like gene sequences demonstrating its potential role in N_2 fixation (Valdès et al. 2005; Trujillo et al. 2006, 2010; Carro et al. 2012). To our knowledge, most works dealing with abiotic stress tolerance are aimed to select only known symbiotic bacteria, i.e., rhizobia and *Frankia*, and very little work has addressed the role of other nodule-colonizing bacteria. Special attention should be drawn on isolating these bacteria, particularly those tolerant to stress conditions. Their use as bioinoculants in combination with rhizobia or *Frankia* strains could help to emerge a new dimension into the application of microbial inoculants to N_2-fixing plants under abiotic stress conditions.

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