Chapter 4
Role of Root Nodule Bacteria in Improving Soil Fertility and Growth Attributes of Leguminous Plants Under Arid and Semiarid Environments

S.K. Singh, Rakesh Pathak, and Anjly Pancholy

4.1 Introduction

In the late seventeenth century, tools of modern science began to reveal the secrets of plant nutrition, and nitrogen, phosphorus, and potash were identified as essential nutrients for plant growth. The soil was assumed to be the source of phosphorus and potash, but it has been a matter of argument up to nineteenth century that either plants absorb nitrogen from the air or extract it from the soil. Schultz-Lupitz (1881) revealed that the plants required more nitrogen than any other soil nutrient and leguminous plants were able to accumulate large amounts of nitrogen. The nodules attached to the roots of leguminous plants were responsible for converting nitrogen gas of the atmosphere into soluble nitrogenous compounds (Hellriegel 1887; Hellriegel and Wilfarth 1888). Presently, this marvelous piece of natural chemistry is known as the symbiotic association between leguminous plants and a soil bacterium. Plant–bacteria interactions in the rhizosphere are the determinants of plant health and soil fertility (Hayat et al. 2010). It is now well established that the leguminous plants enhance soil fertility and non-leguminous plants deplete it.

The intensive use of chemical fertilizers has degraded soil fertility resulting in severe health and environmental hazards such as soil erosion, water contamination, pesticide poisoning, falling groundwater table, water logging, and depletion of biodiversity. The plant–microbe interactions in the rhizosphere play an essential role in transformation, mobilization, solubilization of nutrients, and uptake of essential nutrients by plants. The soil bacteria supply nutrients to crops, stimulate plant growth, control or inhibit the activity of plant pathogens, improve soil structure, bioaccumulation, or microbial leaching of inorganics, etc. (Ehrlich 1990), and have been used in crop production for decades (Davison 1988). It has

---

S.K. Singh (✉) • R. Pathak • A. Pancholy
Central Arid Zone Research Institute, Jodhpur, Rajasthan 342 003, India
e-mail: sksingh1111@hotmail.com

A.P. Hansen et al. (eds.), *Rhizobium Biology and Biotechnology*, Soil Biology 50,
DOI 10.1007/978-3-319-64982-5_4

also been used in soil for the mineralization of organic pollutants known as bioremediation of polluted soils (Burd et al. 2000; Zhuang et al. 2007; Zaidi et al. 2008).

This biological approach is becoming more popular as an alternative to chemical fertilizers for improving crop yield, and the application of plant growth-promoting rhizobia (PGPR) has found a vital place in the sustainable agriculture system (Shoebitz et al. 2009). Suhag (2016) advocated replacement of chemical fertilizers by biofertilizers and suggested that the use of biofertilizers naturally activates microorganisms, restores soil natural fertility, and protects it against drought and soil-borne diseases and thereby stimulates plant growth. The PGPRs are also termed as plant health-promoting rhizobacteria or nodule-promoting rhizobacteria. PGPR can be either symbiotic bacteria or free-living rhizobacteria on the basis of their relationships with the plants (Khan 2005). They can be divided into two groups according to their residing sites, bacteria living inside the plant cell, producing nodules (iPGPR or symbiotic bacteria), and bacteria living outside the plant cell, which do not produce nodules (ePGPR or free-living rhizobacteria) promoting plant growth (Gray and Smith 2005).

Rhizobia are the best known iPGPR and produce nodules in leguminous plants. The bacteria that nodulate legumes mainly belong to Proteobacteria families: Rhizobiaceae (*Rhizobium; Ensifer syn. Sinorhizobium*), Bradyrhizobiaceae (*Bradyrhizobium*), Hyphomicrobiaceae (*Azorhizobium*; *Devosia*), Methylobacteriaceae (*Methylobacterium*), Brucellaceae (*Ochrobactrum*), Phyllobacteriaceae (*Mesorhizobium*; *Phyllobacterium*), and Burkholderiaceae (*Burkholderia*; *Cupriavidus*).

The PGPRs function in different ways (Glick 2001); they synthesize compounds (Zahir et al. 2004), enable the uptake of nutrients (Çakmakçi et al. 2007), and decrease or prevent the plant diseases (Saravanakumar et al. 2008). Rhizobia inoculation has been reported to activate host genes involved in the production of phenolic compounds/phytoalexins, and higher levels of these compounds benefit the plant by restricting disease development (El Hadrami et al. 2007). The antagonistic and plant growth promotion properties of *R. leguminosarum* b.v. *phaseoli* against root rot caused by *F. solani* f.s. *phaseoli* in bean (Buonassisi et al. 1986) and *R. japonicum* against root rot diseases caused by *F. solani* and *M. phaseolina* in soybean have been demonstrated (Al-Ani et al. 2012).

The mechanism for enhancement of plant growth and yield by the PGPR may be due to the ability to produce an enzyme (1-aminocyclopropane-1-carboxylate) (Li et al. 2000), hormones like indole acetic acid (Patten and Glick 2002), abscisic acid (Dobbelaere et al. 2003), gibberellic acid, and cytokinins (Dey et al. 2004); symbiotic nitrogen fixation (Kennedy et al. 2004); antagonism against phytopathogenic bacteria by producing siderophores, β-1,3-glucanase, chitinases, antibiotic, fluorescent pigment, and cyanide (Glick and Pasternak 2003); solubilization and mineralization of nutrients (Richardson 2001; Banerjee and Yasmin 2002); enhancing resistance to drought (Alvarez et al. 1996), salinity, waterlogging (Saleem et al. 2007), and oxidative stress (Stajner et al. 1997); and production of vitamin B, niacin, pantothenic acid, thiamine, riboflavin, and biotin (Sierra et al. 1999; Revillas et al. 2000).

The root nodule bacteria are gram-negative bacteria and belong to a diverse group of soil-inhabiting bacteria (O'Hara 2001). Probably, all the agricultural soils contain some bacteria capable of nodulating some legumes. However, all these bacteria may not be able to nodulate legumes or if they can they may not form an effective symbiosis. It is very common situation when new legumes are introduced to new lands (O'Hara et al. 2002). Howieson et al. (2000b) suggested probable situations including more and less number of nodules on the uninoculated legume and an effective, competitive inoculant strain of root nodule bacteria adapted to the soil conditions when a new legume is introduced to new lands. The uninoculated legume may form effective nodules in good quantity due to the presence of a large population of effective root nodule bacteria. The formation of less number of nodules on uninoculated legumes indicates absence or little presence of population of root nodule bacteria in the soil that are able to nodulate the particular host legume. These two situations revealed that the competitive inoculant strain of root nodule bacteria should be selected that are adapted to the soil conditions (O'Hara et al. 2002).

The productivity of a legume crop depends on the effective *Rhizobium* population available in the soil and well-nodulated plants. At the early stage of seedling, generally higher nodules and nodulation are found. The large and pink color nodules are considered the effective and better nodules, whereas small and white nodules are indicative of a poor symbiosis (Duseja and Shrivastav 2015). This chapter covers the role of root nodule bacteria in improving soil fertility and growth attributes of leguminous plants under arid and semiarid environments via direct and indirect mechanisms.

4.2 Legumes

Arid and semiarid areas cover about 45% of the Earth's land surface (Schimel 2010). Since nodulation and N fixation use significant amounts of plant photosynthate, it may be inferred that nodulation is a useful attribute in a N-poor environment (Sprent and Gehlot 2010). The plant family Leguminosae (Fabaceae) is the third largest family in the Angiosperms dominating range of arid and extreme ecosystems. They are of ecological importance in sand dune stabilization, soil fixation, and revegetation of semiarid and arid ecosystems (Rodríguez-Echeverría et al. 2012). Over 100 agriculturally important legumes in symbiotic associations with rhizobia contribute nearly half of the annual quantity of biological nitrogen fixation (BNF) entering soil ecosystems and they provide an easy and inexpensive way to enhance soil fertility and agricultural productivity (Zahran 2011).

Legumes bear seeds in pods and fix the atmospheric nitrogen with the help of bacteria in their root nodules (Vance 1997). They are rich in protein and more than 16,000 species of legumes including herbs, shrubs, and trees are known worldwide out of which only about 200 are cultivated. The legumes improve soil quality through favorable effects on soil biological, chemical, and physical conditions (Howieson et al. 2000a). They have long been recognized and valued as soil-building crops. The properly managed legumes enhance the nitrogen supplying power of soils, increase the soil reserves of organic matter, stimulate the soil biological activity, improve soil structure, reduce soil erosion, increase soil aeration, improve soil water-holding capacity, and make the soil easier to till (Graham and Vance 2000).

Generally, soils contain large *Rhizobium* populations which readily nodulate common leguminous plants and trees, but there are situations in which the production of well-nodulated plants is difficult. Some crops need different Rhizobial species which may not be present in all soils or the rhizobia present in the soil fix little nitrogen. Such problems can be overcome by mixing specially selected Rhizobium cultures before sowing. *Acacia senegal* and *Prosopis cineraria* are the most important dryland resources of Western Rajasthan desert ecosystem (Tewari et al. 1998; Jindal et al. 2000) among leguminous arid zone tree species. *A. senegal* is highly drought-tolerant multipurpose tree species and is an important forest resource of gum arabic, fuel wood, human food, and fodder for livestock (Aoki et al. 2007). Whereas *P. cineraria* grows very well in dryland agroforestry systems and plays an important role in controlling soil erosion, sand dune stabilization, improving soil fertility, providing fuel energy resources, supplying feed and forage for grazing animals, firewood, furnishing timber and furniture wood, and supplementing food for humans (Tewari et al. 1998; Manzano and Navar 2000; Zare et al. 2011). Clusterbean, cowpea, moth bean, and horse gram are important annual legumes grown in arid and semiarid region (Chhillar 2009).

4.3 Factor Affecting Nodulation

There are several factors including moisture stress, salinity, unfavorable soil pH, nutrient deficiency, mineral toxicity, temperature extremes, inadequate photosynthesis, plant diseases, trace element deficiencies, etc., which inhibit nodulation and impose limitations on the vigor of the host legume (Brockwell et al. 1995). The effects of salt stress on nodulation and nitrogen fixation of legumes have been examined in several crops including legumes (Delgado et al. 1994; Nithyakalyani et al. 2016; Rao et al. 2002). The water stress modifies the rhizobial cells and leads to a reduction in infection and nodulation of legumes, while high soil temperatures delay nodulation or restrict it to the subsurface parts (Graham 1992). Nodulation of soybean was markedly inhibited at higher temperatures (Chibeba et al. 2015). Piha and Munnus (1987) reported that bean nodules formed at 35 °C were small and had low specific nitrogenase activity. The soils of $\text{pH} < 5$ make some rhizobia unable to persist resulting in failure of nodulation in the legumes (Bayoumi et al. 1995). Taylor et al. (1991) reported that acidity had more severe effects on rhizobial multiplication. The low-medium pH (<4.5) affected the number of nodules, the nitrogenase activity, the nodule ultrastructure, and the fresh and dry weights of nodules to a greater extent (Vassileva et al. 1997), while in the acidic soils with pH of >5.0, where heavy metal activity is relevant, the presence of available aluminum inhibits nodulation (Bordeleau and Prevost 1994). Both pH (4.5) and aluminum (100 mM) caused delays in nodulation of *Vigna unguiculata*, particularly at low Ca^{2+} levels (0.3 mM), while at a high calcium concentration (3.0 mM), nodulation was improved (Hohenberg and Munns 1984).

Soares et al. (2014) assessed the symbiotic efficiency of symbiotic nitrogen-fixing bacteria with *V. unguiculata* and their tolerance to pH and aluminum. They contrarily reported that three strains (UFLA03-164, UFLA03-153, and UFLA03-154) yielded higher values for dry weight production of the aerial part. These strains grow at varied pH levels of 5, 6, and 6.8 and at high aluminum concentration levels. While strains UFLA03-84, UFLA03-153, and UFLA03-164 can tolerate up to 20 $mmol_c\ dm^{-3}$ of Al^{+3} (Soares et al. 2014). The nodulation, growth, and shoot nitrogen in some grain legumes were adversely affected on addition of bicarbonate (Tang and Thomson 1996). Nodulation of *Arachis hypogaea* was also inhibited when plants grew in nutrient solution containing carbonate (Tang et al. 1998). Similarly, heavy metals had adverse effects on nodulation and nitrogen fixation of legumes (Klimek-Kopyra et al. 2015).

Nitrogen fertilization is needed as starter nitrogen to achieve a substantial yield of legumes at the stage when the symbiotic nitrogen fixation is unable to provide enough nitrogen (Buttery and Dirks 1987), but excess amounts of soluble nitrogen in the soil generally restrict or reduce nodulation and nitrogen fixation in the legumes (Afza et al. 1987). Thies et al. (1995) reported suppressed nodulation with the application of urea (90 kg of nitrogen ha^{-1}) in soybean plants. Similarly, the root system of *Cajanus cajan* was poorly developed after application of nitrogen fertilizer (up to 60 kg of N nitrogen ha^{-1}) and adversely affected the nodule number, nitrogenase activity, nodule dry weight, shoot weight, and root and shoot nitrogen (Kaushik et al. 1995). Herbicides have also been reported to affect the growth of *B. japonicum* under in vitro conditions (Mallik and Tesfai 1993) and reduced the nodulation of soybeans under greenhouse conditions (Mallik and Tesfai 1985). However, some of the herbicides including sethoxydim, alachlor, fluazifop butyl, and metolachlor did not have detrimental effects on nitrogen fixation or seed yields when added at the recommended rates for weed control in soybean (Kucey et al. 1988).

The climatic conditions of arid and semiarid regions are often characterized by hot, dry summers, subhumid monsoon, and cold dry winter. The climatic conditions in this region restrict the buildup of soil organic matter and soils are generally deficient in nitrogen (Kackar et al. 1990). The soil of region is sandy loam with $pH > 8.1$ and low nutrient levels, with 0.23% organic carbon, 0.03% nitrogen, and 0.02% phosphorus (Dhir 1984). The rhizobial population can survive under limited moisture levels of desert soils and can perform effective nodulation of legumes growing therein (Jenkins et al. 1987, 1989; Tate 1995), but their densities are lowest under the most desiccated conditions and it may increase as the moisture stress is relieved (Waldon et al. 1989).

The major problem is the increase in salinity levels of the soil that causes reduction in plant growth and yield in irrigated arid and semiarid regions (Parida and Das 2005). Symbiotic effectiveness depends on the specific combination of compatible legume and rhizobium under the saline conditions (Faghire et al. 2013). Therefore, identification and application of salinity-tolerant rhizobia in legume cropping area helps in the formation of effective nodules and efficient nitrogen fixation. Plant productivity is considerably reduced due to osmotic inhibition of water uptake by roots or specific ion effects (Mayak et al. 2004). To improve plant growth under stress conditions, it is important to improve salt stress tolerance in crops. Zohra et al. (2016) characterized slow-growing rhizobial strains isolated from *Retama monosperma* root nodules from Algeria resistant to alkaline pH up to 9 and salinity equal to 2% (w/v) NaCl. Younesi et al. (2013) reported positive response of *Pseudomonas fluorescence* and *Rhizobium meliloti* co-inoculation on nodulation and mineral nutrient contents in alfalfa under salinity stress conditions. Sobti et al. (2015) showed that the rhizobia isolated from the desert soils are able to survive, grow, and effectively nodulate their leguminous hosts even at high salt concentrations. The tolerance to high levels of salinity and the survival and persistence in severe and harsh desert conditions make these rhizobia highly valuable inocula to improve productivity of the leguminous plants cultivated under extreme environments.

4.4 Inoculation

There are two basic components in a good inoculant including dense Rhizobium population that can produce higher number of nodules and can fix appreciable amount of nitrogen and the inoculant should have good shelf-life so that can be easily packaged and distributed. It is essential that the inoculum should produce an economic response in the field. Several authors have shown that it is possible to improve the growth of leguminous trees by inoculation with effective rhizobia (Wolde-Meskel and Sinclair 1998; Bogino et al. 2006; Maia and Scotti 2010).

Singh et al. (2011) molecularly characterized the diverse groups of plant growth-promoting rhizobacteria (PGPRs) in the rhizosphere and root nodules of native *A. senegal* and *P. cineraria* of western Rajasthan by direct sequencing of *16S rRNA* gene to detect genetic diversity in field populations of PGPRs and reported that the treatments with *Bacillus licheniformis* or *S. kostiense,* either inoculated individually or as co-inoculants, had positive effect on phenotypic traits of germination. Chagas-Junior et al. (2012) carried out a greenhouse experiment to evaluate the effect of natural nodulation in the development of Pacara Earpod Tree (*Enterolobium contortisiliquum*) and Leucaena (*Leucaena leucocephala*) using soil samples of woods, cultivated areas, and degraded areas. They observed better nodulation occurring in soil cultivation, providing a higher accumulation of biomass in both species.

In spite of inoculation with certain rhizobial cultures, no nodulation was reported because the strains used for inoculation became exopolysaccharide deficient due to mutation or any unspecified reason (van Rhijn et al. 2001). The success and efficiency of inoculants for agricultural crops are affected by various factors, viz., ability of rhizobia to colonize plant roots, the exudation by plant roots, and the soil health. The root colonization efficiency of the rhizobia is closely associated with microbial competition and survival in the soil, as well as with the modulation of the expression of several genes and communication between cells (Meneses et al. 2011; Alquéres et al. 2013; Beauregard et al. 2013). Soil health is another important factor that affects the inoculation efficiency, due to several characteristics such as soil type, nutrient pool and toxic metal concentrations, soil moisture, microbial diversity, and soil disturbances caused by management practices. The roots of plant secrete a wide range of compounds that interfere with the plant–bacteria interaction and react to different environmental conditions. It is considered an important factor in the efficiency of the inoculants (Cai et al. 2012; Carvalhais et al. 2013).

4.5 Role of Root-Nodulating Bacteria in Soil Health and Plant Growth

The root-nodulating bacteria have the ability to inhibit certain soil-borne plant pathogens along with nitrogen fixation. Due to their dual role in plant growth promotion and disease control, they have become the valuable part of sustainable agriculture (Mallesh 2008). The antagonistic and plant growth promotion properties of *R. leguminosarum* b.v. *phaseoli* against root rot caused by *F. solani* f.s. *phaseoli* in bean (Buonassisi et al. 1986) and *R. japonicum* against root rot diseases caused by *F. solani* and *M. phaseolina* in soybean have been demonstrated (Al-Ani et al. 2012).

Nitrogen is essential for the synthesis of enzymes, proteins, chlorophyll, DNA, and RNA in the cell; hence, it is very important for plant growth and production of food and feed. It is provided through rhizobial bacteroids for nodulating legumes. The BNF accounts for 65% of the total nitrogen utilized in agriculture (Matiru and Dakora 2004). The biochemical reactions of BNF take place mainly through symbiotic association of nitrogen-fixing microorganisms with legumes that converts atmospheric elemental nitrogen into ammonia (Shiferaw et al. 2004).

The nitrogen-fixing genes of Rhizobia and regulation of nitrogenase activity in the nodules depend on the genes of host plant genotype compatibility which determines symbiotic effectiveness. Cross talk between plant host and rhizobia from recognition of partners, through functional nodule formation and nitrogen reduction, determines effective symbiosis (Long and Ehrhardt 1989). The host plant responds chemotactically to flavonoid molecules and induces the expression of nodulation (nod) genes in Rhizobia that in turn respond to the lipo-chitooligosaccharide signals and trigger mitotic cell division in roots, leading to nodule formation (Dakora 2003; Lhuissier et al. 2001; Matiru and Dakora 2004). It is a molecular dialogue between the host plant and a compatible strain of *Rhizobium* which serves as an initiative for the development of nodules (Murray et al. 2007). Besides, there are a number of factors including host–microsymbiont compatibility, physicochemical conditions of the soil, and the presence of both known and unknown biomolecules such as flavonoids, polysaccharides, and hormones that affect the nodulation on legume roots (Zafar-ul-Hye et al. 2007).

The nitrogen fixed by Rhizobia in legumes also benefits the associated non-leguminous plants growing in intercrops through direct transfer of biologically fixed nitrogen (Snapp et al. 1998) or to subsequent crops grown in crop rotation (Hayat et al. 2008a, b). Besides nitrogen fixation, species of *Rhizobium* and *Bradyrhizobium* produce abscisic acids, auxins, cytokinins, lumichrome, riboflavin, lipo-chitooligosaccharides, and vitamins that enhance plant growth (Hayat and Ali 2004; Hayat et al. 2008a, b). In many low-input agriculture systems including grasses, crops depend on the nitrogen fixed by the legume counterparts for their nitrogen requirements and protein (Hayat and Ali 2010). Other plant growth-promoting traits of Rhizobia and *Bradyrhizobia* include phytohormone production (Arshad and Frankenberger 1998), siderophore release (Jadhav et al. 1994), solubilization of inorganic phosphorus (Chabot et al. 1996a), and antagonism against plant pathogenic microorganisms (Ehteshamul-Haque and Ghaffar 1993). Application of *B. japonicum* to the crop of radish increased about 15% plant dry matter (Antoun et al. 1998). Chabot et al. (1996b) applied bioluminescence from *Rhizobium leguminosarum* bv. *phaseoli* strain having lux genes to visualize in situ colonization of roots by Rhizobia in maize. Further, Yanni et al. (2001) also reported similar findings on maize root colonization and infection by Rhizobia.

The in vitro studies on Rhizobia-infected cereal roots revealed that Rhizobia are brought into closer contact with cereal roots during legume–cereal rotations and/or mixed intercropping and may result in non-legume root infection by native rhizobial populations in the soil. Various researchers have isolated natural endophyte *Rhizobium* from the roots of non-leguminous species including rice (Yanni et al. 1997), cotton, sweet corn (Mclnroy and Kloepper 1995), maize (Martinez-Romero et al. 2000), wheat (Biederbeck et al. 2000), and canola (Lupwayi et al. 2000) either grown in rotation with legumes or in a mixed cropping system involving symbiotic legumes. Wiehe and Holfich (1995) demonstrated that the strain R39 of *R. leguminosarum* bv. *trifolii* multiplied under field conditions in the rhizosphere of host legumes as well as non-legumes including corn, rape, and wheat.

The plant growth-promoting ability of Rhizobia inoculation varies with soil properties and crop rotation (Hilali et al. 2001), and the inoculation response mainly depends on the soil moisture, available nitrogen, yield potential, and the richness and effectiveness of native Rhizobia (Venkateswarlu et al. 1997). Rao (2001) reported 10–25% yield benefits with inoculation in the trials conducted on arid legumes like *Cyamopsis tetragonoloba*, *Vigna aconitifolia*, and *V. radiata*. The positive effects of chickpea field co-inoculation with *Mesorhizobium* sp. and *Pseudomonas aeruginosa*, which accounted for an increase of 32% in grain yield, compared to the uninoculated control (Verma et al. 2013). Fenugreek bio-inoculated with *R. meliloti* strain FRS-7 resulted in 36.8 and 45.9% increased yields over control for two consecutive years under semiarid conditions saving about 20 kg ha^{-1} nitrogen accompanied with better crop yield and soil health (Singh and Patel 2016). Rhizobia-inoculated plants produced significantly higher nodule number, nodule dry weight, grain yield, and yield components than non-inoculated non-fertilized plants. Inoculation of field-grown lentil with rhizobia strain Lt29 and Lt5 enhanced seed yield by 59% and 44%, respectively (Tena et al. 2016). Bhargava et al. (2016) studied the phenotypic, stress tolerance, and plant growth-promoting characteristics of rhizobial isolates from selected wild legumes of semiarid region and suggested that the functional diversity displayed by the isolates can be utilized for the legume crop production by cross inoculation.

4.5.1 Symbiotic Nitrogen Fixing Bacteria

The BNF is the most efficient process to supply the large amounts of nitrogen required by legumes to produce high-yielding crops. There are approximately 700 genera and ~13,000 species of legumes; only about 20% (Sprent and Sprent 1990) have been examined for nodulation and shown to have the ability to fix nitrogen. The legume plants enter into a symbiotic partnership with certain bacteria called rhizobia. The rhizobia present in the soil or added as seed inoculum enter into the root hairs and move through an infection thread toward the root of legume plants. It multiplies rapidly in the root, causing the swelling of root cells to form nodules (Limpens et al. 2003).

The plant transports carbohydrate to the nodules which is used as a source of energy by the rhizobia. Some of the carbohydrates are also used by the rhizobia as a source of hydrogen for the conversion of atmospheric nitrogen to ammonia (Gopalakrishnan et al. 2015). The symbiotic relationship between root nodule bacteria and legumes has high degree of specificity for effective nodulation. It operates at both levels of the symbiosis, i.e., the nodulation and nitrogen fixation by the exchange of specific chemical signals between the two partners (Perret et al. 2000). The symbiosis is controlled at a molecular level and is possible due to the presence of appropriate genes (Schlaman et al. 1998; Perret et al. 2000).

The volume of nitrogen fixed by various leguminous plants varies according to its species and varieties. It is directly related to the dry matter yield within a species. Some leguminous crops can fix up to 400 kg nitrogen ha^{-1} during the best years. Clover can fix over 200 kg, while Lucerne can fix 300 kg nitrogen ha^{-1}. Most of the grain legumes obtain 50–80% of their total nitrogen requirements through biological fixation, while some legumes, viz., faba bean, fix up to 90%. Faba bean in association with rhizobia can fix up to 120 kg nitrogen ha^{-1} (Danso 1992). The rate of rhizobia survival, the extent of effective nodulation, and plant growth factors are responsible for the potential nitrogen fixation. Similarly adverse soil condition or environmental stress restricts the nitrogen fixation process. High level of soil nitrogen reduces nitrogen fixation as legumes specially use most of the available soil nitrogen before beginning of the nitrogen fixation, while lower nitrogen levels can also reduce plant growth. During the period of formation of nodules and beginning of nitrogen fixation, legume requires a minimum quantity of nitrogen (about 20–22 kg ha^{-1}), from other sources depending on growing conditions (Anglade et al. 2015). Symbiotic nitrogen fixation is carried out by selective species of bacteria specific to particular legume species (Chandrasekar et al. 2005; Qureshi et al. 2009).

The nitrogen fixation is a key factor in low-input agricultural systems to sustain long-term soil fertility. It has immense importance under the areas where there is high farm land pressure and the fallow system cannot be followed. Under such situations, biologically fixed nitrogen improves soil nitrogen content and increases the yield of subsequent crops in cropping systems (Habtegebrial and Singh 2006).

4.5.2 Non-symbiotic Nitrogen-Fixing Bacteria

A number of PGPRs associate with various C3 and C4 plants including rice, wheat, maize, sugarcane, and cotton and increase their vegetative growth and grain yield (Kennedy et al. 2004). Azotobacter species are free-living heterotrophic and have been reported to increase the yield of rice (Yanni and El-Fattah 1999), cotton (Anjum et al. 2007), and wheat (Barassi et al. 2000). Similarly some obligatory anaerobic heterotrophs, viz., Clostridia, are able to fix atmospheric nitrogen under complete absence of oxygen (Kennedy et al. 2004). *Azospirillum* species are aerobic heterotrophs and have the ability to fix nitrogen under microaerobic conditions (Roper and Ladha 1995). They have been reported in the rhizosphere of gramineous plants (Kennedy and Tchan 1992; Kennedy et al. 2004). Their beneficial effects have been reported on the grain yield of wheat under both greenhouse and field conditions (Ganguly et al. 1999). The association of *Azospirillum* with plant leads to improved development and yield of host plants (Fallik et al. 1994) due to improved root development by increased water and mineral uptake and BNF (Okon and Itzigsohn 1995). *Azospirillum* influences the root respiration rate, metabolism, and root proliferation of the host by the synthesis of phytohormones (Okon and Itzigsohn 1995).

4.5.3 Phosphate Solubilization

Phosphorus is the structural component of nucleic acids, phospholipids, and adenosine triphosphate and is an essential and key nutrient for the metabolic and biochemical pathways for various reactions including BNF and photosynthesis (Ehrlich 1990; Richardson and Simpson 2011). It exists as organic and inorganic phosphates forms in the soil and is absorbed by the plant in both mono- and dibasic forms (Glass 1989), but it remains in insoluble forms, hence not available for plant nutrition. The availability of this element depends on its solubility that may be enhanced by the root activity of plant and microorganisms available in the soil. Plant growth-promoting rhizobia have the capacity to convert both organic and inorganic insoluble phosphates compounds in a form accessible to the plant (Igual et al. 2001; Rodríguez et al. 2006). Phosphate-solubilizing bacteria constitute approximately 1–50% of the total population of cultivable microorganisms in the soil (Khan et al. 2009) and solubilize inorganic soil phosphates through the production of organic acids, siderophores, and hydroxyl ions (Rodríguez et al. 2006, Sharma et al. 2013). Several phosphate-solubilizing bacteria belonging to genera *Pseudomonas, Bacillus, Rhizobium, Burkholderia, Achromobacter, Agrobacterium, Microccocus, Aerobacter, Flavobacterium*, and *Erwinia* have been isolated from the roots and rhizospheric soil of various plants (Rodríguez et al. 2006; Ambrosini et al. 2012; Farina et al. 2012; Costa et al. 2013; Souza et al. 2013, 2014) and have the ability to solubilize insoluble mineral phosphate, viz., tricalcium phosphate, dicalcium phosphate, hydroxyl apatite, and rock phosphate (Goldstein 1986; Rodríguez and Fraga 1999; Rodríguez et al. 2006).

4.5.4 Other Plant Growth Promotion Mechanisms

The bacteria present in the rhizosphere affect the plant growth directly or indirectly. They improve the uptake of nutrients, produce plant growth-promoting compounds, and protect plant root surfaces from colonization by pathogenic microbes (Mantelin and Touraine 2004). The symbiotic and non-symbiotic bacteria promote plant growth directly through production of hormones (Vivas et al. 2005), plant growth regulators, and other plant growth-promoting activities (Dobbelaere et al. 2003). PGPR has also been used to remediate contaminated soils (Zhuang et al. 2007; Huang et al. 2005) and mineralize organic compounds in association with plants (Saleh et al. 2004). Some of the important genera of bacteria used in bioremediation include *Bacillus, Pseudomonas, Methanobacteria, Ralstonia*, and *Deinococcus,* etc. (Milton 2007). It has been observed that the application of certain rhizobacteria can increase the uptake of nickel from soils (Rai et al. 2006).

4.5.5 Soil Health

Soil is a heterogeneous mixture of different organisms in which different organic and mineral substances are present in solid, liquid, and gaseous phases (Kabata-Pendias 2004), and the soil structure including size of soil aggregates, their arrangement, and stability is the result of physical forces and natural grouping of particles (Lynch and Bragg 1985). The soil aggregation is influenced by several factors including soil mineralogy, cycles of wetting and drying, soil pH range, and clay and organic material contents (Majumder and Kuzyakov 2010; Vogel et al. 2014). The fine spatial heterogeneity of soils results in a complex mosaic of gradients selecting for or against bacterial growth (Vos et al. 2013).

Soil health is the capacity of soil to function as a vital living system, within ecosystem. It sustains plant and animal productivity and maintains or improves water and air quality simultaneously promoting the plant health (Doran and Zeiss 2000). The soil type, climate, cropping patterns, use of pesticides and fertilizers, availability of carbon substrates and nutrients, toxic material concentrations, and the presence or absence of specific assemblages and types of organisms are major factor affecting the soil health (Doran and Zeiss 2000; Kibblewhite et al. 2008). The microbial communities provide many potentially indicators for sustainable management of soils (Pulleman et al. 2012), and the flexibility of soil functional is governed by the effects of the physicochemical structure on the composition and physiology of microbial community (Griffiths and Philippot 2012).

4.5.6 Biofertilizer

The soluble nitrogen is one of the most important soil nutrients and its supply to the plants is always a matter of concern and is mainly fulfilled by the chemical fertilizer. Although the atmospheric air contains enormous nitrogen gas, it cannot be used by the plants directly. It must be converted into nitrogenous compounds for the use of plants. Microbes have the ability to perform this action naturally, and *Rhizobium* is the most important microorganism of choice that enters into the roots of legumes and stimulates the plant to form nodules. The plant and the bacteria live in symbiotic coordination. The bacteria get carbohydrates from the host and in return it gives soluble nitrogen to the plant. The plant switches genes in the *Rhizobium* present in the nodule that enables it to convert nitrogen gas into nitrogenous compounds, and this process is known as nitrogen fixation. This was the most important source of nitrogen for agriculture until the early years of this century until the discovery of chemical process for ammonia synthesis in 1909. Gradually farming was mechanized and traditional legume cereal rotations were abandoned as relatively cheap nitrogen fertilizers became commonly available and they appeared more efficient than legumes in increasing grain yields. However, legumes are able to supply more nitrogen for most systems if they were called upon to do so. The worldwide population growth, economic growth, and agricultural productions are the major issues that demanded the use of chemical fertilizers in agriculture (Morel et al. 2012).

The chemical fertilizers have become essential components of modern agriculture, and the current agricultural production depends on the large-scale use of these fertilizers for nitrogen, phosphorus, and potassium (Wartiainen et al. 2008; Adesemoye et al. 2009). However, the higher use of chemical fertilizers can cause unanticipated environmental impacts (Adesemoye et al. 2009). The plant growth-promoting bacteria-based inoculation method may be utilized as an alternative to the chemical fertilizers. The inoculants containing plant growth-promoting bacteria is getting interest worldwide due to expensive nitrogen fertilizers and its adverse effect on the environment, water contamination, acidification of soils, and greenhouse effects (Hungria et al. 2013).

Drawbacks of intensive farming practices and environmental costs of nitrogen and phosphorus fertilizers have renewed interest in biofertilizers. Rhizobia species have been well studied due to their symbiotic relationship with leguminous plants and their agronomical application as inoculants in the cultivation of economic crops (Alves et al. 2004; Torres et al. 2012). The results of in vitro and in vivo efficacy of *S. saheli* strains suggest that their co-inoculation with PGPRs can not only reduce the use of chemical fertilizers but also can significantly enhance yields by increasing plant growth and suppressing soil-borne plant pathogenic fungi (Gautam et al. 2015). The soybean *Bradyrhizobium* association is a good example of the efficiency of BNF, and *B. elkanii* and *B. japonicum* are species commonly used to inoculate this leguminous plant (Alves et al. 2004). PGPRs with ACC deaminase trait usually improve yield and plant growth and thus are good candidates for biofertilizer formulation (Shaharoona et al. 2006a, b). The nitrogen provided by the root-nodulating bacteria is less prone to leaching, volatilization, and denitrification and is therefore considered an important biological process that contributes to sustainable agriculture (Dixon and Kahn 2004).

4.6 Conclusions

Intensive use of chemical fertilizers and pesticides has led to soil fertility degradation and severe health and environmental hazards. By contrast, researches have demonstrated that biofertilizers naturally activate the microorganisms found in the soil being cheaper, effective, and environmental friendly. They are gaining importance for use in crop production, restore the soil's natural fertility, and protect it against drought and soil diseases and therefore stimulate plant growth. Further exploitation of functional diversity, legume–rhizobia gene interactions, and tolerance of efficient rhizobial isolates to the adversities of arid and semiarid regions environments in combination with compatible PGPRs can improve soil health and legume crop production. Concurrently, a better understanding of the interactions of leguminous plants vis-à-vis soil fertility, pest, and diseases shall create opportunities for better, low-cost biological management of soil fertility in arid and semiarid regions environments to make agriculture practices more sustainable and economical. It is right time to generate more supplementary investigations to develop biofertilizer technology to introduce superior strains to produce industrial and commercial biological fertilizers.

References

Adesemoye AO, Torbert HA, Kloepper JW (2009) Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb Ecol 58:921–929

Afza R, Hardarson G, Zapata F (1987) Effect of delayed soil and foliar N fertilization on yield and N2 fixation of soybean. Plant Soil 97:361–368

Al-Ani RA, Adhab MA, Mahdi MH, Abood HM (2012) *Rhizobium japonicum* as a biocontrol agent of soybean root rot disease caused by *Fusarium solani* and *Macrophomina phaseolina*. Plant Prot Sci 48(4):149–155

Alquéres S, Meneses C, Rouws L, Rothballer M et al (2013) The bacterial superoxide dismutase and glutathione reductase are crucial for endophytic colonization of rice roots by *Gluconacetobacter diazotrophicus* PAL5. Mol Plant Microbe Interact 26:937–945

Alvarez MI, Sueldo RJ, Barassi CA (1996) Effect of *Azospirillum* on coleoptile growth in wheat seedlings under water stress. Cereal Res Commun 24:101–107

Alves BJR, Boddey RM, Urquiaga S (2004) The success of BNF in soybean in Brazil. Plant Soil 252:1–9

Ambrosini A, Beneduzi A, Stefanski T, Pinheiro FG et al (2012) Screening of plant growth promoting rhizobacteria isolated from sunflower (*Helianthus annuus* L.). Plant Soil 356:245–264

Anglade J, Billen G, Garnier J (2015) Relationships for estimating N2 fixation in legumes: incidence for N balance of legume-based cropping systems in Europe. Ecosphere 6(3):1–24

Anjum MA, Sajjad MR, Akhtar N, Qureshi MA et al (2007) Response of cotton to plant growth promoting rhizobacteria (PGPR) inoculation under different levels of nitrogen. J Agric Res 45 (2):135–143

Antoun H, Beauchamp CJ, Goussard N, Chabot R et al (1998) Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: effect on radishes (*Raphanus sativus* L.). Plant Soil 204:57–68

Aoki H, Katayama T, Ogasawara T, Sasaki Y et al (2007) Characterization and properties of *Acacia senegal* (L.) Willd var. *senegal* with enhanced properties (Acacia (sen) SUPER GUM TM). Part 5: factors affecting the emulsification of *Acacia senegal* and Acacia (sen) SUPER GUM TM. Food Hydrocoll 21:353–358

Arshad M, Frankenberger WT Jr (1998) Plant growth regulating substances in the rhizosphere. Microbial production and function. Adv Agron 62:46–51

Banerjee MR, Yasmin L (2002) Sulfur oxidizing rhizobacteria: an innovative environment friendly soil biotechnological tool for better canola production. In: Proceeding of Agroenviron, Cairo, Egypt, 26–29 October, pp 1–7

Barassi CA, Creus CM, Casanovas EM, Sueldo RJ (2000) Could *Azospirillum* mitigate abiotic stress effects in plants? Auburn University. Web site: http://www.ag.auburn.edu/argentina/pdfmanuscripts/brassi.pdf

Bayoumi HEA, Biro B, Balazsy S, Kecskes M (1995) Effects of some environmental factors on *Rhizobium* and *Bradyrhizobium* strains. Acta Microbiol Immunol Hung 42:61–69

Beauregard PB, Chai Y, Vlamakis H, Losick R et al (2013) *Bacillus subtilis* biofilm induction by plant polysaccharides. Proc Natl Acad Sci USA 110:E1621–E1630

Bhargava Y, Murthy JSR, Rajesh Kumar TV, Narayana Rao M (2016) Phenotypic, stress tolerance and plant growth promoting characteristics of rhizobial isolates from selected wild legumes of semiarid region, Tirupati, India. Adv Microbiol 6:1–12

Biederbeck VO, Lupwayi NZ, Haanson KG, Rice WA et al (2000) Effect of long-term rotation with lentils on rhizosphere ecology and on endophytic Rhizobia in wheat. In: Abstract of the 17th North American conference on symbiotic nitrogen fixation. Laval University Quebec, Canada, July 2000, pp 23–28

Bogino P, Banchio E, Rinaudi L, Cerioni G et al (2006) Peanut (*Arachis hypogaea*) response to inoculation with *Bradyrhizobium* sp. in soils of Argentina. Ann Appl Biol 148:207–212

Bordeleau LM, Prevost D (1994) Nodulation and nitrogen fixation in extreme environments. Plant Soil 161:115–124

Brockwell J, Bottomley PJ, Thies JE (1995) Manipulation of rhizobia microflora for improving legume productivity and soil fertility: a critical assessment. Plant Soil 174:143–180

Buonassisi AJ, Copeman RJ, Pepin HS, Eaton GW (1986) Effect of *Rhizobia* spp. on *Fusarium* f. sp. *phaseoli*. Can J Plant Pathol 8:140–146

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

Buttery BR, Dirks VA (1987) The effect of soybean cultivar, *Rhizobium* strain and nitrate on plant growth, nodule mass and acetylene reduction rate. Plant Soil 98:285–293

Cai Z, Kastell A, Knorr D, Smetanska I (2012) Exudation: an expanding technique for continuous production and release of secondary metabolites from plant cell suspension and hairy root cultures. Plant Cell Rep 31:461–477

Çakmakçi R, Erat M, Erdoğan ÜG, Dönmez MF (2007) The influence of PGPR on growth parameters, antioxidant and pentose phosphate oxidative cycle enzymes in wheat and spinach plants. J Plant Nutr Soil Sci 170:288–295

Carvalhais LC, Dennis PG, Fan B, Fedoseyenko D et al (2013) Linking plant nutritional status to plant-microbe interactions. PLoS One 8:e68555

Chabot R, Antoun H, Cescas MP (1996a) Growth promotion of maize and lettuce by phosphate-solubilizing *Rhizobium leguminosarum* biovar *phaseoli*. Plant Soil 184:311–321

Chabot R, Antoun H, Kloepper JW, Beauchamp CJ (1996b) Root colonization of maize and lettuce by bioluminescent *Rhizobium leguminosarum* biovar *phaseoli*. Appl Environ Microbiol 62:2767–2772

Chagas-Junior AF, dos Santos GR, de Lima Melo RC, de Oliveira AG et al (2012) Effect of natural nodulation in the development of leguminous trees on soils of cerrado in Tocantins. J Biotechnol Biodivers 3(1):38–44

Chandrasekar BR, Ambrose G, Jayabalan N (2005) Influence of biofertilizers and nitrogen source level on the growth and yield of Echinochloa frumentacea (Roxb.) Link. J Agric Technol 1:223–234

Chhillar BS (2009) Management of insect pests and diseases of pulses with special reference to arid legumes. In: Kumar D, Henry A, Vittal KPR (eds) Legumes in dry areas Indian Arid Legume Society. Scientific Publishers, Jodhpur, pp 447–449

Chibeba AM, de Fátima GM, Brito OR, Nogueira MA et al (2015) Co-Inoculation of soybean with *Bradyrhizobium* and *Azospirillum* promotes early nodulation. Am J Plant Sci 6:1641–1649

Costa P, Beneduzi A, Souza R, Schoenfeld R et al (2013) The effects of different fertilization conditions on bacterial plant growth promoting traits: guidelines for directed bacterial prospection and testing. Plant Soil 368:267–280

Dakora FD (2003) Defining new roles for plant and rhizobial molecules in sole and mixed plant cultures involving symbiotic legumes. New Phytol 158:39–49

Danso SKA (1992) Biological nitrogen fixation in tropical agro systems. Twenty years of biological nitrogen fixation in Africa. In: Mulogoy K, Guye M, Spence DSC (eds) Biological nitrogen fixation and sustainability of tropical agriculture. Proceedings of the 4th International conference of Africa Association for Biological nitrogen fixation (AABNF) held at International Institute of Tropical Agriculture. Wiley, Chichester, New York, Toronto, pp 336–362

Davison J (1988) Plant beneficial bacteria. Biotechnology 6:282–286

Delgado MJ, Ligero F, Lluch C (1994) Effects of salt stress on growth and nitrogen fixation by pea, faba-bean, common bean and soybean plants. Soil Biol Biochem 26:371–376

Dey R, Pal KK, Bhatt DM, Chauhan SM (2004) Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L) by application of plant growth promoting rhizobacteria. Microbiol Res 159:371–394

Dhir RP (1984) Soils of arid and semi-arid regions, their characteristics and properties. In: Shankarnarayana KA (ed) Agroforestry in arid and semi-arid zones. Central Arid Zone Research Institute, Jodhpur, pp 20–29

Dixon R, Kahn D (2004) Genetic regulation of biological nitrogen fixation. Nat Rev Microbiol 2:621–631

Dobbelaere S, Vanderleyden J, Okon Y (2003) Plant growth promoting effects of diazotrophs in the rhizosphere. Crit Rev Plant Sci 22:107–149

Doran JW, Zeiss MR (2000) Soil health and sustainability: managing the biotic component of soil quality. Appl Soil Ecol 15:3–11

Duseja U, Shrivastav A (2015) Isolation of physic-chemical characterization of Rhizobium strains isolated from Vigna fields of gird region of Madhya Bharat. J Acad Ind Res 4(5):155–159

Ehrlich HL (1990) Geomicrobiology, 2nd edn. Dekker, New York, p 646

Ehteshamul-Haque S, Ghaffar A (1993) Use of Rhizobia in the control of root diseases of sunflower, okra, soybean and mungbean. J Phytopathol 138:157–163

El Hadrami AA, Mabrouk Y, Sifi B, Boudabous A et al (2007) Treatment of chickpea with Rhizobium isolates enhances the expression of phenylpropanoid defense-related genes in response to infection by *Fusarium oxysporum* f. sp *ciceris*. Plant Physiol Biochem 45:470–479

Faghire M, Mohamed F, Taoufiq K, Fghire R et al (2013) Genotypic variation of nodules' enzymatic activities in symbiotic nitrogen fixation among common bean (*Phaseolus vulgaris* L.) genotypes grown under salinity constraint. Symbiosis 60:115–122

Fallik E, Sarig S, Okon Y (1994) Morphology and physiology of plant roots associated with *Azospirillum*. In: Okon Y (ed) *Azospirillum*-plant associations. CRC Press, Boca Raton, pp 77–84

Farina RA, Beneduzi A, Ambrosini A, Campos SB et al (2012) Diversity of plant growth-promoting rhizobacteria communities associated with the stages of canola growth. Appl Soil Ecol 55:44–52

Ganguly TK, Jana AK, Moitra DN (1999) An evaluation of agronomic potential of *Azospirillum brasilense* and *Bacillus megaterium* in fibre-legume-cereal system in an Aeric haplaquept. Indian J Agric Res 33:35–39

Gautam R, Singh SK, Sharma V (2015) Suppression of soil-borne root pathogens of arid legumes by *Sinorhizobium saheli*. SAARC J Agric 13(1):63–74

Glass ADM (1989) Plant nutrition: an introduction to current concepts. Jones and Bartlett Publishers, Boston, p 234

Glick BR (2001) Phytoremediation: synergistic use of plants and bacteria to clean up the environment. Biotechnol Adv 21(3):83–393

Glick BR, Pasternak JJ (2003) Plant growth promoting bacteria. In: Glick BR, Pasternak JJ (eds) Molecular biotechnology principles and applications of recombinant DNA, 3rd edn. ASM Press, Washington, pp 436–454

Goldstein AH (1986) Bacterial solubilization of microbial phosphates: a historical perspective and future prospects. Am J Altern Agric 1:51–57

Gopalakrishnan S, Sathya A, Vijayabharathi R, Varshney RK et al (2015) Plant growth promoting rhizobia: challenges and opportunities. Biotech 5:355–377

Graham PH (1992) Stress tolerance in *Rhizobium* and *Bradyrhizobium*, and nodulation under adverse soil conditions. Can J Microbiol 38:475–484

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

Gray EJ, Smith DL (2005) Intracellular and extracellular PGPR: commonalities and distinctions in the plant–bacterium signaling processes. Soil Biol Biochem 37:395–412

Griffiths BS, Philippot L (2012) Insights into the resistance and resilience of the soil microbial community. FEMS Microbiol Rev 37:112–129

Habtegebrial K, Singh BR (2006) Wheat responses in semiarid northern Ethiopia to N2 fixation by *Pisum sativum* treated with phosphorus fertilizers and inoculants. Nutr Cycl Agroecosyst 75:247–255

Hayat R, Ali S (2004) Potential of summer legumes to fix nitrogen and benefit wheat crop under rainfed condition. J Agron 3:273–281

Hayat R, Ali S (2010) Nitrogen fixation of legumes and yield of wheat under legumes-wheat rotation in Pothwar. Pak J Bot 42(4):2317–2326

Hayat R, Ali S, Ijaz SS, Chatha TH et al (2008a) Estimation of N2-fixation of mung bean and mash bean through xylem ureide technique under rainfed conditions. Pak J Bot 40(2):723–734

Hayat R, Ali S, Siddique MT, Chatha TH (2008b) Biological nitrogen fixation of summer legumes and their residual effects on subsequent rainfed wheat yield. Pak J Bot 40(2):711–722

Hayat R, Ali S, Amara U, Khalid R et al (2010) Soil beneficial bacteria and their role in plant growth promotion: a review. Ann Microbiol 60:579–598

Hellriegel H (1887) Welche Stickstoffquellen stehen der Pflanze zu Gehote? Landw Verso Sta 33:464465

Hellriegel H, Wilfarth H (1888) Untersuchungen iiber die Stickstoff· nahrung der Gramineen und Leguminosen. Beilagebeft zu der Ztschr. Ver. Riibenzucker·lndustrie Deutschen Reichs, p 234

Hilali A, Prevost D, Broughton WJ, Anloun H (2001) Effects of inoculation avec des souches de *Rhizobium leguminosarum* biovar *trifolii* sur la croissance du ble dans deux sols du Maroc. Can J Microbiol 47:590–593

Hohenberg JS, Munns DN (1984) Effect of soil acidity factors on nodulation and growth of *Vigna unguiculata* in solution culture. Agric J 76:477–481

Howieson JG, O'Hara GW, Carr SJ (2000a) Changing roles for legumes in Mediterranean agriculture: developments from an Australian perspective. Field Crop Res 65:107–122

Howieson JG, O'Hara GW, Loi A (2000b) The legume-rhizobia relationship in the Mediterranean Basin. In: Sulas L (ed) Legumes for Mediterranean forage crops, pastures and alternative uses, cahiers options Mediterraneennes, vol 45. pp 305–314

Huang XD, El-Alawi Y, Gurska J, Glick BR et al (2005) A multi-process phytoremediation system for decontamination of persistent total petroleum hydrocarbons (TPHs) from soils. Microchem J 81:139–147

Hungria M, Nogueira MA, Araujo RS (2013) Co-inoculation of soybeans and common beans with rhizobia and azospirilla: strategies to improve sustainability. Biol Fertil Soils 49:791–801

Igual JM, Valverde A, Cervantes E, Velázquez E (2001) Phosphate solubilizing bacteria as inoculants for agriculture: use of updated molecular techniques in their study. Agronomie 21:561–568

Jadhav RS, Thaker NV, Desai A (1994) Involvement of the siderophore of cowpea *Rhizobium* in the iron nutrition of the peanut. World J Microbiol Biotechnol 10:360–361

Jenkins MB, Virginia RA, Jarrel WM (1987) Rhizobial ecology of the woody legume mesquite (*Prosopis glandulosa*) in the Sonoran Desert. Appl Environ Microbiol 33:36–40

Jenkins MB, Virginia RA, Jarrel WM (1989) Ecology of fast growing and slow-growing mesquite-nodulating rhizobia in Chihuahua and Sonoran desert ecosystems. Soil Sci Soc Am J 53:543–549

Jindal SK, Singh M, Pancholy A, Kackar NL (2000) Performance of *Acacia senegal* (L.) Willd. accessions for tree height at rocky rangelands of the Thar desert. J Arid Environ 45:111–118

Kabata-Pendias A (2004) Soil-plant transfer of trace elements—an environmental issue. Geoderma 122:143–149

Kackar NL, Jindal SK, Solanki KR, Singh M (1990) Nodulation in seedlings of *Prosopis cineraria* (L.) Druce. Nitrogen Fixing Tree Res Rep USA 8:152–153

Kaushik UK, Dogra RC, Dudeja SS (1995) Effects of fertilizer N on nodulation, acetylene-reducing activity, and N uptake in pigeon pea (*Cajanus cajan*). Trop Agric 72:76–79

Kennedy IR, Tchan Y (1992) Biological nitrogen fixation in no leguminous field crops: recent advances. Plant Soil 141:93–118

Kennedy IR, Choudhury AIMA, KecSkes ML (2004) Non-symbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better exploited? Soil Boil Biochem 36(8):1229–1244

Khan AG (2005) Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. J Trace Elem Med Biol 18:355–364

Khan MS, Zaidi A, Wani PA (2009) Role of phosphate solubilizing microorganisms in sustainable agriculture – a review. Agron Sustain Dev 27:29–43

Kibblewhite MG, Ritz K, Swift MJ (2008) Soil health in agricultural systems. Philos Trans R Soc Lond B Biol Sci 363:685–701

Klimek-Kopyra A, Baran A, Zając T, Kulig B (2015) Effects of heavy metals from polluted soils on the roots and nodules formation. Bulgarian J Agr Sci 21(2):295–299

Kucey RMN, Chaiwanakupt P, Arayangkool T, Snitwongse P et al (1988) Nitrogen fixation (^{15}N dilution) with soybeans under Thai field conditions. II. Effect of herbicides and water application schedule. Plant Soil 108:87–92

Lhuissier FGP, de Ruijter NCA, Sieberer BJ, Esseling JJ et al (2001) Time course of cell biological events evoked in legume root hairs by Rhizobium Nod factors: state of the art. Ann Bot 87:289–302

Li J, Ovakin DH, Charles TC, Glick BR (2000) An ACC deaminase minus mutant of *Enterobacter cloacae* UW4 no longer promotes root elongation. Curr Microbiol 41:101–105

Limpens E, Franken C, Smit P, Willemse J et al (2003) LysM domain receptor kinase regulating rhizobial nod factor-induced infection. Science 302:630–633

Long SR, Ehrhardt DW (1989) New route to a sticky subject. Nature 338:545–546

Lupwayi NZ, Rice WA, Clayton GW (2000) Endophytic Rhizobia in barley and canola in rotation with field peas. In: Abstracts, 17th North American conference on symbiotic nitrogen fixation, University of Laval, Quebec, Canada, 23–28 July 2000, p 51

Lynch JM, Bragg E (1985) Microorganisms and soil aggregate stability. In: Stewart BA (ed) Advances in soil science. Springer, New York, pp 133–171

Maia J, Scotti MR (2010) Growth of *Inga vera* Willd. subsp. affinis under rhizobia inoculation. RC Suelo Nutr Veg 10(2):139–149

Majumder B, Kuzyakov Y (2010) Effect of fertilization on decomposition of 14C labelled plant residues and their incorporation into soil aggregates. Soil Tillage Res 109:94–102

Mallesh SB (2008) Plant growth promoting rhizobacteria, their characterization and mechanisms in the suppression of soil borne pathogens of coleus and ashwagandha. Ph. D thesis, Department of Plant Pathology, College of Agriculture, Dharwad, University of Agricultural Sciences, Dharwad, India, p 160

Mallik MAB, Tesfai K (1985) Pesticidal effect on soybean-rhizobia symbiosis. Plant Soil 85:33–41

Mallik MAB, Tesfai K (1993) Compatibility of *Rhizobium japonicum* with commercial pesticides *in vitro*. Bull Environ Contam Toxicol 31:432–437

Mantelin S, Touraine B (2004) Plant growth-promoting bacteria and nitrate availability: impacts on root development and nitrate uptake. J Exp Bot 55:27–34

Manzano MG, Navar J (2000) Processes of desertification by goats overgrazing in the *Tamaulipan thornscrub* (matorral) in northeastern Mexico. J Arid Environ 44(1):1–17

Martinez-Romero E, Gutierrez-Zamora ML, Estrada P, Caballero-Mellado J et al (2000) Natural endophytic association between *Rhizobium Etli* and maize. In: Abstracts, 17th North American conference on symbiotic nitrogen fixation, University of Laval, Quebec, Canada, 23–28 July 2000, p 51

Matiru VN, Dakora FD (2004) Potential use of rhizobial bacteria as promoters of plant growth for increased yield in landraces of African cereal crops. Afr J Biotechnol 3(1):1–7

Mayak S, Tirosh T, Glick BR (2004) Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiol Biochem 42:565–572

Mclnroy JA, Kloepper JW (1995) Survey of indigenous endophytes from cotton and sweet corn. Plant Soil 173:337–342

Meneses CH, Rouws LF, Simões-Araújo JL, Vidal MS et al (2011) Exopolysaccharide production is required for biofilm formation and plant colonization by the nitrogen-fixing endophyte *Gluconacetobacter diazotrophicus*. Mol Plant Microbe Interact 24:1448–1458

Milton HSJ (2007) Beneficial bacteria and bioremediation. Water Air Soil Pollut 184:1–3

Morel MA, Braña V, Castro-Sowinski S (2012) Legume crops, importance and use of bacterial inoculation to increase production. In: Goyal A (ed) Plant crop. Intech, Rijeka, pp 217–240

Murray JD, Bogumil JK, Shusei SH, Satoshi T et al (2007) A cytokinin perception mutant colonized by *Rhizobium* in the absence of nodule organogenesis. Science 315(5808):101–104

Nithyakalyani V, Kannan M, Anandan R (2016) Insecticide and salt tolerance of plant growth promoting root nodule bacteria. Int J Curr Microbiol App Sci 5(4):942–956

O'Hara GW (2001) Nutritional constraints on root nodule bacteria affecting symbiotic nitrogen fixation: a review. Aust J Exp Agric 41:417–433

O'Hara G, Yates R, Howieson J (2002) Selection of strains of root nodule bacteria to improve inoculant performance and increase legume productivity in stressful environments. In: Herridge D (ed) Inoculants and nitrogen fixation of legumes in Vietnam, ACIAR proceedings, vol 109e. pp 75–80

Okon Y, Itzigsohn R (1995) The development of *Azospirillum* as a commercial inoculant for improving crop yields. Biotechnol Adv 13(3):415–424

Parida AK, Das AB (2005) Salt tolerance and salinity effect on plants: review. Ecotoxicol Eviron Saf 60:324–349

Patten CL, Glick BR (2002) Role of *Pseudomonas putida* indole-acetic acid in development of the host plant root system. Appl Environ Microbiol 68:3795–3801

Perret X, Staehelin C, Broughton WJ (2000) Molecular basis of symbiotic promiscuity. Microbiol Mol Biol Rev 64:180–201

Piha MI, Munnus DN (1987) Sensitivity of the common bean (*Phaseolus vulgaris* L.) symbiosis to high soil temperature. Plant Soil 98:183–194

Pulleman M, Creamer R, Hamer U, Helder J et al (2012) Soil biodiversity, biological indicators and soil ecosystem services—an overview of European approaches. Curr Opin Environ Sustain 4:529–538

Qureshi MA, Ahmed MJ, Naveed M, Iqbal NA et al (2009) Coinoculation with *Mesorhizobium ciceri* and *Azotobacter chroococcum* for improving growth, nodulation and yield of chickpea (*Cicer arietinum* L.). Soil Environ 28:124–129

Rai AB, Angle JS, Chaney RL (2006) Bacterial inoculants affecting nickel uptake by *Alyssum murale* from low, moderate and high Ni soils. Soil Biol Biochem 38:2882–2889

Rao DLN (2001) BNF research progress 1996-2000: all India coordinated research project on biological nitrogen fixation. IISS, Bhopal

Rao DLN, Giller KE, Yeo AR, Flowers TJ (2002) The effects of salinity and sodicity upon nodulation and nitrogen fixation in chickpea (*Cicer arietinum*). Ann Bot 89(5):563–570

Revillas JJ, Rodelas B, Pozo C, Martinez-Toledo MV et al (2000) Production of B-group vitamins by two Azotobacter strains with phenolic compounds as sole carbon source under diazotrophic and adiazotrophic conditions. J Appl Microbiol 89:486–493

Richardson AE (2001) Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Aust J Plant Physiol 28:897–906

Richardson AE, Simpson RJ (2011) Soil microorganisms mediating phosphorus availability. Plant Physiol 156:989–996

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

Rodríguez H, Fraga R, Gonzalez T, Bashan Y (2006) Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant Soil 287:15–21

Rodríguez-Echeverría S, Fajardo S, Ruiz-Díez B, Fernandez-Pascual M (2012) Differential effectiveness of novel and old legume–rhizobia mutualisms: implications for invasion by exotic legumes. Oecologia 170:253–261

Roper MM, Ladha JK (1995) Biological N2-fixation by heterotrophic and phototrophic bacteria in association with straw. Plant Soil 174:211–224

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

Saleh S, Huang XD, Greenberg BM, Glick BR (2004) Phytoremediation of persistent organic contaminants in the environment. In: Singh A, Ward O (eds) Soil biology, applied bioremediation and phytoremediation. Springer, Berlin, pp 115–134

Saravanakumar D, Lavanya N, Muthumeena B, Raguchander T et al (2008) *Pseudomonas fluorescens* enhances resistance and natural enemy population in rice plants against leaf folder pest. J Appl Entomol 132(6):469–479

Schimel DS (2010) Drylands in the earth system. Science 327:418–419

Schlaman HRM, Phillips DA, Kondorosi E (1998) Genetic organisation and transcriptional regulation of rhizobial nodulation genes. In: Spaink HP, Kondorosi A, PJJ H (eds) The *Rhizobiaceae*. Kluwer, Dordrecht, pp 361–386

Schultz-Lupitz A (1881) Reinertrage auf leichtem Boden, ein Wort der Erfahrung, zur Abwehr der wirtschaftlichen. Noth Landw Jahrb 10:778–848

Shaharoona B, Arshad M, Zahir ZA (2006a) Effect of plant growth promoting rhizobacteria containing ACC-deaminase on maize (*Zea mays* L.) growth under axenic conditions and on nodulation in mung bean (*Vigna radiata* L.). Lett Appl Microbiol 42:155–159

Shaharoona B, Arshad M, Zahir ZA, Khalid A (2006b) Performance of *Pseudomonas* spp. containing ACC-deaminase for improving growth and yield of maize (*Zea mays* L.) in the presence of nitrogenous fertilizer. Soil Biol Biochem 38:2971–2975

Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA (2013) Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. SpringerPlus 2:1–14

Shiferaw B, Bantilan MCS, Serraj R (2004) Harnessing the potential of BNF for poor farmers: technological policy and institutional constraints and research need. In: Serraj R (ed) Symbiotic nitrogen fixation; prospects for enhanced application in tropical agriculture. Oxford & IBH, New Delhi, p 3

Shoebitz M, Ribaudo CM, Pardo MA, Cantore ML et al (2009) Plant growth promoting properties of a strain of Enterobacter ludwigii isolated from *Lolium perenne* rhizosphere. Soil Biol Biochem 41(9):1768–1774

Sierra S, Rodelas B, Martinez-Toledo MV, Pozo C et al (1999) Production of B-group vitamins by two *Rhizobium* strains in chemically defined media. J Appl Microbiol 86:851–858

Singh NK, Patel DB (2016) Performance of fenugreek bioinoculated with *Rhizobium meliloti* strains under semi-arid conditions. J Environ Biol 37:31–35

Singh SK, Pancholy A, Jindal SK, Pathak R (2011) Effect of plant growth promoting rhizobia on seed germination and seedling traits in *Acacia senegal*. Ann For Res 54(2):161–169

Snapp SS, Aggarwal VD, Chirwa RM (1998) Note on phosphorus and genotype enhancement of biological nitrogen fixation and productivity of maize/bean intercrops in Malawi. Field Crop Res 58:205–212

Soares BL, Ferreira PAA, de Oliveira-Longatti SM, Marra LM et al (2014) Cowpea symbiotic efficiency, pH and aluminum tolerance in nitrogen-fixing bacteria. Sci Agric 71(3):171–180

Sobti S, Belhadj HA, Djaghoubi A (2015) Isolation and characterization of the native Rhizobia under hyper-salt edaphic conditions in Ouargla (southeast Algeria). Energy Proc 74:1434–1439

Souza R, Beneduzi A, Ambrosini A, Costa PB et al (2013) The effect of plant growth-promoting rhizobacteria on the growth of rice (*Oryza sativa* L.) cropped in southern Brazilian fields. Plant Soil 366:585–603

Souza R, Meyer J, Schoenfeld R, Costa PB et al (2014) Characterization of plant growth-promoting bacteria associated with rice cropped in iron-stressed soils. Ann Microbiol 65:951–964

Sprent JI, Gehlot HS (2010) Nodulated legumes in arid and semi-arid environments: are they important? Plant Ecol Divers 3(3):211–219

Sprent JI, Sprent P (1990) Nitrogen fixing organisms. Pure and applied aspects. Chapman & Hall, London

Stajner D, Kevreaan S, Gasaić O, Mimica-Dudić N et al (1997) Nitrogen and *Azotobacter chroococcum* enhance oxidative stress tolerance in sugar beet. Biol Plant 39:441–445

Suhag M (2016) Potential of biofertilizers to replace chemical fertilizers. Int Adv Res J Sci Eng Technol 3(5):163–167

Tang C, Thomson BD (1996) Effects of solution pH and bicarbonate on the growth and nodulation of a range of grain legumes. Plant Soil 186:321–330

Tang C, Barton L, Raphael C (1998) Pasture legume species differ in their capacity to acidify soil. Aust J Agr Res 49:53–58

Tate RL (1995) Soil microbiology (symbiotic nitrogen fixation). Wiley, New York, pp 307–333

Taylor RW, Williams ML, Sistani KR (1991) Nitrogen fixation by soybean-*Bradyrhizobium* combinations under acidity, low P and high Al stresses. Plant Soil 131:293–300

Tena W, Meskel EW, Walley F (2016) Symbiotic efficiency of native and exotic rhizobium strains nodulating lentil (*Lens culinaris* Medik.) in soils of southern Ethiopia. Agronomy 6:2–10

Tewari JC, Pasiecznik NM, Harsh LN, Harris PJC (1998) *Prosopis* species in the arid and semi-arid zones of India. The Prosopis society of India and Henry Doubleday Research Association, Jodhpur and West Midlands, p 127

Thies JE, Woomer PL, Singleton PW (1995) Enrichment of *Bradyrhizobium* spp. populations in soil due to cropping of the homologous host legume. Soil Biol Biochem 27:633–636

Torres AR, Kaschuk G, Saridakis GP, Hungria M (2012) Genetic variability in *Bradyrhizobium japonicum* strains nodulating soybean *Glycine max* (L.) Merrill. World J Microbiol Biotechnol 28:1831–1835

van Rhijn P, Fujishige NA, Lim PO, Hirsch AM (2001) Sugar-binding activity of pea lectin enhances heterologous infection of transgenic alfalfa plants by Rhizobium *leguminosarum* biovar *viciae*. Plant Physiol 126:133–144

Vance CP (1997) Enhanced agricultural sustainability through biological nitrogen fixation. In: Legocki A, Bothe H, Puhler A (eds) Biological fixation of nitrogen for ecology and sustainable agriculture. Springer, Berlin, pp 179–186

Vassileva V, Milanov G, Ignatov G, Nikolov B (1997) Effect of low pH on nitrogen fixation of common bean grown at various calcium and nitrate levels. J Plant Nutr 20:279–294

Venkateswarlu B, Hari K, Katyl JC (1997) Influence of soil and crop factors on the native rhizobia populations in soils under dry land farming. Appl Soil Ecol 7:1–10

Verma JP, Yadav J, Tiwari KN, Kumar A (2013) Effect of indigenous *Mesorhizobium* spp. and plant growth promoting rhizobacteria on yields and nutrients uptake of chickpea (*Cicer arietinum* L.) under sustainable agriculture. Ecol Eng 51:282–286

Vivas A, Barea JM, Azcón R (2005) *Brevibacillus brevis* isolated from cadmium- or zinc-contaminated soils improves in vitro spore germination and growth of *Glomus mosseae* under high Cd or Zn concentrations. Microb Ecol 49:416–442

Vogel C, Babin D, Pronk GJ, Heister K et al (2014) Establishment of macro-aggregates and organic matter turnover by microbial communities in long term incubated artificial soils. Soil Biol Biochem 79:57–67

Vos M, Wolf AB, Jennings SJ, Kowalchuk GA (2013) Micro- scale determinants of bacterial diversity in soil. FEMS Microbiol Rev 37:936–954

Waldon HB, Jenkins MB, Virginia RA, Harding EE (1989) Characteristics of woodland rhizobial populations from surface and deep soil environment of the Sonoran desert. Appl Environ Microbiol 55:3058–3064

Wartiainen I, Eriksson T, Zheng W, Rasmussen U (2008) Variation in the active diazotrophic community in rice paddy-*nifH* PCR-DGGE analysis of rhizosphere and bulk soil. Appl Soil Ecol 39:65–75

Wiehe W, Holfich G (1995) Survival of plant growth promoting rhizosphere bacteria in rhizosphere of different crops and migration to non-inoculated plants under field conditions in north-east Germany. Microbiol Res 150:201–206

Wolde-Meskel E, Sinclair FL (1998) Variations in seedling growth, nodulation and nitrogen fixation of *Acacia nilotica* inoculated with eight rhizobial strains. For Ecol Manage 104:239–247

Yanni YG, El-Fattah FKA (1999) Towards integrated biofertilization management with free living and associative dinitrogen fixers for enhancing rice performance in the Nile delta. Symbiosis 27:319–331

Yanni YG, Rizk RY, Corich V, Squartini A et al (1997) Natural endophytic association between *Rhizobium leguminosarum* bv. *trifolii* and rice roots and assessment of its potential to promote rice growth. Plant Soil 194:99–114

Yanni YG, Rizk RY, Abd El-Fattah FK, Squartini A et al (2001) The beneficial plant growth-promoting association of *Rhizobium leguminosarum* bv. *trifolii* with rice roots. Aust J Plant Physiol 28:845–870

Younesi O, Baghbani A, Namdari A (2013) The effects of *Pseudomonas fluorescence* and *Rhizobium meliloti* co-inoculation on nodulation and mineral nutrient contents in alfalfa (*Medicago sativa*) under salinity stress. Int J Agric Crop Sci 5(14):1500–1507

Zafar-ul-Hye M, Zahir ZA, Shahzad SM, Irshad U et al (2007) Isolation and screening of Rhizobia for improving growth and nodulation of lentil (*Lens culinaris* Medic) seedlings under axenic conditions. Soil Environ 26(1):81–91

Zahir AZ, Arshad M, Frankenberger WT Jr (2004) Plant growth promoting rhizobacteria: application and perspectives in agriculture. Adv Agron 81:97–168

Zahran HH (2011) Condition for successful Rhizobium-Legume symbiosis saline environment. Biol Fertil Soils 12:73–80

Zaidi S, Usmani S, Singh BR, Musarrat J (2008) Significance of *Bacillus subtilis* strains SJ-101 as a bioinoculant for concurrent plant growth promotion and nickel accumulation in *Brassica Juncea*. Chemosphere 64:991–997

Zare S, Tavili A, Darini MJ (2011) Effect of different treatments on seed germination and breaking seed dormancy of *Prosopis koelziana* and *Prosopis juliflora*. J For Res 22(1):35–38

Zhuang XL, Chen J, Shim H, Bai Z (2007) New advances in plant growth-promoting rhizobacteria for bioremediation. Environ Int 33:406–413

Zohra HF, Mourad K, Meriem KH (2016) Preliminary characterization of slow growing rhizobial strains isolated from *Retama monosperma* (L.) Boiss. root nodules from Northwest coast of Algeria. Afr J Biotechnol 15(20):854–867