Chapter 5
Diversity, Nitrogen fixation, and Biotechnology of Rhizobia from Arid Zone Plants

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5.1 Introduction

Some microbes have the ability to stimulate the seedling emergence and further growth in poor structured soils and hence can be used as agrochemicals. They react with various metabolites released by plant roots. This interaction helps in nutrient uptake of plants, adaptation of plants to adverse soil chemical conditions, and susceptibility to disease (Bouhmouch et al. 2005). The beneficial soil microorganisms have been widely studied due to their potential for agricultural productivity (Davranova et al. 2013). These microbes in the rhizosphere are important for their beneficial effect on plant growth, especially under stress conditions (Zahran 2011).

The term rhizobia generally refers to members of the genus *Rhizobium*, but in true sense it includes all bacteria that are capable of nodulation and fixing the nitrogen in association with leguminous crop (Willems 2006; Tiana et al. 2012). Rhizobia are soil-inhabiting heterogeneous group of diverse bacteria (Lema et al. 2012) with the potential to stimulate nodule formation with the roots of both leguminous and non-leguminous plants (Schwieger and Tebbe 2000; Nelson and Sadowsky 2015). These bacteria enter into the root tissues via root hairs or directly via wounded tissues during specific interactions with the host and induce nodule formation on roots and/or shoots (Zakhia et al. 2006). The rhizobia fix atmospheric nitrogen into ammonia through effective nodules and support plant growth. In return, the rhizobia obtain nutrition and protection by the host in symbiotic manner (Lodwig and Poole 2003; Bhattacharjee and Sharma 2015). The ineffective nodules do not fix nitrogen, but nutrients are absorbed by the rhizobia; in this situation, the rhizobia could be

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considered parasitic (Denison and Kiers 2004; Fujita et al. 2014). This symbiotic relationship for nitrogen fixation has been extensively studied in agriculture for improving soil health and crop yields (Zahran 2009; Mus et al. 2016).

Rhizobia have a large amount of homospermidine and are found in both free-living form in soils and inside of a host showing their adaptable approach for survival, by inhabiting soils or root nodules developed through rhizobia–legume interactions (Fujihara 2009). Phylogenetically rhizobia are very diverse, representing several lineages, comprised of 12 genera and more than 90 species of α- and β-proteobacteria (Tiana et al. 2012) including *Rhizobium*, *Bradyrhizobium*, *Azorhizobium* (Benson et al. 2015), *Mesorhizobium*, and *Sinorhizobium* (Chriki-Adeeb and Chriki 2016), and belong to the family Rhizobiaceae. Rhizobia are gram-negative, rod-shaped (~0.5–0.9 μm in width and 1.2–3.0 μm in length), heterotrophic (Prescott et al. 1996; Ahmed and Abdelmageed 2015), and nonspore formers. The dormant or stationery phase bacteria can survive under environmental stress conditions, whereas actively growing bacteria usually die under stress conditions (Feng et al. 2014). With the advent of modern biotechnological tools and techniques such as rDNA sequencing, 16S diversity, DNA–rRNA hybridizations, and rRNA catalogues, more diversity of rhizobia could be exposed and their relationships with other groups of bacteria could be discovered which led to a gradual increase in the number of its genera. Bakhoum et al. (2014) reported high diversity among root-nodulating bacteria of *A. senegal* and found its association with arid and semiarid regions. The study of the diversity and distribution of rhizobial strains may be exploited in the formulation of inoculants to combat adverse environmental conditions of the arid regions. The symbiotic association of legume–rhizobia contributes approximately 80% biologically fixed nitrogen including 25–30% of the worldwide protein intake. The ability of rhizobia to inhibit certain soil-borne plant pathogens has extended the importance of rhizobia and is receiving increasing attention in sustainable agriculture (Gautam et al. 2015). This chapter provides an overview of the recent developments and concerns associated with the diversity, nitrogen fixation, and biotechnology of rhizobia from arid zone plants.

5.2   Arid Region and Rhizobia

The climate of arid zones is often characterized as hot and dry summers, subhumid monsoon, and cold dry winters. The high temperature, low relative humidity, high evaporation rate, and scanty rainfall are major features of arid regions. The soils of these regions are generally deficient in nitrogen and organic matter (Rajasekar et al. 2015). These ecosystems are characterized by lack of moisture and nitrogen, but drought and salt stresses are probably main factors that inhibit the growth of organisms in arid and semiarid regions (Fita et al. 2015; Rajasekar et al. 2015). Higher soil temperatures (35–40 °C) and environmental conditions prevailing in arid regions generally result in ineffective nodulation and restrict the plant–microbe symbioses in the arid zone plants (Requena et al. 2001). Rhizobia have potential to restore soil fertility and sand dune stabilization in arid regions. The natural rhizobia

of leguminous plants growing in arid zones exhibit higher tolerance to prevailing adverse conditions like salt stress, elevated temperature, and drought. Several rhizobial strains have been reported to be heat tolerant and establish effective symbioses with their host (McIntyre et al. 2007) showing their relevance in arid climates. The biological nitrogen fixation (BNF) is the best way for nitrogen input into desert ecosystems, and *Rhizobium*–legume symbiosis represents the major mechanism of BNF in arid lands (Zahran 2001).

5.3 Biological Nitrogen Fixation in Arid Regions

The effective management of nitrogen is essential for sustainable agriculture. Biologically fixed nitrogen plays an important role in this direction as the nitrogen obtained from this phenomenon is less susceptible to volatilization, denitrification, and leaching and is directly used by the plant. About 80% of biological nitrogen in agricultural settings comes from symbioses of leguminous plants and species of *Rhizobium*, *Sinorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Azorhizobium*, and *Allorhizobium* (Graham and Vance 2000). The leguminous plants of arid zone, i.e., *Prosopis* species*, Acacia* species*, Cyamopsis tetragonoloba*, *Vigna unguiculata*, *V. aconitifolia*, *Macrotyloma uniflorum*, *Phaseolus vulgaris*, *Medicago sativa*, *Arachis hypogaea*, etc., have drought resistance mechanisms for their survival in these areas. The osmoadaptation of the host and rhizobia is essential for maintaining effective symbiosis and nitrogen fixation in these regions. The *Rhizobium*–legume symbioses are the important nitrogen-fixing systems and may have the potential to increase nitrogen input in arid lands. The nitrogen fixed by the symbiotic association of *Rhizobium* species and legumes represents a renewable source of nitrogen for agriculture estimating 200–300 kg nitrogen per ha per year (Peoples et al. 1995). The crops planted after harvesting of legumes often yield equivalent to those expected from application of 30–80 kg of nitrogen per ha. The nitrogen fixed for alfalfa, red clover, pea, soybean, cowpea, and vetch is estimated about 65–335 kg (Tate 1995) or 23–300 kg of nitrogen per ha per year (Wani et al. 1995). The BNF is one of the important ways for nitrogen input into desert ecosystems and nearly half of biologically fixed nitrogen is symbiotic (Werner and Newton 2005). *Rhizobium*–legume symbioses represent the major mechanism of BNF in these areas as compared to other nitrogen-fixing bacteria and heterotrophs (Laranjo et al. 2014).

The symbiotic association offers competitive advantage for rhizobial survival by attaching to roots or living in the rhizosphere due to root exudation. One gram of root is estimated to release 50–100 mg of exudate, sufficient to support more than 2000 bacteria (Morgan et al. 2005), and even non-leguminous host can support a considerable number of rhizobia. Yanni et al. (1997) recovered 106 cells of *Rhizobium leguminosarum* bv. *Trifolii* cells from the each gram of internal tissues of fresh rice roots. The rhizobia are symbiotically competent however; symbiotically incompetent rhizobial strains have also been reported (Krol and Becker 2014). Rhizobia with survival ability in desert soils and arid regions that showed effective

symbiotic characteristics with their host legumes have been identified (Jenkins et al. 1989). Effective rhizobia are competitive and able to migrate under scarce moisture conditions (Wadisirisuk et al. 1989). Athar and Johnson (1996) reported two mutant strains of *Rhizobium meliloti* that were competitive with naturalized alfalfa rhizobia and were symbiotically effective under drought stress. They suggested that the nodulation, growth, and nitrogen fixation can be improved by inoculation with competitive and drought-tolerant rhizobia in alfalfa. The seeds of leguminous plants may be inoculated with rhizobia to increase nodulation and nitrogen fixation. The inoculated seeds resulted in improved seed germination and seedling traits than from non-inoculated seeds (Singh et al. 2011; Pancholy et al. 2011). Peoples et al. (1998) compared the annual herbage production under stress and reported that *Medicago*-based pastures could fix more than 70 kg of nitrogen per ha per year, making them efficient and sustainable. Various conditions that influence the competition and saprophytic capability of rhizobia under environmental factors have been studied, and it has been reported that the increasing oxidative flux of proline in bacteroids may provide an agronomically significant yield advantage under uncertain stress conditions (Hayat et al. 2012).

Naturally occurring annual and perennial legumes are well nodulated, and their root nodules are active in nitrogen fixing. Jha et al. (1995) suggested the suitability of rhizobium- inoculated wild herb legumes for providing vegetation cover and improving soil fertility in unreclaimed lands. Studies revealed that *Rhizobium* strains isolated from *Astragalus cicer* and wild desert plants effectively nodulate *M. sativa* and *P. vulgaris* (Zhao et al. 1997) and *Vicia faba* and *Pisum sativum* (Zahran and Sprent 1986), respectively. The rhizobia of wild legumes may have better traits than the homologous rhizobia, and inoculation of effective rhizobia from wild legumes to other legume crops is a novel strategy to improve the effectiveness of the *Rhizobium*–legume symbiosis (Zahran 2001).

5.4 Rhizobia and Stress

The rhizobia establishing reciprocal or symbiotic associations with the roots of the hosts (Schwieger and Tebbe 2000) have to cope with various environmental stresses including drought, low pH, salinity, high temperature, heavy metal toxicity, and nutrition deprivation stresses (Salvagiotti et al. 2008; Laranjo and Oliveira 2011) and that may affect their survival, growth, and/or symbiotic performance in the field (Howieson and Ballard 2004). Low soil pH may alter motility mechanisms of the rhizobia and disturb the molecular signals of the legume symbiosis (Morón et al. 2005); similarly, higher soil pH can also affect the rhizobial growth in soil (Dilworth et al. 2001). The survival, growth, and structure of the rhizobia are badly affected by the water stress, and at severe conditions, the formation and longevity of nodules and its functions are negatively affected leading to the permanent cessation of nitrogen fixation (Nadeem et al. 2014). The soil salinity has been reported to be disadvantageous for the survival and diversity of natural rhizobial populations due

to direct toxicity as well as through osmotic stress (Diouf et al. 2007; Vriezen et al. 2007; Thrall et al. 2008) and affects the distribution of rhizobia in arid soil and rhizospheres. The higher temperatures are detrimental to rhizobia and host and may inhibit the adherence of rhizobia to the root hair and formation of root hair and infection thread. The rhizobial species and strains differ in the high temperature tolerance and selection of strains for temperature tolerance may be a good means of overcoming temperature stress (Bansal et al. 2014).

5.4.1 Drought Stress and Water Deficiency

Understanding legumes–rhizobia responses to drought is of great importance and a fundamental part of making symbiotic combinations stress tolerant (Mouradi et al. 2016; Serraj et al. 1999). As a result of water removal and exposure to the atmosphere, the plant cells become susceptible to the chemical damage and formation of certain molecules can induce the lipid peroxidation, protein denaturation, and nucleic acid damage (Casteriano 2014). Drought not only affects the rhizobial infection, its persistence, survival, and colonization but also limits the nodulation (Mhadhbi et al. 2011). However, some rhizobial species have shown their ability to tolerate and survive under drought and severe water deficit conditions (Abolhasani et al. 2010). These strains can support severe drought conditions by various adaptive strategies and production of a number of compounds including chaperones and sugars, synthesis of stress enzyme 1-aminocyclopropane-1-carboxylic acid, and production of exopolysaccharides, trehalose, phosphate-solubilizing agents, siderophores, phytohormones, etc. (Hussain et al. 2014). These strains can be used to improve drought impact on plants and to assist them to tolerate adverse conditions by producing physical and chemical changes (Yang et al. 2009).

The rhizobia modify its morphological structure under water deficiency conditions, and the changed morphology leads to reduction in rhizobial infection and nodulation of legumes and restriction of nodule development and function. Several legumes, viz., *C. tetragonoloba*, *V. unguiculata, V. aconitifolia, M. uniflorum*, and *Cicer arietinum*, are tolerant to water deficit condition and have developed mechanisms to tolerate inadequate water supply. Biochemical modifications such as increase in sucrose (Lobato et al. 2009), reduction of soluble proteins, and increase in total amino acids (Costa et al. 2011) contribute to osmotic adjustment of these plants. The rhizobia can exist in arid soils, but their density is lowest under the most desiccated conditions; however, it increases as the moisture stress is relieved (Jenkins et al. 1989). Studies on *Rhizobium*–legume symbiosis show that trehalose content in nodules under drought stress correlates positively with an increase in plant tolerance to this stress. Almanza et al. (2010) explored the effect of co-inoculation with mycorrhiza and rhizobia on the nodule trehalose content of different bean genotypes under contrasting moisture conditions. They found that the correlation analysis showed a significantly positive correlation between mycorrhizal colonization and nodule trehalose content.

The wide range of variability can be observed for the rhizobial strains with different sensitivity to soil moisture, and moisture stress-tolerant rhizobial strains can be identified that effectively nodulate arid legumes. Wadisirisuk et al. (1989) observed that osmotolerant rhizobia can migrate even under scarce moisture conditions. Zahran (1999) improved nodulation and nitrogen fixation in *M. sativa* by inoculating plants with drought-tolerant rhizobia. Athar and Johnson (1996) reported that osmotolerant strains of *R. meliloti* performed better than those of the nontolerant alfalfa rhizobia and formed effective symbiotic relationship under drought conditions. It has been reported that the rhizobial strains with ability for increased accumulation of compatible solutes are able to perform better in stressed soil (Straub et al. 1997).

Trees of genera *Acacia* and *Prosopis* are of central importance in rural economy of arid regions (Roshetko 2001) as these trees can grow with rainfall as low as 40–50 mm per year and the long tap roots of trees reach the groundwater during rainless period. These trees contribute toward the soil stabilization and improvement through nitrogen fixation (Fagg and Stewart 1994), agroforestry potential, and nodulation ability (Masutha et al. 1997). Even under water stress conditions prevailing in these regions, the tree legumes could fix 43–581 kg of nitrogen per ha as compared to annual legumes which fix 15–210 kg of nitrogen per ha (Dakora and Keya 1997).

5.4.2 Salt Stress

Salinity reduces the ability of plants to take up water, which in turn reduces growth rate along with several metabolic changes (Munns 2002). Salt stress inhibits the initial steps of rhizobia–legume symbioses by causing root hair curling and reducing nitrogen fixation (Kulkarni and Nautiyal 2000; Laranjo and Oliveira 2011). Soil salinity or acidity is detrimental for the survival and diversity of natural rhizobial populations and also restricts their ability to establish symbiotic associations (Bala et al. 2003; Diouf et al. 2007). Diversity among salt-tolerant rhizobia has been studied. Many rhizobia are fairly salt tolerant and are capable of living under severe moisture deficient conditions (Zahran 1999; Sadowsky 2005). Studies revealed that free-living rhizobia are more salt tolerant than their host legumes (Zahran et al. 2007; Laranjo and Oliveira 2011). A number of workers reported rhizobial isolates with high capacity (1–5% NaCl) for salt tolerance (Zou et al. 1995; Maatallah et al. 2002; Al-Shaharani and Shetta 2011; Zahran et al. 2012). Some rhizobial strains collected from arid and saline areas were reported as highly salt tolerant that withstand at high levels (5–10%) of NaCl (Zahran et al. 2003).

Salinity and drought under arid conditions have adversely affected the growth, development, and yield of *Cicer arietinum* (Khaitov et al. 2016). Salinity inhibited the survival and proliferation of *Rhizobium* spp. in the soil and rhizosphere. Salinity coupled with drought significantly declines plant biomass, nodule development, nitrogenase activity, and yield of the legume crop (Garg and Baher 2013;

Egamberdieva et al. 2014). It has been observed that the survival of rhizobia in the plant root and soil is affected by nutrient deficiency, salinity, drought, and acidity (Slattery et al. 2004). The efficient plant growth-promoting bacteria improve the production of chickpea and overcome these stresses. Inoculation of plants with *Rhizobium* sp. significantly increased shoot, root dry matter, and nodule number as compared to the uninoculated plants (Khaitov et al. 2016). The salt tolerance abilities of rhizobia may have an important effect on the successful *Rhizobium*–legume associations under salinity conditions. Bano et al. (2010) reported that bacterial strains adapted to drought stress are effective in the root-nodule symbiosis and alleviate growth and yield of the legume imposed by drought stress.

The rhizobia, tolerant to extremely higher levels of salts, have been isolated from various crop and wild legumes (Ali et al. 2009). Zeghari et al. (2000) observed some *Acacia* and *Prosopis* strains that are able to tolerate up to 500 mM NaCl. Positive response of *Pseudomonas fluorescence* and *R. meliloti* co-inoculation on nodulation and mineral nutrient contents in alfalfa under salinity stress conditions has been reported by Younesi et al. (2013). It has also been reported that rhizobia from naturally growing tree legumes in the deserts are able to survive, grow, and effectively nodulate their leguminous hosts even at high salt concentrations (Sobti et al. 2015). Inoculation with *Rhizobium* is an effective approach to strengthen nitrogen fixation, increase nutrition, and promote yield in the legume crops. Therefore, inoculation with the effective rhizobial inoculants might be an important approach to improve crop production under salinated soil conditions.

5.4.3 Temperature Stress

The root zone temperatures not only influence the survival of rhizobia in the soil but also affect the exchange of molecular signals between the symbiotic partners (Zahran 1999; Hungria and Vargas 2000) leading to reduced nitrogenase activity (Lira Junior et al. 2005). Rhizobia are mesophilic in nature and require optimum temperatures ranging from 28 to 31 °C for their growth in culture (Graham 2008), while it ranges between 35 and 45 °C for free-living rhizobia (Zahran et al. 2012; Abd-Alla et al. 2014). The heat stress may cause plasmid alterations in cellular polysaccharides and the rhizobia may lose the capacity of infectivity (Zahran 1999). Thus, high soil temperature results in the formation of ineffective nodules; some strains of rhizobia have been reported to be heat tolerant and form effective symbioses with their host legumes. Rhizobia growing in soils in India during the summer season are subjected to high temperature stress. Bansal et al. (2014) studied the symbiotic effectivity of high temperature tolerant mung bean (*Vigna radiata*) rhizobia under different temperature conditions and reported that two rhizobial isolates, namely, MR23 and MS57, were capable of forming nodules even at 49 °C under sterilized Leonard jar conditions, while MR14 did not form any nodule. By contrast, nodulation of soybean was markedly inhibited at higher temperatures (Chibeba et al. 2015).

Some studies revealed that rhizobial strains from *Sesbania aculeata* survived at 50 and 65 °C on yeast mannitol agar at pH 7 up to 2 and 4 h (Kulkarni et al. 2000). Zahran et al. (2012) isolated a number of rhizobial strains growing at 40 and 45 °C, and a strain isolated from *Trifolium resupinatum* showed growth even at 50 °C. The rhizobial strains isolated from hot climatic area had more tolerance to abiotic stress especially temperature (Mishra et al. 2013). Bansal et al. (2014) isolated temperature-tolerant rhizobia from mung bean and evaluated under different temperature regimes under greenhouse conditions. The rhizobial growth at different temperatures showed that all rhizobia exhibited optimum growth between 30 and 40 °C and two isolates MR23 and MS57 showed good growth even at 45 °C. The survival of *Sinorhizobium meliloti* was enhanced when cells were dried in stationary phase with an increasing drying temperature (Vriezen et al. 2006). Such studies may help in development of improved strains for stress conditions.

Trehalose metabolism in rhizobia has important role for signaling plant growth, yield, and adaptation to abiotic stress (Suarez et al. 2008). Increased plant growth, nitrogen content, and nodulation of *P. vulgaris* have been reported under drought stress due to co-inoculation of *R. tropici* and *Paenibacillus polymyxa* (Figueiredo et al. 2008). The *P. vulgaris* plants inoculated with *Rhizobium etli* expressing trehalose-6-phosphate synthase gene had more nodules, higher biomass, and increased nitrogenase activity as compared to plants inoculated with wild-type *R. etli*. Similarly, the plants inoculated with strain overexpressing trehalose-6-phosphate synthase gene revealed upregulation of genes related to the stress tolerance. The high temperature tolerant rhizobial isolates accumulated more trehalose when grown at higher temperature as compared to reference strains at normal temperature (Nandal et al. 2005; Bansal et al. 2014). It has also been suggested that trehalose could function as an osmoprotectant in rhizobia species under stress conditions and protects bacterial cells from heat during the salinity and moisture stress (McIntyre et al. 2007). Figueiredo et al. (2008) reported that the co-inoculation of common bean plants with rhizobia and *P. polymyxa* alleviated the adverse effects of drought stress and maintained plant development and growth.

5.5 *Rhizobium*–Legume Symbioses Under Arid Regions

The legume–*Rhizobium* symbiosis is the most popular association in any ecosystem and contributes an important role in the nitrogen enrichment of soils as compared to other biological nitrogen-fixing systems. But it is sensitive to various environmental stresses, viz., drought, soil pH, temperature, water logging, low phosphorus, and other nutrient limitations (Zahran 1999), as discussed above. The legume cropping systems increase soil fertility, enhance the plant productivity, and prevent erosion and desertification (Egamberdieva et al. 2014). Incorporating of legume in crop rotation has been reported to increase the yield of cotton and wheat (Khaitov et al. 2014). The *Rhizobium*–legume symbiosis has received most attention as it is widely used for sustainable crop yield and soil fertility in the agricultural practices (Egamberdieva et al. 2015). The legumes have the ability to shift the

composition of soil bacterial community (Lorenzo et al. 2010; Bakhoum et al. 2012) and can change the communities responsible for symbiotic nitrogen fixation (Rodríguez-Echeverría 2010).

The nodulation is also influenced by a number of factors, viz., salt and water stress, temperatures, soil type, pH, organic matter content, rhizobial populations, nature of the host, etc. (Dudeja et al. 2012; Bansal et al. 2014), and varies from 0 to 10^5 per gram of soil. Studies reveal that the *Rhizobium*–legume symbioses may occur under severe environmental conditions including exposure to salt, aridity, acidic or alkaline soil, higher temperatures, nutrient deficiency, and soil toxicity (Zahran 1999; Sadowsky 2005). The associated environmental stresses with legume–*Rhizobium* symbiosis have been studied in several legume species (Abd-Alla et al. 2014; Laranjo et al. 2014). Fujishige et al. (2006, 2008) reported that rhizobia establish biofilms on either biotic or abiotic surfaces for its overall fitness in the soil and rhizosphere contributing to an efficient symbiosis (Rinaudi et al. 2006). The biofilms are basically surface-attached bacterial communities of single or multiple species covered within a self-produced extracellular matrix (Stanley and Lazazzera 2004). In this biofilm, rhizobia are protected from various environmental stresses due to lower metabolic rate and exopolymeric matrix. Several examples prove that in spite of the fact that rhizobia are nonspore formers, they remain viable under severe stress (Gorbushina et al. 2007). Some rhizobial species survive in the soil at least 4–5 years without their host (Hirsch 2010).

The leguminous trees have been used for a variety of food, feed, and fuel wood purposes in semiarid regions (Jindal et al. 2000; Aoki et al. 2007), and trees of the genera *Acacia* and *Prosopis* are of immense importance in the rural economy of the arid and semiarid areas due to their resistance to heat, drought, salinity, and alkalinity. The species of *Acacia* and *Prosopis* contribute to soil stabilization and improvement through nitrogen fixation (Rasanen and Lindstrom 2003; Singh et al. 2011) along with providing high-quality animal fodder, timber, fuel wood, charcoal, gums, and other products. *Prosopis* have deep roots and are well nodulated under drought conditions with the potential to fix nitrogen. Chickpea can restore soil fertility due to deep penetrating root system which enables them to utilize the limited available moisture (Tripathi et al. 2015). The nitrogen fixed by various tree legumes ranged from about 20–84% (Al-shaharani and Shetta 2015). Dakora and Keya (1997) reported that the tree legumes fix about 43–581 kg of nitrogen per ha, compared to about 15–210 kg of nitrogen per ha for grain legumes. Wange (1989) obtained effective symbioses between woody rhizobia from *Acacia* and other tree species with peanut and cowpea. He reported that this symbiosis was more effective as compared to the symbiosis between the trees and their homologous rhizobia. Zhang et al. (1991) isolated rhizobia from the trees of *Acacia* and *Prosopis*, grown in arid regions, and reported effective symbiosis with legumes, e.g., *P. vulgaris*, *V. faba*, and *M. sativa*. The rhizobia isolated from *Acacia nilotica*, *Sesbania sesban*, *Alhagi maurorum*, *Melilotus indicus*, and *T. resupinatum* showed more or less efficient symbiotic performance with *V. faba*, *Vigna sinensis*, *P. sativum*, and *M. sativa* (Zahran 1999).

The severe water deficit response in cowpea reduced the nodule mass and affected constituents of nodule structure, while in moderate stress the impact on nodule water content was higher as compared to changes in nodule mass (Figueiredo et al. 1999). They also reported that the inoculation with *Bradyrhizobium* can improve the negative effect of water deficient *V. unguiculata*. The water deficit negatively affected the growth and nodulation parameters in the alfalfa–rhizobia combinations, while combinations involving RhL9 rhizobial strain expressed more tolerance levels than the other combinations (Mouradi et al. 2016). Mishra et al. (2013) isolated 15 rhizobial bacteria nodulating clusterbean from arid and semiarid regions of Rajasthan to identify effective and competitive strains tolerant to various abiotic stresses such as temperature, pH, and salinity and observed that the rhizobial isolates from hot climatic area had more tolerance to abiotic stress, especially temperature. Several authors reported that the growth of leguminous trees can be improved by inoculation with effective rhizobia (Bogino et al. 2006; Maia and Scotti 2010). The *N*-acyl homoserine lactone signals found in many species of legume-nodulating rhizobia regulate all the important issues for successful establishment of a bacteria–plant symbiosis including nodulation, nitrogen fixation, growth rate, and polysaccharide production (González and Marketon 2003).

5.6 Biotechnology

The information on the gene regulation in response to various stresses by rhizobia is scanty, and not much information about the tolerance or stress resistance signaling pathways is available for this group. DNA microarray was employed to examine gene expression in *S. meliloti* cells under increased NaCl or sucrose stress to monitor high salinity and hyperosmotic stress, respectively (Domínguez-Fererras et al. 2006); overlapping effects on gene transcription in response to high salinity and hyperosmotic stress were observed with differential expression of a large number of genes. The rhizobial growth requires pSymB plasmid in response to salt stress, and the plasmid is essential for saprophytic competence of *S. meliloti*. The flagellar biosynthesis genes for *S. meliloti* were downregulated after osmotic upshift, and a number of genes essential for succinoglycan biosynthesis, including *exoP*, *exoM*, and *exoN*, were also strongly upregulated upon the induction of drought stress (Domínguez-Fererras et al. 2006). Cytryn et al. (2007) analyzed the response of *B. japonicum* to drought using a genome-wide transcriptional analysis and observed that many genes responsible for regulation of transcription, DNA repair and cell cycle regulation, cation uptake and heat shock, pili assembly proteins and flagellin, transport of sucrose and other molecules, succinylation of osmoregulated periplasmic glucans, energy transfer, and various aspects of metabolism were upregulated including upregulation of lipopolysaccharide synthesis transferase in *B. japonicum* under drought-stress conditions.

The 16S ribosomal RNA (rRNA) gene is commonly used for the rhizobial phylogeny and taxonomic studies (Větrovský and Baldrian 2013) because it is

usually present in a sufficient size (about 1500 base pairs) as a multigene family or as operons in almost all bacteria, and little change in their function is recorded over time (Patel 2001; Janda and Abbott 2007). Pancholy et al. (2011) molecularly characterized the diverse groups of plant growth-promoting rhizobacteria in the rhizosphere and root nodules of native *Acacia senegal a*nd *Prosopis cineraria* trees of western Rajasthan using *16S rRNA* gene sequencing to reveal the presence of genetic diversity. They identified eight rhizospheric isolates as *Sinorhizobium saheli* out of the nine isolates obtained from *A. senegal*, whereas out of the eight isolates from *P. cineraria* one was identified as *Sinorhizobium kostiense* and five as *S. saheli*. The molecular identification studies revealed that *S. saheli* were found to be associated with the root nodules of *A. senegal* and *S. kostiense* and *S. saheli* with root nodules of *P. cineraria* in the arid region of Rajasthan (Singh et al. 2011). Rasanen et al. (2001) reported *S. saheli* and *S. kostiense* from *Acacia* and *Prosopis* nodules in Sudan and Senegal.

Haukka et al. (1996) performed the sequence analysis of 230-nucleotide segment of 16SrRNA (rDNA) gene and observed one strain belonging to *R. huakuii*, while the rest belong to species of the genus *Sinorhizobium*. Similarly, Khbaya et al. (1998) performed rDNA gene and 16S-23S rRNA analysis in the majority of rhizobia nodulating four *Acacia* species (*A. cyanophylla*, *A. gummifera*, *A. horrida*, and *A. radiana*) and identified them as *Sinorhizobium* and *R. galegae–Agrobacterium tumefaciens* species. Lafay and Burdon (1998) isolated nodules from shrubby legumes, characterized 21 genomic species by small-subunit ribosomal DNA PCR–RFLP, and performed phylogenetic analyses among 745 rhizobial strains. The *R. tropici* dominated among the *Rhizobium* and *Mesorhizobium* isolates of these shrubby legumes, while *Bradyrhizobium* species were the most abundant. Further, Lafay and Burdon (1998) investigated the structure of rhizobial communities' nodulating *Acacia* using molecular approach and characterized 118 isolates from nodule samples of 13 different *Acacia* species collected from 44 sites. The ATP synthase beta-subunit (*atpD*), glutamine synthetase II (*glnII*), and DNA recombinase A (*recA*) were also used along with 16S rRNA for accurate classification of the rhizobia (Gaunt et al. 2001).

Various genes including nodulation (*nod, nol*, and *noe*) and nitrogen fixation (*nif* and *fix*) genes are involved in nitrogen fixation of legume–rhizobia symbiosis. The *nod* genes facilitate the nodulation process by encoding biosynthesis enzymes (Laranjo et al. 2014), while the nitrogen fixation genes catalyze the nitrogen fixation reaction by the synthesis of the enzyme complex (Cummings et al. 2009; Wang et al. 2012). Tan et al. (1999) isolated nodule from 11 species of wild legumes and characterized them on the basis of 16S rRNA gene RFLP and sequence analysis, DNA–DNA hybridization, and restriction pattern of nodDAB and *nif*H genes and reported that most of them belonged to the genus *Mesorhizobium*. Wang et al. (1999) isolated 50 rhizobial isolates from root nodules of *Mimosa affinis* and identified as *R. etli* on the basis of the results of PCR–RFLP and RFLP analyses of small-subunit rRNA genes, multilocus enzyme electrophoresis, and DNA–DNA homology. They proposed a new biovar *mimosae* within *R. etli* obtained from *M. affinis* which formed nitrogen-fixing nodules on the legume tree *L. leucocephala*.

Zakhia et al. (2006) performed 16S ARDRA, SDS-PAGE of total cell proteins, and 16S and ITS rDNA sequencing and reported diverse and externally distributed isolates in both ARDRA and SDS-PAGE analyses.

The modification of bacterial strains for synthesis of the peptide antibiotic trifolitoxin and the transfer of such genes to other less-effective rhizobia is another challenge under biotechnology field. The peptide antibiotic trifolitoxin produced by some strains of *R. leguminosarum* bv. *trifolii* is toxic to a wide range of rhizobia though trifolitoxin (*tfx*) genes expressed by *R. etli* were reported to be more competitive for nodulation in unsterilized soil than its close isogenic strain (Robleto et al. 1997). The chromosomal DNA transferred from the salt-tolerant *Bacillus* species into a strain of *R. leguminosarum* made it salt tolerant (Mabrouk and Belhadj 2012).

5.7 Diversity of Rhizobia from Arid Zone Plants

The rhizobia of arid zone plants nodule various naturally growing trees and herbs, in cultivated and noncultivated lands, including legumes native to arid regions. The majority of rhizobia isolated from arid zone plants have a wide diversity. Many are closely associated with various partners, while some are selective and specific with narrow host range (Perret et al. 2000; Tan et al. 1999). For example, *Leucaena leucocephala* is one of the wide-host range legumes and is nodulated by various strains of bean-nodulating rhizobia (Mhamdi et al. 2000). Similarly, *P. vulgaris* has been reported with diverse strains of rhizobia belonging to different species of *R. leguminosarum*, *R. etli*, and *R. tropici* (Van Berkum et al. 1996). Bakhoum et al. (2014) reported a large diversity among rhizobial strains from arid and semiarid zones associated with *A. senegal* on the basis of IGS 16S–23S rDNA and observed that the distribution of root-nodulating bacteria associated with *A. senegal* was correlated to physical and chemical characteristics of the soils. The characterization and isolation of native rhizobial population is essential for the efficient exploitation of biological nitrogen fixation.

Diversity has great importance in the function of root nodulation and assists the plants to cope up with soil stresses (Bala and Giller 2007). Rhizobial populations' diversity could be greatly influenced by the plant provenance used for trapping, and analysis of rhizobia from different geographic regions along with their host plants is essential to characterize better interactions between rhizobia, legumes, and geographical factors (Liu et al. 2005). *A. senegal* is a multipurpose species and has major importance for the reforestation of arid and semiarid zones and is much valued by rural populations as a source of fodder and timber and gum Arabic (Muller and Okoro 2004). Enormous genetic diversity has been reported among the root-nodulating bacteria that are able to nodulate *A. senegal* (Sarr et al. 2005; Fall et al. 2008).

The clusterbean root nodules provide greater recovery to the crop and help in fixing the atmospheric nitrogen in adequate quantity (Pathak 2015a, b). Strains of

legume bacteria are selective in terms of the crop species they nodulate, and the plant within a cross-inoculation group can be inoculated with a culture of the right kind of bacterial strains (Stafford and Lewis 1980). For example, clusterbean belongs to the cowpea cross-inoculation group and the rhizobial strain that formed nodules on cowpea also formed nodules on cluster bean roots (Hassen et al. 2014; Pathak 2015a, b). Boukhatem et al. (2012) investigated the diversity of rhizobia associated with *Acacia* species and reported that the in vitro tolerances of rhizobial strains to NaCl and high temperature varied regardless of their geographical and host plant origins. They reported that the *A. saligna* was efficiently nodulated with the widest diversity of rhizobia including both fast-growing (*Rhizobium*, *Ensifer*, *Mesorhizobium*) and slow-growing ones (*Bradyrhizobium*). Bakhoum et al. (2014) revealed higher genetic diversity among rhizobial strains of semiarid region than in the arid region and the distribution of root-nodulating bacteria associated with *A. senegal* was influenced by soil physical and chemical characteristics. They further observed that the rhizobial strains nodulating *A. senegal* were closely related to *Mesorhizobium plurifarium*. Araujo et al. (2015) reported high species diversity of rhizobia-nodulating *P. lunatus* on the basis of partial sequence of the 16S rRNA genes analysis.

The rhizobia of the leguminous trees under arid conditions have been classified into different genera including *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, and *Mesorhizobium* (Tan et al. 1999; Wang et al. 1999; Tighe et al. 2000; Zahran et al. 2000) on the basis of phenotypic, genotypic, and molecular analysis. Various workers grouped the rhizobia isolated from *A. senegal* and *P. chilensis*, on the basis of phenotypic characteristics (Zhang et al. 1991), lipopolysaccharide profiles (Lindstrom and Zahran 1993), profiles of proteins, plasmids, and fatty acids, and DNA–DNA hybridization (Zahran 1997) and phylogenetic analyses (Haukka et al. 1996). Doignon-Bourcier et al. (2000) isolated 64 *Bradyrhizobium* strains from nodules of native leguminous plant species in arid regions belonging to the genera *Cassia*, *Crotalaria*, *Sesbania*, etc., and assessed the genotypic diversity. They reported that these strains were diverse and formed 27 groups by amplified fragment length polymorphism and 16 groups by intergenic gene spacers, PCR, and restricted fragment length polymorphism.

5.8   Conclusion

The industrial fertilizer production is costly and requires large inputs of fossil fuel and results in pollution in the form of greenhouse emissions and fertilizer runoff. The symbiotic/biological nitrogen fixation is not only inexpensive but also sustainable process to substantiate or gradually replace chemical fertilizers to improve the production and productivity of legumes. The rhizobia and legume adapt well under different ecological conditions including arid region and fix considerable amounts of nitrogen. The adverse environmental conditions of arid region may affect the quality and quantity of the rhizobia. Identification and use of efficient rhizobial strains and species that could effectively work under stressed soil environment may

improve the productivity of the nutrient-poor arid soils. Breeding for enhanced nitrogen fixation for soil acidity/alkalinity, root rot disease, phosphorus tolerance, soil pH, high temperatures, and scanty soil moisture needs to be addressed.

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