Chapter 11 Rhizobial Inoculants for Sustainable Agriculture: Prospects and Applications



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Abstract Due to continuous growth of world population, there is dire need of serious efforts and innovative approaches to meet food demands through sustainable production practices, improvement in supply chain, and control of food wastage. All these efforts should ensure the access to nutritious food to all suffering from hunger and malnutrition. Due to intensive crop cultivation and use of synthetic fertilizers, soil health is seriously deteriorating. However, soil fertility can be improved by incorporating legumes in the cropping system and/or use of rhizobial inoculants, which not only increase nitrogen fixation but also improve soil fertility and crop production through several other attributes such as phosphate solubilization, siderophores production, phytohormones production, enzymes synthesis, and exopolysaccharides production. Moreover, these bacteria can be helpful for improvement in crop production on marginal lands due to their tolerance against various biotic and abiotic stresses. All these characteristics make rhizobia equally important for non-legumes as for legumes. The use of rhizobial inoculants can ensure improvement in crop productivity and environment sustainability by enhancing soil fertility and reduction in use of synthetic chemical fertilizers. Present review focuses on important plant growth-promoting mechanisms of rhizobia and the use of these rhizobia for sustainable crop production through improvement in crop nutrition, physiology, productivity, and stress tolerance of crop plants. The potential of the synergistic use of rhizobia with other soil microorganisms for sustainable agriculture has also been elucidated with examples, followed by their future prospects.

Keywords Rhizobium \cdot Plant growth promotion \cdot Sustainable agriculture \cdot Soil health and fertility

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11.1 Introduction

Decline in soil fertility and organic matter contents is one of the major constraints of crop production in arid and semiarid regions that is attributed to low rainfall, high temperature, and increase in calcareousness of these soils. As the demand for increase in crop production is rising due to expansion of colonization on agricultural lands, farmers try to use more chemical fertilizer which deteriorates soil biology and environmental quality. The governments all over the globe are prioritizing the development of eco-friendly alternate strategies for crop production. Beneficial soil bacteria have significant impact on the growth and productivity of crop plants (Uren 2007). Among these, rhizobia are a group of bacteria which fix atmospheric nitrogen by developing symbiotic association with legumes (Wang et al. 2018). Rhizobia fix about 50% of the total annually fixed nitrogen in the world (Hatice et al. 2008). They develop special structures within the plant cells, called nodules (Beneduzi et al. 2013; Wang et al. 2018). Soil fertility can be improved by incorporating legumes in the cropping system and/or use of rhizobial inoculants which not only increase nitrogen fixation but also improve soil fertility and crop production through several other attributes (Zahir et al. 2018).

The incorporation of grain legumes in cropping system can also be helpful to improve the productivity of the following cereal crops. Moreover, the rhizobia in root nodules of these crops not only fix atmospheric nitrogen in the presence of legume host (Bhattacharyya and Jha 2012) but also help cereal crops through other growth-promoting characteristics such as phosphate solubilization (Khan et al. 2010), siderophores production (Chandra et al. 2007), phytohormones production (Chi et al. 2010), enzymes synthesis (Duan et al. 2009), and exopolysaccharides production (Monteiro et al. 2012). Rhizobia are ubiquitous microorganisms in soil; however, their diversity and population depend upon different factors including crop species, crop rotation, soil properties, agricultural practices, and the extent and distribution of wild species of leguminous plants (Sadowsky 2005; Roberts et al. 2017).

The efficiency of rhizobia varies greatly among different strains depending upon plant host variety, soil and environmental factors, and their interaction (Allito et al. 2014), so efficient host-cultivar-specific combination is recommended in diverse agro-ecological zones and soils with different fertility status. Although *Rhizobium* inoculation increases the nodulation, nitrogen uptake, physiology, shoot and root growth, and yield of legume crops (Sogut 2006; Ahmad et al. 2013a, b), the effectiveness of these inoculants for nodulation and nitrogen fixation is reduced in the presence of high dose of nitrogen-containing chemical fertilizers (Ogutcu et al. 2008). For example, nitrogen application rates greater than 40 kg N ha⁻¹ decreased the nodulation and nitrogen fixation in field pea (Clayton et al. 2004), an initial dose of nitrogen is however, required for establishment of root system at early stages of crop growth (Simonsen et al. 2015). The organic amendments on the other hand increase the nodulation and yield of peanut (Agegnehu et al. 2015) and thus can be used in integration with rhizobial inoculants (Argaw and Mnalku 2017).

Rhizobial inoculants are cheaper than inorganic fertilizers, so less financial risks are present in using them as source to improve productivity of legume crops (Ronner et al. 2016). Rhizobial inoculation is considered to be effective for symbiotic nitrogen fixation (SNF) and is being advocated to be used in the absence of effective rhizobia for a specific crop, in low population of effective indigenous rhizobial that really slows down the nodulation process, and/or when more effective rhizobial inoculants are available for a specific crop variety to be grown than the indigenous rhizobial species (Giller 2001). The selection of native rhizobia is imperative for the development of effective and affordable rhizobial inoculants to improve productivity of agro-ecosystems (Koskey et al. 2017). Moreover, the compatibility of rhizobial strain and host plant species/variety must be taken into account along with plant growth-promoting characteristics. In the case of the combined use of rhizobia with other beneficial soil microbes, the compatibility of strains should be tested before their use as inoculants.

Under field conditions, the inoculated bacterial strains have survival disadvantage as compared to indigenous microbial populations. In addition to strong plant growthpromoting abilities, the bacterial strains in developed rhizobial inoculants should have the ability to effectively colonize plant roots and capability to compete for nutrients and space with indigenous microorganisms in the soil and rhizosphere (Stephens and Rask 2000). Genetic engineering and strain selection can be helpful in improving the survival competency of rhizobial inoculants (Geetha and Joshi 2013).

The application of rhizobial inoculants to improve crop productivity has potential for sustainability of agriculture systems. The integrated use of these rhizobial inoculants with other soil microbes can be more beneficial to improve plant growth (Figs. 11.1 and 11.2) and for sustainable crop production by meeting the climate



Fig. 11.1 Effect of *Rhizobium* and plant growth-promoting rhizobacteria on *Cicer arietinum* under wire house conditions in pot experiment



Fig. 11.2 Effect of *Rhizobium* and plant growth-promoting rhizobacteria on root growth of *Cicer arietinum* under wire house conditions in pot experiment

change challenges and nutrient depletions and biocontrol of plant pathogens. The combined use helps to increase the efficiency of rhizobial inoculants through synergistic effects and combination of various mechanisms of actions in legumes (Pierson and Weller 1994) and non-legumes.

11.2 Plant Growth-Promoting Mechanisms of Rhizobia

Rhizobia are a diverse group of bacteria which are ubiquitous in all types of soils in different agro-ecological zones. In addition to symbiotic nitrogen fixation in legumes, they can improve soil fertility and crop productivity through a number of growth-promoting characteristics. These characteristics have been summarized in Table 11.1. Moreover, these bacteria can help in improving crop productivity on marginal lands due to their tolerance against various biotic and abiotic stresses.

11.3 Nodulation and Symbiotic Nitrogen Fixation

Legumes are considered as important component of cropping systems for maintaining the soil fertility and productivity. These crops have the ability to fix atmospheric nitrogen by forming symbiotic associations with rhizobia present in root nodules. The symbiotic nitrogen fixation (SNF) accounts for major share of globally fixed nitrogen through all means that can meet about 50–60% of crop nitrogen requirements as reported in the case of soybean (Salvagiotti et al. 2008). The SNF in legumes is a complex process, mediated by chemical signals between legume host

Bacterial species	Plant growth-promoting characteristics	References
Rhizobium sp.	IAA production, P solubilization, N fixation	Shengepallu et al. (2018)
Rhizobium sp.	Improved enzymatic activities, N fixation	Mouradi et al. (2018)
Rhizobium sp.	Antagonistic activity, P solubilization, IAA production, ammonia production, siderophores production, HCN production	Manasa et al. (2017)
Rhizobium hainanense	Nitrogen fixation, IAA production, exopolysaccharides production	Mujahidy et al. (2013)
<i>Rhizobium</i> sp.	IAA production, siderophores production, exopolysaccharides production, HCN production, ammonia production	Ahemad and Khan (2010)
Rhizobium sp.	P solubilization	Sridevi and Mallaiah (2009)
Rhizobium sp.	Exopolysaccharides production	Santaella et al. (2008)
Rhizobium leguminosarum	Exopolysaccharides production	Janczarek et al. (2015)
Rhizobium leguminosarum	P solubilization, IAA production, ACC deaminase activity, siderophores production	Prabha et al. (2013)
Rhizobium leguminosarum	Siderophores production, IAA production, P solubilization, N fixation	Flores-Felix et al. (2012)
Rhizobium sp.	Antimicrobial activity	Bhattacharya et al. (2013)
Rhizobium phaseoli	IAA production	Zahir et al. (2010)
Sinorhizobium sp.	Exopolysaccharides production	Castellane et al. (2015)
Sinorhizobium sp.	Chitinase activity, glucanase activity, IAA production, siderophores production, P solubilization	Kumar et al. (2010)
Sinorhizobium meliloti	IAA production, nitrogen fixation, P solubilization	Bianco and Defez (2010)
<i>Mesorhizobium</i> sp.	IAA production, siderophores production, benzoic acid production, exopolysaccharides production, HCN and ammonia production	Ahemad and Khan (2012)
Mesorhizobium sp.	Siderophores, IAA, ammonia, and HCN production, P solubilization, antifungal activity	Ahmad et al. (2008)
Mesorhizobium ciceri	Siderophores, HCN, and ammonia production	Wani et al. (2007b)
Mesorhizobium loti	Siderophores and IAA production, antagonistic activity, P solubilization	Maheshwari et al. (2007)
Bradyrhizobium sp.	P solubilization, IAA, siderophores, and HCN production	Badawi et al. (2011)
Rhizobium	P solubilization, K solubilization, IAA production	Patel et al. (2017)

 Table 11.1
 Plant growth-promoting characteristics of rhizobial strains

(continued)

Bacterial species	Plant growth-promoting characteristics	References
Bradyrhizobium japonicum	ACC deaminase activity, IAA production	Shaharoona et al. (2006)
Azorhizobium sp.	ACC deaminase activity; IAA, ammonia, and siderophores production; P solubilization; Zn solubilization; S oxidation	Islam et al. (2009)
Rhizobium sp.	Exopolysaccharides production	Marczak et al. (2017)
Bradyrhizobium, Rhizobium	Nitrogen fixation, P solubilization, IAA and siderophores production, production of hydrolyzing enzymes (cellulase and pectinase)	Shamsuddin et al. (2014)
Rhizobium cellulosilyticum, Rhizobium radiobacter,	P solubilization, Zn solubilization, IAA production	Gontijo et al. (2018)
Rhizobium sp.	Production of IAA, GA, flavonoid, and siderophores, Zn and P solubilization	Routray and Khanna (2018)
Rhizobium nepotum Rhizobium tibeticum	P solubilization	Rfaki et al. (2015)
Rhizobium sp.	IAA production	Abrar (2017)
Rhizobium sp.	Nitrogen fixation	Malisorn and Prasarn (2014)
Rhizobium sp.	P solubilization	Karpagam and Nagalakshmi (2014)
Rhizobium leguminosarum, Bradyrhizobium japonicum, Mesorhizobium thiogangeticum	P solubilization, IAA production	Singha et al. (2016)

Table 11.1 (continued)

and rhizobia that facilitate nodulation and nitrogen fixation. Complex oxidation and reduction reactions occur during the process of nodulation which consume high amount of metabolic energy, thus reducing atmospheric dinitrogen to ammonia. During the nodulation process, the flavones are released by host plant in the rhizosphere where they trigger the *nod* (nodulation) genes in rhizobia (Subramanian et al. 2006). The activated *nod* genes mediate the production of *nod* (nodulation) factors by rhizobia (D'Haeze and Holsters 2002) which signal the host plant for curling and deformation of root hairs, thus trapping the rhizobia within these special structures (Gage 2004). Infection threads are developed in root hairs through which rhizobia enter in to the inner cortex of plant roots (Jones et al. 2007). Once bacteria enter into the cortical cells of nodule primordium (Mylona et al. 1995), they differentiate into nitrogen-fixing forms "the bacteroids." The bacteroids multiply in the root nodules and fix nitrogen. On nodule senescence, some of these bacteria may enter back into the soil (Denison and Kiers 2011). Bacteria live in the root

nodules, supply fixed nitrogen to plant, and get carbon compounds from plant in return (Lodwig and Poole 2003; Andrews et al. 2009) which are being utilized by these rhizobia as carbon and energy source for respiration and nitrogen fixation, in the form of adenosine triphosphate (ATP) (Lodwig et al. 2003; Hungria and Kaschuk 2014). The SNF can contribute significantly to sustainable crop production.

Rhizobia are very specific to their host plants where they can form nodules and fix atmospheric nitrogen. For decades, scientists were of the opinion that each legume can make symbiotic association with only one rhizobial strain. For example, for decades *Bradyrhizobium japonicum* has been thought to be the only strain that can make symbiotic association with soybean (Rodriguez-Navarro et al. 2010). Later literature reports that there are a number of strains from different genera such as *Bradyrhizobium, Rhizobium, Sinorhizobium*, and *Mesorhizobium* which can also develop successful symbiosis with soybean, thus fixing atmospheric nitrogen in soybean crop (Biate et al. 2014). Beijerinck, a Dutch microbiologist and botanist, in 1901, reported the process of biological nitrogen fixation (BNF) for the first time (Wagner 2011). The SNF is the major process that contributes plant-available nitrogen; however, nitrogen-fixing efficiency of different crops varies with soil physicochemical conditions (Thies et al. 1992; Giller 2001), the mineral nitrogen status of soil (Thies et al. 1991), indigenous rhizobial population, soil organisms, and environmental factors (Al-Falih 2002; Liu et al. 2011).

11.4 Phosphate Solubilization

Phosphorus (P) is the second most limiting plant nutrient after nitrogen that has a major role in plant metabolic processes such as photosynthesis, respiration, energy transfer, transmission of phosphorus-associated heredity material, cell division and development, and synthesis of nucleic acid and phospholipids (Fernandez et al. 2007; Richardson and Simpson 2011). Farmers use synthetic chemical fertilizer for meeting the crop P requirements (Turan et al. 2006). Plants absorb P in the form of primary and secondary orthophosphates (Bhattacharyya and Jha 2012). When P fertilizer is applied in the soil, it becomes unavailable to plants due to complexation with calcium carbonate in alkaline calcareous soils under arid and semiarid climate (Leytem and Mikkelson 2005) and with sesquioxide in acidic soils (McLaughlin et al. 2011).

Soil microbes play an important role in the availability of phosphorus in soils (Sharma et al. 2013) which use different P-solubilizing mechanisms such as lowering of soil pH by production of low molecular weight organic acids, siderophores production, and release of hydroxyl ions (OH⁻) and enzymes (Barroso et al. 2006; Rodriguez et al. 2006; Glick 2012). The microorganisms are also involved in the mineralization of phosphorus through decomposition of organic compounds, thus making P available to plants (Rodriguez et al. 2006) through the production of phosphatases (Aseri et al. 2009) and phytases (Maougal et al. 2014).

Rhizobia have the ability to make available the fixed inorganic P through solubilization and organic P through decomposition (Tao et al. 2008) by above-described mechanisms. A number of rhizobial strains have been documented which solubilize inorganic and mineralize organic P compounds in soil (Afzal and Bano 2008; Khan et al. 2010). Rhizobial species from the genera *Rhizobium* (Egamberdiyeva et al. 2004), *Bradyrhizobium* (Egamberdiyeva et al. 2004; Afzal and Bano 2008), *Sinorhizobium* (Bianco and Defez 2010), and *Mesorhizobium* (Rodrigues et al. 2006; Chandra et al. 2007) have been reported to solubilize P through production of low molecular weight organic acids.

11.5 Siderophores Production

Siderophores are low molecular weight organic compounds which have high affinity for Fe and other metals. These compounds are released by soil microbes especially bacteria in iron-deficient soils, make complexes with Fe, and make it available to plants (Raymond and Dertz 2004; Skaar 2010). Siderophores may chelate with ferric iron, making it available to crop plants and microorganisms (Ahmed and Holmstrom 2014); however, pathogenic fungi are unable to use chelated iron. Iron plays an important role in chlorophyll synthesis and respiration (Kobayashi and Nishizawa 2012). It is also essential for ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), metabolism of oxygen, transfer of electron, and catalysis/enzymatic processes in plants (Aguado-Santacruz et al. 2012). Iron is an important component of nitrogenase complex ferredoxin and leghemoglobin thus helps in nitrogen fixation (Raychaudhuri et al. 2005).

Iron converts into oxyhydroxides and hydroxides; the insoluble forms, under aerobic conditions, thus become unavailable to plants and microorganisms (Rajkumar et al. 2010). Soil pH also affects Fe availability to plants and microorganisms (Masalha et al. 2000). So, under such conditions, siderophores help the microorganisms and plants to meet their Fe needs. Siderophores also make complexes with other essential elements such as molybdenum, cobalt, nickel, and manganese, thus enhancing their availability to microorganisms and plants (Bellenger et al. 2008; Braud et al. 2009). Siderophores complex with heavy metals such as cadmium, copper, and aluminum and radioactive elements like neptunium and uranium (Neubauer et al. 2000) and thus alleviate the heavy metal stress.

It is a well-established fact that rhizobial strains from the genera *Azorhizobium* (Islam et al. 2009), *Rhizobium* (Carson et al. 2000; Arora et al. 2001; Mehboob et al. 2011; Prabha et al. 2013; Manasa et al. 2017; Routray and Khanna 2018), *Bradyrhizobium* (Badawi et al. 2011; Shamsuddin et al. 2014), *Mesorhizobium* (Chandra et al. 2007; Ahmad et al. 2008), and *Sinorhizobium* (Carson et al. 2000; Ahmad et al. 2008) can produce siderophores which chelate with ferric ion under iron-limiting soil conditions (Ahemad and Khan 2011a) and make it available to crop plants.

11.6 Phytohormones Production

Phytohormones are organic molecules, involved in important physiological processes of plants, and thus improve their growth and development. They are synthesized within the plant body at one point and transport to some other place for performing physiological functions (Saharan and Nehra 2011). Phytohormones when applied exogenously are termed as plant growth regulators, due to their involvement in plant growth regulation. They are classified in five major classes as cytokinins, gibberellins, auxins, abscisic acid, and ethylene (Khalid et al. 2006; Saharan and Nehra 2011).

Auxins are involved in root and shoot growth especially at seedling stage (Patten and Glick 1996). Indole-3-acetic acid (IAA), one of the important auxins, is involved in cell division, cell differentiation, gene regulation (Ryu and Patten 2008), apical dominance, cell enlargement, root development (Khan et al. 2014), and nodulation (Remans et al. 2007). It has been well documented that most of the rhizobial strains isolated from root nodules produce indole-3-acetic acid in the presence and absence of L-tryptophan, the immediate precursor of auxins (Ahmad 2011). A number of studies report the production of IAA by rhizobial strains from the genera *Azorhizobium* (Naidu et al. 2004), *Rhizobium* (Dazzo et al. 2005; Weyens et al. 2009; Abrar 2017; Shengepallu et al. 2018), *Mesorhizobium* (Ahemad and Khan 2012), *Bradyrhizobium* (Badawi et al. 2011), and *Sinorhizobium* (Bianco and Defez 2010). The auxins produced by bacteria are involved in production of branches, and the surface area of roots) and more adventitious roots (Dazzo and Yanni 2006; Solano et al. 2010).

Cytokinins are involved in plant cell division, development of roots, formation of root hairs, shoot and branching, chloroplast development, and leaf senescence. It also controls cell division in embryonic as well as mature plants (Srivastava 2002; Oldroyd 2007). Cytokinin is important for regulating the number of nodules in a symbiotic relationship between *Rhizobium* and legume crops. It is reported to play a critical role in the activation of nodule primordial, thus, a positive regulator of nodulation (Kisiala et al. 2013).

Cytokinins produced by bacteria stimulate shoot growth and reduce root/shoot ratio in drought-stressed plants (Arkhipova et al. 2007). Different rhizobial species such as *Rhizobium leguminosarum* (Zahir et al. 2010), *Sinorhizobium meliloti*, *Sinorhizobium fredii*, *Sinorhizobium medicae*, and *Mesorhizobium loti* (Kisiala et al. 2013) have the ability to produce cytokinins. Moreover, *Rhizobium* regulates the expression of signaling pathway and activates cortical cells to divide in plants and enhances the endogenous cytokinin production in plants (Oldroyd 2007).

Gibberellins (GA) play a role in leaf expansion and stem elongation of plants. Exogenous application of gibberellins helps to promote bolting of the plants and parthenocarpy in fruits, increases the number of buds and fruit size, and is involved in breaking of tuber dormancy. Soil microorganisms have been studied to produce gibberellins which help to improve plant growth. Bacterially produced gibberellins affect plant growth and nodulation positively as well as negatively. They induce nodule organogenesis however and inhibit the nodulation at infection stage (McAdam et al. 2018). A number of rhizobial strains from the genera *Rhizobium* (Bottini et al. 2004), *Bradyrhizobium* (Morrone et al. 2009; Afzal et al. 2010), and *Sinorhizobium* (Boiero et al. 2007) have been reported to produce the gibberellins (Mirza et al. 2007).

Abscisic acid (ABA) plays an important role in seed germination, leaf development, root growth, and stomatal closure (De Smet et al. 2006). Its production is mostly prominent in stress conditions like drought stress, where it is in guard cells and stimulates stomatal closure and prevents water loss through transpiration. Its role is also reported during salt stress, resistance against pathogen, and developmental processes, such as seed dormancy and germination (Goggin et al. 2009; Rodriguez-Gacio et al. 2009). The ABA also regulates nodulation in legumes (Suzuki et al. 2004). Rhizobial species from different genera including *Rhizobium* and *Bradyrhizobium* have been reported to produce abscisic acid (Dobbelaere et al. 2003; Boiero et al. 2007) and help in plant growth regulation.

11.7 Enzyme Synthesis

Enzyme production is an important attribute of soil bacteria including rhizobia. During recent years, a number of rhizobial strains have been reported to produce extracellular enzymes. Important rhizobial enzymes include chitinase, phosphatase, cellulase, catalase, and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (Prabha et al. 2013; Mouradi et al. 2018) which help plants to cope with biotic and abiotic stresses (Ahmad 2011). It has been well documented that ACC deaminase produced by bacteria in soil lowers the ethylene levels in plant body by cleaving the ACC, the immediate precursor of ethylene (Shaharoona et al. 2007). The lower concentration of ethylene is required for regulation of physiological processes in plants (Arshad and Frankenberger 2002; Owino et al. 2006); its higher concentration, however, under stress negatively affects plant growth (Zahir et al. 2008).

The bacterial ACC deaminase converts ACC into ammonia and α -ketobutyrate for use by bacteria as carbon and nitrogen source (Saleem et al. 2007; Singh et al. 2015). Inoculation of crop plants with bacteria containing ACC deaminase enzyme increases mineral uptake, nodulation, and seedling growth of plants (Ahmad et al. 2011, 2013b) leading to improved growth and productivity (Glick 2012; Ahmad et al. 2014). The ACC deaminase-containing bacteria help plants to cope with damaging effects of stresses such as salinity (Nadeem et al. 2007; Ahmad et al. 2011), heavy metals (Khan et al. 2013), flooding (Grichko and Glick 2001), drought (Zahir et al. 2008), and pathogenic stress (Wang et al. 2000).

A number of rhizobial strains having ACC deaminase activity from the genera *Azorhizobium* (Islam et al. 2009), *Rhizobium* (Mirza et al. 2007; Hafeez et al. 2008; Duan et al. 2009), *Bradyrhizobium* (Shaharoona et al. 2006), and *Sinorhizobium* (Ma et al. 2004) have been reported. Rhizobia also produce some other enzymes

such as catalase (Bumunang and Babalola 2014), urease (Deshwal and Chaubey 2014; Nosheen and Bano 2014), and chitinase (Saha et al. 2012) and protect plants under stresses along with enhancing nutrient availability. Rhizobial strains also produce lipase, cellulase, protease, β -1,3-glucanase (Gopalakrishnan et al. 2014), and oxidase (Gauri et al. 2011). All these enzymes are important in nutrient availability and induction of tolerance against biotic and abiotic stresses.

11.8 Exopolysaccharides Production

Exopolysaccharides (EPSs) are complex polymers of high molecular weight which are released by soil microbes including rhizobia (Vijayabaskar et al. 2011; Rao et al. 2013). The bacterial EPSs include humic acids, nucleic acids, phospholipids, proteins, glycoproteins, and polysaccharides (Flemming et al. 2007). Exopolysaccharides are involved in biofilms formation (Sutherland 2001) and protect microorganisms against toxic effects of osmotic stress, desiccation (Sandhya et al. 2009), salinity (Ashraf et al. 2004; Qurashi and Sabri 2012), bacteriophage attacks, and poisonous compounds (Sutherland 2001). Exopolysaccharides improve root and shoot growth and increase fertilizer use efficiency through better water use (Alami et al. 2000).

Rhizobia have the ability to produce exopolysaccharides which help in biofilm formation. The EPSs-producing bacteria can better survive against environmental extremities and can efficiently utilize water and nutrients. Rhizobial exopoly-saccharides increase soil aggregation, help plant roots to adhere with soil, and improve water holding capacity of soil and nutrient availability in the root zone (Donot et al. 2012; Hussain et al. 2014). The EPSs also help in the establishment of symbiotic association between plants and rhizobia (Skorupska et al. 2006). The EPS-producing species from different rhizobial genera including *Rhizobium* (Zafarul-Hye et al. 2013; Janczarek et al. 2015; Marczak et al. 2017), *Sinorhizobium* (Castellane et al. 2015), *Mesorhizobium* (Castellane et al. 2015), and *Brady-rhizobium* (Ahemad and Khan 2011b) have been reported.

11.9 Production of Other Compounds

Nitrogen is an essential element for plant and microbial growth that is involved in the synthesis of a number of compounds including nucleic acids, amino acids, and proteins. Certain rhizobial strains have the ability to produce ammonia and thus help plants in mineral nutrition (Goswami et al. 2014) and improve plant growth and biomass (Mia et al. 2005). The ammonia-producing bacteria also help in biological control of fungi (Al-Mughrabi 2010; Jha et al. 2012) and reduce the growth of competing microflora, thus increasing the growth of nitrifying bacteria in soil (Angus et al. 1999). Rhizobial species from the genera *Rhizobium* (Zafar-ul-Hye

et al. 2013), *Bradyrhizobium* (Wani et al. 2007a, b; Ahemad and Khan 2011c), and *Mesorhizobium* (Ahmad et al. 2008; Ahemad and Khan 2012) have been reported as ammonia producers.

Lumichrome helps in plant growth (Zhang et al. 2002; Dakora 2003) by improving net carbon assimilation especially under water-stressed conditions (Matiru and Dakora 2005). Inoculation of plants with lumichrome-producing rhizobial strains induces water stress tolerance in plants through minimizing the stomatal conductance of water and transpiration losses in leaves (Mehboob et al. 2009). Riboflavin is a component of bacterial flavin coenzymes which are the typical cofactors of flavoproteins. These flavoproteins are important for various cellular processes, such as for energy production, DNA repairing, redox reactions, biosynthesis, and light emission (Burgess et al. 2009). Riboflavin also affects the rhizobial symbiotic relationship, rhizobial survival in the rhizosphere, and their ability to colonize plant roots (Victor et al. 2013).

Several strains of rhizobia including species from the genera *Rhizobium* and *Sinorhizobium* have been recognized as riboflavin producing which act as plant growth promoter (Yang et al. 2002). Riboflavin produced by bacteria can reduce Fe^{+3} into its more soluble Fe^{+2} forms where it acts as electron donor (Crossley et al. 2007). Rhizobia can also produce zeatin (Boiero et al. 2007), hydrogen cyanide, tensin, viscoinamide, pyrrolnitrin (Bhattacharyya and Jha 2012), and antibiotics (Chandra et al. 2007) such as phenazines (Krishnan et al. 2007) and thus help plants in biocontrol of pathogenic bacteria (Triplett et al. 1994). Rhizobia have the ability to produce bio-stimulatory agents which induce systemic resistance in the plant body (Yanni et al. 2001; Singh et al. 2006).

11.10 Rhizobial Inoculants for Sustainable Crop Production

Using rhizobial inoculants is an emerging technology not only for the improvement of leguminous crops but also for non-legumes due to their cost-effectiveness and environment-friendly nature. The specific group of rhizobia makes symbiotic relation with specific legume plant but may improve plant growth without making symbiotic association in non-legumes. Therefore, the inoculation with rhizobia improves plant growth and productivity in the most significant manner under both normal and stressed conditions as summarized in below sections and Table 11.2.

11.10.1 Crop Nutrition

Rhizobia have positive influence on soil nutrients and thus improve nutrient uptake (Allito et al. 2014) through phosphate solubilization (Khan et al. 2010), siderophores

Crop	Rhizobial strain	Growth conditions	Effects on plants	References
Soybean	Bradyrhizobium sp.	Field experiment	Increased N, P, and S contents and improved seed and straw yield	Raja and Takankhar (2018)
Soybean	Bradyrhizobium sp.	Field study	Increased number of pods, pods weight, and grain yield	Galindo et al. (2018
Soybean	Bradyrhizobium sp.	Field experiment	Increased phosphorus use effi- ciency and plant N and P uptake	Fituma et al (2018)
Soybean	Bradyrhizobium japonicum	Field experiment	Increased nodulation, dry matter production, and nitrogen uptake	Solomon et al. (2012
Soybean	Bradyrhizobium	Field experiment	Increased nodulation, shoot nitrogen accumulation, and improved plant growth	Cerezini et al. (2016
Peanut	Bradyrhizobium sp.	Field experiment	Increased plant N and P uptake and nodulation	Argaw (2018)
Peanut	Rhizobium sp.	Field conditions	Improved shoot growth and nodulation under saline conditions	Akhal et al. (2013)
Groundnut	Rhizobium sp.	Field study	Increased growth, oil contents, protein contents, and yield parameters	Mohammed and Sahid (2016)
Chickpea	Rhizobium sp.	Field study	Improved plant growth and yield	Laabas et al (2017)
Wheat	Rhizobium sp.	Pot study	Improved shoot length, shoot and root dry weight	Kamran et al. (2017
Maize	Azospirillum brasilense Rhi- zobium tropici	Greenhouse	Enhanced plant height, stem diameter, dry biomass of shoots and roots, and N accumulation in shoots	Picazevicz et al. (2017
Legumes	Rhizobium sp.	In vitro	Improved plant growth, enhanced plant defense mecha- nisms, and resistance against herbivores	Thamer et al. (2011)
Chickpea	Rhizobium sp.	Field study	Increased growth and yield parameters and concentration of nitrogen and organic matter in soil	Zaman et al (2011)
Common bean	Rhizobium sp.	Greenhouse Field study	Significant effect on chlorophyll contents, photosynthesis, intercellular CO_2 concentration, and the transpiration rate	Bambara and Ndakidemi (2009)
Pepper Tomato	Rhizobium phaseoli	In vitro Pot study	Promoted growth at different stages, increased yield and qual- ity of seedlings and fruits	Garcia- Fraile et al. (2012)
Mung bean Mash bean	Rhizobium japonicum	Greenhouse Field study	Increased height, root and shoot growth, pod number, pod length, nodulation, and seed weight	Ravikumar (2012)

 Table 11.2
 Effect of rhizobial inoculants on growth, nutrient uptake, and yield of different crops under in vitro, pot, and field conditions

(continued)

Crop	Rhizobial strain	Growth conditions	Effects on plants	References
Carrot Lettuce	Rhizobium leguminosarum	In vitro Pot study	Promoted plant growth by increasing dry matter of shoots and roots and increased N, P, and Ca uptake	Flores-Felix et al. (2012)
Pea	Rhizobium leguminosarum	Pot study	Decreased disease severity, increased seed fresh and dry weights, and better seed filling in pods	Wienkoop et al. (2017)
Pea Lentil	Rhizobium leguminosarum	Field study	Increased seed yield and effec- tive in disease control	Huang and Erickson (2007)
Kidney bean	Rhizobium etli	Pot study	More nodules with increased nitrogenase activity and higher biomass	Suarez et al. (2008)
Lettuce	Bradyrhizobium japonicum	Axenic conditions	Reduced heavy metal stress and increased shoot and root length	Seneviratne et al. (2016)
Cowpea	Bradyrhizobium sp.	Greenhouse	Increased biological nitrogen fixation, plant growth, and crop productivity	Rodrigues et al. (2015)
Cowpea	Bradyrhizobium japonicum	Field study	Increased plant height and chlo- rophyll contents	Nyoki and Ndakidemi (2014)
Peanut	Bradyrhizobium sp.	Axenic conditions	Improved plant growth, nodule number, and nitrogen contents	Castro et al. (2012)
Wheat	Azorhizobium caulinodans	Axenic conditions	Increased number and weight of leaves and roots	Liu et al. (2017)
Black medic	Sinorhizobium meliloti	Pot study	Increased biomass production under metal stress	Fan et al. (2011)
Chickpea	Mesorhizobium sp.	Field conditions	Improvement in symbiotic parameters leading to enhanced growth and yield	Kaur et al. (2015)
Chickpea	Mesorhizobium mediterraneum	Field study	Capable to nodulate in stress conditions and increased nodule number and grain yield	Romdhane et al. (2009)
Bean	Rhizobium	Field conditions	Increased growth and yield parameters and protein contents	Yadegari et al. (2010)
Bean	Rhizobium sp.	Hydroponic culture	Higher nodulation and increased phosphatase and phytase activity	Mandri et al. (2012)
Soybean	Bradyrhizobium japonicum	Field study Glasshouse experiment	Enhanced plant height, number of leaves, leaf chlorophyll con- tent, stem girth, leaf area, and leaf area index	Tairo and Ndakidemi (2013)
Soybean	Bradyrhizobium japonicum		Increased N content of inocu- lated plants and increased root nodulation and yield	Dhami and Prasad (2009)
Kidney vetch	Mesorhizobium metallidurans	In vitro	Enhanced tolerance to high con- centrations of heavy metals	Vidal et al. (2009)

Table 11.2 (continued)

production (Chandra et al. 2007), and phytohormones production (Chi et al. 2010), in addition to improvement in nitrogen uptake through SNF of atmospheric nitrogen. Rhizobial inoculation can minimize the dependence on chemical fertilizers as it enhances the nutrient uptake of crop plants. For example, Soumaya et al. (2016) conducted an experiment to study the effect of *Rhizobium* inoculation on mineral contents of sulla (*Hedysarum coronarium* L.) crop grown on calcareous soil and reported a significant increase in nutrients uptake leading to improved performance of crop in terms of growth and nodulation.

Rhizobium inoculation improves the nutrient (P, K, Ca, and Mg) uptake in different plant parts such as leaves, shoots, roots, and pods (Makoi et al. 2013), enhances the availability of macro- and micronutrients, and thus improves the nutritional quality of different plant components (Tairo and Ndakidemi 2014). Nyoki and Ndakidemi (2014) observed that inoculation of Bradyrhizobium *japonicum* in cowpea resulted in greater uptake of macronutrients such as N. P. K. Mg, Ca, and Na as compared to control. Similar results were obtained by Tairo and Ndakidemi (2014) where they reported that *B. japonicum* inoculation significantly enhanced the uptake of N, P, K, and Na within the roots, pods, shoots, and whole plant of cowpea (Vigna unguiculata (L.). In another study, rhizobial inoculation increased nitrogen fixation which resulted in increased root growth enabling it to acquire more nutrients (Rokhzadi and Toashih 2011; Das et al. 2012). It has been reported that *Mesorhizobium* inoculation not only improves growth and nutrient uptake, but it also positively affected the yield attributes, symbiotic relationship, and enhanced quality of chickpea grains (Singh and Singh 2018). The increased nitrogen content resulted in higher protein content which was also due to *Rhizobium* inoculation (Kumar et al. 2014). In another study, it was observed that Mesorhizobium sp. enhanced N and P uptake in both grain and shoot in chickpea as compared to uninoculated control (Sahai and Chandra 2011). Similarly, Chandra and Pareek (2015) reported 0.6%, 6.5%, and 4.3% increase in organic carbon, available N, and available P, in chickpea plant after *Rhizobium* inoculation. Further, Kaur et al. (2015) reported higher protein contents and increase in N and P contents after Mesorhizobium inoculation in chickpea. The application of Rhizobium improves the N and P content of soil which can be utilized by the next crop after harvesting of crop (Abdalla et al. 2013; Tagore et al. 2013). Studies revealed that Mesorhizobium inoculation increased the soil microbial biomass carbon (Bhattacharjya and Chandra 2013) that resulted in more crop biomass and subsequently higher return of organic matter into the soil, thus increasing microbial biomass and activities (Babu et al. 2015).

It has been well documented that rhizobial inoculation separately and in combination with other bacterial strains can improve the nodulation and nutrient uptake in crop plants (Ahmad et al. 2013a). For example, Elkoca et al. (2010) studied the effect of *Rhizobium leguminosarum* by. *phaseoli* separately and in combination with *Bacillus subtilis* and *Bacillus megaterium* on nitrogen fixation and nutrient uptake of the common bean (*Phaseolus vulgaris* L. cv. "elkoca-05") and reported that the triple inoculation of *Rhizobium leguminosarum*, *Bacillus subtilis*, and *Bacillus megaterium* increased the plant N (52.1%), K (25.6%), Mg (97.6%), and sulfur (282.4%) as compared to uninoculated control. Similarly, it also improved the seed protein (30.1%), K (25.8%), Mg (95.5%), and S (282.8%) contents in seed of the common bean when compared with uninoculated control. The improvement in micronutrient contents (Zn and Cu in plant and seed) was also observed by inoculation with *Rhizobium leguminosarum* in combination with *Bacillus subtilis* and *Bacillus megaterium*.

11.10.2 Crop Physiology

Nitrogen is an essential nutrient that needs to be applied as a fertilizer for plant growth and development. Chlorophyll also contains nitrogen which is an integral component of photosynthesis. The biological nitrogen fixation (BNF) accounts for about 60% of the total fixed nitrogen (Bano and Iqbal 2016). In BNF, nodulating bacteria gain carbon and other energy resources from photosynthesis and in turn provide nitrogen. This mechanism depends on the activity of chloroplasts which is a structural component of photosynthesis (White et al. 2009). Besides nitrogen fixation, rhizobia that make symbiotic association with plants may also improve physiological status of plants by improving nutrient bioavailability and uptake (Afzal and Bano 2008), phytohormones production (Chandra et al. 2007), siderophores and osmolytes production (Grover et al. 2010; Saidi et al. 2013), and regulation of ACC deaminase (Duan et al. 2009). Rhizobial inoculation has the ability to improve chlorophyll contents of crop plants (Elkoca et al. 2010) and thus can improve the photosynthetic activity and productivity of crop plants. Hussain et al. (2018) found that Rhizobium phaseoli-RS-1 and Mesorhizobium ciceri-RS-8 improved the transpiration rate, photosynthetic rate, stomatal conductance of water, intrinsic water use efficiency, relative water contents, chlorophyll contents, and nutrients uptake of maize crop under normal and stressed conditions.

Rhizobium inoculation improves physiological characters of plants which direct toward maximum growth and yield. In a study rhizobium alone as well as in combination with *Pseudomonas* strains reduced the adverse effects of salinity by significantly improving the transpiration rate, photosynthetic rate, stomatal conductance of water, C assimilation rate, relative water contents, and chlorophyll contents in mung bean (Ahmad et al. 2013b). They improved the physiology, growth, and quality of plant by adapting several mechanisms mainly by lowering endogenous level of ethylene (Ahmad et al. 2011). The rhizobial inoculation enhanced leaf chlorophyll contents in both glasshouse and field conditions when compared with control treatment (Bambara and Ndakidemi 2009).

Literature reports the increased photosynthetic leaf area, chlorophyll content, and relative water contents due to inoculation of ACC deaminase- and IAA-producing or phosphate-solubilizing rhizobium strains (Saghafi et al. 2018). In another study, Jimenez-Gomez et al. (2018) observed that *Rhizobium laguerreae* possessing several plant growth-promoting abilities showed positive results for vegetative parameters of leafy vegetable which include leaf number, size and weight, as well as

chlorophyll and nitrogen contents as compared to uninoculated control. *Rhizobium* inoculation in legumes enhances the leaf chlorophyll contents of crops (Tairo and Ndakidemi 2013). For example, in the case of soybean and cowpea, it was observed that *B. japonicum* inoculation and phosphorus supplementation significantly increased leaf chlorophyll contents both in field and glasshouse experiments (Makoi et al. 2013; Nyoki and Ndakidemi 2014). The increase in chlorophyll contents results in increased photosynthetic processes (Sylvie and Patrick 2009), and as a result plant produces more sugars for its growth and development.

11.10.3 Crop Productivity

Rhizobial inoculation has been well documented to improve productivity of legume crops under normal (Anjum 2011; Shurigin et al. 2015; Khaitov et al. 2016; Woldemeskel et al. 2018) and stressed conditions (Aamir et al. 2013; Ahmad et al. 2014; Sistani et al. 2017). For example, it has been observed that rhizobial inoculation improved growth, yield, and nitrogen fixation in chickpea (Kyei-Boahen et al. 2002; Fatima et al. 2008), pea (Huang and Erickson 2007), and the common bean (Argaw 2016). Similarly, Sharma et al. (2011) observed significant improvement in nitrate reductase activity, number of effective nodules, and leghemoglobin contents in groundnut due to inoculation with *Rhizobium* strains. The improvement in plant height, straw yield, and grain yield was observed in lentil in response to rhizobial inoculation (Haque et al. 2014).

Indigenous population and cropping history affect the performance of cropspecific symbionts under field conditions. Higher population of indigenous rhizobia in soil where the same legume is being grown in previous years suppresses the influence of inoculated rhizobial strains, while, in the case of low indigenous population, rhizobial inoculants have the ability to improve production of legume crops. For example, about 57% higher seed yield of inoculated plots of soybean was observed by Martyniuk et al. (2018) when compared with uninoculated control. They studied the influence of rhizobial inoculation on productivity of soybean, pea, and yellow lupine in a soil with higher populations of indigenous pea and lupine symbionts and low population of soybean rhizobia. The improvement in grain yield of soybean might be due to higher nodulation in inoculated plots (169%) as compared to uninoculated plots. In the case of soil with relatively high populations of indigenous rhizobia of pea and yellow lupine, no response of inoculation was observed on yield contributing parameters of these crops.

It has been observed that inoculation of *Bradyrhizobium japonicum* improved the root and shoot growth, grain yield and yield-related parameters, and grain nitrogen contents of mash bean (Hussain et al. 2011). Similarly, the inoculation with *Mesorhizobium* strains improved the grain yield in *Cicer arietinum* (Wolde-meskel et al. 2018). In another study, Bhatt and Chandra (2014) also observed that the inoculation with *Mesorhizobium* improved the straw yield, grain yield, nodulation, and phosphorus and nitrogen uptake in chickpea. Alam et al. (2015) found that in

soybean plant, inoculation with *Rhizobium* sp. BARIRGm901 increased the nodule weight, nodule number, plant height, root biomass, shoot biomass, nitrogenase activity, nitrogen fixation and assimilation, strove yield, and seed yield as compared to uninoculated control. Argaw and Muleta (2017) reported that rhizobial inoculation improved the number of nodules, dry mass of nodules, and total biomass yield and grain yield of *Phaseolus vulgaris*.

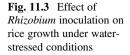
The use of the most efficient rhizobial strain for specific host variety can maximize the profitability of inoculants, thus capitalizing the maximum productivity of crops (Allito et al. 2014). For example, Kulasooriya et al. (2017) conducted an experiment on *Trifolium repens* L. with the objective to develop cost-effective and eco-friendly technology for crops to minimize the use of nitrogenous fertilizers. They prepared inoculants by using efficient rhizobial strains. They observed significant improvement in biomass of inoculated *Trifolium* plants as compared to plants which were fertilized with urea, under field conditions. They attributed the increased biomass with significant increase in root nodulation of inoculated plants. In another study, Tena et al. (2016) studied the efficiency of different rhizobial strains on nodulation in lentil (*Lens culinaris* Medik.) under field conditions. They evaluated six rhizobial strains and reported a significant increase in nitrogen fixation and grain yield as compared to uninoculated control; however, these strains varied in their ability to improve grain yield of lentil under field conditions.

11.10.4 Stress Tolerance in Crop Plants

Heavy metals are among the main inorganic soil pollutants that are added from agrochemicals, industrial wastes, and mining (Marchiol et al. 2004). The persistence and non-degradable nature of heavy metals pose enormous harmful impacts on microorganisms (Broos et al. 2005; Krujatz et al. 2011), plants (Wani et al. 2008; Wani and Khan 2010), and ecosystem (Cheung and Gu 2007). For example, cadmium (Cd) negatively affects nitrogenase activity of rhizobia and photosynthesis activity of legume host, thus reducing nodulation efficiency (Ahmad et al. 2012). In another study, zinc toxicity adversely affected the symbiotic association between *Rhizobium leguminosarum* by. *viciae* and pea by decreasing rhizobial population, thus reducing the nodulation and plant growth (Chaudri et al. 2000).

Using rhizobia under stress is not only beneficial for legume crops but can also improve growth of non-legumes (Fig. 11.3) and help in phytoremediation of contaminated soils. The use of rhizobia in combination with legumes is useful in phytoremediation and is recommended as eco-friendly, cost-effective, and easy-touse approach under adverse soil conditions (Kang et al. 2018). They used *Sinorhizobium saheli* YH1 for reducing the uptake of metal by *Leucaena leucocephala* in mine tailings and metal-polluted soils. It was observed that *S. saheli* YH1 improved plant health of *L. leucocephala* by reduction in metal uptake by plants under heavy metal-polluted soils and recommended to use the approach for phytoremediation of Cd- or Mn-polluted soils.





Rhizobial growth, survival, and distribution in soil are affected by environmental stresses including salinity (Tate 1995). Indigenous population can easily adapt to the local environmental conditions, so they are comparatively more efficient and competitive (Mrabet et al. 2005); however, inoculated rhizobial strains have been well documented to improve plant growth under normal as well as stressed conditions (Ahmad et al. 2014; Allito 2015; Khaitov et al. 2016). The strains vary in their growth under stressed environment with some strains showing more growth even at higher levels of stress that might be owing to stress tolerance ability of these rhizobial strains (Sgroy et al. 2009; Ahmad et al. 2011). Rhizobial strains use different mechanisms to deal with salinity stresses. Inoculation of crop plants with salt-tolerant rhizobia has the ability to improve crop productivity under salt stress (Ahmad et al. 2012, 2014).

Beneficial soil bacteria including several species of *Pseudomonas*, *Rhizobium*, and *Bacillus* have been reported to improve disease resistance in crop plants (Kang et al. 2006; El-Batanony et al. 2007; Samavat et al. 2011) through production of different antimicrobial compounds and hydrolytic enzymes and inducing plant defense mechanisms (Duan et al. 2009). For instance, El-Batanony et al. (2007) reported that *Rhizobium leguminosarum* in combination with AM fungi was effective in biocontrol of *Fusarium solani*, *F. oxysporum*, and *Rhizoctonia solani* in faba bean. In another study, Gao et al. (2012) reported that inoculation with AM fungi and rhizobia directly inhibited the growth and reproduction of pathogen and activated the overall defense system of plant by enhancing PR gene expressions and recommended it for controlling soybean red crown rot in acid soils.

11.11 Synergistic Effects of Rhizobial Inoculation with Other Soil Microbes and Organic Sources

Rhizobial strains can be used in combination with other soil microbes to develop inoculants having two or more strains: the co-inoculation or consortium inoculants. It has been observed that AM fungi in combination with Rhizobium improved the mineral nutrition of legume crops (Tavasolee et al. 2011). Similarly, Guo et al. (2010) conducted a study on udorthent to evaluate the efficacy of Sinorhizobium meliloti separately and in combination with arbuscular mycorrhiza and lime on growth, nodulation, and nutrient uptake of lucern. It was observed that integrated use was better in improving the nodulation and growth of lucern, as compared to alone application of rhizobial strain. The combined use also improved the nitrogen and phosphorus uptake in lucern crop as compared to uninoculated plants. In another study, the combined use of AMF fungi and Rhizobium enhanced productivity, nutrient use efficiency, and profitability of pea crop in addition to saving of about 25% N and P fertilizers in Himalayan acid Alfisol (Bai et al. 2016). The integrated use of Rhizobium and AM fungi can also be effective to enhance symbiotic nitrogen fixation under stressed conditions (Chalk et al. 2006). For instance, the integrated use of Rhizobium and AM fungi has been well documented to improve plant growth and control of pathogens under field conditions (Akhtar et al. 2011).

The use of *Rhizobium* in combination with plant growth-promoting bacteria can better improve the crop productivity under normal as well as marginal soil conditions. For example, use of consortium developed from *Rhizobium tropici* (CIAT 899), *Paenibacillus polymyxa* Loutit (L), and *P. polymyxa* (DSM 36) improved growth, phytohormone levels, nitrogen content, and nodulation in the common bean (*Phaseolus vulgaris* L.) under drought-stressed conditions, thus having the ability to induce drought stress tolerance in crop plants (Figueiredo et al. 2008).

Rhizobial inoculation in combination with other organisms has also been found beneficial for agriculture ecosystem. Co-inoculation of *Rhizobium* and *Pseudomonas fluorescens* in the common bean increased root and shoot growth, nitrogenase activity, nodulation, and chlorophyll contents in leaves. It also increased the nitrogen and phosphorus uptake by crop plants (Samavat et al. 2012). Similarly, the increase in plant growth and nodulation was observed due to the combined use of *Bradyrhizobium* and ACC deaminase-containing PGPR in mung bean (Shaharoona et al. 2006). The co-inoculation of *Cicer arietinum* with rhizobium and phosphate-solubilizing bacteria significantly improved the seed yield, strove yield, nodule number, and protein content in grain as well as in straw. This co-inoculation also improved the uptake of nitrogen and phosphorus in seed and straw (Singh et al. 2018). Similarly, *Rhizobium* in combination with phosphate-solubilizing bacterial inoculants increased the grain and straw yield, thousand-seed weight, pod number plant⁻¹, seed number pod⁻¹, nodule leghemoglobin content and its number, and fresh and dry biomass (Tagore et al. 2013).

The integrated use of rhizobial inoculants with organic sources can be helpful to increase the productivity of crop plants in soils with poor nutrient contents. The

Cron	Dhizohiol on	Synergizing	Effects on plant growth	Deferences
Crop Wheat	Rhizobial sp. Rhizobium sp.	organism Azospirillum and	Effects on plant growth Increased zinc contents	ReferencesShah et al.
		Pseudomonas	in plant at different growth stages	(2016)
Wheat and soybean	Bradyrhizobium	Azotobacter	Increased nitrogen contents	Rawat et al. (2013)
Maize	Rhizobium tropici	Azospirillum sp.	Improved shoot dry weight, total N con- tents, and grain yield	Mark et al. (2015)
Rice	Rhizobium sp.	Azospirillum brasilense	Increased plant growth	Hahn et al. (2016)
Rice	Bradyrhizobium, Rhizobium	Lysinibacillus, Alcaligenes, and Bacillus	Early growth and vigor of rice	Shamsuddir et al. (2014)
Soybean	Rhizobium japonicum	Azotobacter chroococcum and Azospirillum	Improved membrane stability and chloro- phyll contents	Zahedi et al (2013)
Chickpea, pea, and lentil	Rhizobium	Pseudomonas fluorescens, Anabaena laxa	Enhances soil polysac- charide content and plant dry weight	Babu et al. (2015)
Black gram	Rhizobium	<i>Azotobacter</i> sp.	Increased shoot length, root length, fresh and dry biomass, number of leaves, root nodules per plant, chlorophyll con- tents, and reducing and non-reducing sugar contents	Gaur et al. (2017)
Chickpea	Rhizobium sp.	Pseudomonas fluorescens, Azoto- bacter chroococcum, and Bacillus megaterium	Significant increase in nodule number, dry weight of nodules, root and shoot growth, nitrogen and phospho- rus contents, and grain and straw yield	Verma et al (2010)
Chickpea	Sinorhizobium ciceri	Pseudomonas sp.	Increased nodulation and plant dry matter	Messele and Pant (2012)
Chickpea	Mesorhizobium ciceri	Bacillus sp.	Increased seed yield and grain protein contents	Wani et al. (2007b)
Chickpea	Mesorhizobium sp.	Pseudomonas fluorescens, Azoto- bacter chroococcum, and Bacillus megaterium	Increased root and shoot dry weight and nodulation	Werma et a (2012)

 Table 11.3
 Synergistic effect of rhizobial inoculants with other soil microbes on growth, nutrient uptake, and yield of different crops

(continued)

Crop	Rhizobial sp.	Synergizing organism	Effects on plant growth	References
Cowpea	Bradyrhizobium sp.	Paenibacillus graminis	Increased plant growth, enhanced efficiency of symbiotic association	Rodrigues et al. (2015)
Rajmash	Rhizobium leguminosarum	Pseudomonas lurida, Pseudomo- nas putida	Enhanced plant bio- mass and increased uptake of N, P, K, Zn, and Fe contents	Mishra et al. (2014)
Lentil	Rhizobium sp.	Rhizobacteria	Increased shoot length, root length and total biomass, and nodulation	Zafar-ul-Hye et al. (2013)
Lentil	R. leguminosarum	Pseudomonas fluorescens	Improved plant growth and nodulation	Khanna et al (2011)
Pea	Rhizobium leguminosarum	Arbuscular mycor- rhizal fungi	Increased plant bio- mass, photosynthetic rate, and N fixation activity	Geneva et al. (2006)
Common bean	Rhizobium sp.	Paenibacillus polymyxa and Bacillus megaterium	Enhanced shoot and nodule weight	Korir et al. (2017)
Common bean	Rhizobium sp.	Paenibacillus polymyxa and Bacillus megaterium	Increased plant growth and nodulation	Korir et al. (2017)
Pigeon pea	Rhizobium sp.	Arbuscular mycor- rhizal fungi	Increased growth, nutrition, and chloro- phyll contents	Havugimana et al. (2016)
Pigeon pea	Sinorhizobium fredii	Pseudomonas fluorescens	Enhanced growth and yield and potential bio- control agent against <i>Fusarium</i> wilt	Kumar et al. (2010)
Soybean	Bradyrhizobium sp.	Azospirillum sp.	Increased grain yield and nodulation and enhanced nitrogen contents	Ferri et al. (2017)
Soybean	Bradyrhizobium elkanii	Streptomyces griseoflavus	Significantly increased plant growth, nodula- tion, N ₂ fixation, N uptake, and yield	Htwe et al. (2018)
Soybean	Bradyrhizobium sp.	Rhizobium	Enhanced drought tol- erance IAA production, EPS production, nodu- lation, and nodule N contents of plants	Uma et al. (2013)

 Table 11.3 (continued)

(continued)

Crop	Rhizobial sp.	Synergizing organism	Effects on plant growth	References
Soybean	Rhizobium sp.	Arbuscular mycor- rhizal fungi	Enhanced shoot dry weight and increased plant N and P contents	Wang et al. (2011)
Peanut	Bradyrhizobium sp.	Fungal endophyte, Phomopsis liquidambar	Increased nodule num- ber, shoot nitrogen contents, and flavonoid synthesis	Zhang et al. (2016)
Peanut	Bradyrhizobium sp.	Serratia marcescens and Trichoderma harzianum	Increased number and mass of root nodules	Badawi et al. (2011)
Peanut	Bradyrhizobium sp.	Ochrobactrum intermedium	Promoted growth and tolerance against high temperature and salinity stress	Paulucci et al. (2015)
Corn and Soybean	Bradyrhizobium japonicum	Azospirillum brasilense	Promoted seed germi- nation, nodule forma- tion, and early seedling development	Cassan et al. (2009)
Soybean	Bradyrhizobium japonicum	Bacillus amyloliquefaciens	Better root colonization and increased number of nodules	Masciarelli et al. (2014)

Table 11.3 (continued)

effectiveness of the combined use of *Rhizobium* and different levels of vermicompost to improve the growth and productivity of faba bean was investigated by Argaw and Mnalku (2017) under field conditions. The integrated use of *Rhizobium* and vermicompost significantly improved all parameters of faba bean including number of nodules plant^{-1} , nodule dry weight plant^{-1} , and grain yield as compared to uninoculated control. They recommended using *Rhizobium* inoculant in combination with 8 tons ha⁻¹ of vermicompost to boost the productivity of faba bean under field conditions in Haramaya, Ethiopia. More examples on the effectiveness of rhizobia in combination with other soil microbes for improving the productivity of different crops have been summarized in Table 11.3.

11.12 Conclusion and Future Prospects

It is evident from the above literature that rhizobia improve the productivity of cropping systems which not only increase nitrogen fixation but also improve soil fertility and crop production through several other attributes such as phosphate solubilization, siderophores production, phytohormones production, enzymes synthesis, and exopolysaccharides production. Moreover, these bacteria can be helpful for improvement in crop production on marginal lands due to their tolerance against various biotic and abiotic stresses. Their sole application and co-application with other plant growth-promoting rhizobacteria have the synergistic effects on crop plants both under normal and stressed environmental conditions.

Integrating legumes in the existing cropping systems and/or use of rhizobial inoculants can give better economic returns to farmers and contribute in maintaining soil fertility status for future use. Keeping in view the importance of rhizobia in sustainability of cropping systems, future research should focus on understanding the mechanisms involved in rhizobial-induced growth promotion. Strategies for improvement in plant-rhizobia interactions through molecular genetics, bioinformatics, and modeling tools should also be developed for sustainable crop production.

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