Nonsymbiotic and Symbiotic Bacteria Efficiency for Legume Growth Under Different Stress Conditions

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Abstract

In order to achieve maximum crop yields, excessive amounts of expensive fertilizers are applied in intensive farming practices. However, the biological nitrogen fixation via symbiotic and nonsymbiotic bacteria can play a significant role in increasing soil fertility and crop productivity, thereby reducing the need for

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chemical fertilizers. It is well known that a considerable number of bacterial species, mostly those associated with the plant rhizosphere, are able to exert a beneficial effect on plant growth. The use of those bacteria, often called plant growth-promoting rhizobacteria (PGPR), as biofertilizers in agriculture has been the focus of research for several years. The beneficial impact of PGPR is due to direct plant growth promotion by the production of growth regulators, enhanced access to soil nutrients, disease control, and associative nitrogen fixation. Legumes play a crucial role in agricultural production due to their capability to fix nitrogen in association with rhizobia. Inoculation with nodule bacteria called rhizobia has been found to increase plant growth and seed yields in many legume species such as chickpea, common bean, lentil, pea, soybean, and groundnut. However, both rhizobia and legumes suffer heavily and adversely from various abiotic factors. The impact of different stress factors on both PGPR and legume production is critically reviewed and discussed.

16.1 Introduction

Legumes are plants that belong to the family Fabaceae (approximately 700 genera and 18,000 species) and are categorized into two groups as cool season and warm or tropical season legumes (Toker and Yadav 2010; Miller et al. 2002). Broad bean (*Vicia faba*), lupins (*Lupinus* spp.), lentil (*Lens culinaris*), chickpea (*Cicer arietinum*), grass pea (*Lathyrus sativus*), common vetch (*Vicia sativa*), and dry pea (*Pisum sativum*) are placed in the cool season food legume group (FAOSTAT 2009; Andrews and Hodge 2010). In contrast, cowpea (*Vigna unguiculata*), soybean (*Glycine max* L.), mung bean (*Vigna radiata*), urd bean (*Vigna mungo*), and pigeon pea (*Cajanus cajan*) are included in the warm season food legume group (Latef and Ahmad 2015). Symbiotic relationship between legumes and rhizobia transforms atmospheric N into ammonium (Geurts et al. 2012) which is used as nutrient by legumes (Howard and Rees 1996) and other subsequent or intercropped crops (Liu et al. 2010). After cereals and oilseeds, legumes rank third in world production (Graham and Vance 2003). One-third (20–40%) of all dietary proteins are provided by legumes which are a primary source of amino acids (Zhu et al. 2005; Kudapa et al. 2013). Pulses are generally used as foods (Rebello et al. 2014). The low energy density and nutrient dense abilities make legumes a valuable food option to fulfill the requirement of undernourished or underserved populations (FAO 1994).

The frequent legume consumption reduces the risk of coronary heart disease by 22% and cardiovascular disease (CVD) risk by 11% (Flight and Clifton 2006). High intake of legumes protects from obesity and related disorders (Papanikolaou and Fulgoni 2008). The legumes also lower blood glucose and insulin responses (Mollard et al. 2012; Jenkins et al. 1980; Nestel et al. 2004) and increase sensitivity of insulin (Nestel et al. 2004). Since legumes are rich in sodium and low in potassium (Rebello et al. 2014), the probability of suffering from these disorders becomes low even when legume consumption is high. Phytochemicals, enzyme inhibitors, phytoestrogens, phytohemagglutinins (lectins), saponins, phenolic compounds, and oligosaccharides are also reported in the majority of legumes (Rebello et al. 2014). Legumes are low in fat content and rich in proteins (Campos-Vega et al. 2010) and complex carbohydrates (Kalogeropoulos et al. 2010) making legume an important and qualified food source. In addition, high content of fibers, polyunsaturated fatty acids, magnesium, and low glycemic index are other valuable properties of legumes (Bouchenak and Lamri-Senhadji 2013).

Biological nitrogen fixation (BNF) plays an important role in land improvement. Leguminous plants and rhizobia together form a symbiotic relationship (Freiberg et al. 1997; Zahran 2001) and have a great quantitative effect on the soil N pool (Ohyama et al. 2009; Abd-Alla et al. 2013). On the other hand, the N deficiency severely limits the plant growth. A prosperous BNF, however, increases agricultural productivity while minimizing soil loss and ameliorating adverse edaphic conditions. However, abiotic stresses have harmful impacts on plant development, including legumes (Singleton and Bohlool 1984; Subba Rao et al. 1999). Drought, salinity/alkalinity, unfavorable soil pH, nutrient deficiency, changes in temperature, inadequate or extreme soil moisture, and decreased photosynthetic activity conspire against a prosperous symbiotic process. In order to overcome these stress conditions, numerous inoculants have been developed to produce symbiotic legume-microbe formulations. In addition to this, experiments are performed in order to formulate new solutions supplemented with plant and microbe exudates which contain flavonoids, sugars, amino acids, and other low molecular weight molecules that are involved in microbe-plant interaction (Garg and Geetanjali 2009; Skorupska et al. 2010; Morel et al. 2012). By using these exudates, symbiotic relationships between bacteria and plants could be mimicked for plant development. Among symbiotic bacteria, rhizobia live in the rhizosphere of legumes and produce root nodules (Foth 1990; Abd-Alla et al. 2013). Structurally, rhizobia are small and rod-shaped Gram-negative bacteria which belong to *Rhizobiaceae* family (Long 1989) and spread over subclass *Alphaproteobacteria* and *Betaproteobacteria*. *Rhizobium*, *Mesorhizobium*, *Ensifer* (formerly *Sinorhizobium*), *Azorhizobium*, *Methylobacterium*, *Bradyrhizobium*, *Phyllobacterium*, *Devosia,* and *Ochrobactrum* are some of the notable genera. Briefly, the PGPR involving rhizobia promote the growth of legumes by stimulating the production of ACC deaminase and hormones as auxins, cytokinins, gibberellins, and certain volatiles; symbiotic nitrogen fixation; solubilization of mineral like phosphorus and other nutrients; etc. (Bashan and Holguin 1997; Ahmad et al. 2008). The growth of rhizobia and its nitrogen-fixing ability, however, are negatively impacted by several environmental factors (Singleton et al. 1982; Sherren et al. 1998; Abd-Alla et al. 2013). The effect of abiotic stresses on legume growth and nonsymbiotic/symbiotic bacteria efficiency and nodulation and nitrogen fixation is discussed in the following section.

16.2 Bacteria Involved in Legume Growth Under Stress Environment

16.2.1 Symbiotic Bacteria

Among symbiotic bacteria, rhizobia associate essentially with leguminous plants (Long 1989; Sprent 2001), and the other one *Frankia*, grouped in *Actinobacteria*, interacts with plants of eight different families (Huss-Danell 1997; Franchee et al. 2009). In land-based systems, symbiotic relationship between *Rhizobium* and legumes is the primary source of fixed N, and more than half of the biological N is supplied by BNF. Symbiotic bacteria infect the legume roots and form nodules (West et al. 2002). During preinjection stage, it is necessary for rhizobia to recognize the roots of the appropriate host in order to be able to colonize. During nodule formation, three root tissues (epidermis, pericycle, and cortex) must be transformed (Geurts et al. 2012). The roots secrete flavonoids, and when bacteria encounters flavonoids, bacterial nodulation genes (*nod/nol/noe*) are activated (Ovtsyna and Staehelin 2003). Nodulation genes in turn regulate the synthesis of nodulation factors which triggers the formation and deformation of root hairs, formation of nodule primordia, induction of early nodulin gene expression, ion flux changes, depolarization of membrane potential, and intra-extracellular alkalization (Broughton et al. 2000; Perret et al. 2000).

16.2.2 Nonsymbiotic Bacteria

The term “nonsymbiotic” could be defined as having an interdependent relationship. Nonsymbiotic bacteria also fix atmospheric nitrogen and in association with symbiotic bacteria increase plant growth. Nonsymbiotic nitrogen-fixing bacteria (free living, associative, and endophytes) are cyanobacteria, *Azospirillum*, *Azotobacter*, *Gluconacetobacter diazotrophicus* and *Azocarus*, etc. (Bhattacharyya and Jha 2012). Due to the inefficiency of suitable carbon and energy sources for free-living organisms, their role in nitrogen fixation is considered as minor (Wagner 2011). On the other hand, associative nitrogen fixer, *Azospirillum*, located predominantly on the root surface of the plant fixes remarkable amount of nitrogen within the rhizosphere of the host plants. Even if their nitrogen-fixing amount is outstanding, the level of the nitrogen fixation is determined by several factors. Soil temperature, low oxygen pressure, availability of photosynthates, efficiency of nitrogenase enzyme, and competitiveness of the bacteria are some of the factors that limit the nitrogen fixation process. *Azotobacter* is another aerobic bacterium with genomic content G-C of 63–67.5% and fixes nitrogen nonsymbiotically (Becking 2006). Soil, water, and sediments are the habitat of *Azotobacter* (Torres et al. 2004, 2005). *Azotobacter* facilitates plant growth by synthesizing IAA and other growth-promoting substances (Ahmad et al. 2005). Also, nodulation and nitrogen fixation in legumes have been found significantly increased following dual inoculation of *Rhizobium* and *Azospirillum* or another PGPR such as *Azotobacter* (Rodelas et al. 1996, 1999).

16.3 Impacts of Abiotic Stresses on PGPR and Legumes

16.3.1 Salinity Stress

Salinity is one of the biggest problems which decreases quality and productivity of crops worldwide. Approximately, 10% of the world's crop fields and 27% of irrigated lands are affected by the salinity stress. When precipitation is insufficient to leach the ions from the soil profile, salts accumulate and cause soil salinity (Blaylock 1994). In hot and dry climate conditions, the level of soil salinity is increased. Soil salinity has a negative impact on growth and yields of crops including legumes (Singleton et al. 1982; Kumari and Subbarao 1984). The level of salt toxicity, however, depends on plant species and concentration and composition of salts (Delgado et al. 1994). For soybean, it was reported that nodulation, total N content, and yields were reduced by soil salinity (Singleton and Bohlool 1984). Similarly, plant height of peanut (*Arachis hypogaea* cv. NC-7) decreased by 21.6% and fresh weight by 21.4% after application of 4 dS/m salinity levels, whereas root length decreased by 30% after 8 dS/m salinity levels (Aydınşakir et al. 2015). Salinity level also affects the net photosynthetic rate of plants. As an example, Stoeva and Kaymakanova (2008) revealed that the net photosynthetic rate (PN) of beans (*Phaseolus vulgaris*) measured on the seventh day of treatment was reduced to 65% at 50 mM NaCl, 56% at 50 mM Na_2SO_4, and 40% and 20% by 100 mM each of NaCl and Na_2SO_4, respectively. However, plants adapt to their environment to maintain their survival. In this regard, Moriuchi et al. (2016) found that *Medicago truncatula* plants merely adapted to the environment and removal of salinity stress led to lower growth potential for saline-adapted plants suggesting that adaptation to high salinity is inherited from parents to the offsprings.

Under salinity stress, legumes are not able to maintain their regular nitrogen fixation and nodulation abilities. In a study conducted with alfalfa cultivated in saline environment, it was observed that a number of active nodes and nitrogen fixation were decreased (Nabizadeh et al. 2011). Nodule structure is also affected by salinity stress. Serraj et al. (1995) found out that treatment with 100 mM NaCl had adverse impacts on the soybean nodules by turning nucleus into a lobed structure and with different chromatin distribution and enlarged periplasmic space after 2 h exposure. Changes in the nucleus lead to differences in gene expression that could be seen in phenotype as decreased nitrogen fixation activity. The sensitivity of nitrogen fixation process to saline conditions could be related with the tolerance level of the bacteria. Velagaleti and Marsh (1989) reported that salinity resulted in decreased rhizobia colonization and shrinkage of root formation, while salt-tolerant *Bradyrhizobium* symbiosis with soybean revealed lower inhibitory impact of salinity in N_2 fixation. Bacteria have evolved several mechanisms to counter salinity stress (Shrivastava and Kumar, 2015). And hence, symbiotic relationship of rhizobia and legume plants is helpful in adapting to the salinity stress. For example, *Rhizobium* and *Pseudomonas* when used as mixed inoculant enhanced the growth and nodulation of mung bean grown under salinity stress by providing auxin and ACC deaminase (Ahmad et al. 2012). Pro-betaine and proline are involved in salt stress tolerance in *Medicago sativa* (Trinchant et al. 2004). Under osmotic stress, nitrogen-fixing bacteria, *Sinorhizobium meliloti,* regulates the expression of BetS gene which has a role in Gly-betaine/Pro-betaine transporter (Boscari et al. 2002). Use of *S. meliloti* would be a useful method to overcome salinity stress. In another perspective, it is suggested that creating a symbiosis between a salt-tolerant plant genotype and a rhizobia maintains salt tolerance and effective nitrogen fixation activity (Zahran 1999; Keneni et al. 2010). Obtaining sucrose from phloem is significant for nodule nitrogen fixation (Gordon et al. 1987). However, the presence of C source on the roots of legumes is not enough for nitrogen fixation. Enzymatic activity is required to supply C to the bacteroides. López et al. (2008) detected more enzymatic activity of PEPC (phosphoenolpyruvate carboxylase), MDH (malate dehydrogenase), and ICDH (isocitrate dehydrogenase) in *Lotus japonicas*, nodulated by *M. loti*, than *Medicago truncatula*, nodulated by *S. meliloti*. *Lotu japonicus* nodule C metabolism was shown to be less sensitive to salinity than in *M. truncatula* since the enzymes that had a role in C supply could fuel the bacteroides for processing the nitrogen fixation. However, the nitrogenase activity in *L. japonicus* nodules was inhibited by salinity. Even some strategies have been developed to find better breeds, they are long drawn and cost intensive.

16.3.2 Cold Stress

Temperature is another important factor essentially required for proper growth and development of plants. However, if a plant is exposed to a colder temperature for a longer duration, it may suffer from cold stress which could lead to loss of flower, decrease in photosynthetic activity, reduced activity of conductive tissue and enzymatic activity, and slowing down of the growth rate. In order to avoid such harmful cold temperature effects, plants need to develop certain mechanisms. In this context, soluble sugar is even sensitive to abiotic stresses, but reserve of sugar has a role to fight against stress conditions. Sugar protects cells from damage by serving as osmoprotectant, nutrient, and primary messengers in signal transduction (Yuanyuan et al. 2009). Proline is yet another important biomolecule (an amino acid) that acts as osmoprotectant and protects plants from stress conditions and hence accelerates the plant recovery. For instance, proline content increased in roots and shoots of lentil grown under cold stress conditions (Oktem et al. 2008). The length and fresh weight of shoots were decreased significantly resulting in the loss of yield. Hekneby et al. (2001) exposed the 21-day-old *Medicago truncatula* plants to 20/15 °C or 10/5 °C (day/night temperatures) for 40 days. The results revealed a significant increase in root/shoot ratio of *M. truncatula* plants grown under cold environment while total dry matter, leaf area, and specific leaf area ratio did not differ between two temperature treatments showing the tolerance degree of *M. truncatula* to cold stress. Exposure of plants to cold stress can also affect *Rhizobium*-legume symbiosis resulting in poor nodulation and nitrogen fixation. As an example, Lidström et al. (1985) found out that population density of *Rhizobium* strains was decreased from 3×10^8 to 1×10^5/g after −5 °C in soil acidity conditions. Also, the nitrogen fixation was decreased which was attributed due to cold stress rather than soil acidity and caused by the reduction of bacterial numbers in soil after cold treatment. Lastly, molecular aspects of the cold stress response and adaptation to cold stress have also been reported for soybean (Zhang et al. 2014). As an example, molecular signal exchanges between rhizobia and the legume are affected by the temperature causing reduction in nodulation process. There are inter-organismal signaling between rhizobia and its symbiotic partners, and this could be inhibited by low temperature. Low temperatures inhibit biosynthesis and secretion of signal molecules so that the interaction between plant and bacterial symbiotic relationship is interrupted. For instance, genistein secretion from soybean roots, which is required for the induction of *nod* genes of *B. japonicum*, is retarded (Abd-Alla 2001, 2011).

16.3.3 Nutrient Deficiency Stress

Nutrients are required by plants to live, grow, and reproduce. Deficiency of nutrients restricts the growth of plants (Table 16.1). Plant nutrients are divided mainly into two groups: macronutrients (Ca, P, N, K, S, and Mg) and micronutrients (B, Cl, Mn, Fe, Zn, Cu, Mo, and Ni). The critical concentration of these nutrients required

Table 16.1 The effect of different nutrient deficiencies on plants and legume-rhizobia symbiosis

Element	Nutrient deficiency symptoms/ damage	Importance in legume-rhizobia symbiosis
Nitrogen	Yellowing of older leaves while the rest of plant remain light green	Inhibits nodule formation and nitrogenase activity (Sprent et al. 1988)
Phosphorus	Leaf tips have a burnt look, older leaves turn dark green or reddish-purple	In case of deficiency, nitrogen fixation and symbiotic interactions are damaged (Weisany et al. 2013)
Potassium	Wilt of older leaves, interveinal chlorosis, and scorching inward from leaf margins	In case of deficiency, restrict rhizobial growth (Vincent et al. 1977)
Boron	Witches' broom formation and terminal buds die	Number of rhizobia infecting the host cell and number of infection thread are reduced during boron deficiency (Bolanos et al. 1996)
Molybdenum	Yellowing of older leaves (bottom of plant) while rest of the plant remain light green	Fe-Mo cofactor for most nitrogenases (Weisany et al. 2013)
Sulfur	Firstly, younger leaves turn yellow and sometimes this could be followed by older leaves	In case of deficiency, limited growth of rhizobia (O'Hara et al. 1987)
Calcium	Distorted or irregular shape of new leaves that are on the top of plant. It can cause blossom-end rot	In case of deficiency, nitrogen fixation in nodules is decreased (Banath et al. 1966) and nodulation and nodule development reduced (Banath et al. 1966)
Iron	Yellowing happens between the veins of young leaves	Fe-Mo cofactor for most nitrogenases (Weisany et al. 2013)

Modified from Guide to Symptoms of Plant Nutrient Deficiencies, Bradley and Hosier (1999) for optimum growth of plants, however, varies from genotypes to genotypes and from organs to organs. The impact of nutrient deficiency on legumes is discussed in the following section.

16.3.3.1 Phosphorus Stress

Among plant nutrients, phosphorus (P) is an important element and is involved in numerous biochemical processes, particularly in energy acquisition, storage, and utilization (Epstein and Bloom 2005). N_2-fixing nodules have high requirement of P. Unlike N, the P resources are not renewable, and therefore, it is expected that high-grade rock phosphates (RP) will be depleted gradually. As a result, the production of legumes in P-deficient soil is likely to suffer heavily (Sulima et al. 2015). However, phosphate-solubilizing bacteria (PSB) belonging to genera *Bacillus*, *Pseudomonas*, *Achromobacter*, *Alcaligenes*, *Brevibacterium*, *Corynebacterium*, *Serratia*, *and Xanthomonas* can be useful in supplying soluble P to plants (Khan et al. 2007). The impact of PSB, however, differs from species to species when inoculated with symbiotic *Rhizobium* bacteria. Rosas et al. (2006), for example, designed an experiment to assess the impact of *Pseudomonas* when co-inoculated with *S. meliloti 3DOh13* against alfalfa and *B. japonicum TIIIB* against soybean. The results demonstrated no significant differences between *S. meliloti 3DOh13*-inoculated alfalfa plants and *S. meliloti 3DOh13*+ *Pseudomonas* co-inoculation. However, the number and dry weight of soybean nodules was greater for co-inoculation with *B. japonicum TIIIB* and *Pseudomonas* compared to the sole application of *B. japonicum TIIIB*. Considering these, it is suggested that PSB in combination with other PGPR including rhizobia could be useful for enhancing legume production.

16.3.3.2 Sulfur Stress

Sulfur (S) is yet another important nutrient element for plants. Sulfur plays an important role in development and functioning of nodules. However, the deficiency of S limits N_2 fixation. Sulfur-oxidizing bacteria, for example, *Beggiatoa*, *Chromatium*, *Chlorobium*, *Thiobacillus*, *Sulfolobus*, *Thiospira*, and *Thiomicrospira*, are used to fulfill the sulfate requirement of plants. Under sulfur-deficient conditions, these bacteria could be used to transform elemental S into sulfate that plants can utilize. For groundnut, Anandham et al. (2007) investigated the impact of co-inoculation of *Thiobacillus,* sulfur-oxidizing bacteria, and *Rhizobium* under S-deficient soil. The results indicated that the nodule number, nodule dry weight, and biomass were significantly increased, and pod yield was enhanced by 18%.

16.3.3.3 Iron Stress

Plants growing in calcareous soils suffer from iron deficiency. Some soil bacteria synthesize ferric chelate reductase (FC-R) enzyme and release organic acids that decrease apoplastic pH of root and leaf cells. Ferric chelate reductase reduces Fe^{3+} to available form (Donnini et al. 2009). Many experiments have shown that the increased FC-R activity helps plants to take up Fe while growing under Fe-deficient conditions (López-Millán et al. 2001; Manuel and Alcántara 2002). FC-R activity can be utilized for determination of Fe-chlorosis-tolerant rootstocks (Bavaresco et al. 1991; Romera et al. 1991). Furthermore, the other way for Fe acquisition from soil is releasing of organic acids such as citrate and malate (Jones 1998; Abadía et al. 2002). Many researches demonstrated that organic acid excretion makes iron available to plants under Fe-starved conditions (Jones et al. 1996; Abadía et al. 2002).

High lime in soil affects Fe nutrition detrimentally in many ways. At first, availability of Fe in soil is decreased under lime and high pH conditions. Fe is trapped in bicarbonate soils and becomes unavailable for uptake by plants. Due to increased bicarbonate concentration, Fe acquisition is deteriorated (Nikolic and Roemheld 2003). However, some treatments can help to alleviate lime-induced Fe deficiency of soils. Afterwards, Fe entered into root apoplast must be carried into xylem. However, some part of Fe^{3+} remains in the root apoplast under lime-contained soil conditions and cannot be carried into plant shoot as a result of high pH in root apoplast (Kosegarten and Koyro 2001; Molassiotis et al. 2005). It has been proposed that some part of Fe absorbed from soil remains in the root apoplast (Bienfait et al. 1983). In an experiment it was exhibited that chlorosis and root Fe content of chlorotic plants could be related to removing of root Fe into plant shoots. Iron (Fe^{3+} citrate)-loaded xylem must be distributed into the leaf from veins after removal from the leaf (Mengel 1995). There must be re-reduction of Fe^{3+} citrate into Fe^{2+} for distribution in leaves (Brüggemann et al. 1993; Mengel 1994; Toselli et al. 2000; Bohórquez et al. 2001). Iron present in leaf apoplast must enter cell in order to maintain distribution of Fe in the leaf vein to the leaf. Mengel (1994) reported that during Fe chlorosis in the leaves, active Fe concentration is lower than non-chlorosis plants, but total Fe concentration is the same in both plant leaves. Therefore, leaf FC-R enzyme possesses a remarkable importance for elevating Fe availability in the leaves.

Rhizobacteria lowers the rhizosphere pH by releasing organic acids which in turn increases FC-R activity. Many researchers have suggested that bacterial treatments cause a decrease in soil pH and an increase in nutrition availability in soil (Sharma and Johri 2003; Orhan et al. 2006; Karlidag et al. 2007; Zhang et al. 2009). Also, increase in root and leaf Fe concentration has been reported. Iron is available in soil complexes with many organic acids such as citrate and malate that increases availability of insoluble ferric oxyhydroxides (Jones et al. 1996). Thus, increase of active iron (Fe^{2+}) in soil may have increased Fe uptake by plant from soil. Root inoculations considerably influenced root FC-R activity. Fe^{2+} is returned into Fe^{3+} after loading to xylem and is transported to shoots as Fe^{3+}-citrate with complexing with citrate. Transportation type of Fe in xylem is mainly Fe^{3+} citrate complex. Therefore, increase of citrate in xylem helps Fe transportation from root to shoots. Therefore, distribution of Fe to leaves and regreening were maintained as a result of a decrease in leaf apoplastic pH. Leaf apoplastic pH may have been decreased with many treatments such as spraying diluted acid or citric acid (Tagliavini and Rombola 2001) to leaves or ammonium fertilizer application to soil; thus iron in veins can be distributed in leaves. In this regard, decrease in leaf apoplastic pH can be achieved by uptake and translocation of organic acids released by bacteria in rhizosphere.

16.3.4 Drought Stress

The long exposure of plants to water-insufficient conditions, often called drought stress, has an adverse impact on plants (Zahran 1999) including legumes (Sangakkara and Hartwig 1996; Marino et al. 2007). Therefore, the assessment of drought impact on legume-*Rhizobium* symbiosis efficiency under abiotic stress conditions becomes highly critical. Ureides are nitrogenous compounds contributing to nitrogen recycling which accumulate in shoots and nodules of legumes under drought stress and consequently decline symbiotic nitrogen fixation (SNF) rapidly (Vadez et al. 2000). In addition, decreased transpiration rate diminishes N demand of shoot that lowers the rate of xylem translocation and reduces enzymatic activities which leads to decrease in nitrogen fixation rate (Valentine et al. 2011). Moreover, initiation of nodules, nodule growth, development, and function are affected by drought (Smith et al. 1988; Vadez et al. 2000; Streeter 2003). Drought situation also decreases photosynthetic activity which in turn adversely affects the SNF (Ladrera et al. 2007; Valentine et al. 2011).

In a study, Purcell et al. (1997) compared the nodulation patterns of two different soybeans: one tolerant to drought while the other was sensitive to drought. Drought-tolerant soybean was referred as “Jackson,” while drought-sensitive one was referred as “SCE82-303.” Even though the mass and number of nodules differed among two cultivars resulting, the nodule mass increased in “Jackson,” while it decreased in “SCE82-303.” Similarly, the impact of drought on SNF efficiency of *Rhizobium* was variable (Marino et al. 2007). For this, pea plants were grown in a split root system where one of the half was able to reach water, while the other half lacked water. Application of water-deficient conditions revealed decreased N_2 fixation. Furthermore, cell redox was imbalanced due to the reduction in the water potential of nodules. Besides, feedback signaling for systemic nitrogen did not work in the absence of water since the N_2 fixation was active and maintained at control values for half of the roots that were able to reach the water. This finding thus suggests that split root system controls the N_2 fixation at the local level rather by a systemic nitrogen signal. Considering these and other related studies, it becomes important to develop strategies that could protect both legumes and rhizobia from the negative impact of drought stress.

16.3.5 High Temperature and Heat Stress

Temperature is another important factor that affects N_2 fixation process among legumes. However, the temperature requirement of legumes varies from species to species or from cultivars to cultivars. For instance, the optimum temperature for N_2 fixation in clover and pea is 30 °C, while it is 35–40 °C for guar, soybean, peanut, and cowpea (Michiels et al. 1994). For beans, optimum temperature for nodule function is 25–30 °C, while 30–33°C temperature restricts nodule activity (Piha and Munnus 1987). However, nitrogen fixation by legumes is a main problem while growing at high temperatures in tropical and subtropical regions (Michiels et al. 1994). Infection of root hair, differentiations of bacteroides, structure of nodules, and legume root nodule function are affected by temperature (Zahran 1999). Additionally, photosynthetic rate, membrane stability, relations with water, and respiration are also impacted negatively by increased temperatures, which also regulate hormone levels and primary and secondary metabolite production. Heat stress also lowers the synthesis of ureides and decreases levels of nitrate reductase and glutamate synthase in legumes (Hungria and Vargas 2000; Christophe et al. 2011; Latef and Ahmad 2015). The decreased nitrogenase activity results in the reduction of N_2 fixation or accelerated nodule senescence leading to decreased nodule endurance (Bordeleau and Prevost 1994; Hungria and Vargas 2000; Christophe et al. 2011; Latef and Ahmad 2015). There are reports where increase in root temperatures has been found to adversely affect the bacterial infection and N_2 fixation of legumes, for example, soybean (Munevar and Wollum 1982), guar (Arayankoon et al. 1990), peanut (Kishinevsky et al. 1992), cowpea (Rainbird et al. 1983), and beans (Piha and Munnus 1987; Hungria et al. 1993). Plants have, however, evolved mechanisms to cope high temperature through heat-shock protein expression and other stress-related proteins and reactive oxygen species (ROS) production (Bhattacharya and Vijaylaxmi 2010; Hasanuzzaman et al. 2013).

16.3.6 Soil Acidity Stress

Globally, acidity covers nearly 40% of the lands that are available for farming (Valentine et al. 2011). An area which is larger than 1.5 Giga hectares is under acidity threat limiting the agricultural production (Graham and Vance 2000; Abd-Alla et al. 2014b). Soil acidity is increased by the impacts of global warming and agricultural applications that limit the legume crop productivity. However, alkalinity and acidity are the two extreme situations for any soil that may hamper growth, survival, and nitrogen fixation ability of rhizobia (Lapinskas 2007). During *Rhizobium*-legume symbiosis, *Rhizobium* was found more sensitive to acidic conditions than legumes. Virtually, since rhizobia are incapable of persisting and surviving under acidic conditions, this could reduce the effectiveness of symbiosis and concomitantly loss in legume productivity. Therefore, selection and application of acid-tolerant rhizobia become important for enhancing the production of legumes under acid stress environment. In this regard, mutants of *R. leguminosarum* that grew at pH as low as 4.5 (Chen et al. 1993) and *S. meliloti* which grew at pH level below 5.5 (Foster 2000) are reported. In addition, some rhizobial species can grow at a wide range of pH. For instance, *S. fredii* can grow at pH levels between 4 and 9.5 (Fujihara and Yoneyama 1993). Like acidity, alkalinity stress also destructs the growth of *Rhizobium* (Monica et al. 2013) and their symbiotic relationship with legumes (Zahran 1999). Therefore, it is also important to select *Rhizobium* isolates, which could survive the alkalinity stress and be capable of nitrogen fixation and, hence, the legume production (Abd-Alla et al. 2014a). Apart from rhizobia, yields and growth of legumes are also impacted by soil acidity (Ferguson et al. 2013). However, soil acidity helps to adjust the availability of mineral nutrients (e.g., phosphorus) and severity of some phytotoxic elements (e.g., aluminum, manganese, and iron) in natural/degraded ecosystems (Muthukumar et al. 2014).

Conclusion

Rhizobacteria including both symbiotic and nonsymbiotic bacteria are one of the important classes of soil microbiota which augment crop production including those of legumes in different agronomic regions. The application of PGPR provides a comparable yield and quality by supplying essential nutrients and hormones to legumes. Also, the application of PGPR helps to alleviate several stress conditions as drought, salinity, nutrient stress, and low/high temperature stress. The tolerance to high levels of stresses and the survival and persistence of PGPR in severe and harsh conditions make these bacteria a highly valuable organism to enhance legume production in extreme environmental conditions. However, further studies are needed to evaluate the performance of PGPR in different stressed conditions choosing a range of legume crops. The mineral nutrition and fertilization effects of N_2-fixing and other free-living PGPR should be examined regularly and carefully before they are recommended for application by farming communities.

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