

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

# 16

Metin Turan, Nurgül Kitir, Erdal Elkoca, Deniz Uras, Ceren Ünek, Emrah Nikerel, Bahar Soğutmaz Özdemir, Leyla Tarhan, Ahmet Eşitken, Ertan Yildirim, Negar Ebrahim Pour Mokhtari, Şefik Tüfenkçi, M. Rüştü Karaman, and Adem Güneş

## 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

M. Turan (✉) • N. Kitir • D. Uras • C. Ünek • E. Nikerel • B. Soğutmaz Özdemir • L. Tarhan  
Department of Genetics and Bioengineering, Faculty of Engineering, Yeditepe University,  
Istanbul, Turkey  
e-mail: [m\\_turan25@hotmail.com](mailto:m_turan25@hotmail.com)

E. Elkoca  
Department of Agronomy, Faculty of Agriculture, Ataturk University, Erzurum, Turkey

A. Eşitken  
Horticulture and Viticulture Department, Faculty of Agriculture, Selcuk University,  
Konya, Turkey

E. Yildirim  
Department of Horticulture, Faculty of Agriculture, Ataturk University, Erzurum, Turkey

N.E.P. Mokhtari  
Organic Farming Department, Islahiye Vocational School, Gaziantep University,  
Gaziantep, Turkey

Ş. Tüfenkçi  
Faculty of Agriculture, Department of Biosystem Engineering, Yüzüncü Yıl University,  
Van, Turkey

M.R. Karaman  
Vocational School of Medicals and Aromatics, Afyon Kocatepe University, Afyon, Turkey

A. Güneş  
Agricultural Faculty, Soil and Plant Nutrition Science, Erciyes University, Kayseri, Turkey

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<sub>2</sub>SO<sub>4</sub>, and 40% and 20% by 100 mM each of NaCl and Na<sub>2</sub>SO<sub>4</sub>, 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<sub>2</sub> 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 japonicus*, 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  $3 \times 10^8$  to  $1 \times 10^5$ /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<sub>2</sub>-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<sub>2</sub> 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<sup>3+</sup> 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  $\text{Fe}^{3+}$  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 ( $\text{Fe}^{3+}$  citrate)-loaded xylem must be distributed into the leaf from veins after removal from the leaf (Mengel 1995). There must be re-reduction of  $\text{Fe}^{3+}$  citrate into  $\text{Fe}^{2+}$  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 ( $\text{Fe}^{2+}$ ) in soil may have increased Fe uptake by plant from soil. Root inoculations considerably influenced root FC-R activity.  $\text{Fe}^{2+}$  is returned into  $\text{Fe}^{3+}$  after loading to xylem and is transported to shoots as  $\text{Fe}^{3+}$ -citrate with complexing with citrate. Transportation type of Fe in xylem is mainly  $\text{Fe}^{3+}$  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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub>-fixing and other free-living PGPR should be examined regularly and carefully before they are recommended for application by farming communities.

## References

- Abadía J, López-Millán AF, Rombolà A, Abadía A (2002) Organic acids and Fe-deficiency: a review. *Plant Soil* 241:75–86
- Abd-Alla MH (2001) Regulation of nodule formation in soybean-*Bradyrhizobium* symbiosis is controlled by shoot or/and root signals. *Plant Growth Regul* 34:241–250
- Abd-Alla MH (2011) Nodulation and nitrogen fixation in interspecies grafts of soybean and common bean is controlled by isoflavonoid signal molecules translocated from shoot. *Plant Soil Environ* 57:453–458
- Abd-Alla MH, El-enany AE, Bagy MK, Bashandy SR (2014) Alleviating the inhibitory effect of salinity stress on gene expression in ? fenugreek ( ) symbiosis by isoflavonoids treatment. *J Plant Int* 9(1):275–284
- Abd-Alla MH, El-Enany AE, Nafady NA, Khalaf DM, Morsy FM (2014a) Synergistic interaction of *Rhizobium leguminosarum* bv. *viciae* and arbuscular mycorrhizal fungi as a plant growth promoting biofertilizers for faba bean (*Vicia faba* L.) in alkaline soil. *Microbiol Res* 169:49–58
- Abd-Alla MH, Issa AA, Ohyama T (2014b) Impact of harsh environmental conditions on nodule formation and dinitrogen fixation of legumes. *Agricultural and biological sciences “advances in biology and ecology of nitrogen fixation”*. ISBN: 978-953-51-1216-7
- Ahmad F, Ahmad I, Khan MS (2005) Indole acetic acid production by the indigenous isolates of *Azotobacter* and fluorescent pseudomonas in the presence and absence of tryptophan. *Turk J Biol* 29:29–34
- Ahmad F, Ahmad I, Khan MS (2008) Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiol Res* 163:173–181
- Ahmad M, Zahir ZA, Asghar HN, Arshad M (2012) The combined application of rhizobial strains and plant growth promoting rhizobacteria improves growth and productivity of mung bean (*Vigna radiata* L.) under salt-stressed conditions. *Ann Microbiol* 62:1321–1330
- Anandham R, Sridar R, Nalayini P, Poonguzhali S, Madhaiyan M, Sa T (2007) Potential for plant growth promotion in groundnut (*Arachis hypogaea* L.) cv. ALR-2 by co-inoculation of sulfur-oxidizing bacteria and *Rhizobium*. *Microb Res* 162:139–153
- Andrews M, Hodge S (2010) Climate change, a challenge for cool season grain legume crop production. In: *Climate change and management of cool season grain legume crops*. Springer, Netherlands, pp 1–9

- Arayankoon T, Schomberg HH (1990) Nodulation and N<sub>2</sub> fixation of guar at high room temperature. *Plant Soil* 126:209–213
- Aydınşakir K, Büyüktaş D, Dinç N, Karaca C (2015) Impact of salinity stress on growing, seedling development and water consumption of peanut (*Arachis hypogaea* cv. NC-7). *Akdeniz Univ Ziraat Fak Derg* 28:77–84
- Bashan Y, Holguin G (1997) Azospirillum–plant relationships: environmental and physiological advances (1990–1996). *Can J Microbiol* 43:103–121
- Banath CL, Greenwood EAN, Loneragan JF (1966) Effects of calcium deficiency on symbiotic nitrogen fixation. *Plant Physiol* 41(5):760–763
- Bavaresco L, Fregoni H, Frascini P (1991) Investigations on iron uptake and reduction by excised roots of different grapevine rootstocks and a *V. vinifera* cultivar. In: Chen Y, Hadar Y (eds) Iron nutrition and interactions in plant. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 139–143
- Becking J (2006) The family Azotobacteraceae. *Prokaryotes* 6:759–783
- Bhattacharya A, Vijaylaxmi (2010) Physiological responses of grain legumes to stress environments. In: Yadav SS (ed) Chickpea breeding and management. CAB International, Wallingford, pp 35–86
- Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World J Microbiol Biotechnol* 28:1327–1350
- Bienfait HE, Bino RJ, VanderBlick AM, Duivenvoorden JF, Fontaine FM (1983) Characterization of ferric reducing activity in roots of Fe-deficient *Phaseolus vulgaris*. *Physiol Plant* 59:196–202
- Blaylock AD (1994) Soil salinity, salt tolerance and growth potential of horticultural and landscape plants. Co-operative Extension Service, University of Wyoming, Department of Plant, Soil and Insect Sciences, College of Agriculture, Laramie, Wyoming
- Bohórquez JM, Romera FJ, Alcántara E (2001) Effect of Fe<sup>3+</sup>, Zn<sup>2+</sup> and Mn<sup>2+</sup> on ferric reducing capacity and regreening process of the peach rootstock Nemaguard [*Prunus persica* (L.) Batsch]. *Plant Soil* 237:157–163
- Bolanos L, Brewin NJ, Bonilla I (1996) Effects of boron on Rhizobium-legume cell-surface interactions and nodule development. *Plant Physiol* 110(4):1249–1256
- Bordeleau LM, Prevost D (1994) Nodulation and nitrogen fixation in extreme environments. *Plant Soil* 161:115–125
- Boscari A, Mandon K, Dupont L, Poggi MC, Le Rudulier D (2002) BetS Is a major glycine betaine/proline betaine transporter required for early osmotic adjustment in *Sinorhizobium meliloti*. *J Bacteriol* 184:2654–2663
- Bouchenak M, Lamri-Senhadj M (2013) Nutritional quality of legumes, and their role in cardio-metabolic risk prevention: a review. *J Med Food* 16:185–198
- Bradley L, Hosier S (1999) Guide to Symptoms of Plant Nutrient Deficiencies ISO 690
- Broughton WJ, Jabbouri S, Perret X (2000) Keys to symbiotic harmony. *J Bacteriol* 182:5641–5652
- Bruggemann W, Maas-Kantel K, Moog PR (1993) Iron uptake by leaf mesophyll cells: The role of the plasma membrane-bound ferric-chelate reductase. *Planta* 190(2)
- Campos-Vega R, Loarca-Pina G, Dave Oomah B (2010) Minor components of pulses and their potential impact on human health. *Food Res Int* 43:461–482
- Chen H, Richardson AE, Rolfe BG (1993) Studies of the physiological and genetic basis of acid tolerance in *Rhizobium leguminosarum* biovar *trifolii*. *Appl Environ Microbiol* 59:1798–1804
- Christophe S, Jean-Christophe A, Annabelle L, Alain O, Marion P, Anne-Sophie V (2011) Plant N fluxes and modulation by nitrogen, heat and water stresses: a review. Based on comparison of legumes and non legume plants. In: Shanker AK, Venkateswarlu B (eds) Abiotic stress in plants—mechanisms and adaptations. InTech, Croatia, pp 79–119
- Delgado MJ, Ligerio F, Lluch C (1994) Effects of salt stress on growth and nitrogen fixation by pea, faba-bean, common bean and soybean plants. *Soil Biol Biochem* 26:371–376
- Donnini S, Castagna A, Ranieri A, Zocchi G (2009) Differential responses in pear and quince genotypes induced by Fe deficiency and bicarbonate. *J Plant Physiol* 166:1181–1193

- Epstein E, Bloom AJ (2005) Mineral nutrition of plants: principles and perspectives, 2nd edn. Sinauer Associates, Sunderland, MA
- FAO (1994) Pulses and derived products
- FAOSTAT (2009) Food and Agriculture Organization of the United Nations, Rome
- Ferguson BJ, Lin MH, Gresshoff PM (2013) Regulation of legume nodulation by acidic growth conditions. *Plant Signal Behav* 8:e23426
- Flight I, Clifton P (2006) Cereal grains and legumes in the prevention of coronary heart disease and stroke: a review of the literature. *Eur J Clin Nutr* 60:1145–1159
- Foster JW (2000) Microbial responses to acid stress. In: Storz G, Hengge-Aronis R (eds) Bacterial stress response. ASM Press, Washington, DC, pp 99–115
- Foth HD (1990) Fundamentals of soil science. Wiley, New York
- Franche C, Lindström K, Elmerich C (2009) Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. *Plant Soil* 321(1–2):35–59
- Freiberg C, Fellay R, Bairoch A, Broughton WJ, Rosenthal A, Perret X (1997) Molecular basis of symbiosis between *Rhizobium* and legumes. *Nature* 387:394–401
- Fujihara S, Yoneyama T (1993) Effects of pH and osmotic stress on cellular polyamine contents in the soybean *Rhizobia fredii* P220 and *Bradyrhizobium japonicum* A1017. *Appl Environ Microbiol* 59:1104–1109
- Garg N, Geetanjali G (2009) Symbiotic nitrogen fixation in legume nodules: process and signaling: a review. In: Lichtfouse E, Navarette M, Véronique S, Alberola C (eds) Sustainable agriculture. Springer, Netherlands, pp 519–531
- Geurts R, Lillo A, Bisseling T (2012) Exploiting an ancient signalling machinery to enjoy a nitrogen fixing symbiosis. *Curr Opin Plant Biol* 15:1–6
- Gordon AJ, Mitchel DF, Ryle GJA, Powell CE (1987) Diurnal production and utilization of photosynthate in nodulated white clover. *J Exp Bot* 38:84–98
- Graham PH, Vance CP (2000) Nitrogen fixation in perspective: an overview of research and extension needs. *Field Crops Res* 65:93–106
- Graham PH, Vance CP (2003) Legumes: importance and constraints to greater use. *Plant Physiol* 13:872–877
- Hasanuzzaman M, Gill SS, Fujita M (2013) Physiological role of nitric oxide in plants grown under adverse environmental conditions. In: Tuteja N, Gill SS (eds) Plant acclimation to environmental stress. Springer Science + Business Media, New York, pp 269–322
- Hekneby M, Antolin MC, Sanchez-Diaz M (2001) Cold response of annual mediterranean pasture legumes. In: Delgado I, Lloveras J (eds) Quality in lucerne and medics for animal production. *Options Méditerranéennes* 45. CIHEAM, Zaragoza, pp 157–161
- Howard JB, Rees DC (1996) Structural Basis of Biological Nitrogen Fixation. *Chem Rev* 96(7):2965–2982
- Hungria M, Franco AA, Sprent JI (1993) New sources of high-temperature tolerant rhizobia for *Phaseolus vulgaris* L. *Plant Soil* 149(1):103–109
- Hungria M, Vargas MAT (2000) Environmental factors affecting N<sub>2</sub> fixation in grain legumes in the tropics, with an emphasis on Brazil. *Field Crops Res* 65:151–164
- Huss-Danell K (1997) Actinorhizal symbioses and their N<sub>2</sub> fixation. *New Phytol* 136:375–405
- Jenkins DJ, Wolever TM, Taylor RH, Barker HM, Fielden H (1980) Exceptionally low blood glucose response to dried beans: comparison with other carbohydrate foods. *Br Med J* 281:578–580
- Jones DL, Darrah PR, Kochian LV (1996) Critical evaluation of organic acid mediated iron dissolution in the rhizosphere and its potential role in root iron uptake. *Plant Soil* 180:57–66
- Kalogeropoulos N, Chiou A, Ioannou M, Karathanos VT, Hassapidou M, Nikolaos K, Andrikopoulos NK (2010) Nutritional evaluation and bioactive microconstituents (phytosterols, tocopherols, polyphenols, triterpenic acids) in cooked dry legumes usually consumed in the Mediterranean countries. *Food Chem* 121:682–690
- Karlidag H, Esitken A, Turan M, Sahin F (2007) Effects of root inoculation of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient element contents of leaves of apple. *Sci Hortic* 114(1):16–20



- Keneni AF, Assefa PC, Prabu (2010) Characterization of acid and salt-tolerant rhizobial strains isolated from faba bean fields of Wollo Northern Ethiopia. *J Agric Sci Technol* 12:365–376
- Khan MS, Zaidi A, Wani PA (2007) Role of phosphate solubilizing microorganisms in sustainable agriculture – a review. *Agron Sustain Dev* 27:29–43
- Kishinevsky BD, Sen D, Weaver RW (1992) Effect of high root temperature on Bradyrhizobium-peanut symbiosis. *Plant Soil* 143:275–282
- Kosegarten H, Koyro HW (2001) Apoplastic accumulation of iron in the epidermis of maize (*Zea mays*) roots grown in calcareous soil. *Physiol Plant* 113:515–522
- Kudapa H, Ramalingam A, Nayakoti S, Chen X, Zhuang WJ, Liang X, Varshney RK (2013) Functional genomics to study stress responses in crop legumes: progress and prospects. *Func Plant Biol* 40:1221–1233
- Kumari MSL, Subbarao NS (1984) Root hair infection and nodulation of lucerne as influenced by salinity and alkalinity. *Plant Soil* 40:261–268
- Ladrera R, Marino D, Larrainzar E, González EM, Arrese-Igor C (2007) Reduced carbon availability to bacteroids and elevated ureides in nodules, but not in shoots, are involved in nitrogen fixation response to early drought in soybean. *Plant Physiol* 145:539–546
- Lapinskas EB (2007) The effect of acidity on the distribution and symbiotic efficiency of rhizobia in Lithuanian soils. *Eurasian Soil Sci* 40:419–425
- Latef AAHA, Ahmad P (2015) Legumes and breeding under abiotic stress: an overview. In: *Legumes under environmental stress yield, improvement and adaptations*. John Wiley & Sons Ltd, Hoboken, NJ, pp 1–20
- Lindström K, Sorsa M, Polkunen J, Kansanen P (1985) Symbiotic nitrogen fixation of *Rhizobium* (Galega) in acid soils, and its survival in soil under acid and cold stress. *Plant Soil* 87:293–302
- Liu J-Q, Allan DL, Vance CP (2010) Systemic signaling and local sensing of phosphate in common bean: Cross-Talk between photosynthate and microRNA399. *Mole Plant* 3(2):428–437
- Long SR (1989) *Rhizobium* genetics. *Annu Rev Genet* 23:483–506
- López M, Herrera-Cervera JA, Iribarne C, Tejera NA, Lluch C (2008) Growth and nitrogen fixation in *Lotus japonicus* and *Medicago truncatula* under NaCl stress: nodule carbon metabolism. *J Plant Physiol* 165:641–650
- López-Millán AF, Morales F, Abadía A, Abadía J (2001) Iron deficiency-associated changes in the composition of the leaf apoplastic fluid from field-grown pear (*Pyrus communis* L.) trees. *J Exp Bot* 52:1489–1498
- Manuel D, Alcántara E (2002) A comparison of ferric-chelate reductase and chlorophyll and growth ratios as indices of selection of quince, pear and olive genotypes under iron deficiency stress. *Plant Soil* 241:49–56
- Marino D, Frendo P, Ladrera R, Zabalza A, Puppo A, Arrese-Igor C, González EM (2007) Nitrogen fixation control under drought stress. Localized or systemic? *Plant Physiol* 144:1233
- Mengel K (1994) Iron availability in plant tissues—iron chlorosis on calcareous soils. *Plant Soil* 165:275–283
- Mengel K (1995) Iron availability in plant tissues—iron chlorosis on calcareous soils. In: Abadía J (ed) *Iron nutrition in soils and plants*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 389–397
- Michiels J, Verreth C, Vanderleyden J (1994) Effects of temperature stress on bean nodulating *Rhizobium* strains. *Appl Environ Microbiol* 60:1206–1212
- Miller PR, McConkey BG, Clayton GW, Brandt SA, Staricka JA, Johnston AM, Neill KE (2002) Pulse crop adaptation in the northern Great Plains. *Agron J* 94:261–272
- Molassiotis AN, Diamantidis GC, Therios IN, Tsirakoglou V, Dimassi KN (2005) Oxidative stress, antioxidant activity and Fe (III)-chelate reductase activity of five *Prunus* rootstocks explants in response to Fe deficiency. *Plant Growth Regul* 46:69–78
- Mollard RC, Zykyus A, Luhovyy BL, Nunez MF, Wong CL, Anderson GH (2012) The acute effects of a pulse-containing meal on glycaemic responses and measures of satiety and satiation within and at a later meal. *Br J Nutr* 108:509–517



- Monica NISTE, Roxana VIDICAN, Ioan ROTAR, Rodica POP (2013) The effect of pH stress on the survival of *Rhizobium trifolii* and *Sinorhizobium meliloti* in vitro. *Bull UASMV Ser Agric* 70(2):449–450
- Morel MA, Braña V, Castro-Sowinski S (2012) Legume crops, importance and use of bacterial inoculation to increase production. In: Goyal A (ed) *Crop plant*. InTech, Croatia. doi:10.5772/37413
- Moriuchi KS, Friesen ML, Cordeiro MA, Badri M, Vu WT, Main BJ et al (2016) Salinity adaptation and the contribution of parental environmental effects in *Medicago truncatula*. *PLoS One* 11(3):e0150350
- Munevar F, Wollum AG (1982) Response of soybean plants to high root temperature as affected by plant cultivar and Rhizobium strain. *Agron J* 74:138–142
- Muthukumar T, Priyadharsini P, Uma E, Jaison S, Pandey RR (2014) Role of arbuscular mycorrhizal fungi in alleviation of acidity stress on plant growth. In: Miransari M (ed) *Use of microbes for the alleviation of soil stresses*. Springer Science + Business Media, New York, pp 43–71
- Nabizadeh E, Jalilnejad N, Armakani M (2011) Effect of salinity on growth and nitrogen fixation of Alfalfa (*Medicago sativa*). *World Appl Sci J* 13:1895–1900
- Nestel P, Cehun M, Chronopoulos A (2004) Effects of long-term consumption and single meals of chickpeas on plasma glucose, insulin, and triacylglycerol concentrations. *Am J Clin Nutr* 79:390–395
- Nikolic M, Roemheld V (2003) Nitrate does not result in iron inactivation in the apoplast of sunflower leaves. *Plant Physiol* 132:1303–1314
- O'Hara GW, Franklin M, Dilworth MJ (1987) Effect of sulfur supply on sulfate uptake, and alkaline sulfatase activity in free-living and symbiotic bradyrhizobia. *Arch Microbiol* 149(2):163–167
- Ohyama T, Ohtake N, Sueyoshi K, Tewari K, Takahashi Y, Ito S, Nishiwaki T, Nagumo Y, Ishii S, Sato T (2009) *Nitrogen fixation and metabolism in soybean plants*. Nova Science Publishers, Inc., New York
- Oktem HA, Eyidoan F, Demirba D et al (2008) Antioxidant responses of lentil to cold and drought stress. *J Plant Biochem Biotechnol* 17:15–21
- Orhan E, Esitken A, Ercisli S, Turan M, Sahin F (2006) Effects of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient contents in organically growing raspberry. *Sci Hortic* 111:38–43
- Ovtsyna AO, Staehelin C (2003) Bacterial signals required for the Rhizobium-legume symbiosis. In: Pandalai SG (ed) *Recent research developments in microbiology, Part II, vol 7*. Research Signpost, Trivandrum, India, pp 631–648
- Papanikolaou Y, Fulgoni VL III (2008) Bean consumption is associated with greater nutrient intake, reduced systolic blood pressure, lower body weight, and a smaller waist circumference in adults: results from the National Health and Nutrition Examination Survey 1999–2002. *J Am Coll Nutr* 27:569–576
- Perret X, Staehelin C, Broughton WJ (2000) Molecular basis of symbiotic promiscuity. *Microbiol Mol Biol Rev* 64:180–201
- Piha MI, Munnus DN (1987) Sensitivity of the common bean (*Phaseolus vulgaris* L.) symbiosis to high soil temperature. *Plant Soil* 98:183–194
- Purcell LC, Silva M, King CA, Kim WH (1997) Biomass accumulation and allocation in soybean associated with genotypic differences in tolerance of nitrogen fixation to water deficits. *Plant Soil* 196:101–103
- Rainbird RM, Akins CA, Pate JJS (1983) Effect of temperature on nitrogenase functioning in cowpea nodules. *Plant Physiol* 73:392–394
- Rebello CJ, Greenway FL, Finley JW (2014) A review of the nutritional value of legumes and their effects on obesity and its related co-morbidities. *Obes Rev* 15:392–407
- Rodelas B, González-López J, Salmerón V, Pozo C, Martínez-Toledo MV (1996) Enhancement of nodulation, N<sub>2</sub> fixation and growth of faba bean (*Vicia faba* L.) by combined inoculation with *Rhizobium leguminosarum* bv. *viciae* and *Azospirillum brasilense*. *Symbiosis* 21:175–186
- Rodelas B, González-López J, Martínez-Toledo MV, Pozo C, Salmerón NV (1999) Influence of *Rhizobium/Azotobacter* and *Rhizobium/Azospirillum* combined inoculation on mineral composition of faba bean (*Vicia faba* L.) *Biol Fertil Soils* 29:165–169

- Romera FJ, Alcantara E, De La MDG (1991) Characterization of the tolerance to iron chlorosis in different peach rootstocks grown in nutrient solution. *Plant Soil* 130:115–125
- Rosas S, Andres J, Rovera M, Correa N (2006) Phosphate-solubilizing *Pseudomonas putida* can influence the rhizobia–legume symbiosis. *Soil Biol Biochem* 38:3502–3505
- Sangakkara UR, Hartwig UA (1996) Soil moisture and potassium affect the performance of symbiotic nitrogen fixation in faba bean and common bean. *Plant Soil* 184:123–130
- Serraj R, Fleurat-Lessard P, Jaillard B, Drevon JJ (1995) Structural changes in the innercortex cells of soybean root nodules are induced by short-term exposure to high salt or oxygen concentrations. *Plant Cell Environ* 18(4):455–462
- Sharma A, Johri BN (2003) Growth promoting influence of siderophore-producing *Pseudomonas* strains GRP3A and PRS 9 in maize (*Zea mays* L.) under iron limiting conditions. *Microbiol Res* 158:243–248
- Sherren A, Ansari R, Naqvi SSM, Soomaro AQ (1998) Effect of salinity on *Rhizobium* species, nodulation and growth of soybean. *Pak J Bot* 1:75–81
- Singleton PW, Bohlool BB (1984) Effect of salinity on nodule formation by soybean. *Plant Physiol* 74:72–76
- Singleton PW, Swaify SA, Bohlool BB (1982) Effect of salinity on *Rhizobium* growth and survival. *Appl Environ Microbiol* 44:884–890
- Skorupska A, Wielbo J, Kidaj D, Marek-Kozaczuk M (2010) Enhancing *Rhizobium* legume symbiosis using signaling factors. In: Khan MS, Zaidi A, Musarrat J (eds) *Microbes for legume improvement*. Springer-Verlag, New York, pp 27–54. ISBN: 978-3-211
- Smith DL, Dijak M, Hume DJ (1988) The effect of water deficit on N<sub>2</sub> (C<sub>2</sub>H<sub>2</sub>) fixation by white bean and soybean. *Can J Plant Sci* 68:957–967
- Sprent JI, Stephens JH, Rupela OP (1988) Environmental effects on nitrogen fixation. In *World crops: cool season food legumes*. Springer Netherlands, pp. 801–810
- Sprent JI (2001) *Nodulation in Legumes*. Cromwell Press, Royal Botanical Gardens, Kew
- Stoeva N, Kaymakanova M (2008) Effect of salt stress on the growth and photosynthesis rate of bean plants. *J Cent Eur Agric* 9:385–392
- Streeter JG (2003) Effects of drought on nitrogen fixation in soybean root nodules. *Plant Cell Environ* 26:1199–1204
- Subba Rao GV, Johnsen C, Kumarrao JV, Jana MK (1999) Response of the Pigeon pea *Rhizobium* symbiosis to salinity stress: variation among *Rhizobium* strain in symbiotic ability. *Biol Fertil* 9:49–53
- Sulima AS, Zhukov VA, Shtark OY, Borisov AY, Tikhonovich IA (2015) Nod-factor signaling in legume-rhizobial symbiosis. In: El-Shemy H (ed) *Plants for the future*. InTech, Croatia. doi:10.5772/61165
- Tagliavini M, Rombola AD (2001) Iron deficiency and chlorosis in orchard and vineyard ecosystems. *Eur J Agron* 15:71–92
- Tejera N, Lluch C, Martínez M, González J (2005) Isolation and characterization of *Azotobacter* and *Azospirillum* strains from the sugarcane rhizosphere. *Plant Soil* 27:223–232
- Toker C, Yadav SS (2010) Legumes cultivars for stress environments. In: *Climate change and management of cool season grain legume crops*. Springer, Netherlands, pp 351–376
- Torres M, Valencia S, Bernal J, Martínez P (2004) Isolation of *Enterobacteria*, *Azotobacter* sp. and *Pseudomonas* sp., producers of indole-3-acetic acid and siderophores, from Colombian rice rhizosphere. *Rev Latin Microbiol* 42:171–176
- Toselli M, Marangoni B, Tagliavini M (2000) Iron content in vegetative and reproductive organs of nectarine trees in calcareous soils during the development of chlorosis. *Eur J Agron* 13(4):279–286
- Trinchant JC, Boscarri A, Spennato G, van de Sype G, le Rudulier D (2004) Proline betaine accumulation and metabolism in alfalfa plants under sodium chloride stress. Exploring its compartmentalization in nodules. *Plant Physiol* 135:1583–1594
- Vadez V, Sinclair TR, Serraj R (2000) Asparagine and ureide accumulation in nodules and shoot as feedback inhibitors of N<sub>2</sub> fixation in soybean. *Physiol Plant* 110:215–223

- Valentine AJ, Benedito VA, Kang Y (2011) Legume nitrogen fixation and soil abiotic stress: from physiology to genomic and beyond. *Annu Plant Rev* 42:207–248
- Velagaleti RR, Marsh S (1989) Influence of host cultivars and *Bradyrhizobium* strains on the growth and symbiotic performance of soybean under salt stress. *Plant Soil* 119:133–138
- Vincent JM (1977) *Rhizobium*: General microbiology. In R.W.F. Hardy and W.S. Silver (eds.) *A Treatise on Dinitrogen Fixation Section III Biology*. John Wiley & Sons, New York, p 277366
- Wagner SC (2011) Biological nitrogen fixation. *Nat Educ Knowl* 3:15
- Weisany W, Raei Y, Allahverdiipoor KH (2013) Role of some of mineral nutrients in biological nitrogen fixation. *Bull Env Pharmacol Life Sci* 2(4):77–84
- West SA, Kiers ET, Pen I, Denison RF (2002) Sanctions and mutualism stability: when should less beneficial mutualists be tolerated? *J Evol Biol* 15:830–837
- Yuanyuan M, Yali Z, Jiang L, Hongbo S (2009). Roles of plant soluble sugars and their responses to plant cold stress. *African J Biotechnol* 8(10)
- Zahran HH (1999) *Rhizobium*-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. *Microbiol Mol Biol Rev* 63:968–989
- Zahran HH (2001) Rhizobia from wild legumes: diversity, taxonomy, ecology, nitrogen fixation and biotechnology. *J Biotechnol* 91:143–153
- Zhang H, Sun Y, Xie X, Kim MS, Dowd SE, Paré PW (2009) A soil bacterium regulates plant acquisition of iron via deficiency-inducible mechanisms. *Plant J* 58:568–577
- Zhang XN, Li X, Liu JH (2014) Identification of conserved and novel cold-responsive microRNAs in trifoliolate orange (*Poncirus trifoliata* (L.) Raf.) using high-throughput sequencing. *Plant Mol Biol Rep* 32:328–341
- Zhu H, Choi HK, Cook DR, Shoemaker RC (2005) Bridging model and crop legumes through comparative genomics. *Plant Physiol* 137:1189–1196