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Potential of Rhizobia in Improving Nitrogen Fixation and Yields of Legumes

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Additional information is available at the end of the chapter

Abstract

Strong demand for food requires specific efforts by researchers involved in the agricultural sector to develop means for sufficient production. While, agriculture today faces challenges such as soil fertility loss, climate change and increased attacks of pathogens and pests. The production of sufficient quantities in a sustainable and healthy farming system is based on environmentally friendly approaches such as the use of biofertilizers, biopesticides and the return of crop residues. The multiplicity of beneficial effects of soil microorganisms, particularly plant growth promotion (PGP), highlights the need to further strengthen the research and its use in modern agriculture. Rhizobia are considered as PGP comes in symbiosis with legumes taking advantage of nutrients from plant root exudates. When interacting with legumes, rhizobia help in increased plant growth through enriching nutrients by nitrogen fixation, solubilizing phosphates and producing phytohormones, and rhizobia can increase plants' protection by influencing the production of metabolites, improve plant defense by triggering systemic resistance induced against pests and pathogens. In addition, rhizobia contain useful variations to tolerate abiotic stresses such as extreme temperatures, pH, salinity and drought. The search for rhizobium tolerant strains is expected to improve plant growth and yield, even under a combination of constraints. This chapter summarizes the use of rhizobia in agriculture and its benefits.

Keywords: rhizobia, PGP, biocontrol, induced resistance, stress tolerance

1. Introduction

Agricultural productivity is significantly affected by nitrogen and phosphorus deficiencies, which are essential for plant growth. In addition, it is related to the physical and biological

properties of the soil, pest and disease attacks and abiotic stresses. For sustainable agriculture, it would be interesting to carry out an efficient management of nitrogen in the environment. This usually involves the use of microorganisms biologically fixing nitrogen that is used directly by the plant and is, therefore, less susceptible to volatilization, denitrification and leaching. In agricultural settings, perhaps 80% of this biologically fixed N comes from symbiosis involving leguminous plants and one of the *Rhizobia* species [1]. Legumes are able to establish a symbiotic *interaction* with soil bacteria termed *Rhizobia*. *These bacteria in association with legumes can fix atmospheric N* and through this feature, they are introduced into agricultural systems to improve soil fertility, plant growth and limit the use of chemical fertilizers [2]. However, the anticipated benefits of the nitrogen fixing bacteria may be positive or negative depending on rhizobium species and its interaction with the environment [3]. Isolation and selection of rhizobia stress-tolerant strains may enhance the plant growth through nodulation and nitrogen fixation ability of plants under stress conditions [4]. Selection of effective *Rhizobium* strains is the most critical aspect to have maximum benefits from this technology [5]. Regardless of their functions in direct plant growth promotion, rhizobia can act by protecting

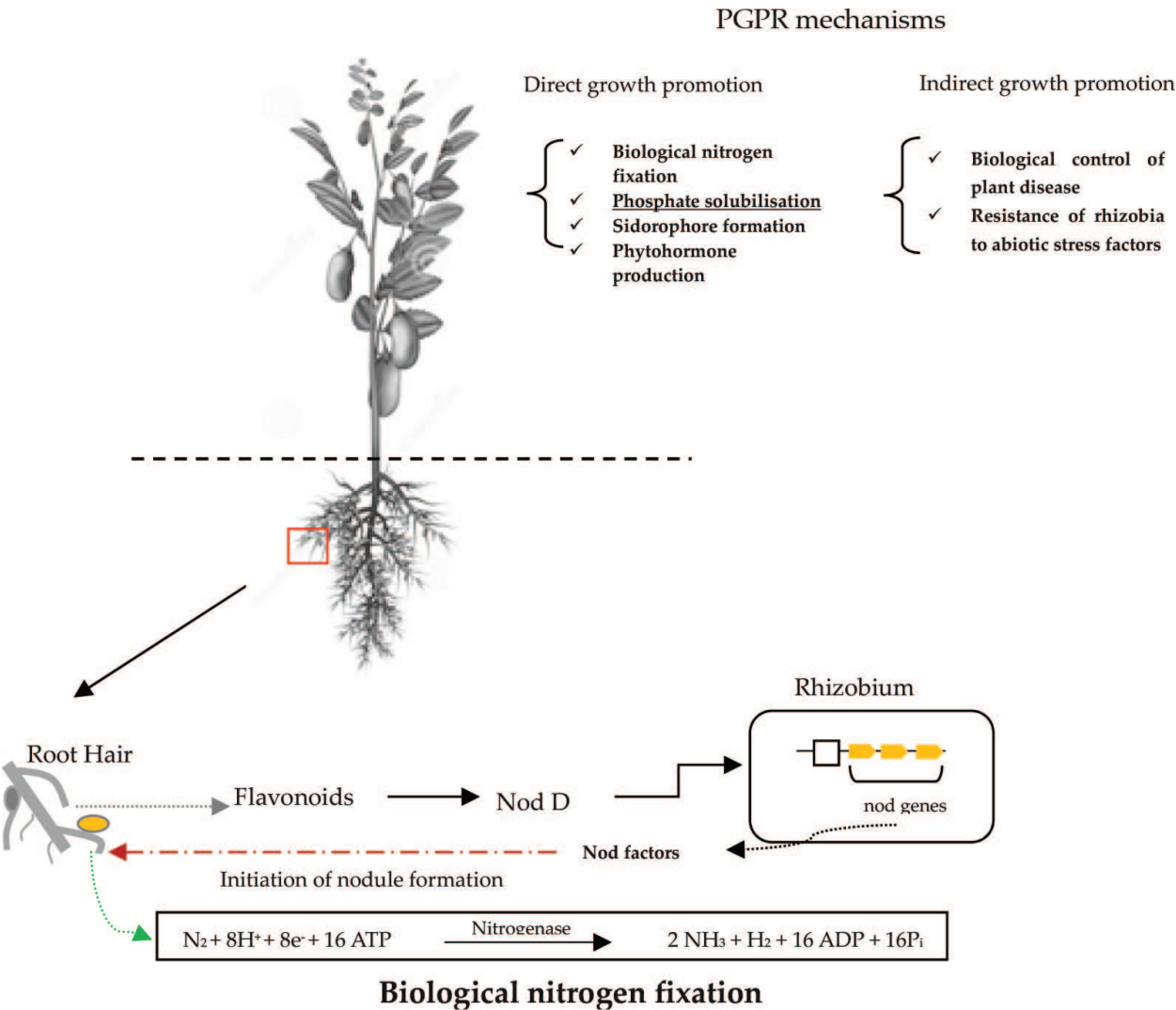


Figure 1. Schematic overview of the nodulation process and plant growth promotions by rhizobia.

host plant against pests and diseases. Different mechanisms can be involved in plant protection by rhizobium inoculation such as competition for nutrients, antibiosis or induced resistance in host plant. This chapter presents an overview highlighting the enhancement of plant growth by rhizobia. Different mechanisms of plant growth promotion by rhizobia were described. Rhizobia can act directly by facilitating plant nutrients acquisition or influencing plant hormone levels or indirectly by attenuating the inhibitory effects of pathogens (**Figure 1**).

2. Plant growth promotion by rhizobia inoculation

Rhizobia can enhance plant growth promotions by both direct and indirect ways. Several mechanisms are involved in the plant growth promotions by rhizobia, different mechanisms involved are discussed as follows.

2.1. Direct plant growth promotions

2.1.1. Biological nitrogen fixation

Nitrogen is a vital element for plant growth; it is required for synthesis of macromolecules such as amino acids, nucleic acids and chlorophyll. In agriculture, fertilization with nitrogen products is practiced to increase the production yield of food [6, 7]. About 78% of the atmospheric air is N, this gaseous substance cannot be used in this form by most living organisms until it has been fixed, that is, reduced (combined with hydrogen) to ammonia. Biological nitrogen fixation (BNF) accounts for about 60% of nitrogen used in agriculture. Significant growth in fertilizer-N usage has occurred in both developed and developing countries [8]. The requirements for fertilizer-N are predicted to increase further in the future [9]; however, the use of high doses of fertilizers has a negative and unpredictable impact on the environment and contaminates the soil, water and natural areas. These effects are considered a threat to human and animal health affecting the quality of life. In addition, developing countries must use cheaper and environmentally friendly alternative methods. Legumes are BNF capable and meet their own needs. The use of legume crops substantially reduces the N requirement from external sources [10]. For more than 100 years, BNF has commanded the attention of scientists concerned with plant mineral nutrition, and it has been exploited extensively in agricultural practice [11]. However, its efficiency varies, and depends on the host genotype, rhizobial efficiency, soil conditions and climatic factors [8]. Currently, the use of microorganisms capable of fixing atmospheric nitrogen is of great practical importance because it makes it possible to bridge the limits of chemical fertilization, which has resulted in unacceptable levels of water pollution [12]. In addition to pollution problems, especially in the water supply, the application of chemical fertilizers is carried out in excess, which becomes very expensive for farmers, whereas the biological fixation of nitrogen through microorganisms can be adapted to the needs of the plant [12]. In legume-*Rhizobium* symbiosis, rhizobia induce nodules formation on the roots of leguminous plants. In this process, N₂ which is chemically inert and makes up approximately 80% of the volume present in the Earth's atmosphere is reduced to ammonia by the bacterial enzyme nitrogenase. The nitrogenase enzymes are irreversibly damaged by exposure to atmospheric levels of oxygen. To protect the nitrogenase from the negative effects

of oxygen, the plants provide a microaerobic environment to ensure the proper functioning of the nitrogenase. In addition, plants exude carbohydrates to support the metabolism of bacterial endosymbionts. In return, bacteria through symbiotic fixation of atmospheric nitrogen provide forms of nitrogen used by the plant for the synthesis of organic nitrogen compounds to meet its nutritional needs. Most of the N added naturally to soils is from biological fixation, that is, symbiotic or nonsymbiotic in nature. BNF is an efficient source of nitrogen [8]. It has been estimated that about 100 Tg N, valued at \$US 40 billion, is required annually for the production of the world's grain and oilseed crops [13], 20% comes from biological nitrogen fixation, and 26% from soil sources could also have originated from pasture or crop legume residues. The other sources are mainly from lightning discharges, burning of fossil fuels and forest and from the emission of magmatic gases. This N is added to soils as nitrate and ammonium in precipitation. If N-fertilizer derived from fossil fuels rises in price, the enhancement of BNF in agriculture will become more important. It has been reported that throughout the world, several areas of land have been degraded, and there is a need for reflection to develop new methods to stop land destruction and to institute a serious reversal of land degradation. Among the alternatives, the BNF can be used in land remediation. Legumes are well known for their ability to fertilize soils through their symbiotic relationship with specific nitrogen-fixing bacteria known as rhizobia, a name that portrays root and stem nodulating bacteria. There are approximately 700 genera and about 13,000 species of legumes, only a portion of which shown to have the ability to fix atmospheric nitrogen [12]. Soil fertilization is carried out in part by the BNF, each year, half of the amount of nitrogen fixed by microorganisms is provided by a 100 legumes in association with rhizobial strains [14]. Legumes are very important both nutritionally and agriculturally because they are very rich in protein, and are responsible for soil fertilization through symbiotic nitrogen fixation in association with rhizobia. The annual N-value of legume symbioses is about 70 million tons [15]. The accumulation of proteins in plants and the enrichment of soil in N result from the fixation of atmospheric nitrogen. Growing plants on soils that are low in mineral nitrogen often limits the growth of these plants, so the yields are affected. The need for nitrogen has meant that symbiotic relationships are evolving between plants and a variety of nitrogen-fixing organisms [16]. Nitrogen input to soils by the BNF is considered a renewable source of N for agriculture [8]. The quantities of N supplied per year and per hectare vary from 200 to 300 kg, such impressive quantities are sufficient to ensure a good yield [8, 17].

2.1.2. Phosphate solubilization

Phosphorus (P) is the most limiting element for plant growth after nitrogen. There are several forms that are inorganic (bound, fixed or labile) and organic (bound), and the concentration depends on the source. The concentration ranges from 140 ppm in carbonate rocks to over 1000 ppm in volcanic materials [18]. The majority of P applied as fertilizer enters into the immobile pools through precipitation reaction with highly reactive Al^{3+} and Fe^{3+} in acidic soils, and with Ca^{2+} in calcareous soils [19, 20]. The availability of phosphorus for plants is influenced by several conditions such as soil pH, aeration, temperature, texture and organic matter, extent of root systems of plants and secretions of root exudates and microbes. Soil microorganisms play a key role in soil P dynamics and subsequent availability of P to plants [10]. Although chemical fertilizer supplies plants with P requirements, excessive application of

P fertilizers is costly for the farmer and harmful to the environment. The content of phosphorus in plants varies from 0.2 to 0.8% dry weight, but only 0.1% of this phosphorus is available to plants [21]. The main source of P for the plant remains in the soil solution. The P content values of agricultural soil solutions are generally very low and remain unsuitable for the needs of the host plant. With the ability to solubilize phosphate, the microbial system can compensate for the amounts of P required for growth of the host plant [22]. Several rhizobia species may solubilize phosphorus, including *R. leguminosarum*, *R. meliloti*, *M. mediterraneum*, *Bradyrhizobium* sp. and *B. japonicum* [23]. These bacteria solubilize phosphorus by the production of low molecular weight organic acids that act on inorganic phosphorus. A large number of strains of *Rhizobium* were able to solubilize phosphorus in liquid culture [24]. The importance of this ability to solubilize phosphorus in plant growth by some rhizobia has been demonstrated in chickpeas and barley [25].

2.1.3. Siderophore formation

Iron is considered an essential micronutrient of plants and is present in the soil with a significantly different distribution. Iron can be present in different forms, either in divalent (ferrous or Fe^{2+}) or trivalent (ferric or Fe^{3+}) states. Soil pH and Eh (redox potential) and the availability of other minerals determine the state of iron in the soil [26]. In aerobic environments, iron exists as insoluble hydroxides and oxyhydroxides, which are not available to plants and microbes [27]. In general, bacteria have the ability to synthesize siderophores, low molecular weight compounds capable of sequestering Fe^{3+} . These siderophores have a high affinity for Fe^{3+} , making iron available to plants. Siderophores are soluble in water and exist in extracellular and intracellular environments. Fe^{3+} ions are reduced to Fe^{2+} and released into cells by Gram-positive and -negative rhizobacteria. This reduction leads to the destruction/recycling of siderophores [27]. Siderophores can also form a stable complex with heavy metals such as Al, Cd, Cu, and so on and with radionuclides including U and NP [28]. Thus, plant inoculation by siderophore-producing bacteria protects them from stress caused by heavy metals and helps them absorb iron. Several rhizobial species nodulating various legumes are known for their production of siderophores [29].

2.1.4. Phytohormone production

Substances that stimulate plant growth at low concentrations, less than or equal to micromolar concentrations are called phytohormones. These molecules include indole-3-acetic acid (IAA) (auxin), cytokinins, gibberellins and abscisic acid.

Indole-3-acetic acid (IAA): IAA is the most advanced phytohormone that enhances root growth resulting in accelerated growth and plant development. IAA is involved in cell division, differentiation and vascular beam formation and plays a key role in nodule formation. Several of the isolated rhizosphere bacteria have been shown to produce IAA. IAA production in rhizobia is via indole-3-pyruvic acid and the indole-3-aldehyde acetic pathway. Inoculation of vetch roots with certain strains of *R. leguminosarum* bv. *viciae* shows a 60-fold increase in IAA in nodules [30]. One of the highest productions of IAA has been described by Mishra et al. [31] with the inoculation of *B. japonicum*-SB1 with *B. thuringiensis*-KR1. Co-inoculation of *Pseudomonas* with *R. galegae* bv. *orientalis* has shown that it produces AIA that has contributed to

increased nodule numbers, root and root growth and nitrogen content. Environmental (acidic pH, osmotic and matrix stress and carbon limitation) and genetic stressors (auxin biosynthetic genes and expression mode) influence the biosynthesis of AIA [32].

Cytokinins: Cytokinin stimulates plant cell division and in some cases, root development and the formation of absorbent hairs [33]. Most rhizospheric microorganisms have been reported to release cytokinins [34]. *Rhizobium* strains are also capable of producing cytokinins [35].

Gibberellins: Gibberellins are considered as plant hormones ensuring the lengthening of the stems and the expansion of the leaves. Some types of dwarfism have been attributed to gibberellin deficiency, but this has no effect on the roots. Many plant growth promoting bacteria are reported to produce gibberellins [36], including *Rhizobium* and *S. meliloti* [37].

Absciscic acid: Several constraints such as low temperatures and lack of water increase the production of abscisic acid. Biosynthesis is regulated indirectly by the production of carotenoids. Unlike auxin, the movement of abscisic acid in plants has no polarity and the transport of abscisic acid can occur in both phloem and xylem tissues [38]. It has been reported that abscisic acid stimulates stomatal closure, inhibits shoot growth without affecting or even promoting root growth, inducing seeds to store proteins and dormant, inducing gene transcription of proteinase inhibitors, and thereby, providing a defense against pathogens and gibberellins [39]. *Rhizobium* sp. and *B. japonicum* produced abscisic acid [36, 37].

2.2. Indirect plant growth promotions

2.2.1. Biological control of plant disease

In addition to their plant growth promoting effects, *Rhizobium* spp. have been increasingly associated with disease suppressive effects in the recent literature [40, 41]. Improvements in plant health are mediated by two different ecological mechanisms: (1) antagonism of pest and pathogens and (2) stimulation of plant host defenses.

2.2.2. Antagonistic effects of rhizobia to pathogens and pest

Antagonism of pest and pathogen populations by *Rhizobium* spp. takes several forms wherein species are pathogens of fungi, bacteria, nematodes and/or parasitic plants. There is evidence that a strain of *Bradyrhizobium japonicum* can cause up to a 75% decrease in sporulation of *Phytophthora megasperma*, 65% in *Pythium ultimum*, 47% in *Fusarium oxysporum* and 35% in *Ascochyta imperfecta* [42]. These findings suggest that only one bacterial strain will control a population of a multitude of pathogenic strains, thus potentially providing bioprotection for the host plant. It is clear from these findings that rhizobia show great potential for use against plant diseases, and therefore, deserve more attention in future studies of cropping systems.

Several studies on the mode of action of *Rhizobium* spp. have shown that the growth inhibition of plant pathogens is ensured by the production of toxic compounds. Early work has allowed the characterization of antimicrobial activities related to extracellular compounds of *Rhizobium* spp. such as trifolitoxin [43] indicating that antibiosis may be part of their reported biocontrol efficacy. Mabrouk et al. [44, 45] have recently demonstrated that the beneficial effect on growth and N-fixation efficiency in pea is evident for some *Rhizobium* isolates.

In addition to pea nodulation, inoculation with rhizobia significantly protect pea against parasitic plant (*O. crenata*) infection. Induced resistance in inoculated peas was characterized by reduction in seed germination of broomrape, radicle growth, parasite attachment to pea roots and finally tuber growth blockage on host roots. These observations have been attributed to the lignification and accumulation of toxic substances in pea roots following inoculation by rhizobial strains [44, 45].

2.2.3. Induction of plant defense by rhizobia against pests and diseases

Rhizobium populations may also promote plant health by stimulating the plant host. The presence of *Rhizobium* spp. would in this case indirectly stimulate the plant to activate its defense mechanisms when challenged with a pathogen through the production of plant defense compounds (phenolics, flavonoids or other phytoalexins, in particular). Induced resistance against *Orobanch*e in peas inoculated with some rhizobial strains was found to be associated with significant changes in levels of the defense enzymes such as peroxidase, polyphenoloxidase and oxidative lipooxygenase (Lox), and in the accumulation toxins derived, including phenolic acids and pisatin and pea phytoalexin. These modifications were attributed to the activation of defense genes following inoculation of pea plants with rhizobia [44–45, 47, 48]. The work of Arfaoui et al. [50] identified some isolates of *Rhizobium* spp. activating the defense in chickpeas against *Fusarium oxysporum* f. sp. in reducing the severity of the disease developed in the host plant. They showed that inoculation of chickpea plants with *Rhizobium* strains, a few days before the attack by *Fusarium oxysporum* f.sp. ciceris, allows the reduction of the incidence of wilting resulting from the significant increase in the activities of several defense-related enzymes such as peroxidases and polyphenoloxidases, resulting in the accumulation of phenolic compounds and the expression of genes related to phenylpropanoid defense [51, 52]. Induced resistance by the bacteria of the rhizosphere has been described against several pathogens such as viruses, bacteria and fungi in several species of plants. However, induction mechanisms and metabolites involved in the induction of plant defense are highly variable depending on the bacterial strain and pathosystems. Several studies have shown that salicylic acid produced by bacteria can induce resistance in many plant species. Several studies have shown that lipopolysaccharides (LPS) of rhizobia are involved in triggering induced systemic resistance (ISR). Some authors have shown that the elicitation/triggering of ISR in potato against the *Globodera pallida* cyst nematode results from LPS of *R. etli* [53, 54]. In pea, systemic resistance induced by *O. crenata* infection was triggered by heat-killed cells and purified LPS of *Rhizobium leguminosarum* [46, 48, 49].

2.2.4. Resistance of rhizobia to abiotic stress factors

In the *Rhizobium*-legume symbiosis, which is a N₂-fixing system, the physiological state of the host plant is a determining factor in the process of atmospheric nitrogen fixation. Therefore, limiting agents do not allow the tolerant and competitive rhizobium strains to express its full nitrogen-binding capacity, which affects the vigor of the host legume. In Tunisia, several factors may limit the symbiotic nitrogen fixation, particularly drought, especially since Tunisia is located in semiarid, arid and Saharan climatic zones where annual rainfall ranges from 100 to 300 mm [55]. Drought affected the crop yields of pulses in Tunisia, which led farmers to

abandon this crop in some areas. In addition to drought, legume crops are affected by salinity, soil pH, nutrient deficiency, mineral toxicity, extreme temperatures, diseases and pests [44].

2.2.5. Soil salinity

Salinity is considered a limiting factor in nodulation and nitrogen fixation in legume-Rhizobium associations, which can adversely affect the yield of legume crops [56]. Rhizobia can tolerate high concentrations unlike legume plants. The growth of certain strains is inhibited by 100 mM NaCl [57, 58], whereas other strains such as *R. meliloti* and *R. fredii* support saline concentrations greater than 300 mM [59, 60]. Therefore, in saline soils, the multiplication of these strains will not be affected in the rhizosphere of the plant host. The accumulation of K ions with several ranges of low molecular weight organic solutes is involved in the osmoadaptation of most microorganisms, in order to balance the osmotic pressure of the growth medium and to maintain the turgor pressure and allowing the cell extension [61].

2.2.6. Water deficiency and drought

Water deficiency is a major limiting factor of symbiotic nitrogen fixation in many arid regions of the Mediterranean basin. One of the immediate responses of rhizobia to water deficiency concerns the morphological changes [62, 63]. Water stress allows the reduction of legume root infection by rhizobia, hence the reduction of nodulation. In addition, the water deficit also restricts the development and function of nodules [59, 64]. The development of effective nodules in desert soils highlights that some strains can tolerate extreme conditions in soils with limited moisture levels [65, 66, 67].

2.2.7. High temperature and heat stress

In temperate regions, the free life and symbiotic life of rhizobia is affected [68]. The optimal temperature range for growth of rhizobial strains varies from 28 to 31°C. Some rhizobial strains cannot grow at 38°C, while others that survive heat stress can lose their nodulation power due to alteration of compounds involved in the infective process such as plasmid hardening or alterations of cellular polysaccharides [68]. Nodules formed at high soil temperature (35–40°C) are usually ineffective formation; however, some strains of rhizobia, such as *R. leguminosarum* *bv.* *phaseoli*, were heat-tolerant and formed effective symbioses with their host plants [69, 70]. These associations will be of great interest for cultivation in arid climates.

2.2.8. Acid soils and soil acidification

Acid soils constrain agricultural production in worldwide [71], with the scope of the problem likely to increase as the result of acid rain, long-term N fertilization and legume N₂ fixation. Legumes are particularly affected, acidity limiting both survival and persistence of nodule bacteria in soil, and the process of nodulation itself [72]. The absence of nodules has been noted in legumes grown in acidic soils, particularly in soils with a pH below 5. The susceptibility of certain rhizobial strains to these conditions is a cause of inhibition of nodule formation [73–75]. Nodules are absent even when a viable population of Rhizobium can be demonstrated [76, 77]. Some researchers have observed that nodulation of *P. sativum* was 10 times more sensitive to acidity than rhizobial multiplication or plant growth [78]. Recent reports indicated

that by selection of acid-soil tolerance in both symbiotic partners [79, 80], annual medics such as *Medicago murex* can be grown symbiotically on soils as acidic as pH 4.3 [81]. Meanwhile, the genetic control of acid tolerance in *Sinorhizobium* is becoming increasingly understood [82]. The establishment of legume symbioses requires the interaction of specific recognition signal molecules produced by both bacterial and plant partners [83]. It has been shown that pH affects the exchange or recognition of these signal molecules by both plant and bacterial partners in both the medic symbiosis [84] and the clover symbiosis [84, 85].

3. Conclusions

Rhizobia produce multiple beneficial effects on plant growth stimulation, host defense against disease and survival under stress with many other unknown benefits. This chapter describes the potential of rhizobia for the promotion of plant growth and highlights the different mechanisms of growth stimulation and the spectrum of resistance available against various abiotic stresses in several crops. In sustainable agriculture, the biological fixation of nitrogen is an important process, particularly in the legume farming system. To benefit from leguminous crops, it would be interesting to select symbiotic pairs adapted to severe conditions and to fix considerable quantities of nitrogen. The importance of the *Rhizobium*-legume interaction is not limited to their symbiotic nitrogen fixation activity or several other activities in the soil, possibly improving soil fertility and plant growth, but some strains of rhizobia can be used to protect plants against attack by pests and pathogens. However, further studies on the precise mode of action and adaptation to the different ecophysiological conditions of these microorganisms may help to maximize the benefits of rhizobia for improving plant growth and health.

Conflict of interest

Authors confirm there are no conflicts of interest.

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References

- [1] Vance CP. Legume symbiotic nitrogen fixation: Agronomic aspects. In: Spaink HP et al., editors. *The Rhizobiaceae*. Dordrecht: Kluwer Academic Publishers; 1998. pp. 509-530
- [2] Ouma EW, Asango AM, Maingi J, Njeru EM. Elucidating the potential of native rhizobial isolates to improve biological nitrogen fixation and growth of common bean and soybean in smallholder farming systems of Kenya. *International Journal of Agronomy*. 2016;1-7
- [3] Sessitsch A, Howieson JG, Perret X, Antoun H, Martínez-Romero E. Advances in *Rhizobium* research. *Critical Reviews in Plant Science*. 2002;**21**:323-378
- [4] Zou N, Dart PJ, Marcar N. Interaction of salinity and rhizobial strain on growth and N₂ fixation by *Acacia Ampliceps*. *Soil Biology and Biochemistry*. 1995;**27**:409-413
- [5] Biswas S, Das RH, Sharma GL. Isolation and characterization of a novel cross-infective rhizobial from *Sesbania aculeata* (Dhaincha). *Current Microbiology*. 2008;**56**:48-54
- [6] Xu G, Fan X, Miller AJ. Plant nitrogen assimilation and use efficiency. *Annual Review Plant Biology*. 2012;**63**:153-182
- [7] Avila-Ospina L, Moison M, Yoshimoto K, Masclaux-Daubresse C. Autophagy, plant senescence, and nutrient recycling. *Journal of Experimental Botany*. 2014;**65**(14):3799-3811. DOI: 10.1093/jxb/eru039
- [8] Peoples MB, Herridge DF, Ladha JK. Biological nitrogen fixation: An efficient source of nitrogen for sustainable agricultural production. *Plant and Soil*. 1995;**174**:3-28
- [9] Subba-Rao NS. Crop responses to microbial inoculation. In: Subba Rao NS, editor. *Recent Advances in Nitrogen Fixation*. London, United Kingdom: Edward Arnold; 1980. pp. 406-420
- [10] Bhattacharyya PN, Jha DK. Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. *World Journal of Microbiology and Biotechnology*. 2012;**28**:1327-1350
- [11] Krasova-Wade T, Diouf O, Ndoye I, Elimane Sall C, Braconnier S, Neyra M. Water condition effects on rhizobia competition for cowpea nodule occupancy. *African Journal of Biotechnology*. 2006;**5**(16):1457-1463
- [12] Sprent JL, Sprent P. *Nitrogen Fixing Organisms. Pure and Applied Aspects*. London, United Kingdom: Chapman & Hall; 1990
- [13] David H, Ian R. Breeding for enhanced nitrogen fixation in crop legumes. *Field Crops Research*. 2000;**65**:229-248
- [14] Tate RL. *Soil Microbiology (Symbiotic Nitrogen Fixation)*. New York: John Wiley & Sons, Inc.; 1995
- [15] Brockwell J, Bottomley PJ, Thies JE. Manipulation of rhizobia microflora for improving legume productivity and soil fertility: A critical assessment. *Plant and Soil*. 1995;**174**:143-180

- [16] Freiberg C, Fellay R, Bairoch A, Broughton WJ, Rosenthal A, Perret X. Molecular basis of symbiosis between *Rhizobium* and legumes. *Nature*. 1997;**387**:394-401
- [17] Wani SP, Rupela OP, Lee KK. Sustainable agriculture in the semi-arid tropics through biological nitrogen fixation in grain legumes. *Plant and Soil*. 1995;**174**:29-49
- [18] Gray EJ, Smith DL. Intracellular and extracellular PGPR: Commonalities and distinctions in the plant–bacterium signaling processes. *Soil Biology and Biochemistry*. 2005;**37**:395-412
- [19] Gyaneshwar P, Kumar GN, Parekh LJ, Poole PS. Role of soil microorganisms in improving P nutrition of plants. *Plant and Soil*. 2002;**245**:83-93
- [20] Hao X, Cho CM, Racz GJ, Chang C. Chemical retardation of phosphate diffusion in an acid soil as affected by liming. *Nutrient cycling in. Agro-Ecosystems*. 2002;**64**:213-224
- [21] Zhou K, Binkley D, Doxtader KG. A new method for estimating gross phosphorus mineralization and immobilization rates in soils. *Plant and Soil*. 1992;**147**:243-250
- [22] Sharma P, Padh H, Shrivastava N. Hairy root cultures: A suitable biological system for studying secondary metabolic pathways in plants. *Engineering in Life Sciences*. 2013;**13**: 62-75
- [23] Marra LM, de OliveiraII SM, Soares CRFS, de Souza Moreira FM, Solubilisation of inorganic phosphates by inoculant strains from tropical legumes. *Scientia Agricola* 2011; **68** (5):603–609
- [24] Halder AK, PRKR C. Solubilization of inorganic phosphate by rhizobium. *Folia Microbiologica*. 1993;**38**(4):325-330
- [25] Hemissi I, Abdi N, Bargaz A, Bouraoui M, Mabrouk Y, Saidi M, Sifi B. Inoculation with phosphate solubilizing *Mesorhizobium* strains improves the performance of chickpea (*Cicer aritenium* L.) under phosphorus deficiency. *Journal of Plant Nutrition*. 2015;**38**(11): 1656-1671
- [26] Bodek I, Lyman WJ, Reehl WF, Rosenblatt DH. Environmental inorganic chemistry: Properties, processes, and estimation methods. In: Walton BT, Conway RA, editors. SETAC Special Publication Series. New York: Pergamon Press; 1988
- [27] Rajkumar M, Ae N, Prasad MNV, Freitas H. Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. *Trends in Biotechnology*. 2010;**28**:142-149
- [28] Neubauer U, Furrer G, Kayser A, Schulin R. Siderophores, NTA, and citrate: Potential soil amendments to enhance heavy metal mobility in phytoremediation. *International Journal of Phytoremediation*. 2000;**2**:353-368
- [29] Chabot R, Antoun H, Cescas MC. Growth promotion of maize and lettuce by phosphate solubilizing *Rhizobium leguminosarum* bv. *phaseoli*. *Plant and Soil*. 1996;**184**:311-321
- [30] Camerini S, Senatore B, Lonardo E, Imperlini E, Bianco C, Moschetti G, Rotino GL, Campion B, Defez R. Introduction of a novel pathway for IAA biosynthesis to rhizobia alters vetch root nodule development. *Archives of Microbiology*. 2008;**190**:67-77

- [31] Mishra PK, Mishra S, Selvakumar G, Bisht JK, Kundu S, Gupta HS. Co-inoculation of *Bacillus thuringiensis* -KR1 with *Rhizobium leguminosarum* enhances plant growth and nodulation of pea (*Pisum sativum* L.) and lentil (*Lens culinaris* L.). *World Journal of Microbiology and Biotechnology*. 2009;**25**:753-761
- [32] Spaepen S, Vanderleyden J. Auxin and plant-microbe interactions. *Cold Spring Harbor Perspectives in Biology*. 2011;**3** a001438
- [33] Frankenberger WTJ, Arshad M. *Photohormones in Soil: Microbial Production and Function*. New York: Dekker; 1995
- [34] Nieto KF, Frankenberger WT Jr. Influence of adenine, isopentyl alcohol and *Azotobacter chroococcum* on the vegetative growth of *Zea mays*. *Plant and Soil*. 1991;**135**:213-221
- [35] Senthilkumar M, Madhaiyan M, Sundaram SP, Kannaiyan S. Intercellular colonization and growth promoting effects of *Methylobacterium* sp. with plant-growth regulators on rice (*Oryza sativa* L. CvCO-43). *Microbiological Research*. 2009;**164**:92-104
- [36] Dobbelaere S, Vanderleyden J, Okon Y. Plant growth promoting effects of diazotrophs in the rhizosphere. *Critical Reviews in Plant Sciences*. 2003;**22**:107-149
- [37] Boiero L, Perrig D, Masciarelli O, Penna C, Cassan F, Luna V. Phytohormone production by three strains of *Bradyrhizobium japonicum* and possible physiological and technological implications. *Applied Microbiology and Biotechnology*. 2007;**74**:874-880
- [38] Walton DC, Li Y. Plant hormones: Physiology, biochemistry and molecular biology. In: *Abscisic Acid Biosynthesis and Metabolism*. Dordrecht: Kluwer; 1995. pp. 140-157
- [39] Davies PJ. The plant hormones: Their nature, occurrence and functions. In: Davies PJ, editor. *Plant Hormones: Physiology, Biochemistry and Molecular Biology*. Dordrecht: Kluwer Academic Publishers; 1995. pp. 1-12
- [40] Huang HC, Erickson RS, Hsieh TF. Control of bacterial wilt of bean (*Curtobacterium flaccumfaciens* pv. *Flaccumfaciens*) by seed treatment with *Rhizobium leguminosarum*. *Crop Protection*. 2007;**26**:1055-1061
- [41] Siddiqui ZA, Baghel G, Akhtar MS. Biocontrol of *Meloidogyne Javanica* by rhizobium and plant growth-promoting rhizobacteria on lentil. *World Journal of Microbiology and Biotechnology*. 2007;**23**:435-441
- [42] Tu JC. Protection of soybean from severe *Phytophthora* root rot by *Rhizobium*. *Physiology and Plant Pathology*. 1978;**12**:233-240
- [43] Malajczuk N, Pearse M, Litchfield RT. Interactions between *Phytophthora cinnamoni* and *Rhizobium* isolates. *Transactions of the British Mycological Society*. 1984;**82**:491-500
- [44] Mabrouk Y, Zourgui L, Sifi B, Delavault P, Simier P, Belhadj O. Some compatible *Rhizobium leguminosarum* strains in peas decrease infections when parasitised by *Orobanche crenata*. *Weed Research*. 2007a;**47**:44-53
- [45] Mabrouk Y, Simier P, Arfaoui A, SIFI B, Delavault P, Zourgui L, Belhadj O. Induction of phenolic compounds in pea (*Pisum sativum* L.) inoculated by *Rhizobium leguminosarum* and infected with *Orobanche crenata*. *Journal of Phytopathology*. 2007b;**155**:728-734

- [46] Mabrouk Y, Zourgui L, Sifi B, Belhadj O. Induction du mécanisme de résistance systémique par des rhizobiums contre le parasitisme de l'*Orobanche crenata* sur le pois. *Revue de l'IRA medenine*. 2008;**3**:1234-1240
- [47] Mabrouk Y, Simier P, SIFI B, Delavault P, Delgrage S, Zourgui L, Belhadj O. Molecular and biochemical mechanisms of defense induced in pea by *Rhizobium leguminosarum* against broomrape (*Orobanche crenata*). *Weed Research*. 2007c;**47**:452-460
- [48] Mabrouk Y, Mejri S, Belhadj O. Biochemical mechanisms of induced resistance by rhizobial lipopolysaccharide in pea against crenate broomrape. *Brazilian Journal of Botany*. 2016a;**39**:107-114
- [49] Mabrouk Y, Hemissi I, Mejri S, Belhadj O. Biochemical analysis of induced resistance in chickpea against broomrape (*Orobanche foetida*) by rhizobia inoculation. *Phytopathologia Mediterranea*. 2016b;**55**(1):54-61
- [50] Arfaoui A, Sifi B, Boudabous A, El Hadrami I, Chérif M. Identification of *Rhizobium* isolates possessing antagonistic activity against *Fusarium oxysporum* f.Sp. *ciceris*, the causal agent of Fusarium wilt of chickpea. *Journal of Plant Pathology*. 2006;**88**:67-75
- [51] Arfaoui A, Sifi B, El Hassni M, El Hadrami I, Boudabous A, Chérif M. Biochemical analysis of chickpea protection against Fusarium wilt afforded by two *Rhizobium* isolates. *Plant Pathology J*. 2005;**4**:35-42
- [52] Arfaoui A, El Hadrami A, Mabrouk Y, Sifi B, Boudabous A, El Hadrami I, Daayf F, Chérif M. Treatment of chickpea with *Rhizobium* isolates enhances the expression of phenylpropanoid defense-related genes in response to infection by *Fusarium oxysporum* f. Sp. *ciceris*. *Plant Physiology and Biochemistry*. 2007;**45**:470-479
- [53] Reitz M, Rudolph K, Schröder I, Hoffmann-Hergarten S, Hallman J, Sikora RA. Lipopolysaccharides of *Rhizobium etli* strain G12 act in potato roots as an inducing agent of systemic resistance to infection by the cyst nematode *Globodera pallida*. *Applied and Environmental Microbiology*. 2000;**66**:3515-3518
- [54] Reitz M, Oger P, Meyer A, Niehaus K, Farrand SK, Hallmann J, Sikora RA. Importance of the O-antigen, core-region and lipid a of rhizobial lipopolysaccharides for the induction of systemic resistance in potato to *Globodera pallida*. *Nematology*. 2002;**4**:73-79
- [55] Ben Romdhane S, Trabelsi M, Aouani ME, de Lajudie P, Mhamdi R, The diversity of rhizobia nodulating chickpea (*Cicer arietinum*) under water deficiency as a source of more efficient inoculants. *Soil Biology and Biochemistry*. 2009; **41**: 2568–2572
- [56] Mohammad RM, Akavan-Karazian M, Campel WF, Rumpbaugh MD. Identification of salt and drought tolerant rhizobium meliloti strains. *Plant and Soil*. 1991;**134**:271-276
- [57] Singleton PW, Elswaify SA, Bohlool BB. Effect of salinity on rhizobium growth and survival. *Applied and Environmental microbiology*. 1982;**44**:884-890
- [58] Yelton MM, Yang SS, Edie SA, Lim ST. Characterzation of an effective salt tolerant fast growing strain of *Rhizobium japonicum*. *Journal of General Microbiology*. 1983;**129**:1537-1547

- [59] Sauvage D, Hamelin J, Larher F. Glycine betaine and other structurally related compounds improve the salt tolerance of *rhizobium meliloti*. *Plant Science Letters*. 1983;**31**: 291-302
- [60] Zhang X, Harper R, Karsisto M, Lindström K. Diversity of *Rhizobium* bacteria isolated from the root nodules of leguminous trees. *International Journal of Systematic Bacteriology*. 1991;**41**:104-113
- [61] Welsh DT. Ecological significance of compatible solute accumulation by microorganisms: From single cells to global climate. *FEMS Microbiology Reviews*. 2000;**24**:263-290
- [62] Shoushtari NH, Pepper IL. Mesquite rhizobia isolated from the Sonoran desert: Competitiveness and survival in soil. *Soil Biology and Biochemistry*. 1985;**17**:803-806
- [63] Busse MD, Bottomley PJ. Growth and nodulation responses of *Rhizobium meliloti* to water stress induced by permeating and nonpermeating solutes. *Applied and Environmental Microbiology*. 1989;**55**:2431-2436
- [64] Serraj R, Sinclair TR, Purcell LC. Symbiotic N₂ fixation response to drought. *Journal of Experimental Botany*. 1999;**50**:143-155
- [65] Jenkins MB, Virginia RA, Jarrel WM. Ecology of fast-growing and slow-growing mesquite-nodulating rhizobia in Chihuahua and Sonoran desert ecosystems. *Soil Science Society of America Journal*. 1989;**53**:543-549
- [66] Waldon HB, Jenkins MB, Virginia RA, Harding EE. Characteristics of woodland rhizobial populations from surface and deep-soil environment of the Sonoran Desert. *Applied and Environmental Microbiology*. 1989;**55**:3058-3064
- [67] Fuhrmann J, Davey CB, Wollum AG. Desiccation tolerance in clover rhizobia in sterile soils. *Soil Science Society of America Journal*. 1986;**50**:639-644
- [68] Zahran HH. *Rhizobium*-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. *Microbiology and Molecular Biology Reviews*. 1999;**63**:968-989
- [69] Hungria M, Franco AA. Effects of high temperature on nodulation and nitrogen fixation by *Phaseolus vulgaris* L. *Plant and Soil*. 1993;**149**:95-102
- [70] Michiels J, Verreth C, Vanderleyden J. Effects of temperature stress on bean-nodulating *Rhizobium* strains. *Applied and Environmental Microbiology*. 1994;**60**:1206-1212
- [71] Edwards DG, HAH S, MNM Y, Grundon NJ, Shamshuddin J, Norhayati M. The management of soil acidity for sustainable crop production. In: Wright RJ et al., editors. *Plant-Soil Interaction at Low pH*. Dordrecht: Kluwer Academic Publishers; 1991. pp. 383-396
- [72] Correa OS, Barneix AJ. Cellular mechanisms of pH tolerance in *Rhizobium loti*. *World Journal of Microbiology and Biotechnology*. 1997;**13**:153-157
- [73] HEA B, Biro B, Balazsy S, Kecskes M. Effects of some environmental factors on *Rhizobium* and *Bradyrhizobium* strains. *Acta Microbiologica et Immunologica Hungarica*. 1995;**42**:61-69

- [74] Carter JM, Gardner WK, Gibson AH. Improved growth and yield of faba beans (*Vicia faba* cv. Fiord) by inoculation with strains of *Rhizobium leguminosarum* biovar *viciae* in acid soils in south-west Victoria. *Australian Journal Agricultural Research*. 1994;**45**:613-623
- [75] Graham PH, Viteri SE, Mackie F, Vargas AT, Palacios A. Variation in acid soil tolerance among strains of *Rhizobium phaseoli*. *Field Crops Research*. 1982;**5**:121-128
- [76] Graham PH. Stress tolerance in *Rhizobium* and *Bradyrhizobium*, and nodulation under adverse soil conditions. *Canadian Journal of Microbiology*. 1992;**38**:475-484
- [77] Graham PH, Draeger K, Ferrey ML, Conroy MJ, Hammer BE, Martinez E, Naarons SR, Quinto C. Acid pH tolerance in strains of *Rhizobium* and *Bradyrhizobium*, and initial studies on the basis for acid tolerance of *Rhizobium tropici* UMR1899. *Canadian Journal of Microbiology*. 1994;**40**:198-207
- [78] Evans LS, Lewin KF, Vella FA. Effect on nutrient medium pH on symbiotic nitrogen fixation by *Rhizobium leguminosarum* and *Pisum sativum*. *Plant and Soil*. 1980;**56**:71-80
- [79] Howieson JG, Ewing MA. Acid tolerance in the *Rhizobium meliloti*–*Medicago* symbiosis. *Australian Journal of Agricultural Research*. 1986;**37**:55-64
- [80] Howieson JG, Ewing MA. Annual species of *Medicago* differ greatly in their ability to nodulate on acid soils. *Australian Journal of Agricultural Research*. 1989;**40**:843-850
- [81] Cheng E, Watkin G, O'Hara Howieson J. *Medicago sativa*, *Medicago murex* differ in the nodulation response to soil acidity. *Plant and Soil*. 2002;**238**:31-39
- [82] Dilworth MJ, Howieson JG, Reeve WG, Tiwari RP, Glenn AR. Acid tolerance in legume root nodule bacteria, selecting for it. *Australian Journal of Experimental Agriculture*. 2001;**41**:435-446
- [83] Denarie J, Debelle F, Prome JC. *Rhizobium* lipo-chitooligosaccharide nodulation factors, signaling molecules mediating recognition, morphogenesis. *Annual Review of Biochemistry*. 1996;**65**:503-535
- [84] Howieson JG, Robson AD, Abbot LK. Acid-tolerant species of *Medicago* produce root exudates at low pH which induce the expression of nodulation genes in *Rhizobium meliloti*. *Australian Journal of Plant Physiology*. 1992;**19**:287-296
- [85] Richardson AE, Djordjevic MA, Rolfe BG, Simpson RJ. Effects of pH, Ca, al on the exudation from clover seedlings of compounds that induce the expression of nodulation genes in *Rhizobium meliloti*. *Plant and Soil*. 1988;**109**:37-47

