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Chapter

The Plant-Rhizobial Symbiotic Interactions Provide Benefits to the Host beyond Nitrogen Fixation That Promote Plant Growth and Productivity

Jemaneh Z. Habtewold and Ravinder K. Goyal

Abstract

Rhizobial symbiotic interactions are known for nitrogen fixation, providing commercial crops and other plants with self-sufficiency in nitrogen requirements. An enormous contribution from nitrogen fixation is vital to the global nitrogen cycle. The symbiotic nitrogen reduces the carbon footprint of crop cultivation, which underlines its importance in agricultural sustainability. Extensive research efforts have been made to understand the symbiotic relationship at molecular, physiological, and ecological levels. This led to the isolation and modification of symbiotic strains for enhanced nitrogen efficiency. During the evaluation of strains for nitrogen fixation in exchange for supporting the bacterium in terms of space and resources, it has been observed that the accrued benefits to the host plants extend well beyond the nitrogen fixation. The symbiotic interaction has been advantageous to the host for better growth and development, tolerating a stressful environment, and even keeping the pathogenic microbial enemies at bay. Additionally, it enabled the availability of the mineral nutrients, which otherwise were inaccessible to the host. In this chapter, we bring together the information with a focus on the role of rhizobial symbiotic interactions that promote plant growth and productivity through phytohormone synthesis, by facilitating the availability of mineral nutrients, and by improving the plant tolerance to sub-optimal growth conditions.

Keywords: rhizobia, legumes, symbiosis, growth, plant hormones, environmental adaptability, stress tolerance, heavy metals

1. Introduction

Nitrogen (N) is an essential macro-nutrient that is needed for plant growth. Plants are unable to use N in its gaseous state, which is freely and abundantly available in the atmosphere. The atmospheric nitrogen (N_2) must first be converted chemically or naturally into NO_3^- or NH_4^+ before plants can assimilate it. Nature has given many microorganisms the ability to convert N_2 into a usable form. In microorganisms, the metabolic conversion of N_2 takes place either in their free-living state or through symbiosis with the host plant. Legumes have the unique ability to form symbiotic interactions with rhizobia, which helps meet the plant's N requirement. The symbiotic interaction is very complex, beginning with a chemical conversation between the rhizobia and host plant [1]. The host plant synthesizes specific flavonoids that are perceived through *NodD* genes expressed by a symbiont. Subsequently, through an array of events, a mutualistic relationship is established in which the bacterium imports carbon metabolites from the host in exchange for a usable form of N, or fixed N. Biological N fixation has been studied extensively to understand the evolutionary logistics, chemistry, regulation, and ecological perspectives [2]. The complexity of symbiotic mechanisms indicates the coevolution of a process that led to the development of N-fixing nodules [3]. The question of whether plants selected bacteria, whether bacteria influenced evolution, or whether legumes and rhizobia coevolved is being debated. Unlike mitochondrial and chloroplast evolution, the loss of genes from the rhizobia to the host nucleus, which would have compromised bacterial survival as a free-living organism, did not occur [4]. Nonetheless, the symbiotic arrangement has evolved over time into a secure partnership.

Symbiotically fixed N plays a key role in agricultural sustainability, especially when many of the agricultural practices, including N supplementation, that were instrumental in enhancing productivity and food security may not have been in tune with safeguarding the environment and preserving our ecosystems. Legumes are high in protein content and contribute roughly one-quarter of total grain production; their N self-sufficiency and increasing the N content of soil for subsequent crops have a significant impact on reducing the need for N-fertilizers [5]. Easing the pressure on chemical-N demand is of environmental significance because the majority of the N in N-fertilizers becomes a source of aquatic system pollution [6] or a substrate for nitrous oxide (N_2O) gas in the environment through denitrification. The enormous carbon footprint of fertilizer synthesis and subsequent N-pollution endangers the sustainability of agriculture and the environment. This has generated a significant interest in improving the efficiency of both symbiotic and biological N fixation in legumes and non-legumes, respectively. Many strategies have been employed to improve the symbiotic productivity notwithstanding the challenges of competitiveness of the native strains [7, 8].

Nodulation and N fixation may have been an important factor in the diversification of legumes, enabling them to be one of the largest families of widely distributed plant species [3]. The symbionts developed the capability to synthesize plant hormones that are critical to the growth and development of plants [9]. The biosynthetic pathways of many of the hormones are long and complex, requiring dedicated genetic and cellular machinery. Their involvement in the regulation of nodule development [10] underscores the interdependence of symbiotic partners. The symbiotic interactions are known to enhance plant growth and biomass and increase the availability of micro-elements like iron (Fe) and phosphorus (P) [11]. They enable the host plants to tolerate stressful growth conditions [12], which increases the plant survivability in adverse environmental regimes. It is likely that the enhanced environmental outreach has played a role in the diversification of symbiotic hosts. Although N fixation could be a primary reason for symbiotic evolution, it is not known if the additional beneficial activities were a contributing factor at the early stage. This chapter discusses the benefits of legume-rhizobia symbiosis in plant growth and development, in addition to N fixation, through the production of hormones and increased nutrient availability. The role of symbiosis in stress tolerance and plant defense against pathogens and how this function of symbionts can be improved through molecular tools are also highlighted.

2. Plant growth and development

Plant growth and development, which is greatly influenced by the plant's capacity to carry out photosynthetic activity in favorable and adverse regimes of nutrient availability and environmental conditions, determines the plant productivity. The symbiotic relationship with rhizobia is known to promote plant growth characteristics in legume crops (ref. in [13]). Although N supply is an important factor in plant growth, numerous studies have shown that rhizobia promote plant growth by producing plant hormones. These phytohormones have a profound effect on plant cellular processes and play a critical role in plant development [14]. The symbiotic interactions may act at molecular, physiological, and cellular levels to provide such benefits [15]. Several hormones, such as jasmonates, brassinosteroids, salicylic acid, nitric oxide, strigolactones, etc., that significantly influence plant signaling or other cellular responses are not discussed in this chapter as their impact on the symbiotic process or nodulation has previously been discussed [10]. The other phytohormones, which have been traditionally recognized for their role in dictating plant growth and development, are discussed. Their representative forms are depicted in **Figure 1**.

Auxins: These are a group of phytohormones that regulate processes like growth, seed germination, cell division, elongation and differentiation, root development, and stress response [16]. Plant-associated microbes, including *Rhizobium*, *Microbacterium*, *Mycobacterium*, *Bradyrhizobium* and *Sphingomonas* are among the active producers of indole-3-acetic acid (IAA), which is the major form of auxins in plants [17, 18]. Increased production of IAA by *Bradyrhizobium japonicum* was associated with enhanced soybean yield and survival [19]. A similar stimulatory effect was observed in a different host-rhizobial symbiosis. The IAA-overproducing *Ensifer meliloti* (formerly known as *Sinorhizobium meliloti*) strain was linked with the increased plant biomass and photosynthetic activity in *Medicago sativa*. There was higher nitrogenase activity, which possibly increased the demand for carbohydrates to balance the C/N ratio for an overall positive effect on plant growth [20]. The overproduction of IAA through genetic modification of a *Rhizobium leguminosarum* strain also affected nodule development and increased N fixation [21]. The positive effects of IAA-producing rhizobia on plant growth and productivity have been observed in non-legumes. The inoculation of rice (*Oryza sativa L.)* with Rhizobium bacteria led to about a 20% increase in the dry weight of rice along with enhancements in plant growth characteristics [22, 23]. Many of the symbiotic bacteria have developed genetic infrastructure to synthesize IAA, which clearly promotes the interests of the host plant. What could be the benefit of synthesizing IAA for the symbiotic bacteria other than that the host is healthy? Studies indicate that IAA is involved in nodule development [24, 25]. Nodulated roots are found to be enriched in IAA compared to non-nodulated roots [26]. The exact role of IAA in nodule formation is not known, but the hormone may act as a signal molecule that promotes host-bacterial interaction and attachment of the latter to the host roots [27, 28]. This is supported by an earlier observation that bacteria with a higher level of IAA are found closer to the root rhizosphere than the non-producers that are distantly distributed in the soil [29]. Trehalose (α-D-glucopyranosyl-1,1-α-D-glucopyranoside) synthesis and degradation are tightly

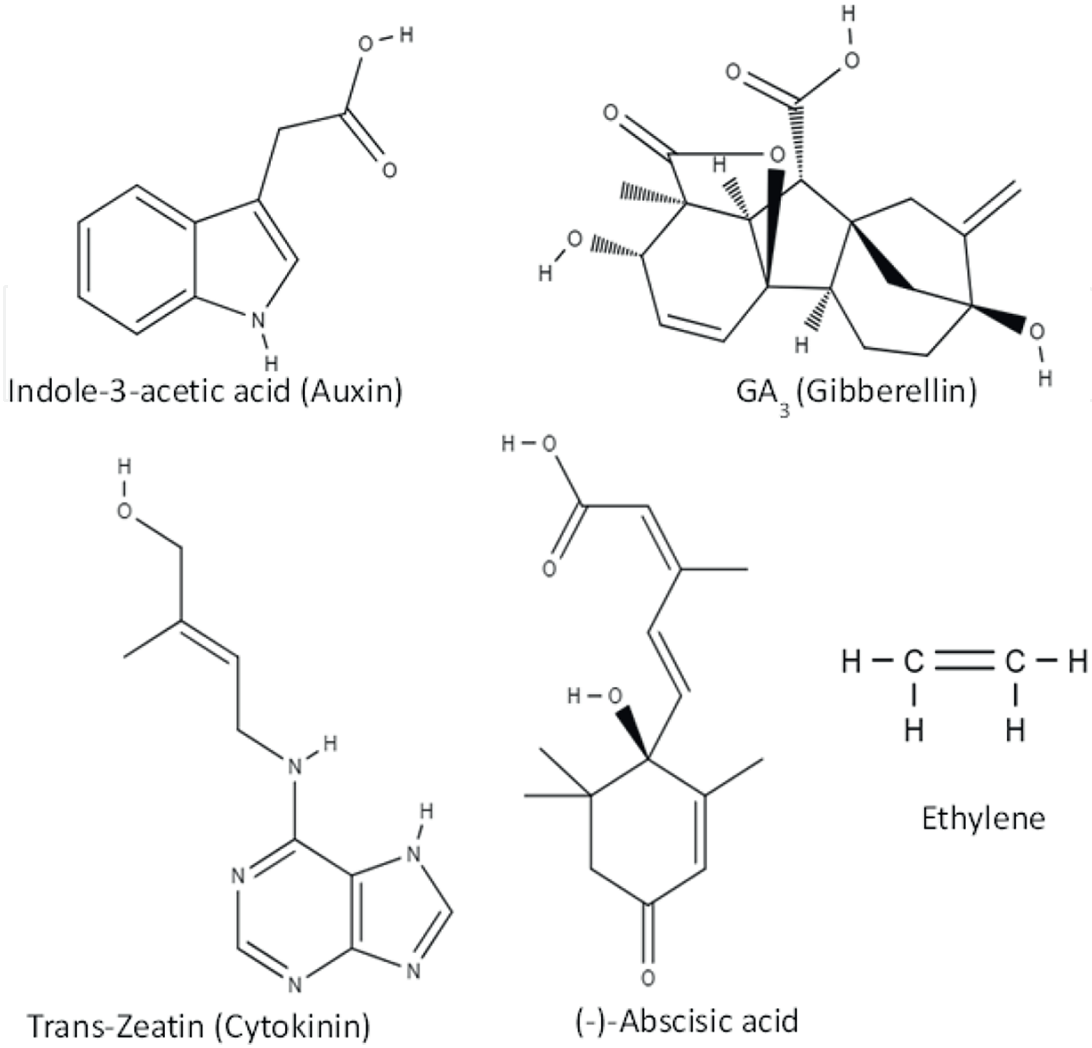

Figure 1.
The representative structure of some plant hormones. Source: PubChem.

regulated during nodule development, suggesting its involvement in nodule formation [30, 31]. The inoculation of soybean with IAA-producing Rhizobium upregulated the expression of the *otsA* gene along with an increase in nodule biomass [32]. The gene encodes trehalose-6-phosphate synthase, which synthesizes the precursor of trehalose. Similarly, the IAA-overproducing *RD64* strain of *E. meliloti* accumulated more trehalose in the nodules of *Medicago truncatula* [33]. The mutualistic benefit of IAA production by rhizobia is also observed through the regulation of N fixation genes by the hormone. There was an up-regulation of the N fixation apparatus by the IAA-overproducing endophytes [34]. A higher level of IAA in the bacteroids was associated with more abundance of transcripts of a N fixation gene, *nifH* [20].

Cytokinins (CKs): They are another group of plant hormones that regulate various aspects of plant growth and differentiation. They are implicated in cell division, seed germination, the formation of roots and shoots, the development of reproductive organs, apical dominance, senescence, stress tolerance, and more, covering almost all aspects of the plant life cycle. Earlier CKs were thought to be synthesized only in roots, but now it has been demonstrated that many plant parts synthesize the hormone. In legume roots, they are involved in nodule organogenesis. The exogenous application of CKs induced nodule-like structures and mutation in CK receptors, histidine kinase in *Lotus japonicus* hit1-1 (lhk1) and *M. truncatula* Cytokinin Response 1 (CRE1) inhibited the development of such structures (reviewed in ref. [10, 35]). The *M. truncatula* genome contains many ATP-binding cassette (ABCG) transporter proteins that enable the movement of hormones to an extracellular environment. One such transporter, MtABCG56, showed root and nodule specific expression, and the transcript level of the gene increased significantly after inoculation with *E. meliloti*, pointing to its involvement in the initial stages of legume-rhizobia symbiosis [36]. CK production was observed in *B. japonicum* [37] and secretion of their bioactive forms was demonstrated in *E. meliloti* [38]. Cytokinins are recognized as signal molecule for nodule development, but they seem to be involved in auto regulation of nodule number (AON). Plants sense the N availability in soil and control nodule formation through AON mechanism. Low N promotes nodulation, however when in abundance, the plant avoids energy intensive symbiotic N and reduce the number of nodules. A reduction in nodule number by a high concentration of CK treatment suggests a role of this hormone in AON [39], but such a possibility needs to be confirmed through further investigation. CKs are synthesized by rhizobia, but the exact role of rhizobial-derived cytokinins is unknown. It has been demonstrated that rhizobial synthesized cytokinins contribute to symbiotic interaction but were not critically required for the interaction as the *Bradyrhizobium sp. strain ORS285* mutant deficient in CK production was able to form nodules [40]. The role of CKs and other hormones in nodule development has been comprehensively reviewed [41]. Although hormones enhance plant growth in soybean and non-leguminous crops (ref. in [42]), it is not clearly established that rhizobial-derived cytokinins are transported to the plant tissue where they influence growth and differentiation.

Gibberellins (GAs): There is a large family of GAs that possess a diterpenoid structure. More than 125 GAs have been identified in plants and other organisms such as fungi and bacteria, but only a small portion of them is biologically active. GAs participates in the regulation of a wide range of plant developmental processes. The most notable are breaking the seed dormancy and enabling its germination; stem elongation; changing the vegetative phase of the plant to the reproductive stage; flower and fruit development; seed development; and senescence. The effect of GAs is mediated by their interaction with DELLA proteins. The hormone is involved in DELLA protein degradation. A certain level of these proteins is required to promote infection through an interaction with the Nod factor mediated pathway. Several mutant studies that interfered with GA biosynthesis, DELLA protein functionality, and the application of the hormone suggested that the GAs can have opposite effects on nodule development depending on the dose and mode of application [43]. In pea, a low concentration of GA application had a positive effect on nodule formation, while a higher amount inhibited the infection thread, which is one of the initial structural changes in the root to acquire the bacterium [44]. The identification of cytochromes P450 in *B. japonicum* indicated the presence of a GA biosynthetic capability in symbiotic organisms, though it was not found to be essential for a symbiotic function [45]. The protein catalyzes the steps of the pathways of many metabolites, including GAs. Subsequently, the genomic analysis of the bacterium identified diterpenoid operon which plays an important role in GA biosynthesis [46]. Later, many rhizobial species were reported to synthesize bioactive GAs (ref. in [47]). A cooperative action of both the symbiont and host led to GA biosynthesis in *Bradyrhizobium diazoefficiens*, where a precursor of a GA is converted to its bioactive form, GA9, by the host GA-3 oxidase [48]. It is speculated that rhizobial GA biosynthesis in cooperation with the host was an evolutionary event that influences nodulation in a mutually beneficial manner. Besides GAs effect on nodule formation, the exogenous application of GA3 in pea delayed the nodule senescence [49]. It is not known if the role of rhizobial-driven GAs is restricted to nodule development and senescence or if they influence other aspects of plant development.

Abscisic acid (ABA) and ethylene: Both of these hormones are central to plant defense mechanism against a variety of stresses. ABA regulates seed dormancy, senescence, stomatal opening and closing, and many other aspects of plant development. It is synthesized as a stress signal, particularly during abiotic stresses, and plays a critical role in downstream responses. Many transcription factors, including DREB2A and 2B, AREB1, RD22BP1 and MYC/ MYB, are known to regulate ABA-responsive gene expression through interactions with cis-acting elements on the DNA. The hormone alters the root architecture under water deficient conditions and modulates the ion influx to change root osmotic potential, providing drought tolerance to the plant [50]. Indirect evidence suggests that ABA might be involved in the host's drought tolerance. The inoculation of chickpea with *R. leguminosarum* led to an enhanced tolerance against drought, which was accompanied by higher synthesis of auxins and GAs with a lower level of ABA [51]. Similarly, in a symbiotic interaction of alfalfa with a IAA-overproducing Rhizobium strain, the host plant showed better adaptation to drought conditions that correlated with a higher level of IAA and downregulation of ABA biosynthesis genes [34]. ABA is known to affect nodule development. Exogenous ABA inhibited nodule formation, which appears to be mediated early in a symbiotic interaction [10]. In a report, GmbZIP1 has been shown to negatively regulate ABA-induced inhibition of nodulation by targeting GmENOD40-1 in soybean [52]. GmbZIP1 is a transcription factor that belongs to the AREB/ABF family, and GmENOD40-1 is a marker gene for nodule formation.

The role of ethylene in nodule formation has been reviewed previously [7, 53]. Ethylene is a negative regulator of nodulation, and interference in ethylene signaling led to stimulation of nodulation in alfalfa [54]. Further, several mutants and transgenic plants with altered ethylene levels and signaling showed variation in nodule size and number [53]. Ethylene is synthesized from its immediate substrate, 1-aminocyclopropane-1-carboxylic acid (ACC), through oxidation. The hormone level could be reduced by the degradation of ACC through a reaction catalyzed by acdS encoded ACC deaminase. A higher level of acdS expression in *B. japonicum* strains was linked with higher numbers of nodules, higher biomass, and delayed senescence of the nodules. The negative effect of ethylene was also demonstrated through over-expressing the ACC deaminase gene in pea and alfalfa (ref. in [7]).

3. Mineral acquisition

Most nutrients available in natural ecosystems are minimally bioavailable to plants since they are bound to inorganic molecules. Microorganisms like bacteria and fungi have the machinery necessary to depolymerize and mineralize the organic forms of these nutrients. With that, inorganic N, P, and S, including ionic species like ammonium, nitrate, phosphate, and sulfate, become available to the plant [55]. Besides the ability to fix N, soil microorganisms also provide essential nutrients by metabolizing recalcitrant forms of N, P and S. As reviewed in [56], these nutrients are needed in several metabolic processes of microorganisms including protein depolymerization and urea catabolism, phosphate and sulfate ester cleavage and phosphonate and sulfonate breakdown. Here, we discuss the role of rhizobial symbionts in P and Fe acquisition by legumes (**Figure 2**).

P availability: P is among the macronutrients essential for plant growth and development; legumes may require a greater amount of P to support N fixation in the nodules. This is likely related to the additional requirement for various metabolic processes, membrane phospholipid biosynthesis, and energy transformations in the nodules initiated by the symbiotic interaction [57]. P limitation can alter the distribution of nodule phosphatidylcholine [58], an essential component of membrane phospholipids [59]. It has also been associated with small nodules and reduced activity of Fe–S cluster enzymes [60], which are important in various regulatory systems for environmental signals [61]. The amount of plant-available P in the soil is usually low. Thus, owing to the high P requirements of legumes along with the low availability

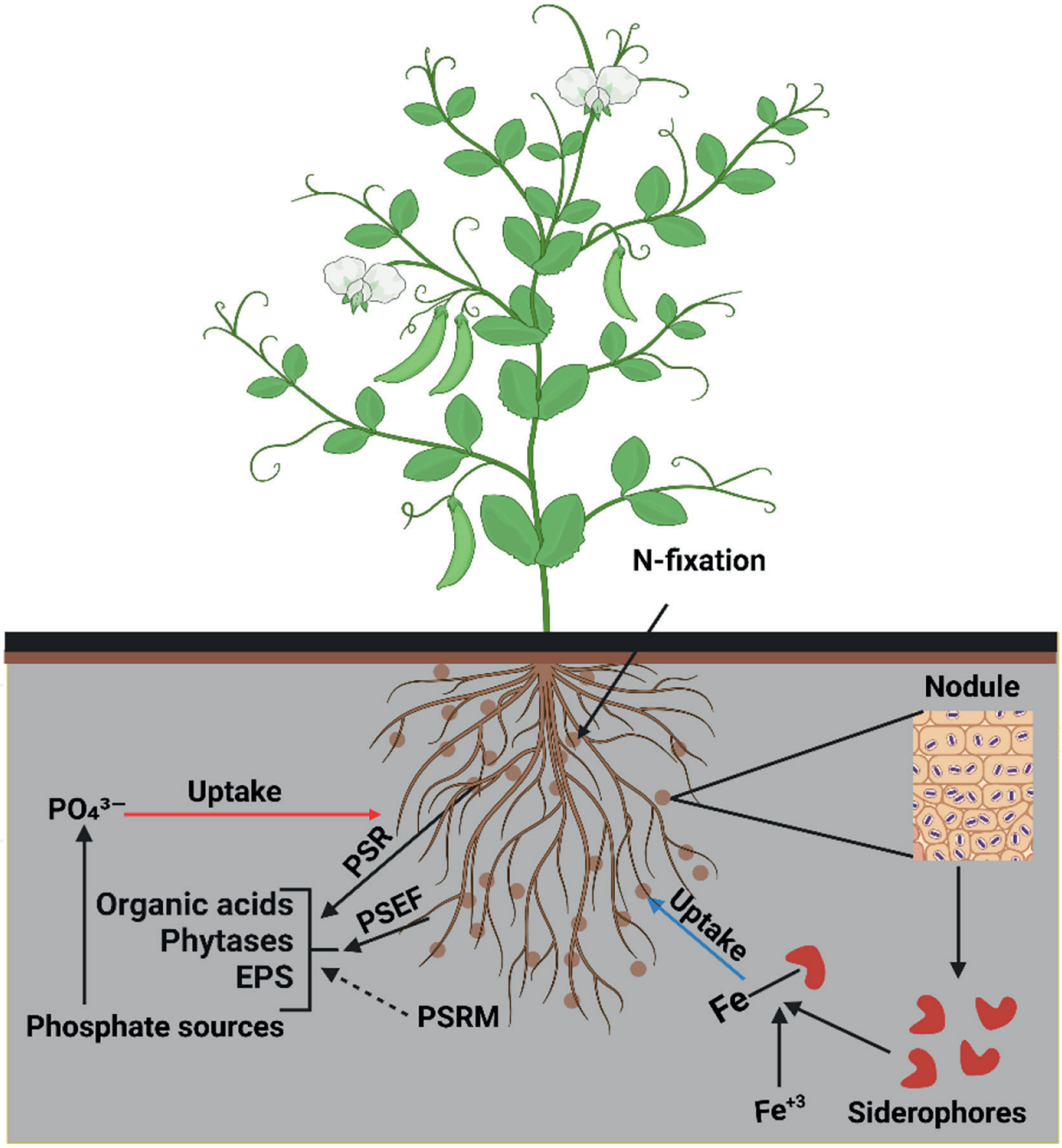

Figure 2.
An illustration depicting the role of root nodulating rhizobia in phosphate and iron acquisition in the rhizosphere. The solubilization process may involve a cooperative action of phosphate-solubilizing rhizosphere microbes (PSRM) and phosphate solubilizing endomycorrhizal fungi (PSEF) with rhizobia, using different organic acids, phytases, and extracellular polymeric substances (EPS). The red and blue arrows show phosphate and iron uptake by plant roots, respectively.

of soluble P in the soil, additional P solubilization and uptake strategies are vital for effective symbiosis. Like many rhizosphere microbes [62], rhizobial symbionts are often associated with P solubilizations [63–68]. By determining the expression level of high-affinity phosphate transporter and mutant analysis, the role of P uptake has been implicated in nitrogen fixation in soybean-*Sinorhizobium fredii* symbiosis and terminal differentiation of nodules in other rhizobial strains [69]. These studies suggest that rhizobial symbionts are important in increasing the availability and uptake of P during legume-rhizobium symbiosis.

Rhizobia-induced phosphate solubilization may involve similar mechanisms that are deployed by other rhizosphere microorganisms, such as the production of acids and phytases, proton extrusion, and extracellular oxidation [70] (**Figure 2**). Although an external application of succinic acid inhibits P solubilization in legumes (e.g., chickpea) [71], several organic acids of microbial origin have been shown to participate in P solubilization in soil [70, 72]. Gluconic acid is one of the P-solubilizing organic acids produced by root-associated rhizobia [63, 68, 73, 74]. Its production by non-rhizobial bacteria including *Azospirillum* and *Nguyenibacter* is involved in the solubilization of calcium phosphate and aluminum phosphate [73, 74]. Besides gluconic acid, the solubilization of Ca and Fe phosphates could also be mediated through other organic acids [68, 75]. Alkaline phosphatases (e.g., *Sinorhizobium meliloti* 104A14) are the other means to solubilize organic phosphates [64, 76]. It is important to note that the presence of non-rhizobial phosphate-solubilizing microorganisms in the rhizosphere can also have a significant impact on the availability of P to legumes, thereby contributing to their increased productivity [77]. Co-inoculation of *Pseudomonas putida* strains and *B. japonicum* TIIIB resulted in greater root and shoot biomass in soybean compared to the rhizobial inoculation alone [78]. Regardless of the role of non-rhizobial microbes, the phosphate solubilization ability of symbiotic rhizobia ensures P availability to support N fixation. During symbiosis, rhizobia might have developed regulation strategies for demand-based P uptake. For example, using a *PhoCDET* phosphate transport system that involves periplasmic binding proteins, *S. meliloti* may ensure P availability when environmental phosphate concentration is below 1 μM [79]. Taken together, rhizobial symbionts are effective in phosphate solubilization, which in turn ensures the availability of P to legume plants.

Iron availability: Fe is an essential element in several metabolic processes in plants such as chlorophyl synthesis, DNA synthesis, and respiration. Despite being abundant in soil, an insoluble form of Fe requires plant strategies to make it absorbable [80]. The limitation of Fe in soil has been shown to affect the growth of many plants including legumes [81]. In legume-rhizobium symbiosis, Fe is important not only for each partner's metabolism but also for maintaining effective symbiotic interaction. Because Fe is a component of several enzymes involved in N-fixation, this mineral is required in a higher amount in a symbiotic interaction than that of the free-living partners. This is evident from the highly sensitive nature of nodulation to Fe limitation [82] and the presence of Fe in higher concentration in nodules than roots, leaves, and seeds [83]. Therefore, legumes have developed strategies for Fe transport into nodules. To meet the high demand of Fe, each of the symbiotic partners is playing a role in Fe uptake. *S. meliloti* was shown to produce volatile organic compounds that function as signals for *Medicago truncatula* to increase its Fe uptake rate [84]. Rhizobial symbionts, on the other hand, released Fe reducing enzymes to acidify the rhizosphere, which facilitated Fe-uptake by the plant [85]. Recently, a study on *M. truncatula* observed an interesting phenomenon: the plant expressed protein signals, which activated the Fe import systems of *S. meliloti* by overriding the Fe-regulating system, *sufT* [86].

One of the common Fe import systems in rhizobia and many other microorganisms is the secretion of siderophores, which are low molecular-mass non-protein molecules that can scavenge Fe^{3+} (**Figure 2**). Under Fe deficiency, rhizobia can secrete siderophores, which form a soluble complex with Fe^{3+} and are imported into the cells via receptors present on the outer membrane surfaces of this Gram-negative bacteria. Rhizobia express genes encoding enzymes involved in siderophore production as well as outer membrane receptor proteins of siderophore-Fe complexes when Fe is limited. For example, enzymes involved in the biosynthesis of a catechol siderophore are encoded by *sid* genes of a rhizobial strain that can nodulate chickpea [87]. Soybean-nodulating *B. japonicum* was also shown to express the *FegA* gene, which encodes an outer membrane receptor protein for siderophore-Fe complexes [88]. From the success in root colonization, biomass, and chlorophyll content enhancement with genetically improved rhizobia, it may now be possible to improve strains having high-affinity siderophore-receptor proteins to increase the Fe uptake in legumes [89].

4. Environmental adaptability

Besides nutrient limitation, other unfavorable conditions such as, high concentrations of heavy metals, drought, soil salinity, extreme pH conditions, and diseases, negatively affect the growth and development of plants, resulting in a significant reduction in the yield of agricultural produce. With increasing anthropogenic activities, the environmental conditions are becoming unfavorable for plant growth. The chemical industries, for example, generate a tremendous amount of heavy metal waste (e.g., Ni, As, Hg, Cd, Pb, Cr, Mn, Si, Fe, and Cu). When left untreated, industrial waste becomes a source of heavy metal contamination in the environment. The negative effects of heavy-metal-contaminated soils (HMCS) on plant productivity are evident. In legumes, it resulted in poor nodulation and plant growth [90]. Nevertheless, some legumes can effectively nodulate and grow in HMCS, suggesting a protective effect of rhizobial symbionts against many of these metals. The *Rhizobium*-induced improvements in nodulation, root and shoot lengths, N-fixation, and seed yield were observed in legumes grown in soil contaminated with Ni (290 mg Ni kg^{-1} soil) [91], As [92–94], Hg [95], Cd [96], Cr [97], Cu [98], Al [99], and Pb [100].

The rhizobia display a natural adaptation to stress, as many of the isolates in HMCS showed a significant potential for heavy metal stress tolerance [101]. A number of the rhizobial strains isolated from Hg-contaminated soils showed tolerance to the metal [102]. The variation in tolerance across the strains was linked with the mercuric reductase activity that converts Hg^{2+} into its less toxic and a volatile form, Hg^0. The adaptive capacity is enhanced through cooperative action among different suites of the microflora. This has been demonstrated in faba bean plants, where a combination of Bacillus strains with those of *Rhizobium* increased the biosorption of Ni and improved plant growth [103]. Similarly, co-inoculation of rhizobia and non-rhizobial bacteria has been shown to improve tolerance against Pb [104], As [94, 105], and Cu [106, 107]. The tolerance mechanism is not well understood, but metal biosorption by rhizobia, accumulation of metals in the roots, volatilization, conversion to less toxic forms, and rhizobia-induced root uptake regulation are some of the ways the symbionts are known to alleviate the metal toxicity. Recently, a molecular investigation into metal tolerance mechanisms revealed changes in m6A RNA methylation in response to Cd stress [101, 108]. The genes that were differentially methylated and expressed were linked to Ca^{2+} homeostasis, ROS pathways, polyamine metabolism, MAPK signaling, hormones, and biotic stress responses. In another study, the primary targets of Al were genes involved in membrane biogenesis, metal ions binding and transport, carbohydrate, and amino acid metabolism and transport [99]. The *Rhizobium* strain B3, which was tolerant to Al toxicity, bound less of the ion compared to the wild-type and maintained membrane integrity. Through recombinant technology, the incorporation of mechanisms of heavy metal tolerance from unrelated species can lead to genetic improvements in the symbiotic rhizobia [109].

Drought stress can reduce nodulation, biomass, and chlorophyll contents of legumes while accumulating ROS that disrupt the structure and function of different biomolecules including DNA [110–112]. Soil salinity negatively impacts rhizobial infections and root nodulations in several legumes: bean [113], soybean [114], pea [115], and chickpea [116]. Some legumes can cope with the adverse conditions, suggesting a positive role for rhizobia in the plant's adaptation to stress. The observed protection against salinity stress by symbionts was due to the production of osmoprotectant molecules (e.g., glutamine, serine, glutamate, and proline) [117], antioxidants [118] and by changing the xylem osmotic potential and amount of aquaporins [119]. Both salinity and drought stress limit the availability of water to plants. Like salinity stress, there are many examples where the protective effect of rhizobia-host symbiosis is evident in the management of drought stress [120]. Both salinity and drought stress responses are complex and may involve non-rhizobial plant growth promoting rhizobacteria and endomycorrhiza [116–119]. On the other hand, extreme pH can have a direct impact on the establishment of symbiosis and thus on the N fixation and productivity of legumes [121, 122]. Soil pH can also influence the diversity and structure of the microbial community around the root rhizosphere, which could affect the legume-rhizobia symbiosis [123–125]. In some legumes, acidic conditions induced the expression of different rhizobial genes (e.g., *actA, typA, atvA, lpiA,* and *ubiF*), which may be playing a role in acid stress tolerance mechanism [126–130].

Symbiotic interactions are known to enhance growth and survival of the host plant against fungal and bacterial pathogens. The anti-phytopathogenic activities of different rhizobial species such as those observed in chickpea against the oomycete pathogen *Phytophthora medicaginis* [131], pigeon pea against *Fusarium* wilt diseases [132], and soybean against root rot diseases [133] have been reported.

5. Strain improvement for enhanced productivity

Advances in molecular biology have allowed genetic improvements in rhizobial strains to increase the symbiotic benefits. Rhizobial strains have been modified to increase N fixation in legumes and some cereals [7, 134] as well as to improve the growth, nutrient supply, and stress tolerance [135, 136]. The overproduction of IAA in *S. meliloti RD64* by introducing a chimeric operon containing genes from *Agrobacterium rhizogenes*, *Pseudomonas syringae pv. savastanoi*, and *Agrobacterium tumefaciens* [137] was associated with the increased production of acid phosphatase and organic acids, resulting in a higher growth of *M. truncatula* under P limitation [138]. Another *Rhizobium meliloti* strain, when modified with extra copies of the *nifA* and *dctABD* genes increased the alfalfa biomass by 13% [139]. The *nifA* gene is involved in nitrogen fixation and *dctABD* gene product is a dicarboxylate transporter. The latter is a primary carbon used by bacteria for energy and other cellular functions. An improvement in symbiotic efficiency can also lead to enhanced plant growth. This has been shown by transferring *Smed_3503*, *Smed_5985*, and *Smed_6456*, into the *S. meliloti Rm1021* strain, which led to a 60% increase in *M. truncatula* biomass [140].

Besides growth and yield, many studies have successfully demonstrated the potential of genetically improved rhizobial symbionts in alleviating adverse environmental conditions in the host legumes. The tolerance mechanisms of these microbes could be exploited for genetic engineering approaches in symbionts. By using this strategy, a *Rhizobium leguminosarum* strain transformed with an *arsenite S-adenosylmethionine methyltransferase* gene of algae, effectively methylated, and volatilized As, to a tolerance against this metal [109]. Similarly, manipulating a superoxide dismutase level reduced Cd stress and increased root and shoot length, biomass, and N content in soybean [135]. The strains have been genetically modified to overproduce cytokinin, IAA, trehalose-6-phosphate synthase, 1-aminocyclopropane-1-carboxylic acid deaminase, high-affinity cytochrome cbb3-type oxidase, etc., to increase salt or drought tolerance or to overcome the waterlogging stress in legumes [32, 34, 54, 137, 138, 141, 142]. Strain improvement followed by extensive evaluation of the strains for survival under field conditions is also critical, as gene modification may reduce the strains' competitiveness against the indigenous strains and hence their survival rate in the nodules [143].

6. Future perspectives

The rhizobial symbiotic interactions are extremely valuable for the sustainability of crop cultivation. This has gained more importance in view of climate change and its imminent threat to the ecological balance. Leguminous crops make a significant contribution to reducing the use of chemical N-fertilizers. The positive impact of commercial and wild symbiotic hosts is enhanced by their growing abilities in diverse environments. The different rhizobial species indirectly increase the N fixation productivity by promoting the growth and productivity of the host plants. A substantial number of benefits other than N fixation point to close coordination between the host and the symbiont while acquiring this capacity. The horizontal transfer of genes appears to have played an important role in improving the symbiotic functionality of the bacterium [144, 145]. Gaining insight into these evolutionary events could provide a broader base for strain improvement. The genetic engineering tools have demonstrated how specific bacterial functions can be transferred across even unrelated species. Further understanding of a symbiotic interaction can bring us closer to developing tailor-made strains.

Acknowledgements

The funding provided by Alberta Pulse Growers and Alberta Results Driven Agriculture Research is acknowledged.

Conflict of interest

The authors do not have any conflict of interests.

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