Chapter

Helping Legumes under Stress Situations: Inoculation with Beneficial Microorganisms

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Abstract

In the upcoming years, legume crops will be subjected to multiple, diverse, and overlapping environmental stressors (raise in global temperatures and CO_2, drought, salinity, and soil pollution). These factors will menace legume productivity and food quality and security. In this context, tolerant plant growth promoting rhizobacteria (PGPR) are useful biotechnological tools to assist legume establishment and growth. In this chapter, tolerant PGPR able to promote legume growth will be revised. Besides, in the era of -omics, the mechanisms underlying this interaction are being deciphered, particularly transcriptomic, proteomic, and metabolomic changes modulated by PGPR, as well as the molecular dialog legume-rhizobacteria.

Keywords: PGPR, legumes, abiotic stress, mechanisms, bacteria

1. Introduction

Plants are colonized by several microorganisms, mainly bacteria, and the number of them sometimes is higher than the number of plant cells [1]. These microorganisms can live inside (endophytes) and outside (epiphytes) the plant tissues, and they do not cause diseases in the host plant [2]; rather, these microorganisms contribute to improve the health and the productivity of the plants [3].

Soils also have many microorganisms with potential to improve plant growth, and overall the rhizosphere. The rhizosphere is an area of interaction between microorganisms and plant roots, and it is inhabited by bacteria, fungi, protozoa, actinomycetes, and algae, with bacteria and mycorrhizal fungi being the main populations [4]. The size of the microbial population in the rhizosphere of plants is influenced by root exudates. The chemicals found in the soil along with the exudates from the plants cause changes in the pH and in the redox potentials that will be determined by the microbial community around the roots [5].

Among the bacteria that colonize the rhizosphere, those promoting plant growth, also known as PGPB (Plant Growth Promoting Bacteria), rhizobacteria or PGPR (Plant Growth Promoting Rhizobacteria), and nitrogen-fixing rhizobia, are the most remarkable because they provide beneficial effects in the development of the plants being able to do it by direct or indirect mechanisms. Regarding legumes, they also interact with rhizobacteria, and the main interaction is the symbiosis between legumes and nitrogen-fixing rhizobia [4]. Rhizobia are bacteria that belong to the family Rhizobiaceae, and the most important genera are *Bradyrhizobium*, *Ensifer*, *Mesorhizobium*, *Rhizobium*, and *Sinorhizobium* [6]. During this interaction, rhizobia invade the root and group into a specialized organ called nodule. Inside the nodules, rhizobia become bacteroids, which transform N_2 to NH_4^+, molecule that can be assimilated by plants [7]. Inoculation of legumes with rhizobacteria produces a bioaugmentation of the microbial population in soils, thus contributing to plants growing bigger and faster [8–10], and this can solve the problem of the rapid growth of the world population that causes a great pressure in the area of land destined for food [11]. Moreover, the arable lands are decreasing because of the climate change and some human activities.

Climate change is one of the most important problems in the planet nowadays. Because of that, temperature and drought are increasing, involving an increase in arid and semi-arid zones and generating a loss of arable soils [12–14]. Drought is an abiotic stress that causes the highest losses in agriculture, so it is a very important factor in crop productivity [15]. Drought and heat involve the appearance of saline soils [16], although some human activities, like the increase of irrigation with bad water quality, are also responsible [17]. Salinity affects around 800 million of hectares in the world, and it is considered a global problem [18] being another stress that limits plant growth, productivity, nitrogen fixation in legumes, and the seed germination [19, 20] due to the uptake excess of NaCl by the plants [21]. Furthermore, the salt excess decreases the organic matter in soils and modifies the microbial population in the rhizosphere [22], so salinity also affects the nodulation negatively [23]. Finally, an additional abiotic stress is heavy metals. The increase of heavy metal concentrations becomes a pollution problem, being humans the main responsible of it [24]. Heavy metals affect plants and soils as the rest of abiotic stress does, and in legumes, they dramatically reduce nodule number and nitrogen fixation [25, 26].

To try to recover these affected soils, phytoremediation is being used, and several studies confirm that it is a very efficient tool, particularly in combination with bacterial inoculation since PGPB improve the potential of plants to phytoremediate soils [27–31]. Legumes belong to the plants used in phytoremediation because this family is one of the most diverse among other plant families in the world, and some of them are able to grow in degraded soils and can be used as pioneer plants in order to repopulate these degraded areas [25, 32]. In fact, legumes are usually used in intercropping with other crops to decrease the amount of pesticides and improve the quality of soils making legumes one of the most promising components of the Climate Smart Agriculture concept [33]. As described above, the symbiosis of legumes with rhizobia improves the growth of legumes and allows them to grow better in the degraded soils, but all the named abiotic stresses interfere with this interaction. For that, authors look for rhizobia resistant to these stresses that able to grow and form nodules even in degraded soils [34–37]. Furthermore, several studies demonstrate that coinoculation of legumes with rhizobia and another PGPR increases nodulation, plant growth, and the potential to phytoremediate soils of plants in the presence of abiotic stresses [38–41]. This improvement in legumes occurs for the interaction between plants and bacteria through different direct and indirect mechanisms that help the plant to grow in the presence of stress.

In this chapter, the different bacterial mechanisms used to improve the plant growth in the presence of the most important abiotic stresses nowadays are been reviewed, in order to help legumes to grow under stress situations and recover the degraded soils using the interaction between legumes and bacteria. Furthermore, the molecular mechanisms involved in these interactions are being described with the transcriptomic, proteomic, and metabolomic studies so far.

2. Effects and mechanisms of plant growth promotion by microorganisms

As mentioned above, some bacteria are capable of promoting plant growth (PGPB and PGPR) through direct (biofertilization) and indirect (biocontrol) mechanisms. The direct mechanisms are based on the direct promotion of plant development, among which are nitrogen fixation, phosphate solubilization, production of phytohormones (auxins, cytokinins, and gibberellins), the enzymatic activity of the aminocyclopropane carboxylic acid (ACC) deaminase, and iron complexation by bacterial siderophores. On the other hand, indirect methods are responsible for inhibiting pathogenic organisms for plants. Among these methods are the synthesis of antibiotics, enzymes that degrade the cell wall, or the induced systemic resistance (ISR) process [42]. The mechanisms carried out by PGPB and PGPR will depend on the host plant and will be influenced in turn by biotic (such as plant defense mechanisms and genotype) and abiotic (such as weather conditions and soil composition) factors [43].

However, bacteria are not the only microorganisms that are able to promote plant growth. Mycorrhizal fungi also can carry out this function, there being a symbiotic association between them and most terrestrial plants [44] favoring micronutrient absorption, resistance to diseases caused by pathogens, or reduction of plant stress caused by environmental factors [45].

2.1 Direct mechanisms

2.1.1 Fixation of nitrogen

Nitrogen is one of the essential elements for life that is present in biochemical structures as important as nucleotides and proteins, but atmospheric nitrogen is mostly nonassimilable for plants since about 78% is in a gaseous state, so it must become ammonia, thanks to the nitrogenase enzyme to be assimilable. This reaction is carried out by rhizobia under symbiosis with legumes, thanks to which the rhizobia obtain carbon provided by the legumes from photosynthesis, and they provide the plant with nitrogen [46].

Atmospheric nitrogen fixation occurs mainly in leguminous plants where rhizobia/plant interactions are highly specific [42]. However, certain free-living bacteria (such as *Frankia* spp. or Actinobacteria) are also able to fix atmospheric nitrogen to a much wider range of plants than rhizobia [47]. For example, coinoculation of *Bradyrhizobium* sp. UFLA 03-84 with *Actinomadura* sp. 183-EL, *Bacillus* sp. IPACC11, or *Streptomyces* sp. 212 in cowpea plants improves the nitrogen fixation even in the presence of salinity conditions [48]. Another example of the improvement of nitrogen fixation in the presence of a different abiotic stress is reported by Saia et al. [49] in which plants of *Trifolium alexandrinum* were inoculated with arbuscular mycorrhizal (AM) fungi leading to an improvement of nitrogen fixation and the plant growth in water stress.

2.1.2 Solubilization of phosphate

Phosphorus is also an essential element for life that is involved in such important processes as energy transfer, respiration, or photosynthesis [50]. After nitrogen, phosphorus is the second most limiting element for plant growth [51]. The content of this element in the soil is 0.05% (w/w) of which only 0.1% can be used by plants, a problem to which the use of phosphorus-based chemical fertilizers that are fixed within the soil must be added and limited the bioavailability of the element [52].

There are a wide variety of microorganisms that can solubilize phosphate to make it assimilable to plants by releasing phosphorus from soil insoluble phosphates. An example of this is the endophytic bacterium *Gluconacetobacter diazotrophicus* that is capable of solubilizing phosphate by acidification [53]. Bacteria also can solubilize phosphates in the presence of different stresses such as *Serratia* sp. J260, *Pantoea* sp. J49, *Acinetobacter* sp., L176, and *Planomicrobium* sp. MSSA-10 that kept this property in the presence of salinity conditions or *Bacillus* sp. L55 in the presence of high temperatures [54, 55]. Besides, other bacteria are able to solubilize organic phosphorous because they produce phytase and phosphatases enzymes that act by dephosphorylating phytates and organic phosphorus [51]. This kind of bacteria can help to legume crops to improve their growth and productivity in these abiotic stress conditions.

2.1.3 Phytohormones production

There are certain microorganisms capable of producing phytohormones, which can promote or modify the development of plants at low concentrations [43]. Among the most common phytohormones are gibberellins, cytokinins, auxins, ethylene, and abscisic acid [53], and their effects can be stimulants or inhibitors of plant growth. The most widely studied are auxins, particularly the indole-3-acetic acid (IAA), being the one that is produced in a greater proportion by plants and PGPR where up to 80% of rhizobacteria synthesize it as a secondary metabolite [56]. This auxin is present in different cellular responses such as cell division, gene expression, or root development and lengthening and affects photosynthesis, pigment formation, and resistance to stress conditions [53].

When the concentration of IAA in plants is adequate, the application of bacterial IAA can have positive, negative, or neutral effects [46], so that this bacterial synthesis will determine the stimulation or inhibition of plant growth and may change the hormone level from optimal to supraoptimal. In this way, the PGPR will stimulate growth when the IAA concentration is below the optimum levels for the plant [42]. It is important that IAA producing bacteria must keep the property even in the presence of any abiotic stress to help plants to grow in these conditions. **Table 1** shows some examples of IAA producing microorganisms that cause improvements in legume crops under stress conditions.

2.1.4 ACC deaminase activity

Ethylene, also known as the stress hormone, is a phytohormone present in all higher plants, making it a key element in a wide range of biological activities, intervening in processes that affect the growth and development of plants where almost all plant tissues and their stages of development are affected by it. It is produced from 1-aminocyclopropane-1-carboxylic acid (ACC) and is catalyzed by the enzyme ACC oxidase [59, 60]. Most abiotic stresses cause a large increase in ethylene concentration causing wilting of the flowers or initiating senescence in the leaves among other consequences, so its increase translates into harmful effects on the growth and health of plants and therefore to a reduction in crop production. However, when its concentration is adequate, it decreases the wilting, stimulates the germination of seeds, and influences the fruit ripening [61]. More recently,

Microorganism	Plant host	Plant improvement	Abiotic stress	References
Ochrobactrum cytisi Azn6.2	*Medicago sativa*	Larger root size	Heavy metals	[37]
Pseudomonas extremorientalis TSAU20 and *P. trivialis* 3Re27	*Galega officinalis*	Improved the growth and increased the nodulation	Salinity	[57]
Bradyrhizobium sp. RM8	*Vigna radiata*	Reduced the uptake of Ni and Zn and increased the nodulation	Heavy metals	[58]
Ensifer meliloti RD64	*M. sativa*	Accumulation of osmoprotectants, greater Rubisco availability	Drought	[8]
Bradyrhizobium sp. RJS9–2	*Stylosanthes guianensis*	Higher salt tolerance and osmoprotectants	Salinity	[9]
Aspergillus japonicus EuR-26	*Glycine max*	Improved the plant biomass and other growth features	Heat	[10]

Table 1.
Beneficial interactions between IAA producing bacteria and legumes under stress conditions.

evidence is being presented showing that ACC deaminase producing microorganisms can facilitate nodulation of legumes under stress conditions, called as nodulating helper bacteria [62, 63].

The great importance of this hormone is the reason for it having being extensively studied, so a wide range of microorganisms capable of secreting the enzyme ACC deaminase is known. ACC deaminase hydrolyses ACC in ammonia and 2-oxobutanoate, thus causing a reduction in the concentration of ethylene in plants [64]. Among ACC deaminase producing microorganisms are the following genera: *Bacillus*, *Pseudomonas*, *Klebsiella*, *Serratia*, *Arthrobacter*, *Azospirillum*, *Streptomyces*, *Microbacterium*, *Achromobacter*, *Acinetobacter*, *Acidovorax*, *Agrobacterium*, *Alcaligenes*, *Enterobacter*, *Agrobacterium*, and *Rhizobium* [59, 60]. The interaction of these microorganisms with legumes enhances plant growth and crop productivity under stress conditions (**Table 2**).

2.1.5 Production of siderophores

Iron is another essential micronutrient for plants that, in the oxygen-rich conditions of the rhizosphere, is in the form of Fe^{+3} that is insoluble for plants and microorganisms [53]. Siderophores, involved in both direct and indirect mechanisms of plant growth promotion, are small molecules of a peptide nature formed by side chains and functional groups that act as ligands with high affinity for the Fe^{+3} ions [65]. A wide range of bacteria and fungi are capable of secreting siderophores that occur in rhizospheric soils in neutral-alkaline pH conditions, where there is a deficiency of this element due to its low solubility in these conditions [66]. These microorganisms can subsequently absorb the siderophore-Fe^{+3} complex by means of a specific receptor and release the Fe in its bioavailable form (Fe^{+2}) to support bacterial growth [61]. The creation of this complex also assumes an important role in the adsorption of Fe by plants, in the presence of other metals such as nickel or cadmium [43]. Another very important function of siderophores is to prevent the proliferation of pathogens by competing for the available iron. In this way, rhizobacteria help plant growth by releasing these biocontrol agents against phytopathogens (antagonism of PGPR against pathogens) [61].

Microorganism	Plant host	Abiotic stress	Benefits for plants	References
Pseudomonas fluorescens TDK1	*Arachis hypogea*	Salinity	Improved plant growth parameters and alleviated saline stress	[67]
Pseudomonas aeruginosa GGRJ21	*V. radiata*	Drought	Increased plant biomass, the relative water content, and osmolytes	[68]
Bacillus subtilis LDR2	*Trigonella foenum-graecum*	Drought	Alleviated ethylene-induced damage and improved nodulation and mycorrhizal fungi colonization	[69]
Arthrobacter protophormiae SA3	*Pisum sativum*	Salinity	Alleviated ethylene-induced damage and improved nodulation and mycorrhizal fungi colonization	[70]
Ochrobactrum pseudogrignonense RJ12, *Pseudomonas* sp. RJ15 and *B. subtilis* RJ46	*Vigna mungo* and *P. sativum*	Drought	Increased seed germination, plant biomass, chlorophyll, and relative water content.	[71]

Table 2.
Beneficial interactions between ACC deaminase producing bacteria and legumes under stress conditions.

Microorganism	Plant host	Plant improvement	Abiotic stress	References
Bradyrhizobium sp. RM8	*V. radiata*	Reduction of Ni and Zn concentrations in plant tissues	Heavy metals	[58]
Bradyrhizobium sp. YL6	*G. max*	Positive effects in photosynthesis and mineral nutrients	Heavy metals	[72]
P. aeruginosa GS-33	*G. max*	Improve plant biomass, chlorophyll content, and reduction of fungal infections	Salinity	[73]
Pseudomonas putida NBRIRA and *Bacillus amyloliquefaciens* NBRISN13	*Cicer arietinum*	Higher growth and yield	Drought	[74]

Table 3.
Beneficial interactions between bacteria that produce siderophores and legumes and plant improvements under stress conditions.

In general, plants can benefit in many ways, thanks to the production of siderophores since they are also involved in the improvement of nitrogen fixation or in the prevention of toxicity by heavy metals [75]. Thus, PGPR able to produce siderophores have a certain competitive advantage over other microorganisms in the rhizosphere [64]. Some of these microorganisms are shown in **Table 3** showing the repercussions caused by the synthesis of iron chelating compounds depending on the crop and the conditions of the plant-microbe interaction.

2.2 Indirect mechanisms

Indirect mechanisms are those processes where PGPR prevent or neutralize the harmful action of phytopathogens by the production of substances that confer greater natural resistance to the host plant, protecting it against infections (biotic stress), although they also help plants to grow actively under conditions of environmental stress (abiotic stress) [43].

2.2.1 Production of antibiotics

Antibiotic production is the main mechanism by which a large and heterogeneous group of bacteria fight the harmful effects of plant pathogens (usually fungi). The antibiotics produced by PGPR are low molecular weight compounds that negatively interfere with the metabolic processes of other microorganisms, thus delaying their growth [64] or inhibiting it [56]. There are some examples of PGPR that produce antibiotics against phytopathogens reflected in **Table 4**.

The effectiveness with which these molecules interfere with pathogen suppression will depend on the metabolite secreted by the PGPR and environmental conditions (mineral content of the soil, osmotic conditions, carbon sources, etc.) [76]. Moreover, some phytopathogens may develop resistance to specific antibiotics by repeated use of the same strain that produces a particular antibiotic, so it is preferable to inoculate plants with PGPR that produce several antibiotics [59]. There are some PGPR that have antagonistic activities against some phytopathogens in addition to improve plant growth in the presence of some stress as it is the case of *Cellulosimicrobium funkei* AR6 that improves the root elongation in crops of *P. vulgare*, *V. radiata,* and *V. mungo* in the presence of Cr(VI) and also has a strong antagonistic activity against *Aspergillus niger* [77]. Another example is *B. thuringiensis* UFGS2 that improves plant growth, physiologic parameters, and the resistance of the soybean against *S. sclerotiorum* under drought stress [78].

2.2.2 Lytic enzymes of the cell wall

Some PGPR produce enzymes that are involved in the lysis of cell walls and neutralization of pathogens by interrupting a particular stage of development or the cell cycle [79], playing an important role in promoting plant growth by protecting them of biotic and abiotic stresses due to the suppression of these pathogens. Among the produced enzymes for this purpose are chitinases, dehydrogenases, β-glucanases, lipases, phosphatases, or proteases [59]. The cell wall of most fungi is formed by residues of β-1,4-N-acetyl-glucoseamine and chitin, so that the bacteria that produce β-1,3-glucanase and chitinase can control the growth of phytopathogen [43]. Furthermore, some PGPR are able to produce this kind of enzymes and protect the crops under abiotic stress like *Bacillus licheniformis* A2 that produces

Microorganism	Plant host	Phytopathogen	Reference
Rhizobium sp. RS12	*C. arietinum*	*Fusarium solani* and *Macrophomina phaseolina*	[80]
Pseudomonas sp. YL23	*G. max*	*Erwinia amylovora* and *Dickeya chrysanthemi*	[81]
Pantoea ananatis RM_2	*P. sativum*	*Trichoderma longibrachiatum* and *Fusarium oxysporum*	[82]
Bacillus sp. B19, *Bacillus* sp. P12, and *B. amyloliquefaciens* B14	*Phaseolus vulgaris*	*Sclerotinia sclerotiorum*	[83]

Table 4.
PGPR that produce antibiotics and their effects as biocontrol agents.

chitinase and protects *A. hypogea* against *Fusarium oxysporum* f. sp. *cubense* under salinity conditions [84]. **Table 5** shows some examples of bacteria capable of producing these types of degrading enzymes.

2.2.3 Induced systemic resistance

There is an important feature of biocontrol that helps plant growth based on two defensive response mechanisms against various external agents known as mechanisms of induced systemic resistance (ISR) and mechanism of acquired systemic resistance (ASR) [56]. ISR is a physiological state of defensive capacity that plants present in response to an environmental stimulus [43] in which nonpathogenic microorganisms, including various PGPB, reduce the negative effects of pathogens of plants by activating a resistance mechanism without the need to target a specific pathogen and can develop this resistance in response to infection by pathogens, to attack by insects, or to a chemical treatment [42].

To stimulate this defensive response mechanism, the ISR uses phytohormones as jasmonic acid (JA) and ethylene (ET) that act as signaling molecules [64], although it has been shown that some organelles (such as flagella) and bacterial molecules (such as lipopolysaccharides of the outer membrane or antibiotics produced by bacteria) can also act as inducing agents activating ISR and generating a rapid accumulation of pathogenesis-related enzymes such as chitinase, β-1,3-glucanase, peroxidase, or liases, among others [85]. It is important to note that the ISR prepares plant to fight the pathogen with an improved defense [56]. A clear example of the ISR elicitor is the effect of *Bradyrhizobium japonicum* in soybean crops, where systemic redox changes are induced in plants [86]. The induction of ISR by *Bacillus* sp. CHEP5 and *Bradyrhizobium* sp. SEMIA 6144 in peanut plants against *S. rolfsii* also has been demonstrated [87]. However, this microbial induction could be limited by abiotic stress like the ISR induction of *B. amyloliquefaciens* S499 in different crops under heat and drought conditions where the response against *Botrytis cinerea* infection was prejudiced [88].

2.2.4 Quorum sensing

Quorum sensing (QS) is a mechanism of genetic regulation in response to cell density mediated by small self-inducing molecules, which are usually secreted out of bacterial cells and act as chemical signals produced by an increase in the cell population. These molecules cause an alteration in bacterial metabolism by activating different sets of genes [89], so that similar bacteria that live in communities and are close to each other will begin to act in a coordinated way. The level of the autoinductors increases proportionally to the population of bacterial cells until it

Microorganism	Host plant	Enzyme	Phytopathogen	References
B. licheniformis A2	*A. hypogea*	Chitinase	*F. oxysporum* f. sp. *cubense*	[84]
Bacillus altitudinis BRHS/S-73	*V. radiata*	Chitinase and protease	*Thanatephorus cucumeris*	[90]
B. subtilis DSM1088, *P. fluorescens* ATCC13525 and *Glommus* spp.	*P. vulgaris*	Chitinase, peroxidase and polyphenol oxidase	*Sclerotium rolfsii*	[91]

Table 5.
Lytic enzymes produced by PGPR and their effect on biocontrol of legume phytopathogens.

exceeds a defined threshold level (*quorum*) where it binds to bacterial cell receptors and triggers a signal transduction cascade leading to changes in the expression of bacterial genes by the action of a group of cells [42].

There are numerous bacterial signaling molecules among which the acyl-homoserine lactone (AHL) produced by Gram-negative bacteria consisting of a common lactone homo resin residue and an acyl chain that can vary between 4 and 18 carbon atoms. There are more than 100 species of proteobacteria that produce AHL, and three types of enzymes are known (LuxI, LuxM, and HdtS) capable of synthesizing AHL *in vivo.* Degradation of AHL can be carried out by various enzymes as is the case of the AHL lactonase that breaks the ester linkage of the lactose ring of the AHL to form N-acyl homoserine reversibly [92]. Most of these signal molecules are of the bacterial origin: *Bacillus* spp., *Klebsiella pneumoniae* KTCTC2241, or *Rhizobium* sp. NGR23 [93]. The detection of this bacterial communication system is interrupted by PGPB, thus preventing pathogen infection through immune responses and preventing plant growth inhibition [94].

3. Molecular mechanisms in the bacteria-plant interactions under abiotic stress

In spite of the abundant literature available on the improvement of plant growth and crop productivity using bacteria under abiotic stress conditions, the molecular mechanisms involved in these interactions remain elusive so far. This area has been studied by some authors to try to understand what changes bacteria elicit on plant gene expression finally leading to enhance the plant resilience to environmental stress. There are not many studies about genes involved in the plant-bacteria interactions, particularly in legumes, but in this section, some examples of these studies are exposed.

Plants have several mechanisms to adapt in the presence of any stress, whose genes are involved in the regulation of transporters, phytochelatins, glutathione reductase, phytohormones, oxidative stress reduction, phenolic compound, osmolytes, and low molecular weight organic acids, among others, and the bacteria role either expressing or repressing these genes has been investigated using real-time quantitative PCR, RNA Seq, and metabolomic and/or transcriptomic analyses. *OsGRAM* family genes are some of the most important in the plant growth and development under stress conditions, and it was demonstrated in rice under an array of stress situations, including salt. The plants inoculated with *B. amyloliquefaciens* SN13 showed overexpression of these genes [95].

Regarding heavy metal stress, one of the main genes expressed or repressed in plants is genes of transporters [96]. In the case of cadmium, *Tatm20* gene codifies a transmembrane transport that is expressed in the presence of Cd in wheat. When wheat plants were inoculated with *Azospirillum brasilense*, this gene was expressed much less than in the absence of the inoculation suggesting that *A. brasilense* helps plants to decrease Cd uptake and accumulation [97]. In this study, we also studied the expression of *TasSOS1* gene involving in keeping the plant cell homeostasis in the presence of high salt concentrations. In contrast to *Tatm20* gene, *TasSOS1* is overexpressed in inoculated plants under salinity stress. This overexpression maintains the homeostasis in plants and makes plant more tolerant to salt excess [97]. In *M. sativa* plants grown under heavy metal conditions, the genes involved in the phytochelatins synthesis were overexpressed in inoculated plants helping plant to detoxify and to grow in the presence of this stress. Moreover, the expression of glutathione reductase was improved by bacteria, so inoculated plants keep the redox status under heavy metal conditions [41]. Other examples are *Bacillus altitudinis*

FD48 that modules the expression of some genes involved in the synthesis of auxins improving root elongation in rice [98] and *Pseudomonas aeruginosa* and *Burkholderia gladioli* that reduced the expression of *CAT*, *GR*, *GPOX*, *APOX*, and *GST* genes in the presence of Cd stress in *Lycopersicon esculentum* plants modulating their antioxidative response [98]. *P. aeruginosa* and *Bacillus gladioli* also elevated the expression of phenolic compound, osmolytes, and molecular weight organic acid (citric acid, malic acid, fumaric acid, and succinic acid) genes, decreasing the physiological damage of *Solanum lycopersicum* plants under Cd toxicity [98].

A global transcriptomic analysis was carried in inoculated *Medicago truncatula* plants grown in the presence of arsenic and inoculated with an arsenic-resistant rhizobial strain. The results showed the overexpression of some defense genes in the inoculated plants (sulfur metabolism, several enzymes of the phytochelatins synthesis pathway, proline, heat shock proteins, and several transcription factors). Besides, secondary metabolism, isoflavonoids and phenylpropanoids, were activated. In contrast, the genes of nodulation were downregulated, particularly those involved in the early stages of the interaction [99, 100].

Under drought environments, *P. putida* MTCC5279 modulates the stress in plants of *Cicer arietinum* by the overexpression of *ACO* and *ACS* (involved in the synthesis of ethylene); *PR1* (synthesis of salicylic acid); *MYC2* (synthesis of jasmonate); *SOD, CAT, APX*, and *GST* (genes that codify the antioxidative enzymes in plants); *DREB1A* (response element to dehydration); *LEA* and *DHN* (dehydrins); and *NAC1* (transcription factors expressed under abiotic stress) genes [101]. In plants of sorghum inoculated with rhizobacterial endophytes, proline (a crucial molecule of maintaining the cellular functions under drought) accumulation was higher than in the noninoculated plants because bacteria induce the overexpression of *sbP5CS2* (pyrroline-5-carboxylate synthase 2) and *sbP5CS1* (pyrroline-5-carboxylate synthase 1) genes under drought stress [102]. This fact also was demonstrated in inoculated chickpea plants, where proline was accumulated by plants under drought conditions besides histidine, citrulline, and threonine [103].

Finally, the molecular mechanisms for plant alleviation in salt stress by bacteria are also reported by some authors. A transcriptome of rice plants inoculated with *Bacillus amyloliquefaciens* SN13 showed that the bacterial inoculation alters gene expression under salt stress. For example, genes of phytohormones, flavonoids, or photosynthesis are found in higher number in inoculated plants [104]. Other example is the inoculation of wheat with *Dietzia natrolimnaea* that causes an overexpression of genes involved in the ABA signaling cascade and in the salt sensitive pathway among others [105].

Recently, some authors have studied the miRNAs induced by PGPR as a possibility to regulate the stress in plants [106, 107]. miRNAs are RNA molecules of 20–24 nucleotides that do not codify proteins, and they get bound to mRNA or any transcriptional factor, regulating the expression of the target gene. However, the only study about miRNAs was performed in chickpea plants under drought stress, where plants were inoculated with *Pseudomonas putida* RA, and this inoculation improves plants adaptation to drought conditions through the regulations of miRNA expression [108].

4. Conclusions and future perspectives

One of the main problems of the rapid increase in the world's population lies in the challenge of having the necessary food for global supply, but the climate change and the pollution decrease the number of the agricultural soil, so a possible solution would be to encourage more widespread use of PGPB. The evidence that PGPR promote the plant growth under stress environments is becoming increasingly true, being even more focused on the study of individual mechanisms than their combined mechanisms, which is why new paths are being opened toward the use of mixed inoculants that act jointly acquiring faster and improving results. There are also investigations in the area of genetics and molecular biology, where studies are being carried out based on the discovery of specific genes capable of motivating greater plant development as well as in the field of nanotechnology where nanoencapsulation and microencapsulation offer an alternative to produce effective formulations against pest control. However, this area needs more investigations and funding to solve the lack of development of new and better storage, shipping, formulation and application techniques of these PGPR, and the development of effective and consistent regulations regarding their use. In this way, the agricultural practice in degraded soils could become an effective and sustainable practice for the benefit of all.

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Conflict of interest

The authors declare no conflict of interest.

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