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Chapter

Legumes and Nodule Associated Bacteria Interaction as Key Factor for Abiotic Stresses Impact Mitigation

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Abstract

Due to climate change, different soil stresses are increasing continuously and they threat the world food security as they limit crop productivity. Therefore, this chapter aims at integrate information about the interaction between legumes and endophytes which will help to: deep understanding of the endophytes-legume relationship, draw attention to the possibilities to exploit this relationship in soil stress mitigation and unraveling what is need to be addressed in the future. The study reviewed the most recent previous scientific works in the field. For legumes tissue colonization, endophytes almost use the same routs which results in their presence in the same niches. Co-inoculation of these bacteria enhances plant growth directly and indirectly. Some endophytes characterized by stress tolerance which interact with legumes and mitigate the adverse effect of soil stresses like salinity, acidity/alkalinity, drought and heavy metal contamination. To reduce stress and enhance plant growth, legume-associated bacteria produce ACC deaminase and other compounds. The interaction process involves induction and expression of many legume-associated bacteria chromosomal and plasmid genes which indicates that this process is a genetic based. So isolation of stress tolerant legume-associated microbes and identification of the gene related to stress tolerance will aid in production of genetic engineered endophytes adaptive to different stresses. It is concluded that all soil stresses can be addressed by application of stress tolerant endophytes to the soil affected with environmental stresses which is sustainable and low cost approach. To maximize the benefit, searching for indigenous stress tolerant endophytes is recommended.

Keywords: Legumes, endophytes, colonization, mechanism, Rhizobium, nodules, stress

1. Introduction

In the last decades the world faced by increasing of food demand due to population increasing. At the same time climate change emerged as a crucial and serious issue which got a global attention [1]. Climate change affects agriculture leading to food insecurity [2]. These problems cannot be resolved unless sustainable

agriculture is practiced, because 36% of the population in the world depends on agriculture for food and as source of economic revenue [3].

Legumes are well-recognized for their impact on the agricultural systems sustainability in addition to their nutritional and health benefits [4]. They are also known for their positive impacts like biological nitrogen fixation, weed suppression, erosion control, improvement of soil health, and eradication of malnutrition in the third-world countries. Therefore, legumes can contribute to meet sustainable food and environmental security objectives [5]. More than that, legumes are also known as "pioneer plants colonizing marginal soils, and as enhancers of the nutritional status in cultivated soils" [6]. All these advantages of legumes make them to be the suitable candidate to address the threatening of climate change which need research approaches to develop crops characterized by the ability to cope with environmental stresses and increasing yield and quality [4]. So using of legumes can lead to sustainable agriculture which "maintains and improves human health, benefits producers and consumers economically, protects the environment, and produces enough food for an increasing world population" [7].

However, sustainable agriculture is faced by abiotic stresses, one of the most important constraints of agricultural production in the world [7]. The most efficient way to face this challenge is using bacteria associated with legumes in the farming systems [8]. These bacteria work together in a team as a community within the root nodule to maintain plant health and survival under harsh conditions and environmental stresses [3, 9]. In addition, the use of these bacteria in agriculture is a low-cost, eco-friendly technology and ethically and socially well accepted [3, 10]. This technology is promising approach due to the increasing recognition that plant tolerance to stress is connected with their associated microbes [11–13]. Among bacteria associated with legumes endophytic bacteria or plant growth promoting bacteria (PGPB) colonize nodules require research focus on exploring their diversity and roles in stress tolerance [3]. So more research on endophytes will enable us to gain insights into the mechanism of colonization and their interactions with plants [3] and best understanding the role they can play in environmental stress mitigation.

Therefore, this chapter aims at review and organize; integrate and evaluate the information about the interaction between legumes and endophytes which will help to: deep understanding of the endophytic bacteria-legume colonization and interaction processes, draw attention to the possibilities to exploit this interaction in soil stress amelioration and unraveling what is need to be addressed in the future studies.

2. The mechanism of legumes nodules colonization by endophytic bacteria

Although root colonization process is very important in nature, till 1987 nothing is known about this process at the molecular level [14] Root colonization is the first step to initiate interaction between the plant and endophytic bacteria. Endophytic bacteria have an affinity with the roots based on several factors including bacterial cross-talk, molecular signaling and quorum sensing (QS) which switch certain genes for using in a variety of plants [15].

The processes of root colonization in *Klebsiella pneumonia*, *Pseudomonas* and *Enterobacter* start by attachment of the bacteria through the fimbriae to root hairs as preferential point, and to the zone of elongation and the root cap mucilage as secondary attachment point without host specificity [16, 17]. For nitrogen-fixing strains it is proposed that type III fimbriae are involved in the adhering to the roots [18]. This mechanism of attachment resembles the adhesion of *Rhizobium japonicum* to soybean roots in which firm attachment was found mediated by pili [19].

The root colonization process probably affected by many factors including "motility, chemotaxis, carbohydrate utilization, and attachment" [17]. Before attachment of endophytes to the plants roots, the plant secretes specific compounds which represent as "chemo-attractant" [20, 21].

For example for attachment of *Pseudomonas* to roots, flagella [14] and other important colonization traits are required like the O-antigen of lipopolysaccharide [22], the ability to synthesize thiamine and high growth rate [23], utilization of organic acids, some amino acids, malic acid and citric acid [20, 21, 24]. However, these traits seem to be characteristic of different *Pseudomonas* species and depend on plant species with which the bacteria associated. This indicates that endophytic colonization is not a passive process, it is an active process controlled by genetic determinants from both partners [25]. It is also reported that cell-surface proteins are involved in the attachment of *Pseudomonas spp*. to plant roots which "include the outer membrane protein OprF of *Pseudomonas fluorescens* OE 28.3" [26] and "an agglutinin isolated from *Pseudomonas putida* a strain Corvallis that mediates agglutination of bacterial cells to a glycoprotein on the plant root" [27].

Following the attraction of endophytes by root exudates and firm attachment, the bacteria distribute along root, the population growth and survival occur, they enter into the root mainly through primary roots and associated lateral roots and tissue wounds, and form micro-colonies [28, 29]. The entry into the root depends "upon the type and availability of nutrients in a tissue, their abundance in the soil and environmental conditions prevailing in that region" [30].

Some routes used by endophyte to colonize plant roots are the same as used by rhizobia in legumes roots colonization which found enter through root hairs and cracks. However, endophytes surpass rhizobia in using more paths to enter plant tissue which make them promising technology as inoculants in sustainable agriculture. This finding supported by the earlier description of endophytes as opportunistic bacteria and "can enter the plant tissue when they find the opportunity either after dissolving the cell wall or through crack entry" [31]. More than that, endophytic associated with legumes nodules were described as "opportunistic bacteria that colonize nodules induced by rhizobia" [32]. Other evidences support the crack entry of endophytes are found in *Klebsiella pneumoniae* 342 which enter the plant after accumulation at lateral roots junctions, which seem reasonable given the nature of this bacterial/host association that does not need the formation of an organized symbiotic structure such as a root nodule as in rhizobia. Before entering the plant, *Klebsiella pneumoniae*342 cells may divide on the rhizosphere or a single cell may enter the plant and then divide in the interior [25].

3. Nodules endophytic bacteria-legumes interaction

After colonization, the endophytes interact with the host plant and beneficial bacteria can significantly affect general plant health and soil quality. Plant growth promotion take place in one of two ways: one way is indirectly by helping plants acquire nutrients through nitrogen fixation, phosphate solubilization or iron chelation, by biocontrol, by outcompeting pathogens for nutrients through siderophore production, or by establishing the plant's systemic resistance. The second way of plant growth promotion is directly by producing phytohormones such as auxin or cytokinin [33] or by producing the enzyme 1-aminocyclopropane-1-carboxylate deaminase, which lowers plant ethylene levels [34]. These processes are achieved by a consortium of different roots or nodules endophytes with eventual coordination with rhizospheric bacteria to help in more nutrient mobilization [30]. For example one of the common plant growth promotion hormones produced by *Pseudomonas*,

Klebsiella and *Enterobacter* spp. is indole acetic acid (IAA) which its production directly associated with plant growth stimulation [35–37]. While both *Pseudomonas* and *Enterobacter* spp. solubilize phosphate and exhibit 1-aminocyclopropane-1-carboxylate-deaminase activity during biotic and abiotic stress and environmental stresses [35, 38]. Some species of endophytes like *Pseudomonas* and *Klebsiella* associated with groundnut nodules were found distinguished by their ability to fix nitrogen [32]. Others like *Enterobacter spp* were found characterized by ammonification and b-1, 3 glucanase activities [35].

The key trait enables interference of endophytes activities with the host plant physiology is production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase by which endophytes might profit from association with the plant because colonization is enhanced, at the same time the plant benefit by stress reduction and increase root growth [12].

However, the process of colonization and interaction between endophytes and different plants is seem to be less complicated and inexpensive regarding energy consumed, if we compared it with colonization and interaction of *Rhizobium*-legumes which includes "pre-infection, root colonization, root adhesion, hair branching, hair curling, infection, nodule initiation, bacterial release, bacteroid development, nodule function, nitrogen fixation, complementary functions, and nodule persistence" [39].

4. Soil stress mitigation

This section shed light on how the interaction between the legumes and their associated endophytes can contribute in addressing the major types of abiotic stress face the plants which include drought, salinity, acidity/alkalinity and heavy metal toxicity [40].

4.1 Using legumes and nodule endophytic bacteria to mitigate soil stress

The application of consortium of proper rhizobia together with plant growth-promoting microorganisms is an effective and environment-friendly approach helps to alleviate different stress conditions such as drought and salinity among others, increase the efficiency of the symbiotic processes and improve the crop yield by different mechanisms of actions under variable conditions [41, 42]. The ability of these bacteria to withstand to high levels of stresses makes them valuable to enhance legume production in harsh environmental conditions [42].

In this regard there are strong evidences that the endophytic bacteria serve host functions like osmolytes [3]. For example Abd-Allah et al. [43] investigated the effect of the endophytic bacterium *Bacillus subtilis* BERA 71 on chickpea plants under saline conditions. They found that application of this endophytic bacterium significantly enhanced the growth of chickpea plants and ameliorate salinity induced oxidative damage. It is also increased macro-nutrients like N, P, K, Ca, and Mg, at the same time decreased sodium accumulation under salinity. Also Barnawal et al. [44] reported that *Arthrobacter protophormiae* strain inhibits the nodule of *Pisum sativum* was found enhanced its growth under high salinity conditions, increasing nodule number and reducing salt stress. Although many investigators have co-inoculated soil isolates and species of *Rhizobium*, fewer studies have co-inoculated nodule associated bacteria and rhizobia [9]. The process of endophyte-*Rhizobium* co-inoculation is promising technology because the association between host and microbiome did not depend solely on N_2-fixing rhizobia, but also required a direct connection between symbiotically linked bacterial communities that resides in the rhizosphere [6].

Co-inoculations of legumes with indigenous rhizobia and salt–tolerant non–rhizobial nodule associated bacteria and rhizosphere bacteria may offer sustainable solution for boosting biological nitrogen fixation and the productivity of legumes in soils affected with different extreme environmental conditions [9, 45]. In the process of co-inoculations, it is not possible to determine exactly which bacterial mechanisms have a more pronounced impact in a given plant–microbe association [46]. However, in multi-microbial interactions local isolates are recommended because of their physiological and genetic adaptation to the environment [41].

Hence to address different soil stress problems, using consortium of locally isolated rhizobia and endophtes is seem to be the most effective and efficient approach than using rhizobium or endphytes alone because in co-inoculation the different plant needs are provided by the different bacteria constitute the inoculum.

4.1.1 Salinity

Soil salinity is one of the major factors destroy environment and limiting the legumes productivity [47]. Soil salinity is increasing continuously due to continuous climate change, and it becomes limiting factor for crop productivity worldwide [3]. It is estimated that more than 6% of land area has affected by salinity [48] and about 10–20% of cultivated and 27–33% of irrigated agricultural lands are afflicted by high salinity [7, 42]. This degradation of the soil results in decreasing the quality and productivity of crops worldwide [42], at the time world population increase which necessitates utilizing lands affected by salinity to meet the food needs [49].

The negative effects of salinity represent in causes osmotic and ionic stresses in plants and constrain the growth. Upon the plant exposed to salinity, osmotic stress occurs immediately because hypertonic conditions outside the cell take place. Ionic stress elevated after several days as a result of the accumulation of Na^+ and Cl^- ion inside the cell. The effects of this osmotic stress are reduction of the "cell turgor pressure, cell elongation and cell division rates" [3]. Other effects include modulation of the cell ion homeostasis which leads to "changes in hormonal status, transpiration, photosynthesis, nutrient translocation" among other metabolic processes [50]. To adapt to the stress, plants have immune system with different physiological mechanisms to induce tolerance. The same role also played by plant-associated microbes [11] which capable to exclude salts and via intracellular accumulate inorganic and/or organic solutes to balance osmotic across the membrane [51]. However, the diversity of microbial properties capable of promoting plant growth makes it difficult to be sure about the importance of particular mechanisms within specific plant–microbe interactions in saline environments [52]. Also to alleviate the effects of salt stress, endophytes play a positive role to adjust cell osmotic, detoxification, regulate phytohormone and nutrient acquisition in plants [3]. The excellent plant growth promoters under stress conditions are endophytes containing ACC deaminase activities due to their ability to block ethylene production at each specific location and "cleaves the ethylene precursor ACC to α-ketobutyrate and ammonia", which metabolized by the bacteria for their growth [53, 54]. These microbes include different genera of *Bacillus*, *Pseudomonas*, *Klebsiella*, *Serratia*, *Arthrobacter*, *Streptomyces*, *Isoptericola*, and *Microbacterium* [55] which show their ACC deaminase properties with high salt concentration [3]. This was verified by Iniguez et al. [56] experiment in which endophytic relationship of the *Klebsiella pneumoniae* strain342 with *Medicago truncatula* was established which indicates that ACC deaminase-producing endophytic bacteria reduce stress ethylene levels in plants and alleviate the damaging effect of this hormone under stress conditions [3]. Microbial volatile organic compounds are among other compounds produced by microbes which play a role in salinity stress conditions due to their

ability to trigger induced systemic resistance in plants [57]. As mentioned before, the symbiotic relationship of rhizobia and legume in presence of non-symbiotic endophytic bacteria, also help in adapting to salinity. For example, *Rhizobium* and *Pseudomonas* when used as co-inoculant promoted mung bean growth under salinity stress by providing auxin and ACC deaminase [58]. This finding indicates that the two bacteria worked in a complementary way, one bacterium provides the plant hormone auxin (probably the *Rhizobium* strain) and the other provides the enzyme ACC deaminase (may be the *Pseudomonas*).

However, still there is no comprehensive review available about exploitation of legume-endophytes relationship to ameliorate salt stress in the soil with concentration in the beneficial effects of endophytes. This necessitates raising scientific community awareness to carry out research in this field to enhance agriculture productivity under saline environments [3].

4.1.2 Acidity/alkalinity

Another problem increased by the impacts of global change is soil acidity or alkalinity which also limits the legumes productivity. During symbiosis process, it is found that rhizobia are more sensitive to acidity than legumes, this means incapability of rhizobia to persist and survive in acidic soils which reduces symbiosis effectiveness and legumes productivity. To overcome this problem, it is important to seek for indigenous acid or alkaline-tolerant rhizobia capable of nitrogen fixation and enhance legumes production under acidic or alkaline conditions [42, 59].

For addressing the problem of acidity or alkalinity of the soil, legumes afford acidity and alkalinity simply can be grown regardless of the growth promoting characteristics and the stress tolerance of their associated bacteria, because some legumes prefer soils pH ranged between light acid to alkaline such as pea, melilot, alfalfa and haricot while clover, lupine and soybean grow well in the acidic soil [60]. Nevertheless, legumes treated with endophytes isolated from acidic or alkaline soils expected to promote their growth in acidic or alkaline soil more than untreated legumes. However, acidic pH (3.8–4.5) was found retarded the development and activity of the bacteria *Rhizobium leguminosarum* and reduces pea yields [60]. At the same time endophytic bacteria like *Klebsiella* isolated from groundnut grown in different regions were found grow at pH ranged between 4 and 8 [61]. Like these endophytes and their leguminous host can be harnessed in co-inoculation process to mitigate acidity or alkalinity of the affected soils.

4.1.3 Drought

Drought is another consequence of climate change and represent major constrain of agriculture. It is estimated that by 2050 drought is expected to cause serious plant growth problems for more than 50% of the arable lands [62]. Among the different environmental stresses, drought is the most destructive factor retarding symbiosis process and rhizobial growth [63].

Legumes and their associated microbes can play role to mitigate the negative effects in the areas affected by drought because microbe live within plant tissues and release various phytochemicals that assist plant to withstand drought stress [1]. The legume associated microbes consortium work in an integrated manner to enhance drought stress tolerance in plants through improve root length and density, root construction to assist in better water and nutrient uptake, enhance soil-water-plant relationships, manipulating phytohormonal signaling, increase

different organic and inorganic solutes, increase the synthesis of osmolytes like proline, increase antioxidant enzymes that scavenge reactive oxygen species (ROS), decrease the regulation of stress-responsive genes and producing drought-tolerant substances like abscisic acid, indole-3-acetic acid, ACC deaminase and volatile organic compounds [1, 8, 52, 57, 64, 65].

Research conducted to study response to drought stress using legumes such as soybean and single endophyte *Pseudomonas simiae* AU showed that inoculation process resulted in expression of their respective genes, induced proline and total soluble sugar content [66]. More drought tolerance characteristics were pronounced when soybean treated with *Bacillus* and *Pseudomonas*, they "improve plant growth, membrane integrity, water status, accumulation of compatible solutes, and osmolytes" [67]. Arshad et al. [68] stated that drought stress on the growth and yield of *Pisum sativum* was significantly decreased by a strain of *Pseudomonas spp*. with ACC deaminase enzyme activity, and concluded that the drought stress induced inhibitory effects of ethylene could be eliminated by application of bacteria containing this enzyme. Likewise, it is reported recently that there is increasing in using rhizobia as biofertilizer to alleviate the effect of drought on legumes growth under stressed environment [63].

It is expected to obtain the best growth conditions in drought affected areas if legumes inoculated with consortium of efficient locally isolated rhizobia and endophytes. The locally isolated strains are more adaptive to the different adverse environmental conditions in the drought areas from where they were isolated, this gives them the advantage to work at maximum rate to mitigate drought.

All the above mentioned advantages of the legume-associated microbes result in positive effects on the overall plant growth which in turn enhance legumes production in the areas affected by drought.

4.1.4 Heavy metal contamination

Heavy metal contamination of the soil is a result of different anthropogenic activities such as mining, modern agricultural practices and industrialization. The deleterious effects of heavy metals discharged from different sources represent in causing potential human risks, accumulation within soils and harm ecosystems, enter food chain, poison plants and seriously affect the beneficial soil microbial compositions and their physiological functions [69]. Soil contamination with heavy metals results in toxic effects on plants [10]. To address this environmental problem, using association of plants with various microorganisms represents a sustainable strategy [40]. However, till now very few studies evaluated the effect of bacterial consortia for heavy metal contamination mitigation [40]. Some studies reported that some bacteria have adapted well to environments polluted with heavy metals and exhibit resistance mechanisms like enhancing the expression of stress related gene, metal bioaccumulation, anti-oxidant activities and alteration of the levels of 1-aminocyclopropane-1-carboxylate (ACC) [10, 70, 71]. Other bacteria adopt different strategies to reduce the toxicity in soil under heavy metal contamination. These strategies include "metal adsorption, bioaccumulation, expulsion of metal outside the cell, biotransformation, release of chelating agents, acidification of adjacent environment, and the ability to change in redox potential" [72].

There are many legumes-associated microbes reported promote plant growth under heavy metal stress like *Trifolium repens* tolerate Fe, Mn, Zn and Cd when associated with *Rhodococcus erythropolis*, *Achromobacter sp*., *Microbacterium sp*. and *Bacillus cereus* [46, 73]. Also *Lupinus luteus* was found grow under high

concentration of Cu, Cd, Pb when associated with *Bradyrhizobium sp*. 750, *Pseudomonas sp*. and *Ochrobactrum cytisi* [74]. In another study *Ochrobactrum* have been used in consortia with nodule-forming bacteria and other plant growth promoting bacteria and *Lupinus luteus* in heavy metal contaminated soils, the result showed increasing of plant biomass and decreasing accumulation of heavy metals [74]. Also when *Vicia faba* cultivated in soil moderately contaminated with Cu and inoculated with consortium of bacteria containing *Rhizobium sp*. CCNWSX0481, *Rhizobium leguminosarum* bv. viciae, *Enterobacter cloacae*, and *Pseudomonas sp*. 2 (2010), significant reduction of accumulated Cu in roots and increase in nodulation, growth and seed yield were observed [75]. These findings strongly indicate that bacterial consortia maximize benefits compared to individual strains in heavy metal stress mitigation [40]. The advantages of using heavy metal-tolerant microbes represent in sustainable and low-cost option to detoxify heavy metal contaminated soils through a process called bioremediation, enhance nitrogen-fixing efficiency, and promote the legumes growth, yields, and grain quality. To realize these benefits, isolation and selection of indigenous metal-tolerant rhizobia are recommended followed by metal resistance genes identification which then can be transferred through genetic engineering to other non-tolerant microbes used in contaminated soils clean up and remediation programs [69].

The legumes-microbes interaction process which results in heavy metal stress mitigation can be useful in coping of legumes with this harsh condition, and at the same time this approach can be useful in bioremediation programs. However, the questions need to be answered through research are: In presence of heavy metal tolerant microbes, Do the legumes able to grow at unlimited concentration of heavy metal or there are limitations? The second question is in case of heavy metal uptake and accumulation in legumes tissue, do legumes able to assimilate the accumulated heavy metals to be useful or at least not harmful? And in which parts of plant more absorbed heavy metals accumulate?

5. The genetic mechanisms involved in nodule endophytic bacteria-legume interaction to mitigate the different stresses

The process of associated bacteria-plant interaction in nature is a complex phenomenon includes biotic, abiotic, and genetic factors. Understanding of this process and the effect of this association is crucial to the agricultural applications [40]. In both plant and associated bacteria, different genes express during the interaction process which start with recognition of the plant and the associated bacteria, passing through colonization and interaction until ending by coping to live in the adverse condition. However, colonizing internal plant tissues differ in endophytes and rhizospheric bacteria due to differences in their genomes [76]. Endophytes protect plants against the inhibitory effects of stresses and at the same time may alter plant gene expression that makes plant less likely to give up to these stresses [77].

Generally, associated microbes have genes responsible for salt stress adaptation [78]. Therefore, during nodule endophytic-legume interaction "ACC-deaminase gene AcdS is expressed and regulated under different stressed environmental conditions" [54]. Significant changes in gene expression take place to mitigate the different environmental stresses. For example in *Sinorhizobium meliloti* 1021 exposed to salt 52 of 137 genes were induced and the remaining 85 were repressed. The long term exposure of this bacterium to salt "activated genes related to polysaccharide biosynthesis and transport of small biomolecules like amino acids, amines, peptides, anions, and alcohols" [79]. Likewise, sudden increase in

salt stress induced genes of unknown functions and repression proteins coding genes. The majority of the regulated genes located in the chromosome and others located on plasmid (pSmbB). This finding suggests the role of *Sinorhizobium meliloti* chromosomal and plasmid genes in the adaptation to salt stress. It is also reported that ribosomal genes and tricarboxylic acid cycle genes are repressed. It is important to show that 25% of genes regulated by salt encode ribosomal proteins [80]. Under osmotic stress, *Sinorhizobium meliloti* regulates the expression of BetS gene which represents a major component of the overall betaine uptake activities in response to salt stress and has a role in Gly-betaine/Pro-betaine transporter [81] involved in salt stress tolerance in *Medicago sativa* [82]. This finding indicates that acdS gene is responsible for salt tolerance and its expression confers host plant the ability to afford salinity. In addition, to overcome salinity stress using of *Sinorhizobium meliloti* would be a useful method [42]. For bacteria induce nodules, genes encoding Nod factors are also included in salt stress. For example in *Rhizobium tropici* CIAT899 46 different Nod factors were identified, of these 14 new Nod factors identified not produced under neutral or acid conditions [47]. Nod factor production increased in the same bacterium when grown under acid conditions [83]. Many other studies used PGPR with leguminous plants confirmed different genes expression under stress conditions. For example in soybean treated with *Pseudomonas simiae* AU, to tolerate drought different genes up-regulated. It is found that different factors involved in the process including "transcription factors (DREB/EREB), osmoprotectants (P5CS, GOLS), and water transporters (PIP and TIP)" [66]. Other studies also reported that stress-related genes may activated to regulate and enhance tolerance toward abiotic stresses through production of Ca^{2+} sensor calcineurin B-like proteins (CBLs) in different legumes such as chickpea [84] and soybean [77]. The definitive targets of these sensors are the abiotic stresses such as drought and salinity [85]. In chickpea the exogenous acdS gene of the salt-sensitive *Mesorhizobium ciceri* strain was found form nodules the same as salt-tolerant strain [86].

For heavy metal tolerance, to enhance the expression of stress response genes or the transcription factors, several signaling pathways activated like reactive oxygen species (ROS) pathway and hormone signaling pathways [85]. *Medicago sativa* produce mitogen-activated protein kinases (MAPKs) when exposed to excess Cu and Cd [87]. In *Medicago truncatula* different concentrations of Hg genes associated with ethylene metabolism and signaling were expressed [88]. From these findings it can be assumed that these genes involved in heavy metal tolerance for *Medicago sativa* and *Medicago truncatula*, and different genes expressed in case of soil contamination with different heavy metals. To address heavy metal stress problem, it is possible to make recombinant bacteria through exploiting different genes including "metal chelators, metal homeostasis, transporters, biodegradative enzymes, metal uptake regulators, and biotic and abiotic stress tolerance" [89].

In spite of the progress of the research in this field, regulatory networks of the interaction of host plant-associated microbes in heavy metal stress are unknown [10] and identification of undiscovered genes involved in endophytism has not been pursued systematically [90]. So efforts should be directed toward identification of different genes of legumes and their associated endophyts involved in the interaction processes, because like these information can benefit in biotechnological applications, recombinant technologies and ensure the efficiency of the interaction between the host legume and its associated bacteria.

The above mentioned findings confirm that the ability of plant growth promoting bacteria to ameliorate stresses is a genetic based, and the genes responsible for these traits induced and expressed once soil stress increased.

6. Strategies to select leguminous plants for future studies related to endophytes and soil stress

The strategy described below can be used as model and applied for legumes although it was suggested by Strobel and Castillo [91] to select plants generally for endophytes isolation. The strategy defined plants distinguished by special characters such as:

i. Plants from unique environmental settings like those characterized by an unusual biology and adopt novel strategies for survival.

ii. Plants have an ethno botanical history.

iii. Endemic plants characterized by an unusual longevity or occupied a certain ancient landmass.

iv. Plants growing in areas of great biodiversity.

7. Future prospective

To understand the endophytes and their interactions with the host legumes, multidisciplinary research include cultivation-independent techniques, the "Omics" fields like genomics, proteomics, metabolomics; and the advancing computational data-mining approaches among others are required. Research focus on isolation and characterization of indigenous rhizobia and endophytes are required combined with studies concentrate in regulatory networks of the interaction of host plant-associated microbes, mechanisms of regulation and expression of already known genes like AcdS gene, and identification of undiscovered genes involved in endophytism can play a crucial role in understanding of this interaction process. Like these studies contribute to obtain the optimum exploitation of legumes and their associated bacteria to mitigate climate change impacts. Also research directed toward using the legumes and their associated endophytes in phytoremidiation programs is highly encouraged to address soil heavy metal contamination which now represents real environmental threat.

8. Conclusions

Legumes and their associated endophytes are one of the key factors in climate change impact mitigation. Bacteria associated with legumes secrete different chemicals and work in social network to alleviate soil stresses and enhance plant growth. The tolerance of these bacteria to different stresses is genetically inherited trait which can be harnessed to produce genetic engineered stress tolerant bacteria used as inoculants in stressed soils. These genetic engineered stress tolerant bacteria will transmit stress tolerance genes through horizontal gene transfer to the indigenous bacteria when applied as inoculants in the stress affected soils, so enrichment of these soils with stress tolerant bacteria will take place eventually. Addressing soil stress problems by using these bacteria is sustainable, eco-friend and cheap approach. To realize the effectiveness and efficiency of this approach, using consortia of locally isolated rhizobia and other endophytes will be more applicable.

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