REVIEW ARTICLE

HARNESSING PGPR MECHANISMS, STRATEGIES AND CHALLENGES IN SELECTION OF SUITABLE BACTERIA FOR DROUGHT STRESS

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Abstract: Plants are continuously exposed to a wide array of environmental stresses. Abiotic stress is one of the foremost limiting factors that are responsible for low agricultural productivity. The incidence of extreme events like prolonged drought, salinity, heavy rain and flooding, heatwave, and frost damage, metal toxicities in problematic soils are increasing day by day under the scenario of changing climate. Crop plants need to acclimatize against adverse external pressure created by environmental and edaphic conditions with their intrinsic biological mechanisms. Drought is one of the significant constraints on agricultural productivity worldwide and is likely to increase further. Several adaptations and mitigation strategies are required to cope with drought stress. Here, microorganisms can come to the rescue in an economical and eco-friendly manner to help plants for better fitness against abiotic stressors. Their interactions with compatible microbes evoke various kinds of local and systemic responses that improve the plant's metabolic capability to fight against abiotic stresses. Root-associated bacterial communities play a vital role in maintaining the health of the plant host. Therefore, it is essential to understand better the mechanisms that influence microbial communities composition and structure and what role the host may play in the recruitment and control of its microbiome. Plant growth-promoting rhizobacteria (PGPR) could play a significant role in alleviating drought stress in plants. These beneficial microorganisms colonize the rhizosphere/endo-rhizosphere of plants and impart drought tolerance by producing exopolysaccharides (EPS), phytohormones, 1-aminocyclopropane- 1-carboxylate (ACC) deaminase, volatile compounds, inducing accumulation of osmolytes, antioxidants, upregulation or downregulation of stress-responsive genes, and alteration in root morphology in the acquisition of drought tolerance. In the present review, we elaborate on the role of PGPR and various mechanisms, which in turn helping plants to cope with drought stress.

Keywords: Abiotic stress, Antioxidants, Microbiome, Osmolytes, Phytohormones, Rhizobacteria

INTRODUCTION

Interactions between plants and soil organisms are crucial for the functioning of terrestrial ecosystems and their response to a changing climate (van der Heijden *et al*., 2008; Cavicchioli *et al*., 2019). Plants and soil organisms interact with several distinct mechanisms. Plants fuel the soil food web through their ground carbon (C) inputs in the form of leaf and root litter and root exudates. Although soil microbes are the primary decomposers of these C inputs, their biomass supports the existence of higher trophic levels; in turn, organisms from these higher trophic levels, such as Collembola and nematodes, stimulate the activity of soil microbes. Together, these organism's actions release nutrients for plant growth and determine the balance between C respiration and stabilization in the soil. But these organisms also interact directly with plants in the rhizosphere by feeding on (or infecting) roots, forming symbiotic relationships such as mycorrhizae, or promoting plant growth through phytohormone production or reducing plant stress signaling. It is well known that different plant species or genotypes can select for other soil communities (Berendsen *et al*., 2012). These selective pressures are incredibly intense in the rhizosphere, the area around the roots directly influenced by root processes and is the home of the rhizosphere microbiome. Recent studies suggest that root exudates have a pivotal role in selecting the rhizosphere microbiome and that choosing a favorable rhizosphere microbiome via altering root exudation patterns might open up new opportunities to increase plant performance, with particular benefits for crop production (Williams and deVries, 2020).

Water deficit is the most common stress affecting plant growth in arid and semiarid regions. Thus, it is necessary to improve the efficiency of plant to capture and use of water and nutrients. Inoculation of plants with native beneficial microorganisms may increase drought tolerance of plants growing in arid or semiarid areas. Plants growing under dangerous environmental conditions, such as those occurring in dry and semiarid soils, undergo water limitation and nutrient deficiencies but have several mechanisms to cope with these adverse factors. Rhizosphere microorganisms are adapted to adverse conditions and compensate for such detrimental conditions (Marulanda *et al*., 2009).

Drought stress is among the most destructive abiotic stresses that increased in intensity over the past decades affecting the world's food security. Drought stress may range from moderate and short to too severe and prolonged duration, restricting the crop yields. Drought is expected to cause serious plant growth problems for more than 50% of the arable lands by 2050 (Kasim *et al*., 2013).

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Impact of drought stress on plant growth

Drought affects plant water potential and turgor, enough to interfere with normal functions changing physiological and morphological traits in plants (Rahdari *et al*., 2012). Growth reduction under drought stress has been studied in several crops such as barley, maize, rice, and wheat (Kalariya *et al*., 2019). Fresh weight and water content are standard growth parameters that are affected by drought. Furthermore, drought stress influences soil nutrient's availability and transport, as nutrients are carried to the roots by water. Therefore, Drought stress decreases nutrient diffusion and mass flow of water-soluble nutrients such as nitrate, sulfate, Ca, Mg, and Si (Selvakumar *et al*., 2012). Drought also induces free radicals affecting antioxidant defenses and Reactive Oxygen Species (ROS) such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals oxidative stress. ROS can damage various levels of the organization at high concentrations, like initiate lipid peroxidation, membrane deterioration and degrade proteins, lipids, and nucleic acids in plants.

Nevertheless, under drought stress, the decrease in chlorophyll content was a symptom of photooxidation (Rahdari *et al*., 2012). Decreasing of chlorophyll content in *Paulownia imperialis*, bean, and *Carthamus tinctorius* was observed under drought stress. Drought also affects biochemical activities like nitrate reductase (NR), due to lower uptake of nitrate from the soil. It also accentuates ethylene's biosynthesis, which inhibits plant growth through several mechanisms (Ali *et al*., 2014).

Drought as multidimensional stress affects various subcellular compartments, cell organs, and whole plant levels. Thus drought negatively affects the quantity and quality of growth in plants. Therefore, to produce more food, the mitigation of drought stresses is vital to achieving the designated goals. Worldwide extensive research is being carried out to develop strategies to cope with drought stress by developing drought-tolerant varieties, shifting the crop calendars, resource management practices, etc. Most of these technologies are cost-intensive. Recent studies indicate that microorganisms can also help plants to cope with drought stress (Vurukonda *et al*., 2016).

Plant growth-promoting rhizobacteria (PGPR)

From the last few decades, PGPR has gained considerable interest in research because of the stimulation of plant growth, increasing crop yields, being less harmful to the environment, and also reducing the cost of chemical fertilizers. PGPR can also be termed as plant health promoting rhizobacteria (PHPR) or nodule promoting rhizobacteria (NPR). Based on their relationship with the plants, PGPR can be divided into symbiotic bacteria and free-living rhizobacteria. There are an array of mechanisms by which PGPR stimulate the growth of plants (Kundan *et al*., 2015).

Role of PGPR under drought stress

The role of microorganisms in plant growth, nutrient management, and biocontrol activity is very well established. These beneficial microorganisms colonize the rhizosphere/ endo rhizosphere of plants and promote plant's development through various direct and indirect mechanisms. Furthermore, the role of microorganisms in the management of biotic and abiotic stresses is gaining importance. The possible explanation for the mechanism of plant drought tolerance induced by rhizobacteria includes (1) production of phytohormones like abscisic acid (ABA), gibberellic acid, cytokinins, and indole -3-acetic acid (IAA); (2) ACC deaminase to reduce the level of ethylene in the roots; (3) induced systemic tolerance by bacterial compounds; (4) bacterial exopolysaccharides (Timmusk *et al*., 2014) (Fig. 1). Therefore, we attempt to throw more light on the mechanisms that operate in microbial species that possess stress alleviating potential and their use in sustainable agriculture. This present review encompasses an overview of the current work reported on PGPR's role in helping plants cope with drought stress (Babou and Lisna, 2019).

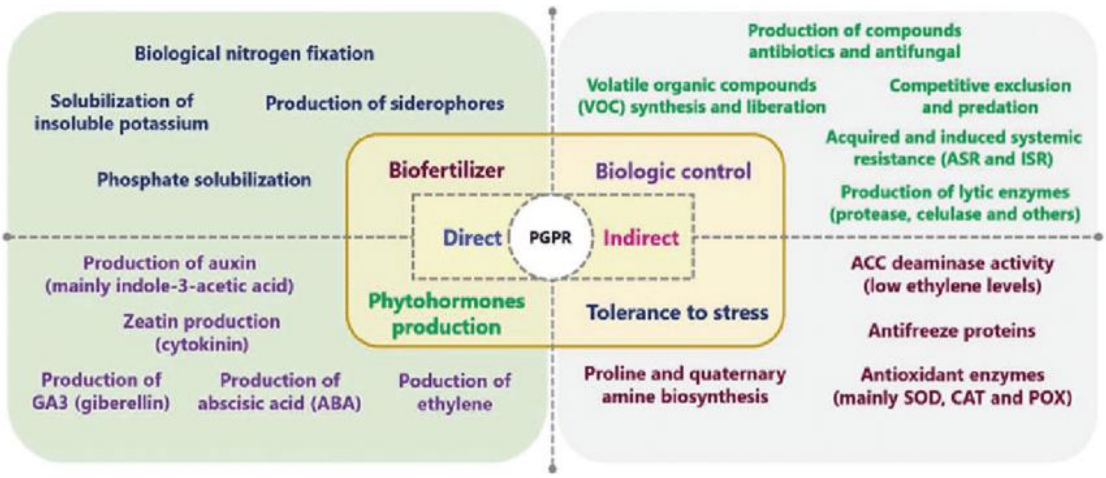

Fig. 1. Direct and indirect mechanisms of plant growth-promoting bacteria (Timmusk *et al*., 2014).

PGPR mediated mechanisms for drought stress tolerance

Several mechanisms have been proposed for PGPR-mediated drought stress tolerance in plants. It includes phytohormonal activity, volatile compounds, alteration in root morphology, ACC deaminase activity, accumulation of osmolytes, EPS production, antioxidant defense and inoculations. The term Induced Systemic Tolerance (IST) has been coined to accommodate the microbial induced physical and chemical changes in plants, which result in enhanced tolerance to abiotic stresses (Vurukonda *et al.*, 2016).

Direct Mechanisms

PGPR's direct mechanism is the foremost step involved to support plant growth in a forward and direct manner. The natural mechanism includes nitrogen fixation, phytohormones production, phosphate solubilization, and increasing iron availability. These mechanisms influence the plant growth activity directly, but the ways it affects will vary from species to species and strain to strain. The presence of PGPR, direct enhancement of mineral uptake has been reported due to increases in specific ion fluxes at the root surface. Organic substances that stimulate plant growth are known as plant growth regulators. They stimulate plant growth by influencing the physiological and morphological processes at very low concentrations. Several microorganisms are capable of producing auxins, cytokinins, gibberellins, ethylene, or abscisic acid. Auxins are produced by several rhizobacterial genera, e.g., *Azospirillum, Agrobacterium, Pseudomonas,* and *Erwinia* (Bertrand *et al*., 2000).

Phytohormone production

Phytohormones are the chemical messengers that play a crucial role in natural growth and occur in low concentration. These phytohormones shape the plant, affecting seed growth, time of flowering, sex of flowers, senescence of leaves, and fruits. They also affect gene expression and transcription levels, cellular division, and growth. In targeted cells, phytohormones also regulate cellular processes, pattern formation, vegetative and reproductive development, and stress responses. Thus, all the major activities like forming a leaf, flowers, and the development and ripening of fruit are regulated and determined by hormones. To decrease the adverse effects of the environmental stressors caused due to growth-limiting ecological conditions, plants mostly attempt to adjust their endogenous phytohormone's levels. While this strategy is sometimes successful, rhizosphere microorganisms may also produce or modulate phytohormones under *in vitro* conditions so that many PGPB can alter phytohormone levels and thereby affects the plant's hormonal balance and its response to stress (Glick *et al.*, 2007).

Indole acetic acid

Indole-3-acetic acid (IAA) is one of the most common and the most studied auxins, and much of the scientific literature considers auxin and IAA to be interchangeable terms. Its main function is cell division, cell elongation, differentiation, and extension. But it has been known that plant responses to IAA vary from plant to plant in terms of sensitivity. Generally, IAA released by rhizobacteria interferes with many plant developmental processes because the endogenous pool of plant IAA may be altered by acquiring IAA that has been secreted by soil bacteria. The IAA production varied among them with *Pseudomonas* (94%), *Azospirillum* spp. (80%), *Azotobacter* spp. (65%) and *Bacillus* spp. (40%). Similarly, the Production of IAA by *Bacillus is* a general characteristic of rhizobacterial isolates (Agrawal and Agrawal, 2013).

IAA is synthesized by several independent biosynthetic pathways and mostly produced in the bud and young plant leaves. In young stems, IAA causes a rapid increase in cell wall extensibility. IAA seems to promote the growth of auxiliary bud and bud formation. There are several ways by which IAA supports the plant. IAA helps in apical dominance and also stimulates lateral and adventitious root development and growth. Besides product, IAA plays a crucial role in leaf and flower abscission. Thus, IAA can be considered significant auxin because it plays an overall role in growth stimulation by being involved in DNA synthesis.

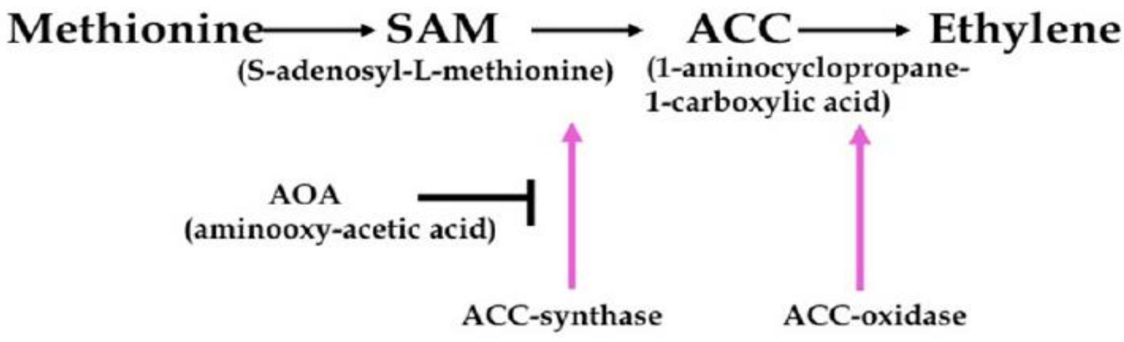

Fig. 2. Formation of ethylene from methionine (Kundan *et al*., 2015).

Tryptophan is an important molecule that alters IAA synthesis which is also identified as the primary precursor for IAA and thus plays a vital role in modulating the level of IAA biosynthesis. Tryptophan stimulates the IAA production and thus regulates the IAA biosynthesis by inhibiting anthranilate, a significant precursor for tryptophan because it seems to reduce IAA synthesis (Figure 2). Therefore tryptophan plays a vital role in IAA production by negative feedback regulation.

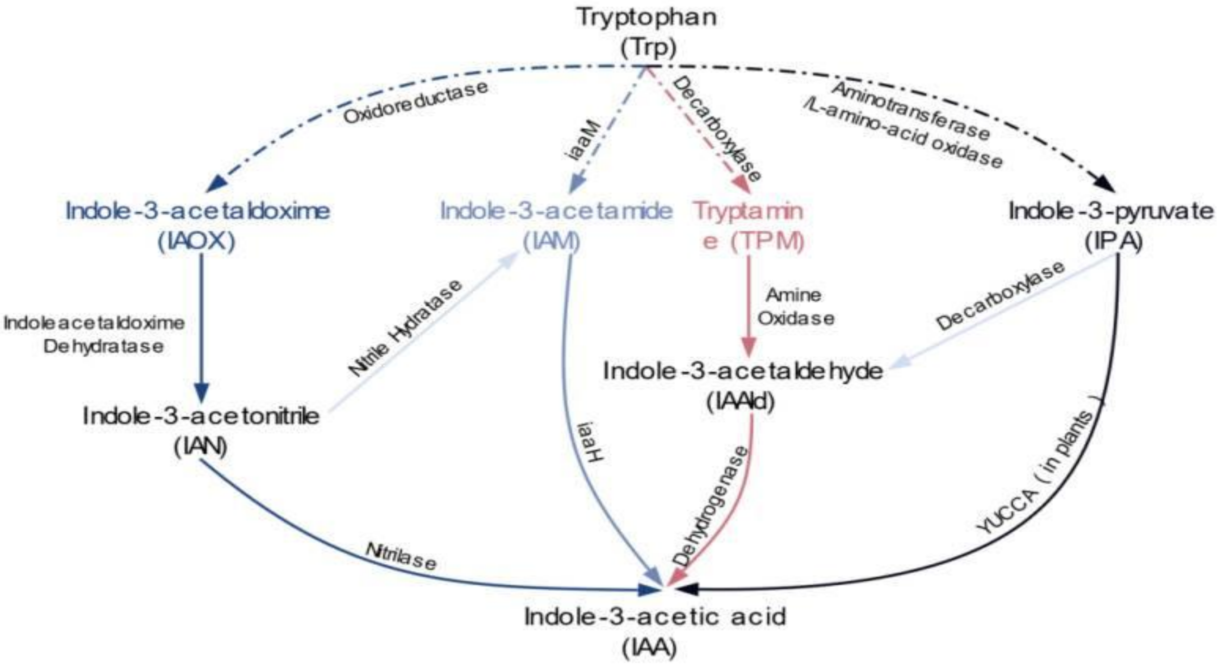

Fig. 3. IAA synthesis by tryptophan-dependent pathway (Zhang *et al*., 2019).

Ethylene

Ethylene is a central plant hormone regulating several aspects of plant growth and development, throughout the whole plant life cycle from germination to senescence. The hormone in the plants is the simplest molecule with a wide range of biological activities. It is produced by plants endogenously and induces different physiological changes in plants at the molecular level. The production of ethylene varies within the plant species and types of tissues. This gaseous hormone is formed by the breakdown of methionine present in all the cells (Figure 3). The production of ethylene is entirely dependent on its rate of production versus its rate of escaping into the atmosphere. It is produced more in dividing cells, mostly in darkness. It affects plant growth by root initiation, fruit ripening, seed germination, and inhibiting root elongation. The primary effect seen is fruit ripening and thus called the aging hormone in plants. Ethylene, because of its simple structure (C_2H_4), influences many aspects of plant growth and development. During severe conditions like extreme temperature, flooding, toxic metals, and radiation exposure, ethylene is synthesized. Under these stressed conditions, ethylene's endogenous production is induced more to harm root growth and eventually on the whole plant. Microbes can potentially influence all regulatory steps of the ethylene pathway. The most direct way of acting on ethylene signaling is to either directly produce or degrade ethylene. Several plant-associated microbes can increase plant ethylene levels by directly synthesizing ethylene or inducing plant ACS activity. Ethylene production by microbes was first reported in the pathogen *Ralstonia solanacearum.*

ACC deaminase production

1-aminocyclopropane-1-carboxylate (ACC) deaminase is a vital enzyme present in plant growth promoting rhizobacteria (PGPR), which regulates ethylene production by metabolizing ACC (an immediate precursor of ethylene biosynthesis in higher plants) into alpha-ketobutyrate and ammonia. Inoculation with PGPR combined with ACC deaminase activity could help promote plant growth and development under stress conditions by reducing stress-induced ethylene production. By lowering the abundance of the ethylene precursor ACC, the PGPR ACC activity is thought to decrease root ethylene production, which in turn can alleviate the repressing effect of ethylene on root growth. Ethylene that is synthesized as a response to various stresses is called "stress ethylene." This increases plant survival in such extreme conditions. Thus, the optimum growth under stressful condition introduction of ACC deaminase genes could be done to maintain ethylene level in plants. Currently, bacterial strains exhibiting ACC deaminase activity have been identified in a wide range of genera such as *Acinetobacter, Achromobacter, Agrobacterium, Alcaligenes, Azospirillum, Bacillus, Burkholderia, Enterobacter, Pseudomonas, Ralstonia, Serratia*and *Rhizobium,* etc., (Ahemad and Kibret, 2014).

Cytokinins and gibberellins

Cytokinins are phytohormones that promote cell division in plant roots and shoots. Their primary function is cell growth and differentiation. As they also affect apical dominance, so they are used by the farmers to increase the overall yield. Cytokinins help the plant by delaying the senescence or aging of tissues and thus affect the leaf growth. The cytokinin balance is influenced by other growth regulator's levels, e.g., auxins and environmental cues. Cytokinins counter the apical dominance induced by auxins; they, in conjunction with ethylene, promote abscission of leaves, flower parts, and fruits. Cytokinins can be produced in soil and pure culture by PGPR. This is an emerging alternative to enhance plant growth to improve yield and quality of crops, playing a crucial role in sustainable development.

Gibberellins are chemicals produced naturally by plants and are involved in several aspects of germination. They stimulate the enzyme (alpha amylase) and help in the hydrolysis of starch present in many seeds into glucose to be used in cellular respiration. Gibberellins are plant hormones that influence and control plant developmental processes like stem elongation, germination, dormancy, flowering, sex expression, and leaf and fruit senescence. Lastly, gibberellins act as a chemical messenger and help by breaking dormancy. Several studies revealed that many soil bacteria, in general, and PGPB in particular, can produce either cytokinins or gibberellins or both (Kundan *et al*., 2015).

Nitrogen fixation

Nitrogen fixation is the conversion of atmospheric nitrogen into utilizable nitrogen that changes to ammonia. This is essential for all life forms because nitrogen is the basic building block of plants. Biological nitrogen fixation generally occurs at mild temperatures by nitrogen-fixing microorganisms, which are widely distributed in nature. The nitrogenase complex is a complex enzyme that carries out the process of N_2 fixation. The nitrogenase structure was elucidated as a two-component metalloenzyme consisting of (i) dinitrogenase reductase, which is an iron protein, and (ii) dinitrogenase consists of a metal cofactor. Dinitrogenase reductase provides electrons with high reducing power, while dinitrogenase utilizes these electrons to reduce N_2 to NH_3.

This process consumes an enormous amount of energy in the form of ATP. The nitrogen fixation process requires the nitrogenase gene (*nif*), which is sensitive to oxygen; therefore, to prevent oxygen from inhibiting nitrogen fixation while at the same time providing sufficient oxygen for the bacteroids within the nodule to respire, it is possible to introduce bacterial hemoglobin, which binds free oxygen. The *nif* genes include structural genes that activate Fe protein, molybdenum, and other regulatory genes that are directly involved in the function and synthesis of enzyme and seem to be present in both symbiotic and free-living systems. The nif genes are genes encoding enzymes involved in the fixation of atmospheric nitrogen.

The primary enzyme encoded by the *nif* genes is the nitrogenase complex which is in charge of converting atmospheric nitrogen to other nitrogen forms such as ammonia, which plants can use for various purposes. Besides the nitrogenase enzyme, the nif genes also encode a number of regulatory proteins involved in nitrogen fixation. The expression of the nif genes is induced as a response to low concentrations of fixed nitrogen and oxygen concentrations (the low oxygen concentrations are actively maintained in the root environment). Nitrogen fixation is regulated by nif regulon, which is a set of seven operons which includes 17 nif genes. Nif genes have both positive and negative regulators. Some of nif genes are: Nif A, D, L,K, F,H S,U,Y,W and Z.

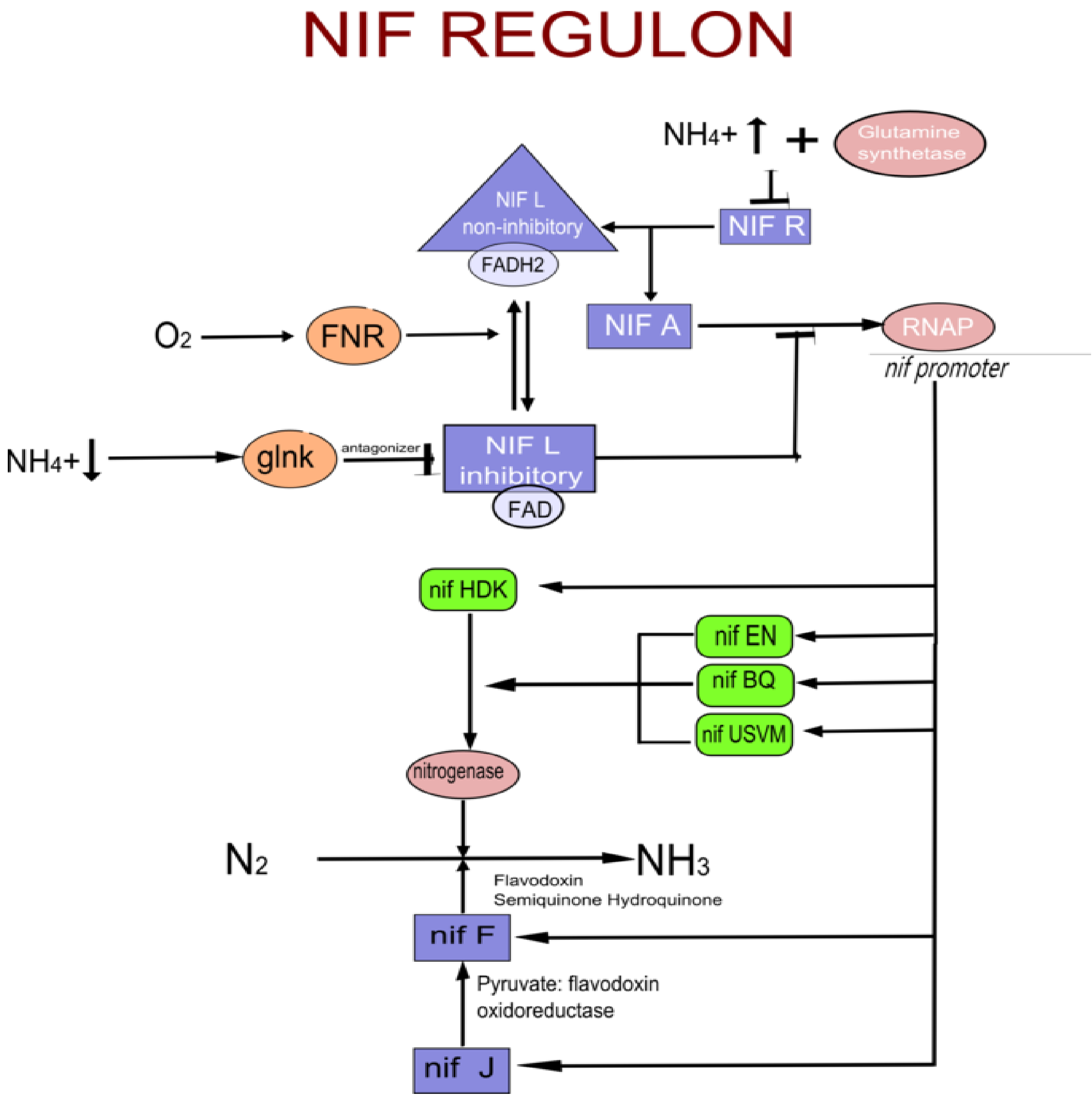

Fig. 4. The proteins in the nif regulon and the pathway of nitrogen fixation (Tilak *et al*., 2005).

Since nitrogen fixation is a very energy-consuming process, requiring at least 16 moles of ATP for each mole of reduced nitrogen, it would be beneficial if bacterial carbon resources are directed toward oxidative phosphorylation, which results in the synthesis of ATP, rather than glycogen synthesis, resulting in the storage of energy in the form of glycogen (Raymond *et al*., 2004).

Nitrogen fixers

A variety of bacterial species belonging to genera *Azospirillum*, *Alcaligenes*, *Arthrobacter*, *Acinetobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Pseudomonas*, *Rhizobium*, and *Serratiacolonize* with the plant rhizosphere can exert many beneficial effects on plant growth (Tilak *et al*., 2005). Biological nitrogen fixation contributes about 180×10^{6} metric tons/year of nitrogen globally, out of which symbiotic association produces 80%, and the rest comes from free-living or associative systems. The nitrogen fixers include symbiotic nitrogen fixers like *Rhizobium* (Figure 4). Inoculation of *Rhizobium* sp. causes a more significant increase in growth and yield of the plant. Also, the number of nodules per root system is significantly larger in plants inoculated with *Rhizobium* sp. than the plants without *Rhizobium* sp. under field condition. Rhizobia also mobilize inorganic and organic phosphorus.

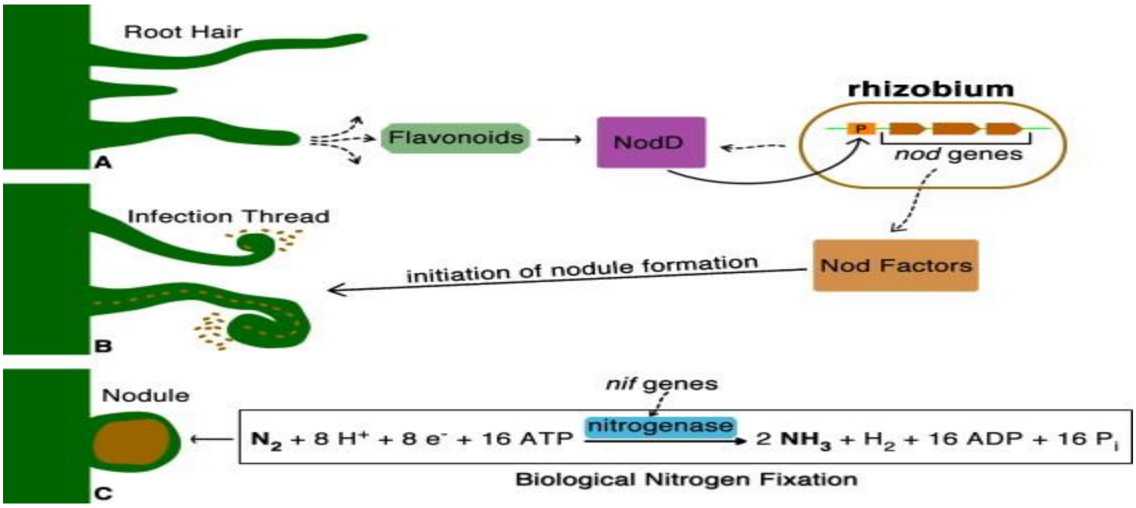

Fig. 5. Biological Nitrogen Fixation by *Rhizobium* sp. (Tilak *et al*., 2005).

Both phosphate-solubilizing strains and N_2-fixing bacterial strains have been found to have great ability to be formulated and used as biofertilizers. It also includes non-symbiotic nitrogen fixers, which have significant economic significance. *Azoarcus* sp, *Gluconacetobacter diazotrophicus*, *Herbaspirillium* sp., and *Azotobacter* sp. are important non-symbiotic nitrogen-fixing bacteria. *Azotobacter* is a free-living aerobic nitrogen fixer and has been reported to increase seedling's seed germination and growth. Out of all the, most abundant species in the rhizosphere is *Bacillus*. These strains release several metabolites that strongly affect the environment by increasing nutrient availability to the plants. *Bacillus subtilis* can maintain regular contact with higher plants and stimulate their growth (Garcia *et al*., 2004). *Pseudomonas* also acts as an efficient PGPR and the essential strain is fluorescent *Pseudomonas* species increases yield if used in combination with biofertilizers. *Pseudomonas putida* is also considered beneficial in growth promotion. Different nitrogen-fixing bacteria, along with their relationship with their host plants, are summarized in Table 1.

Table 1. Nitrogen-fixing bacteria and their relationship with the host plants (Kundan *et al*., 2015).

S.No.	PGPR	Relationship with Host	Suitable host plant
1.	*Azospirillum* sp.	Non-symbiotic	Rice, wheat, maize, sugarcane
2.	*Azotobacter* sp.	Non-symbiotic (aerobic)	*Paspalum notatum* grass, maize, wheat
3.	*Azoarcus* sp.	Non-symbiotic (aerobic/microaerophilic)	Kallar grass, sorghum
4.	*Acetobacter* sp.	Non-symbiotic (obligatory aerobic)	Sugarcane
5.	*Rhizobium leguminosarum*	Symbiotic (endosymbiotic)	Wheat, maize, barley
6.	*Bradyrhizobium betae*	Symbiotic	Sugar beets
7.	*Bradyrhizobium japonicum*	Symbiotic	Cowpeas, mung beans, soybeans
8.	*Burkholderia* sp.	Symbiotic (endo)	Rice

Phosphate solubilization

Phosphorus is another essential nutrient, and plants need an adequate amount of phosphorus for optimum growth. However, phosphorus is present mostly in insoluble forms and hence not able to support plants. Thus, both in PGPB and plant growth-promoting fungi such as mycorrhiza, solubilization, and phosphorus mineralization by phosphate-solubilizing bacteria are essential. The action of low molecular weight organic acids synthesized by various soil bacteria causes solubilization of inorganic phosphorus (Zaidi *et al*., 2009). Conversely, the synthesis of phosphorus, catalyzing phosphoric ester's hydrolysis, causes mineralization of organic phosphorus (Figures 5). Notably, both phosphate solubilization and mineralization can coexist in the same bacterial strain.

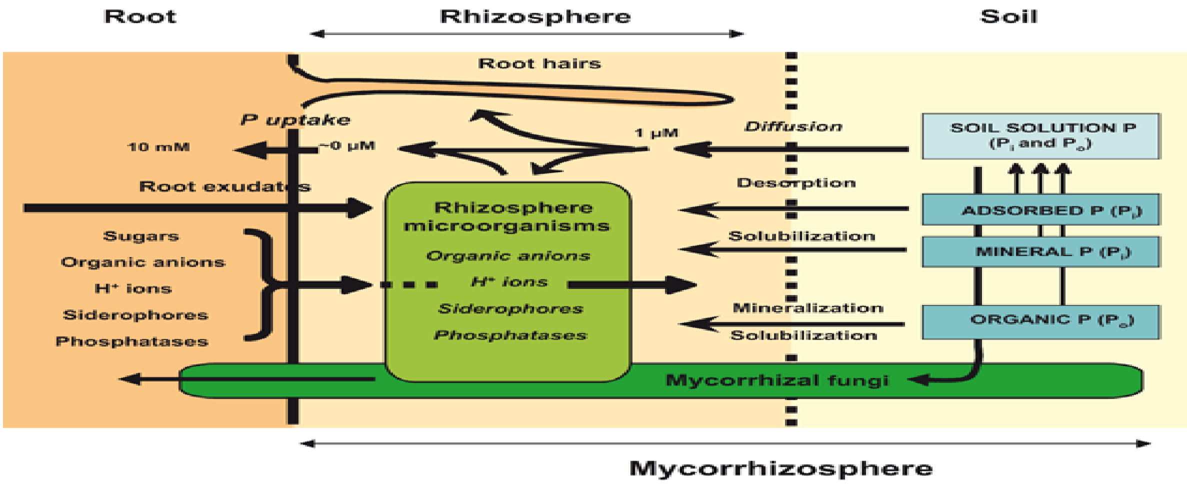

Fig. 6. Phosphate solubilizing bacteria in the rhizosphere zone (Tilak *et al.*, 2005).

The microorganism mediated solubilization of insoluble phosphates is associated with the organic acids detachment, which is often combined with other metabolites, as found in vitro, that the potential for phosphate solubilization by microorganisms is directly related to the production of siderophores, lytic enzymes, and phytohormones. Since most soils are deficient in phosphorus and phosphate fertilizer not affordable by farmers due to its high cost, this has led to the advantage of using soil microorganisms as inoculum for phosphate mobilization. Phosphate solubilizing bacteria are beneficial bacteria capable of hydrolyzing insoluble inorganic phosphorus into soluble organic phosphorus, which is absorbed as a nutrient by the plants. The most efficient phosphate solubilizing bacteria belong to genera *Bacillus*, *Rhizobium*, and *Pseudomonas.*

The form of phosphorus that the plants needed the most for growth and development is present in the inorganic form. It is made available to the plants by converting it into a soluble form by PSB (phosphate solubilizing bacteria), and inoculating plants with PSB increases growth and yield directly. Bacteria generally use two mechanisms to solubilize phosphate, *i.e.*, 1) releasing organic acids and affecting phosphorus mobility through ionic interactions. 2) using phosphatases that help to unbind the phosphate groups from organic matter. These mechanisms are most beneficial in bare soils (Gyaneshwar *et al.*, 2002). In addition to lowering the rhizospheric pH, PSB dissolves the soil phosphate by producing low molecular weight organic acids such as gluconic and ketogluconic acids. The rhizospheric pH is lowered through biotic production of proton/bicarbonate release (anion/cation balance) and gaseous (O_2/CO_2) exchanges. The phosphorus solubilization ability of PSB has a direct correlation with the pH of the medium.

Sequestering iron

Iron is one of the most abundant elements on the earth but still is not readily available to the plants because it is present as ferric ions with very low solubility and is the predominant form of iron in nature. Plants require a large amount of iron. The microorganisms surviving under aerobic conditions also need iron for essential purposes, including heme formation and ATP synthesis. Some bacteria have developed iron uptake systems**.** These systems involve the production of siderophores. Siderophores are microbial Fe-chelating low molecular weight compounds. Siderophores are released by microbes to scavenge iron from these mineral phases by forming soluble Fe^{3+} complexes taken up by active transport mechanisms. Still, the mechanism of siderophores is dynamic only under the low availability of iron. Siderophores are usually stable complexes and can be of different types such as hydroxamates, phenolcatecholates, and carboxylates. Siderophore mediated iron scavenging in gram-negative transport is better studied PGPR than gram-positive PGPR. There are about 500 known siderophores of which chemical structures of 270 of these compounds have been determined (Hider and Kong, 2010). In case of stresses such as heavy metal pollution, siderophores help the plants to bear these stresses besides scavenging iron from the surrounding and making mineral availability to microbes.

Indirect mechanisms

Antibiotic production and lytic enzymes

The indirect mechanism involves the ability of PGPR to reduce the harmful effects of plant pathogens on the growth. This consists of synthesizing the lytic enzymes, including chitinases, cellulases, 1,3-glucanases, proteases, and lipases that can lyse a portion of many pathogenic fungi cell walls. Also, different antibiotics are produced in response to the proliferation of plant pathogens. But excess dependence on antibiotic-producing bacteria as biocontrol agents may be a disadvantage because of the resistance developed against specific antibiotics. The production of one or more antibiotics is the most commonly associated mechanism with plant growth-promoting bacteria to act as antagonistic agents against phytopathogens (Glick *et al*., 2007). The antibiosis mechanism is to produce low molecular weight compounds that are harmful and critical to major enzymes and metabolism of other microorganisms and thus retards the growth.

Induced systemic response (ISR)

There is another mechanism called induced systemic resistance (ISR). This is the mechanism of increased resistance at particular sites of plants at which induction had occurred. The defense mechanism of ISR is activated only when there is an attack of a pathogenic agent. ISR is not specific against particular pathogens but helps the plant to control diseases. ISR involves jasmonate and ethylene signaling within the plant and these hormones stimulate the host plant's defense responses to a range of pathogens (Verhagen *et al.*, 2004). Another mechanism is the siderophore production which prevents plants from some pathogens to acquire an adequate amount of iron and suppresses their ability to grow. It is reported that this mechanism is effective because of the siderophores produced by biocontrol PGPB that show a much greater affinity for iron as compared to fungal pathogens [69]. Therefore the indirect mechanism seems to be beneficial both in terms of understanding the mechanism of biocontrol bacteria and the use of bacterial strains instead of harmful chemical pesticides.

HCN production

The deleterious *Rhizobacteria* act as biocontrol agents of weeds that can colonize plant root surfaces and suppress plant growth. Cyanide being toxic is produced by most microorganisms, including bacteria, algae, fungi, and plants, as a means of survival by competing with their counterparts. Generally, there is no adverse effect on the host plants by inoculation with cyanide-producing bacterial strains, and host-specific *Rhizobacteria* can act as biological weed control agents (Zeller *et al*., 2007). The secondary metabolite produced, which acts as an effective agent for the biocontrol of weeds, is HCN, which is mostly synthesized by *Pseudomonas* and *Bacillus* species. HCN is likely to inhibit the electron transport chain and energy supply to the cell, leading to cells' death. It also seems that PGPR inhibits the proper functioning of enzymes and natural receptors reversible mechanism of inhibition and is also known to inhibit cytochrome oxidase.

Competition

PGPR sometimes competes with the harmful microbes for the nutrient present in trace amounts and can limit the disease-causing agent. This can be explained when there are abundant non-pathogenic microbes in the soil, which would rapidly colonize plants' surfaces and utilize nutrient availability and, therefore, inhibit the growth of pathogenic microbes. Some PGPR with their biocontrol properties is listed in Table 2. These mechanisms are considered critical because they are challenging to study in the system, but competition for the nutrient between PGPR and pathogens is regarded as the most vital interaction that indirectly supports the growth stimulation of the plants by inhibiting the growth of pathogens (Kundan *et al*., 2015).

PGPR induced molecular mechanisms under drought stress

In water deficit conditions, gene induction forms two different proteins: functional proteins and regulatory proteins. Functional proteins include mRNA binding proteins, LEA proteins, water channel proteins, enzymes for osmolytes biosynthesis, proteases. They function directly in abiotic stresses. On the other hand, regulatory proteins include protein kinase, calmodulin-binding protein, phosphatase, and other transcription factors. These are involved in stress-responsive genes expression and signal transduction. HSPS are heat shock proteins that inhibit the misfolding of protein and are classified according to their molecular weight. LEA proteins are the proteins that accumulate during the late embryonic phase in response to abiotic stress. Plants inoculated with PGPR helps in up-regulation of stress tolerance inducing genes. Various molecular strategies have established the mechanism of microbes induced gene expression modulation for abiotic stress tolerance. Molecular networks of signal transduction genes are also involved in drought stress responses. Different molecular techniques give a vast amount of information about induced genes expressions and pathways during plant and rhizobacteria interactions. The methods include high throughput whole-genome gene expression such as microarrays, proteomics, RNA sequencing, 2D-PAGE, differential display. This helps explore the physiological functions of such genes and tolerance induced by PGPR (Paterson *et al*., 2017).

PGPR acts as a biofertilizers

Biofertilizer is a substance that contains living microorganisms that, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the plant's interior and promotes growth by increasing the supply or availability of primary nutrients to the host plant. Biofertilizers have a natural mechanism to supply nutrients to plants by solubilizing phosphorus, nitrogen fixation, and by the synthesis of plant growth-promoting substances. There are microbes present in biofertilizers that increase the soil's natural nutrient cycle and help in building soil organic matter and maintain soil fertility. One of the preferred microorganisms (bacteria) that have gained worldwide acceptance as beneficial bacteria is PGPR. Some bacteria can promote growth by acting as biofertilizers as well as biocontrol agents. The main advantage of using biofertilizers is cheaper and safer than chemical pesticides (Vessey *et al*. (2003).

Co-inoculation of the plant with PGPR

To have a beneficial effect on plants, it is essential to introduce PGPR into the soil. PGPR strains, when inoculated with soil, seem to have a positive impact on the stimulation of growth. PGPR stimulates growth by acting as a biofertilizer for growth promotion and biocontrol agents to control disease management. Climatic variations also influence PGPR effectiveness, but sometimes unfavorable growth conditions in the field are to be expected as the normal functioning of agriculture. **(***Azospirillium*, *Bacillus*, *Burkholderia*, *Klebsiella*, *Pseudomonas* have a direct effect as PGP trait through phytohormones production, nitrogen fixation, and phosphorus uptake. The first step of these bacteria in growth promotion is by colonizing the root. PGPR plays a vital role in the host nodulation response (Remans *et al*., 2007). Besides the direct effect, PGPR affects the plant by controlling pathogens, which are mostly involved in competition and production of metabolites that affect the pathogen directly by siderophores production, lytic enzymes, and antibiotics production and by induced systemic resistance. Induced systemic resistance (ISR) or systemic acquired resistance (SAR) is defined as the activation of the plant host's chemical and physical defenses by an inducer that could be a chemical or a microorganism the control of several pathogens. Therefore, introducing the PGPR beneficial strain into the soil or even treating the root, leaf, or plant part with PGPR will positively affect growth stimulation.

Strategies for enhancing rhizosphere colonization by PGPR Inoculants

Under field conditions, other external factors come into play, and soil bacteria's ability to elicit positive effects on plant growth can be impaired and so that the impact of applying specific PGPM can be variable. The plant rhizosphere is colonized by microorganisms from the soil and the seed. The determinants of soil microorganisms are based on C and N availability, organic matter content, water availability and pH, and biogeographic patterns, including soil type and seasonality. Hence it is necessary to develop strategies for effective inoculation methods so that bacteria of interest gain an advantage in colonization efficiency. Product quality, compatibility, and stability determine effective colonization and consistent inoculum performance under field conditions (Lee *et al*., 2016).

Challenges in PGPR strains selection and characterization

The process of applying *Rhizobacteria* in soil and plant parts to eradicate bacterial and fungal pathogens was pioneered in the Soviet Union in 1958 even though the selection of productive PGPR strains was highly complicated. Selecting the appropriate strain is essential so that the most beneficial bacteria are screened for the experiment to be successful. For this purpose, effective strategies need to be considered. The strategy can be selecting the specific PGPR strain from thousands of root colonizing bacteria and testing their efficacy for plant growth promotion. The plant parts can then be treated with the strain chosen for monitoring the effects. Recently, a mass screening technique has been used to select efficacious PGPR strains (Compant *et al*., 2005). Here physiological, nutritional, and biochemical characteristics, as in Bergey's Manual of Determinative Bacteriology, are used for the primary screening of new isolates. The host plant specificity of adaptation to a particular soil, climatic conditions or pathogen should be considered in selecting the isolation conditions and screening assays. Other approaches can be selected based on traits like antibiotic production, siderophores production, and root colonization. The selection of superior strains can be facilitated by developing high throughput assay systems and adequate bioassays.

CONCLUSION

Drought stress is a severe environmental constraint to agricultural productivity. PGPR plays a vital role in conferring resistance and adaptation of plants to drought stresses and has a potential role in solving future food security issues. The interaction between plant and PGPR under drought conditions affects the plant and changes the soil properties. The mechanisms elicited by PGPR, such as triggering osmotic response and induction of novel genes, play a vital role in ensuring plant survival under drought stress. The development of drought-tolerant crop varieties through genetic engineering and plant breeding is essential, but it is a long drawn process, whereas PGPR inoculation to alleviate drought stresses in plants opens a new chapter in applying microorganisms in dryland agriculture. Taking the current leads available, concerted future research is needed to identify the right kind of microbes and address the issue of delivery systems and field evaluation of potential organisms. The use of bacterial fertilizers has made significant improvements in growth, health, and yield of plants. The mechanism by which PGPR stimulates can be direct or indirect. PGPR also supports development by reducing the phytopathogens, which reduce the yield and growth. The outcome of PGPR inoculation is greatly influenced by plant age and the chemical, physical and biological properties of the soil. Hence prospects can be replaced by chemical fertilizers and supporting the ecosystem in terms of safety. Further understanding of PGPR's complete mechanism could help obtain a more specific strain that will work under more adverse and varying conditions.

REFERENCES

Agrawal, P.K. and Agrawal, S. (2013). Characterization of *Bacillus* sp. strains isolated from rhizosphere of tomato plants (*Lycopersicon esculentum*) for their use as potential plant growth promoting rhizobacteria. *International Journal of*

Current Microbiology and Applied Sciences. 2: 406-417.

Ahemad, M. and Kibret, M. (2014). Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. *Journal of King Saud University- Engineering Sciences*. 26: 1-20.

Ali, S.K.Z., Sandhya, V. and Rao, L.V. (2014). Isolation and characterization of drought tolerant ACC deaminase and exopolysaccharide producing fluorescent Pseudomonas sp. *Annals of Microbiology*. 64: 493-502.

Babou, C. and Lisna, T. (2019). Liquid bio-fertilizer formulated from coconut and its effect on growth and root characteristics of robusta coffee seedlings under drought conditions. *Journal of Plant Development Sciences*. 11 (1): 61-64.

Berendsen, R.L., Pieterse, C.M.J. and Bakker, P.A.H.M. (2012). *Trends in Plant Science*. 17: 478-486.

Bertrand, H., Plassard, C., Pinochet, X., Toraine, B. and Normand, P. (2000). Stimulation of the ionic transport system in *Brassica napus* by a plant growth-promoting rhizobacterium. *Canadian Journal of Microbiology*. 46: 229-236.

Cavicchioli, R., Ripple, W.J., Timmis, K.N., Azam, F., Bakken, L.R., Baylis, M., Behrenfeld, M.J., Boetius, A., Boyd, P.W., Classen, A.T and Crowther, T.W. (2019). Scientists' warning to humanity: microorganisms and climate change. *Nature Reviews Microbiology*. 17 (9): 569-586.

Compant, S., Duffy, B., Nowak, J., Clement, C. and Barka, E.A. (2005). Use of plant growth promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. *Applied and Environmental Microbiology*. 71: 4951-4959.

Garcia, J.A.L., Probanza, A., Ramos, B., Palomino, M.R. and Manero, F.J.G. (2004). Effect of inoculation of *Bacillus licheniformis* on tomato and pepper. *Agronomie for Sustainable Development*. 24: 169-176.

Glick, B.R., Cheng, Z., Czarny, J. and Duan, J. (2007). Promotion of plant growth by ACC deaminase producing soil bacteria. *European Journal of Plant Pathology*. 119: 329-339.

Gyaneshwar, P., Kumar, G.N., Parekh, L.J. and Poole, P.S. (2002). Role of soil microorganisms in improving P nutrition of plants. *Development in Plant and Soil Science*. 95: 133-143.

Hider, R.C. and Kong, X. (2010). Chemistry and biology of siderophores. *Natural Product Reports*. 27: 637-657.

Kalariya, K.S.A., Singh, A.L., Nakar, R., Zala, P.V., Chakraborty, K. and Patel, C.B. (2019). Physiological plasticity of 60 cultivars of *Arachis hypogaea* under natural drought conditions of semiarid region in india. *Journal of Plant Development Sciences*. 11 (1): 29-37.

Kasim, W.A., Osman, M.E., Omar, M.N., Abd El-Daim, I.A., Bejai, S and Meijer, J. (2013). Control of drought stress in wheat using plant growth promoting bacteria. *Journal of Plant Growth Regulation*. 32: 122-130.

Kundan, R., Pant, G., Jadon, N. and Agrawal, P.K. (2015). Plant growth promoting rhizobacteria: mechanism and current prospective. *Journal of Fertilizers and Pesticides*. 6 (2): 155-165.

Lee, S.K., Lur, H.S., Lo, K.J., Cheng, K.C., Chuang, C.C. and Tang, S.J. (2016). Evaluation of the effects of different liquid inoculant formulations on the survival and plant growth promoting efficiency of *Rhodopseudomonas palustris* strain PS3. *Applied Microbiology and Biotechnology*. 100: 7977-7987.

Marulanda, A., Barea, J.M. and Azcon, R. (2009). Stimulation of plant growth and drought tolerance by native microorganisms (AM fungi and bacteria) from dry environments: mechanisms related to bacterial effectiveness. *Journal of Plant Growth Regulation*. 28 (2): 115-124.

Paterson, J., Jahanshah, G., Li, Y., Wang, Q., Mehnaz, S. and Gross, H. (2017). The contribution of genome mining strategies to the understanding of active principles of PGPR strains. *FEMS Microbiology Ecology*. 93: 1-31.

Rahdari, P., Hoseini, S.M. and Tavakoli, S. (2012). The studying effect of drought stress on germination, proline, sugar, lipid, protein and chlorophyll content in Purslane (*Portulaca oleraceae* L.) leaves. *Journal of Medicinal Plants Research*. 6: 1539-1547.

Raymond, J., Siefert, J.L., Staples, C.R. and Blankenship, R.E. (2004). The natural history of nitrogen fixation. *Molecular Biology and Evolution*. 21: 541-554.

Remans, R., Croonenborghs, A., Gutierrez, R.T., Michiels, J. and Vanderleyden, J. (2007). Effects of plant growth promoting rhizobacteria on nodulation of *Phaseolus vulgaris* (L.) are dependent on plant P nutrition. *European Journal of Plant Pathology*. 119: 341- 351.

Selvakumar, G., Panneerselvam, P. and Ganeshamurthy, A.N. (2012). Bacterial mediated alleviation of abiotic stress in crops. In: Maheshwari, D.K. (Ed.), Bacteria in Agrobiology: Stress Management. Springer-Verlag, Berlin Heidelberg. Pp. 205-224.

Tilak, K.V.B.R., Ranganayaki, N., Pal, K.K., De, R. and Saxena, A.K. (2005). Diversity of plant growth and soil health supporting bacteria. *Current Science*. 89: 136-150.

Timmusk, S., Islam, A., Abd El, D., Lucian, C., Tanilas, T. and Kannaste, A. (2014). Drought tolerance of wheat improved by rhizosphere bacteria from harsh environments: Enhanced biomass production and reduced emissions of stress volatiles. *PLoS One*. 9: 1-13.

Van der Heijden, M.G.A., Bardgett, R.D. and Van Straalen, N. M. (2008). *Ecology Letters*. 11: 296-310.

Verhagen, B.W., Glazebrook, J., Zhu, T., Chang, H.S. and Van Loon, L.C. (2004). The transcriptome of rhizobacteria induced systemic resistance in arabidopsis. *Molecular Plant Microbe Interactions*. 17: 895-908.

Vessey, J.K. (2003). Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil*. 255: 571-586.

Vurukonda, S.S.K.P., Vardharajula, S., Shrivastava, M. and SkZ, A. (2016). Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. *Microbiological Research*. 184: 13-24.

Williams, A. and de Vries. F.T. (2020). Plant root exudation under drought: implications for ecosystem functioning. *New Phytologist*. 225: 1899-1905.

Zaidi, A., Khan, M.S., Ahemad, M. and Oves, M. (2009). Plant growth promotion by phosphate solubilizing bacteria. *Acta Microbiologica et Immunologica Hungarica*. 56: 263-284.

Zeller, S.L., Brand, H. and Schmid, B. (2007). Host plant selectivity of rhizobacteria in a crop/weed model system. *PLOS One*. 2: 846.

Zhang, P., Jin, T., Kumar, S.S., Xu, J., Shi, Q., Liu, H. and Wang, Y. (2019). The distribution of tryptophan dependent indole-3-acetic acid synthesis pathways in bacteria unraveled by large scale genomic analysis. *Molecules*. 24 (7): pp 1411-1421.