

Chapter 16

Improving Resilience Against Drought Stress Among Crop Plants Through Inoculation of Plant Growth-Promoting Rhizobacteria



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1 Introduction

Crop plants in arid and semiarid regions come across different abiotic stresses under field conditions such as water shortage, salinity problem, and high temperature (Tester and Bacic 2005; Liu et al. 2020). Along with all these types of stresses, the most severe and adverse factor in the whole world is the water-deficit conditions. It has been estimated that drought stress would result in about 50% loss in crop yield, specifically in the arid and semiarid areas of the world by 2050. Drought-stressed vegetation usually suffers water stress along with less nutrient uptake efficiency, less photosynthesis, disturbance in hormone balance, and enhanced production of reactive oxygen species (ROS) (Husen 2010; Husen et al. 2014; Getnet et al. 2015; Embiale et al. 2016; Siddiqi and Husen 2017, 2019). In response to drought stress, crop plants produce antioxidants and osmoprotectants that impede them in coping with stress conditions. Stress involves hormonal changes, and imbalance results in enhanced production of abscisic acid (ABA), minute reduction in indole acetic acid (IAA), and gibberellins (GA₃) concentration along with a rapid decrease in the zeatin level in plant leaves. The endogenous level of cytokines decreases with an increase in drought stress, resulting in triggered response in enhanced ABA level of shoots and increased ethylene contents of roots.

Numerous microbes exist in the region of the plant roots, that is, rhizosphere that forms a multifarious ecological population, impacting plant growth and productivity by their various metabolic activities and interactions with crop plants (Berg 2009; Lugtenberg and Kamilova 2009; Schmidt et al. 2014). In the vicinity of roots, structural changes occur in bacterial communities associated with the plant that select their assemblage as an adaptation toward abiotic stresses, help to improve resistance toward stress to endorse healthiness and tolerance from drought (Schmidt et al. 2014; Cherif et al. 2015; Naveed et al. 2020; Sabir et al. 2020). Presently, there are many techniques to lessen the drought stress/ water deficit and these are chemical, biological, and physical approaches. Several physicochemical strategies have been recommended by researchers to enhance water stress tolerance among crop plants. Furthermore, for soil water conservation and to make its efficient use, some agronomic practices such as bed planting, deep tillage, mulching, and the cover crops have been adopted. Likewise, foliar spray of glycine betaine osmolytes, proline, and phytohormones such as abscisic acid and cytokinins is found favorable to enhance plant enlargement and facilitate them for the recuperation in the stress, but all of these approaches are found to be costly, vigorous, as well as labor intensive. On the other hand, biological approaches could prove inexpensive and efficient. If we proceed toward the development of stress-tolerant varieties, it may take a long duration to develop a new variety, which will show adaptation to target; on the contrary, there is an alternative to all these time-consuming and expensive approaches that a tiny creature in the soil can do this in a short time and much more economically. Every technique is sound and has its good side along with consequences. However, the use of a biological approach that involves the inoculation of microbes

has been found sustainable and environment-friendly. In this chapter, the main focus has been given to the rhizobacteria involved in improving drought tolerance in crop plants, their mechanisms of action, and prospects for sustainable agriculture under drought stress.

2 Effect of Drought Stress on Crop Plants

Drought stress has negative effects on crop plants, for example, on turgor pressure and plant–water potential, which is sufficient to obstruct regular functioning (Hsiao and Xu 2000), along with changes in the morphological as well as physiological characteristics of the crop plant (Rahdari and Hoseini 2012). Drought stress contributes to about 15–35% variation in the yield of wheat, grain crops, and oilseed crops. Furthermore, it also has an effect on growth stages like flowering and tillering, which need water critically. It is because many physiological and biochemical changes occur in the crops that affect the metabolic activities like reduced water-use efficiency, lessening of the leaf area, poor root growth, and less stem elongation (Farooq et al. 2009). The water-deficit condition also affects the opening and closure of the stomata that can reduce CO₂ levels drastically in the chloroplast (Farooq et al. 2009).

In addition to this, drought results in the reduced vacuole and cytosol volume. In the period of drought, reactive oxygen species have distorted protein and lipids composition results to adversely influence the plant's usual metabolism, ultimately harming plant's growth (Bartels and Sukar 2005). Ma et al. (2012) have practically proved that wheat crop development was adversely influenced by stress caused through drought by the shortage of water as all of the nutrients needed by plants for optimum growth are taken up by plants in dissolved form despite the fact that water is necessary to maintain the turgor pressure of the plant. Other abiotic stresses like salinity along with drought are the foundation for osmotic stress along with imbalance of ions which leads to dehydration, disintegration of the cellular membrane, and in the solute leakage, eventually affecting badly the growth of plant (Niamat et al. 2019; Rizwan et al. 2021). Gill and Tuteja (2010) also revealed that during stress conditions, reactive oxygen species like oxygen (O[·]), superoxide (O₂⁻), hydrogen peroxide (H₂O₂), and the hydroxyl ion (OH⁻) production badly spoil the lipid-protein and the DNA through the oxidation process. The drought stress results in reduction in the seed number and seeds acquiesce at flowering, bud formation, and anthesis stage (Hadi et al. 2012). Yan and Shi (2013) also concluded that wheat fresh weight, dry mass, length of root, and plant height all decline drastically in the drought stress; furthermore, production loss is bloated up as the period of drought increases.

3 Strategies to Combat the Drought Stress in Plants

Presently, many approaches have been found successful under drought stress conditions, for example, application of genetic engineering including various biotechnological approaches and conservative breeding techniques, a combination of diverse strategies along with a selection of variety and microbial usage, especially rhizobacteria with drought stress tolerance.

Conventional breeding is thought to be a classical approach just to make crops tolerant to drought stress. In this technique, genetic variability under drought conditions is defined by testing the germplasm and after that, unlike mating design is followed to introduce beneficial traits in novel cultivars and lines. Thus, conventional breeding has been found useful in mitigating drought stress among crop plants (Howarth 2005). However, it is very costly and time consuming.

The biotechnological approach refers to the manipulation of crop genes into consideration to build up a better variety that could survive in an improved way under different climatic conditions or it could be said that the development of variety that will be better adoptive to climatic changes. Biotechnological approach and molecular breeding have been found better, as these give good results in a shorter period of instance than the other breeding techniques like conventional breeding. It has been chosen as the better technique than conventional breeding, as it lessens the breeding cycle of the plant and advances its selection competence genetically, and therefore boosts the potential of the crop to bear drought and the salinity stress as well (Ashraf 2010; Ditta 2013; Shahzad et al. 2019). At the same time, these techniques are time-taking, costly, and labor-intensive. Furthermore, another genetic method is the management of guard cells to reduce the water use of the product so as to enhance the tolerance against stress (Schroeder et al. 2001). Just to make plants tolerant of diverse abiotic stresses, most scientific research is concentrated on genetic engineering and further molecular techniques, but a combination of these techniques would be beneficial and these are most desirable (Varshney et al. 2011).

Different agronomic approaches are also being adopted to alleviate the negative impact of water-deficit condition on crops like customized irrigation methods as sprinkler and furrow and drip irrigation system. The purpose of these methods is to adopt good agronomic strategies to manage and preserve water from diverse sources like snow and rain. As in the growing season, upper layer residues are used to preserve the soil moisture (Nezhadahmadi et al. 2013). Todd et al. (1991) found that evaporation declines all through the season when residues of wheat are used as mulch just as it slows down the water movement and so reduces the evaporation, which ultimately decreases moisture loss. Crop rotation in addition to this is also a good technique to reduce drought effects such as wheat in winter which decrease the irrigation requirement, as it preserves water required by the plants. Among different approaches, the use of rhizobacteria is thought to be a cost-effective and environment-friendly solution to alleviate drought stress among crop plants.

4 Plant–Microbe Interaction

In agriculture, microbial use, for example, rhizobacteria, can prove a constructive approach to alleviate the undesirable effects of various abiotic stresses such as drought stress. Microbial usage for the alleviation of drought stress is an environment-friendly approach. Plant growth-promoting rhizobacteria (PGPR) take possession of plant roots and augment plant growth by straight and meandering mechanisms (Ditta et al. 2015, 2018a, b; Sarfraz et al. 2019; Ullah et al. 2020). The result of the inoculation of PGPR strains such as *Acinetobacter calcoaceticus* SE370 and *Burkholderia cepacia* SE4 @15% polyethylene glycol (PEG) level of drought showed significant increases in the relative water contents, protein level, and amino acids in cucumber plants (Kang et al. 2014).

Considerable upgradation was established in the potential of water and relative water contents once the seed of wheat crop was inoculated with *Azospirillum brasiliense* (Creus et al. 2004). To alleviate water-deficit conditions by using PGPR, an experiment in the warehouse was carried out, and four pea seeds were sown in each pot coated with two selected strains of PGPR having a 1-aminocyclopropane-1-carboxylate (ACC)-deaminase enzyme. Results demonstrated a reduction in consequence of drought stress on the growth and capitulation of peas just because of inoculation with the PGPR accompanying ACC-deaminase (Zahir et al. 2008). Seedlings of maize inoculated with the *Azospirillum* resulted in additional stress tolerance, accumulation of proline than that of plants that are un-inoculated in the drought stress (Casanovas et al. 2002). In the same way, *Azospirillum lipoferum* strain secluded from the soil water deficit was inoculated on maize seeds and the rhizosphere application scheduled to two maize varieties headed for lessening the drought stress effects. Outcomes have shown that 54.54% amplification in the open amino acids and 63.15% inside the soluble sugar levels took place because of the inoculation of rhizobacteria (Bano et al. 2013). Along with the PGPR, the *Rhizobium* usage to allay stresses like drought stress in the cereals is thought to be a cost-effective and environmentally friendly resolution. Webster et al. (1997) have stated that the *Rhizobium* sp. colonize the rhizosphere of wheat crop and other cereals ultimately promoting the growth of the plant employing various straight and many tortuous mechanisms just as the creation of auxin, metabolites, ACC-deaminase, and siderophores by increasing the action of nitrogenase enzyme proficiently under different types of stress reminiscent of drought. Lettuce inoculated with the mycorrhizal arbuscular fungi (*Glomus mosseae*) along with *Pseudomonas mendocina* amplified the proline buildup, root phosphatase plus the antioxidants (catalase and the peroxidase) working in the stresses like drought (Kohler et al. 2008).

5 PGPR and Crop Plants Under Drought Stress

Rhizobium usage to diminish drought stress is an excellent strategy to improve crop productivity under changing climatic conditions (Saleh Al-Garni 2006). In the rhizosphere, the *Rhizobial* interface among crop plants is incredibly significant. Plants interact with *Rhizobia* in symbiotic or otherwise associative interaction. Usually, atmospheric nitrogen is fixed by *Rhizobia* in the nodules of legumes, which is very beneficial for legumes. However, rhizobacteria that reside in the root zone of non-legumes also have the potential to maintain plant growth and can be used as PGPR (Shakir et al. 2012; Noel et al. 1996; Hussain et al. 2019; Ullah et al. 2019).

Rhizobium belongs to family Rhizobiaceae and is involved in the conversion of atmospheric-N, that is, N_2 into ammonia (NH_3), the process known as biological nitrogen fixation (BNF). On the other hand, apart from the atmospheric N fixation in the legumes, rhizobacteria also play an important role in enhancing the growth and productivity of the non-legumes (Hussain et al. 2009; Ullah et al. 2016). *Rhizobia* bring on forbearance and confrontation to stress among plants that are crucial for plant development under critical environmental conditions such as drought stress. Siderophores manufacturing, which is of low molecular weight and the organic compounds that chelates the iron (Fe) and Zn, production of phytochrome, enzymes biosynthesis to endorse plant development and perk up the nutrients uptake and their accessibility are important mechanisms behind improvement of growth and productivity of non-leguminous crop plants (Zahir et al. 2008; Zeb et al. 2018; Hussain et al. 2020). *Rhizobia* application to non-legumes plants such as maize, wheat, and rice can amplify the drought tolerance via improving the root morphology along with the rate of transpiration improvement that increases the uptake of nutrients under the drought stress conditions to benefit the plant growth.

Rhizobia also activate “Induced Systemic Resistance” (ISR), which helps in provoking various biochemical and physiological transformations among crop plants to bear diverse abiotic stresses such as drought stress (Yang et al. 2009). Two main methods adopted by *Rhizobium* for movement of water in the membrane of cells under osmotic stress are as follows: (1) under the minute osmotic level, solute concentration is sustained by simple diffusion, whereas (2) speedy water movement is synchronized with aquaporin, known as water-specific channels (Bremer and Kramer 2000). *Rhizobium* possesses various mechanisms to enhance abiotic stress tolerance. Among them, primarily, the significant method is the secretion of low-molecular weight compounds for osmotic regulation in the cytoplasm. Further reaction of *Rhizobium* involves fluctuation in concentration of ions, provoking proteins produced under stress, and the osmolytes accumulation just as glycine betaine (Bano and Fatima 2009). Very normally, under drought stress, it is considered with the aim of investigating how *Rhizobium* or the further bacteria come across to conditions like hyper- and hypo-osmosis. In this condition, mechanosensitive channels are used by bacteria to sense the cell membrane tension that allows water and the solutes to break out even with a little difference (Poolman et al. 2002). Similar to legumes, *Rhizobia* also invade into roots of the cereals and act as natural endophyte

where these produce vitamins, riboflavin, and phytohormones, namely auxin, gibberellins, cytokinins, and abscisic acid (ABA) that play a specific role in the maintenance of plant health and vigor under stress conditions (Dakora 2003). Furthermore, Nichols et al. (2005) reported the production of biopolymers just like exopolysaccharides (EPS) which boost the potential of *Rhizobia* to deal with drought stress conditions. These biopolymers help attach *Rhizobia* to the surfaces, give them protection against the antimicrobial agents released by the plants or animals and restrict the dehydration under drought stress. All of these features improve and amplify the capability of *Rhizobia* to live under water stress conditions that ultimately improves plant growth and development under the drought stress conditions.

Rhizobacteria linked with crop plants are categorized into two types, that is, endophytic and the rhizospheric. Endophytic bacteria are capable of living within the plant tissues, and these might inhabit different plant tissues like flowers and leaves along with stem and fruits (phyllosphere) (Naveed et al. 2014, 2020). On the other hand, rhizospheric bacteria are found on the surface of roots (Weyens et al. 2009). There are certain mechanisms through which these bacteria bring about useful effects on plant growth and yield (Yanni et al. 1997). The main mechanisms have been given in Fig. 16.1. The association of bacteria along plants could be helpful toward stimulating the plant strength or these could also be a restrictive aspect; it depends on the colonization of roots and the ability of rhizospheric bacteria (Antoun et al. 1998). Mehboob et al. (2011) have conducted various studies to elucidate the effect of these PGPR on the growth and productivity of cereals like wheat under drought stress. There was the isolation of different rapidly growing strains of *Rhizobia* from the rhizospheric soil of chickpea, nodules of lentils, and mung bean. Results showed that the isolated strains enhanced the growth, nodulation, and grain yield. Furthermore, a considerable rise in nutrient contents of straw and grain samples was also observed. Ultimately, it was recommended that the *Rhizobium* usage as PGPR can prove beneficial in progressing the development and efficiency of cereals under drought stress. Usually, the extended drought stress results in enhanced injuries (an ionic disorder of cell, denaturation of the proteins, and the alteration in the homeostasis of plants) to the crop plants (Manchanda and Garg 2008). Plant-microbe interactions result in enhanced tolerance against abiotic stresses such as drought stresses. Inoculation of cereals with microbes just like *Rhizobia* could prove environment-friendly and cost-effective approach to lessen the negative impact of the drought stress on plant growth; consequently, the use of microbes such as different *Rhizobia* is a beneficial method to reduce the severe effects of stresses via the augmentation of “induced systemic resistance” (ISR) and production of diverse bacterial compounds like osmolytes, antioxidants, enzymes, and phytohormones (Yang et al. 2009). Definite microbes can fight against abiotic stresses (salinity, drought, heavy metals, and nutrient deficiency). Particularly, the bacteria living in the rhizosphere can affect the tolerance of crops against the abiotic stresses more efficiently employing various direct and indirect mechanisms (Fig. 16.1). *Rhizobia* for non-legumes or cereals work as PGPR and reduce the impact of abiotic stresses through a process called induced systemic resistance (ISR) in the course of production of phytohormones such as auxins, gibberellin, cytokinin, and abscisic acid,

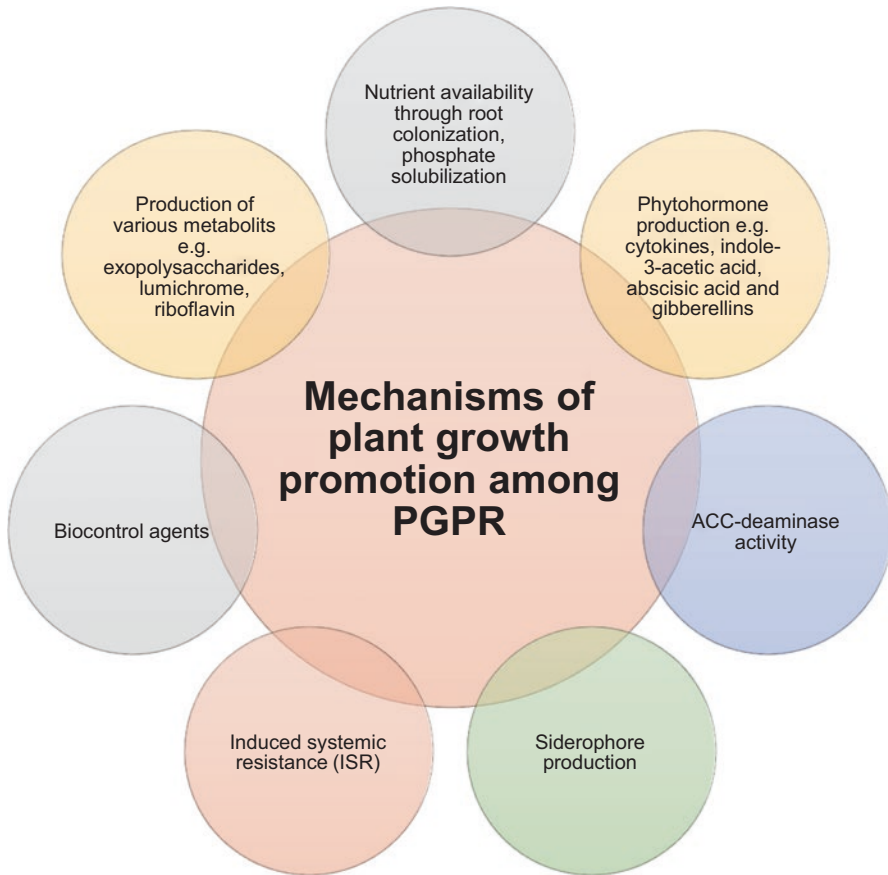


Fig. 16.1 Mechanisms of plant growth promotion among PGPR under drought stress

synthesis of antioxidants and reduction in ethylene levels by producing ACC-deaminase. This method causes a certain type of physical and chemical changes in the plant body that lead to increased tolerance against abiotic stresses (Dimkpa et al. 2009).

Rhizobia mitigate the undesirable impacts of abiotic stresses like salinity, drought, low temperature, high temperature, and the metal toxicity through the production of exopolysaccharides, inducing the resistant genes against stress, enhancing the water circulation and the formation of biofilms, particularly under drought. *Rhizobia* can also generate different osmoprotectants in the rhizosphere during abiotic stresses like drought stress. PGPR enhance plant growth by minimizing disease attack originated from pathogens like nematodes, fungi, viruses, and other types of bacteria (Grover et al. 2011). Literature established that *Bradyrhizobium* makes an association with cereals like wheat, rice, sorghum, barley, and the maize and promote growth through different mechanisms. In the phosphorus-deficient soils, the

inoculation of *Rhizobium leguminosarum* makes it available from organic and inorganic compounds by producing an acid and phosphatase enzyme. *Bradyrhizobium* also increases phosphorus availability via the production of an enzyme named phosphatase (Abd-Alla 1994). Different species of the *Rhizobium* boost up the nutrient availability in the rice rhizosphere and improve the plant growth and productivity. Co-inoculation of the *Rhizobia* with other PGPR such as *Bacillus* also enhances the growth of the cereals like wheat by the making of phytohormones and the antioxidants under different abiotic stresses (Perveen et al. 2002).

Sinorhizobium meliloti is known to produce a variety of polysaccharides, for example, cyclic-glucans (unfettered through NdyB plus NdyA) that play an important role in the development of plant during the interaction of microbe and with the plant host. These PGPR are also known to sequester antibiotics and eliminate toxic rudiments in the rhizosphere (Brencic and Winans 2005). Matiru and Dakora (2004) affirmed that rhizobacteria possess the potential to generate vitamins, cytokinins, lumichrome, auxins, abscisic acid, riboflavin, and lipo-cheto-oligosaccharides under water-deficit conditions that increase the plant intensification, enlargement, and the grain output of cereals like wheat, maize, sorghum, and rice. Also, lipo-cheto-oligosaccharides help during germination of seeds, though lumichrome sustains the plant development, including characteristics, which also help in the uptake of nutrients under abiotic stress. Moreover, the nodule formation in the Parasponia by *Bradyrhizobium* and strains of *Rhizobium* are proof that *Rhizobium* could be a source for nodulation and infection in the cereals just like maize and wheat. The majority of PGPR associated with non-leguminous crop plants manufacture indole-3-acetic acid (IAA) that plays a vital role during root development under abiotic stress such as drought (Hayat et al. 2010). Hayat et al. (2010) revealed that *Rhizobia* can produce siderophore under abiotic stress conditions like pH stress, salinity stress, drought condition, and heavy metals. The siderophores produced confiscate the iron in addition to making Fe accessible for the plant uptake. Blend or consortium of *Rhizobia* with PGPR is also known to enhance plant resistance against drought stress. *Phaseolus vulgaris* L. inoculation with two strains of Paeni bacillus and the *Rhizobium tropici* CIAT 899 reduced the deteriorating impact of drought stress via improving nitrogen level via improved nodulation and growth and productivity in comparison to the un-inoculated control (Marcia et al. 2008).

Rhizobial adaptation toward abiotic stresses is multifaceted with rigid mechanisms because of the contribution of dissimilar genes, involving diverse mechanisms to stand against various stresses such as drought and salinity within the soil (rhizosphere). Bacteria bear the impacts of stress via manufacturing osmoprotectants like glycine, proline, trehalose, as well as the glutamate. Manufacturing these types of compounds provides a shield against stressful conditions (Tobor-Kapłan et al. 2008). Some *Rhizobial* isolates possessing saprophytic and competitive capability are competent to perform and survive well under abiotic stresses in the rhizosphere (Yap and Lim 1983). *Rhizobial* inoculation amplified the stress water resistance of the plant because of the manufacturing of exopolysaccharide (EPS). These EPS guard *Rhizobia* against drought, eventually increasing the tolerance of plants (Sandhya et al. 2009). Water-use efficiency was improved in cereals because

of the increase in root length due to the inoculation of PGPR having the ability to produce ACC-deaminase (Zahir et al. 2008).

Development speed and wheat root colonization were enhanced when inoculated with the NAS206 *Rhizobial* strains, proficient in manufacturing exopolysaccharide. Those biopolymer compounds take part in biofilm formation, attachment of bacteria, and supply of nutrients under stress situations. Biofilms also assist *Rhizobial* colonization and perform like a channel for the water supply among colonies of microbes, genetic material, and transportation of nutrients (Amellal et al. 1998). Schembri et al. (2004) affirmed that beneath deficiency tension, *Rhizobia* make the polysaccharides smoothen the progress of their ordinary working, and therefore increase the development of cereal crops and growth such as wheat via alleviating the critical impact on wheat. Among the *Rhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Allorhizobium*, *Bradyrhizobium*, and *Mesorhizobium*, *Rhizobium* has been commonly used to enhance the growth and development of sunflower, wheat, maize, sorghum, and barley together beneath ordinary plus the stressed circumstances such as drought and salinity. In the same way, soil physical properties and wheat growth could be made better by the inoculation of exopolysaccharide producing PGPR under drought stress (Kaci et al. 2005). Alami et al. (2000) applied the strains of *Rhizobium* YAS34 possessing exopolysaccharide generating the capability in the rhizosphere of sunflower under drought stress. The results revealed increases in root dry biomass up to 70%, while the seeds of sunflower by 100% increase (soil adhering to the root) and enhanced exopolysaccharide manufacturing to increase the bacterial numbers in the rhizosphere. Grover et al. (2011) elaborated that *Rhizobium* sp. within the wheat enhanced nutrient uptake via the production of exopolysaccharide as soil formation is enhanced because of the creation of macro-aggregates in the dearth stresses and limit the uptake of sodium beneath the stress of salinity and, therefore, minimize the harmful effects of abiotic stresses. Plant development was enhanced via the chaperones in maize under water stress conditions as well as the harsh impact of stresses in maize was mitigated by the inoculation of bacteria, that is, *B. subtilis* due to the production of a protein known as CspB (Castiglioni et al. 2008).

PGPR have ACC-deaminase that decrease the intensity of ethylene generated under abiotic stress conditions (Mayak et al. 2004). Kang et al. (2010) found enzyme released from *Rhizobia* under stress and termed it 1-aminocyclopropane-1-carboxylate (ACC) deaminase that probably causes degradation of the ACC into ammonia and the α -ketobutarate, and so lowers the level of ethylene and ultimately reduces the impact of abiotic stress going on the crop plants. Phosphorus is an essential part of phosphor-lipids and phosphoproteins; its availability to the crop plants is enhanced by *Rhizobia*-possessing enzymes called phosphatase (Khan et al. 2012). Nutrient and water utilization for the effectiveness of the pants of sunflower was augmented due to its inoculation with *Rhizobia* YAS34 (Alami et al. 2000). In the sorghum, inoculation of *Rhizobia* amplified phosphorus uptake and growth of plants under variable environmental conditions. It was recommended that an appropriate mixture of cereals with *Rhizobium* would be able to improve the fodder production and grains under field conditions (Matiru and Dakora 2004). Similarly,

Hafeez et al. (2004) observed a positive impact on the yield and growth of cotton when inoculated with *Rhizobium leguminosarum* under controlled conditions. The PGPR inoculation also amplified the uptake of nitrogen and biomass of the cotton. Naveed et al. (2014) found that strains of *Burkholderia phytofirmans* PsJN as well as *Enterobacter* sp. FD17 competently lessened the destructive effects of abiotic stress and enhanced photosynthesis, root biomass, shoot biomass, and the leaf area.

Ansary et al. (2012) affirmed that *Pseudomonas fluorescens* improved the prospective of maize against drought stress because of the joint relation among the rhizobacteria and the maize. For this reason, the yield of maize found under drought was increased. It was found that *R. leguminosarum* also augmented the rice biomass at the stage of vegetation under field conditions (Kennedy et al. 2004). Rashad et al. (2001) inoculated *Bradyrhizobium japonicum* and *Rhizobium leguminosarum* and estimated their perspectives for *Sorghum bicolor* L. under stress conditions. Outcome discovered that appreciably increased production of GA₃ (Gibberellin) and IAA (Indole acetic acid) in shoot and root with co-inoculation as compared to the treatment of control at field capacity level of 100% and 80%. Besides this, sugar contents in leaves, production of siderophore, and solubilization of phosphorus were also increased. Mia and Shamsuddin (2010) established that a few species of *Rhizobium* such as *Azorhizobium caulinodans* can enter the roots of cereals just as wheat, barley, maize, and rice. Their entry into the cortical cells enhances the development and growth of plants directly via hormonal production, that is, abscisic acid, cytokinins, gibberellins, and auxins in the dissimilar situation of surroundings. Abscisic acid plays an important role in the mitigation of various abiotic stresses, for example, temperature, drought, and salinity (Zhang et al. 2006). Also, Aziz et al. (1997) have declared that the plants which are water-stressed manufacture extra molecules like cadaverine that enhance the effectiveness of ABA to alleviate the impacts of drought on the cereal like sorghum, barley, and maize. Bacteria also produce cytokinins that alleviate the effect of dearth by the adjustment of osmotic on the plant.

Rhizobial inoculation proves advantageous for the development and growth of plants while the application of a mixture of dissimilar strains of *Rhizobia* and *Pseudomonas*, *Azospirillum*, and *Bacillus* further enhances this effect. The effectiveness of three strains of rhizobacteria (*Bacillus subtilis* SM21, *Serratia* sp. XY21, and *Bacillus cereus* AR156) inoculated in cucumber was observed under drought stress. The outcome revealed that inoculated plants had more chlorophyll contents and dark leaves than that of control or un-inoculated plants (Wang et al. 2012).

Likewise, an increased level of proline contents in the roots, superoxide dismutase, and enhanced photosynthesis was observed with the inoculation of PGPR (Wang et al. 2012). Akhtar et al. (2013) conducted a field experiment to evaluate the performance of *Rhizobium* and *Bacillus* sp. solely as well as in the combination of growth and yield of wheat crops. Results revealed that the co-inoculation of *Bacillus* sp. and *Rhizobium* appreciably improved grain yield by 17.5% extra compared to the un-inoculated control. Under separate inoculation, *Bacillus* showed the most efficient results as compared to *Rhizobium*. Also, grain proteins, grain numbers, number of tillers, and the biomass were found to be most within co-inoculation. In

soil samples after harvesting, the available phosphorus was improved appreciably with the co-inoculation of both PGPR. Ilyas et al. (2012) took dissimilar type strain, that is, *Azospirillum* from the rhizosphere of maize roots and experimented on maize and wheat under normal and water stress conditions. Results showed that eight *Azospirillum* strains increased the level of zeatin, gibberellins, and auxin more under a well-watered situation compared to the water-stressed one.

In an experiment, strains of *Rhizobia* i.e. *Rhizobium phaseoli* MR-2, *Mesorhizobium ciceri* and *Rhizobium leguminosarum* (LR-30) were isolated from the rhizosphere of *Vigna radiata* L., *Lens culinaris* L. and *Cicer arietinum* L., grown in semiarid and arid regions of Pakistan and their potential to mitigate drought stress in wheat was tested. As a result, colonization of root by these strains, the water-holding capacity, and the nutrient-holding capacity were enhanced beneath the conditions of stress. Also, root length was enhanced because of the manufacturing of auxin via *Rhizobia* under the drought stress (imposed by PEG-6000). These also proved competent to produce plant hormones, and exopolysaccharides and catalase can prove helpful against drought stress in other cereals (Hussain et al. 2014). Colonization and growth promotion of roots of non-legume plants were observed many times via the inoculation of *Rhizobium leguminosarum*. The competent strains of *Rhizobia* should be screened for possible production of auxin as well as the seed of maize be drenched within separate or with the interaction of three *Rhizobia* isolate with L tryptophan. Results showed the inoculation increased the chlorophyll contents, photosynthesis, and transpiration rate greater than un-inoculated control. Also, L tryptophan adding up increased the fresh fodder of maize and dry matter (Qureshi et al. 2013).

A jar experiment was conducted under the axenic environment to find out the potential of chosen strains of *Rhizobia* on yield and growth parameters of the wheat. Results revealed that every strain of *Rhizobia* affected the growth of wheat positively and those strains of *Rhizobia* improved the length of the root by 51.72%, yield of straw by 35.14%, per-plant tiller numbers by 68.76%, the yield of grains by 30.29%, the height of the plant by 28.66%, yield of straw by 35.14%, the weight of the 1000 grains up to 28.40%, percentage of phosphorus within grain and straw up to 66.66% and 23.39, percentage of nitrogen within grain and straw up to 15.07% and 33.16, and potassium within grains and straw up to 51.72% and 21. In conclusion, it was suggested that *Rhizobia* isolated within wheat beneath conditions of axenic can prove effective as well as a useful strategy to improve the growth of wheat and the yield under field conditions both under normal and abiotic stress conditions like drought, salinity, and heavy metal (Mehboob et al. 2011).

A field experiment was performed to elucidate the endurance of *Pseudomonas fluorescens* PsIA12 and *Rhizobium trifolii* R39 within the cereal's rhizosphere (wheat, rape, maize) and the leguminous crop (pea and white lupins) on sandy soil which is loamy. Results confirmed undoubtedly that both of the strains colonize all crops' rhizospheres (non-legumes and legumes). The population of *Pseudomonas* declined with flowering as well as the maturity of the plant, but enlarged legumes during vegetative stages. Both of the strains were also able to survive within the weeds rhizosphere (\log_3 CFU. g^{-1} root) as well as a non-inoculated crop (\log

4.8 CFU/g root). Moreover, the space between non-inoculated and inoculated plants was about 0.6 m. Reason for that research was to approach continued existence of two bacteria under the moderate type of weather though taking into consideration the colonization of roots via both strains, their rearrangement from inoculated to non-inoculated plants, competency for inhabiting plants with the outcome of the bacterial survival and moisture on the plants (Wiehe and Hoflich 1995).

Weavert and Baldani (1992) revealed that the *Rhizobia* acquire cure derivatives to tolerate drought and heat stresses in an improved way than that of parent derivatives. Furthermore, the *Rhizobia* contain precise plasmid, which allows them to stay alive under high temperature as well as under the shortage of moisture content. Plasmid plays a crucial function via different protein syntheses that make *Rhizobia* physically powerful like manufacturing of succinate, catechol, the bacteriocin, lipopolysaccharides, thiamine, lactose, calystegine, melanin, and the succinate along with metabolism of transport of dicarboxylate in the cell of *Rhizobia* under moisture and heat stresses.

6 Multistrain Inoculation

Commercial inoculants of single strain are less effective as compared to multistrain/consortium inoculation. Plant growth with single strain inoculation becomes contradictory due to smaller amount of colonization and inability to bear attacks of pathogens on host plant besides different and variable conditions of environment and soil. Since it has been reported by Klopffer and Raupach (1998), the performance of single PGPR strain might stay nonreliable continually under changing environmental conditions. In contrast, inoculation with a combination of PGPR could exert good results under different types of circumstances, as those require temperature settings, moisture, and diverse pH. Inoculants that are multistrain adjust best under changing environmental conditions existing all through the entire mounting season. Consistent colonization of root, defense against pathogens and the use of a wide range of plant growth enhancing mechanisms are a few prominent features of multistrain inoculants. Co-inoculation of *Bradyrhizobium* and *Rhizobia* operates like partners and has a symbiotic relationship with plants through the fixation of nitrogen within the nodule of the leguminous crop. These plant promoting rhizobacteria also work for non-legumes like sunflower, radish, barley, and wheat, through secretion of plant growth regulators, siderophores, biofilm formation, exopolysaccharides, as well as killing pathogens especially under drought stress. An experiment was conducted for checking the probability of two *Rhizobia* in the improvement of the yield of radish and growth. So, because of this, 266 strains were tested for their auxin potential, solubilization of phosphorus, siderophores, cyanide, and production of siderophores. Result revealed that all strains (83%) were competent to produce siderophores, and only 3% were able to produce cyanide, phosphorus solubilizing strains were 58%, and strains that were capable of producing auxin were 54%. The most distinct outcome found in the case of *Bradyrhizobium*

japonicum strain Soy 213 inoculation was to improve dry biomass of radish by 60%, whereas radish was negatively affected by N44 strain arctic and also it decreased radish dry portion up to 44%. In the next experiment (growth cabinets), *B. japonicum* strain Tal 629 increased the dry stuff of plant by 15%. In the final experimentation, the researchers concluded that a precise *Bradyrhizobium* relation among cereals (maize, sorghum, sunflower, and wheat) has a prospect of improving yield and growth limitation of the plants (Antoun et al. 1998).

Microbes that possess a variety of metabolic activities like P-solubilization, N₂-fixation, antibiotic production, and phytohormone production could replace single-strain inoculation and it would lead to multistrain inoculation. In recent times, Adesemoye et al. (2008) reported that multistrain inoculation could increase the productivity and growth of plants wherever the inoculation of a single strain would be ineffective. Therefore, as compared to single-strain inoculation, plants get great benefits from mixed inoculation (Germida and Xavier 2002). Inoculation of the PGPR agents of multistrain contributes most toward the healthy growth and the high yield because of the wide variety spectrum of their actions, combination of dissimilar quality without linking greater reliability, and genetic engineering (Janisiewicz 1996). Despite ability in the development of multistrain inoculants, the compatibility of microbes is essential, as the microorganisms can have inhibitory effects for one another and can be potential antagonists to each other.

Rhizobial impending to get better non-legume crop growth could perk up by the multistrain inoculation. Berg (2009) has reported that use of inoculation mixture of *Rhizobia* is a hopeful approach. Also, Gunasekaran et al. (2004) confirmed that uptake of plant nutrient was enhanced under nutrient degrading and limiting soil environment through a *Rhizobia*-compatible inoculation in combination with arbuscular mycorrhizal (AM) fungi over inoculation of single microbe. Likewise, enhanced phosphorus uptake, spike length, plant biomass, height of the plant, the yield of grain, leaf sugar and the leaf protein was noted via mixed inoculation of *Rhizobia* (Afzal and Bano 2008). In the same way, Sahin et al. (2004) reported substantial increases within the sugar content plants by the co-inoculation for fixing of N₂ or the P-solubilizing bacteria. In the same way, Sheikh et al. (2006) also confirmed that the seed dressing of *Bacillus thuringiensis* (Bt-10), *Rhizobium meliloti* (R5), and drenching of soil were found to increase the seed germination, height of the plant, plant biomass in okra and length of the root. Furthermore, the health of the plant was found to be enhanced because of the protection which is provided against fungi infections, that is, bioprotection. In addition, beneath the stress of salinity, performance of lettuce was found to be relatively inspiring because its dry and fresh biomass increased by 7.86% and 12.87%, respectively, via joint inoculation of the strain of PGPR of *Serratia* sp. and *Rhizobium* (Han and Lee 2005). Despite the single-strain inoculation of the PGPR, its mixture by the *Rhizobium* and mycorrhizal fungus creates lenience within the plants with limited water conditions (Wang et al. 2012). Besides this, co-inoculation of wheat with *Azotobacter chroococcum* and *Pseudomonas* sp. (E2) relieved water stress by altering the anatomy of plants i.e. by increasing phloem and epidermis thickness, diameter of xylem vessel and root

system dimensions under different levels of field capacity (50, 75 and 25% FC) (El-Afry et al. 2012).

Microbial consortium usage was found to be a proficient plan to improve the wheat crop dearth stresses (Asghar et al. 2015). By the consortium of PGPR, rice growth improved under water stress due to enhanced production of proline contents which lessened oxidative injury (Gusain et al. 2015). Inoculation of the microbial consortium of *P. aeruginosa* (Pa2) and *P. penneri* (Ppl) with EPS production had superior perspectives to enhance tolerance among crop plants under drought stress as compared to the sole strain inoculation of PGPR within the maize (Naseem and Bano 2014).

On the joint beneficial interaction of *Rhizobia*, a few studies have reported the improvement in non-leguminous plants under abiotic stresses. That is why there is a dire need to investigate the prospects of the poly-*Rhizobial* inoculants for improving the productivity and growth of non-leguminous plants under water shortage stress. Various mechanisms of the plant microorganism connections in the rhizosphere are necessary to be elucidated. Complexity pertains to numerous range of processes that are associated with different communities of microbes. Mitigation of stress in crop plants by means of knowing the crosstalk of microorganism's microbes could be optimized and under stress conditions, their capability to stay alive could be enhanced. An abundance of the techniques which are molecular needs to be accessible and will be applied to describe the interaction of the plant microorganisms (Barea 2015).

7 Conclusions

Drought stress has been the foremost threat to food security and sustainable agriculture. Approximately, 38% of agricultural land is currently at the risk of drought stress, and this proportion is being increased because of climate change. Drought stress imposes oxidative stress on the plants by producing the ROS in high amounts that ultimately upset the photosynthetic process of the flora. Conventional methods to develop the drought-tolerant crops are very time consuming, while on other side, total implementation with the latest biotechnology for the product improvement is at a standstill considered by caution. Future climate situation predicts that increased inconsistency in the rainfalls is heading toward more extreme drought actions that would extend faster with the severe intensity. Restricted water accessibility hinders plant growth, resulting in considerable losses in agricultural productivity. Plant growth-promoting rhizobacteria (PGPR) might be used as an economical and environmentally friendly technique to improve crop growth under abiotic stress. PGPR play a significant role in generating rigidity and adjustment in plants toward water-deficit condition and has the capacity to solve the future food security problem. There should be the usage of wide range of methods for leguminous as well as non-leguminous crops growth improvement. For example, PGPR by the production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase amplify the nutrient uptake

of plants by breaking the ACC, thereby stopping ethylene accumulation, and by the production of lumichrome, and riboflavin as indirect mechanisms for non-legumes to tolerate drought stress. Exopolysaccharides formed are another strategy adopted by bacteria that perk up the capacity of soil to retain water. Interaction among the plants and PGPR in drought circumstances influences not only the plants but at the same time also changes the characteristics of the soil. The valuable PGPR improve the construction of the defense system of antioxidant and the osmolytes that lessen the unfavorable ROS impact on the crops. Multistrains inoculation of PGPR is thus important for the improved yield as well as for food security, chiefly in an unfriendly ecological situation. PGPR augment the osmolytes manufacturing that is supportive in reducing the harmful effects of ROS. Multistrain PGPR are potentially competent contenders to reduce the negative impact of the drought on crops, especially in the arid regions. Utilization of plant growth-promoting rhizobacteria as bio-inoculants to maintain vigorous growth of plants is essential in supporting food security, especially in hostile ecological circumstances. The above-highlighted studies provide us better understanding of the relationships among the plants plus useful soil microorganisms that symbolize the forward step to take best out of these dealings. However, the existing information is at the evolution process and for that reason, there is a dire necessity for further research paying attention to plant–microbe interactions at the molecular level to have a mechanism for pathways exploited by microorganisms living in the rhizosphere for the plant development and for the infection inhibition to sustain agricultural productivity. Knowing all that PGPR manufacture the osmolytes such as proline, and glycine-betaine that decrease ROS negative impacts on the plants under drought stress but overexpression of genes responsible for the production of these osmolytes can improve the resistance capability in bacteria and ultimately would enhance stress tolerance capacity of plants. Despite this, the useful activities of the PGPR can be frequently affected by abiotic stresses. The effectiveness of multiple strains can be improved for the better performance by the use of *Rhizobium* as bioinoculants under field conditions employing new tools like nanoencapsulation that advances the colonization to the root hairs by favorable bacterial strains. Even after that, the genes responsible for drought tolerance are multifarious in the plants and require further elucidation through better understanding of their genomics, proteomics, metabolomics, and transcript-omics. Hence, the present studied research can take to an advanced notion to use above-mentioned prospects by using genetic, molecular level strategies, using multistrain PGPR to develop ecologically acceptable biofertilizers to enhance the development and the quality of the harvest grown under drought stress.

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