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Phytobeneficial Traits and Ecophysiological Stress Tolerance of Rhizobia

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Abstract

The microorganisms with the aim of improving nutrients available for plants are an important practice and necessary for agriculture. During the past couple of decades, plant growth-promoting (PGP) rhizobia have been begun to replace the use of chemicals in agriculture, horticulture and environmental cleanup strategies. Scientific researches involve multidisciplinary approaches to understand adaptation of plant growth promoting rhizobacteria (PGPR), their effects on plant physiology and growth, induced systemic resistance, biocontrol of plant pathogens, biofertilization and their tolerance to ecophysiological stresses. This is due to the emerging demand for dependence diminishing of synthetic chemical products, to the growing necessity of sustainable agriculture within a holistic vision of development and to focus on environmental protection. PGP rhizobia are naturally occurring soil bacteria that aggressively colonize plant roots and benefit plants by providing growth promotion and biological nitrogen fixation (BNF). Inoculation of crop plants with certain strains of PGP rhizobia at an early stage of development improves biomass production and yields through direct and indirect effects on roots and shoots growth. In this review, we have discussed the phytobeneficial traits of rhizobia which act as PGPR, and their ecophysiological properties, biocontrolability, mechanisms and the desirable properties exhibited by them.

Keywords

Phytobeneficial traits, Ecophysiology, nitrogen fixation, Phosphate solubilization, siderophore production, rhizosphere.

1. INTRODUCTION

The rhizosphere is a nutrient-rich habitat and harbors a huge variety of bacteria and fungi that each can have neutral, beneficial or harmful effects on the plant (Berendsen *et al.,* 2012). Adesemoye and Kloepper (2009) indicated plant growth and yield stimulation by beneficial soil microorganisms and plant growth promoting rhizobacteria (PGPR) is one among the most effective and best studied soil microorganisms which can promote plant performance. Plants inturn help beneficial soil microbes by giving an auxiliary environment in the rhizosphere and microbes in return also provide several benefits to plants such as growth promotion and stress relief. Hence plant roots ooze different organic nutrients such as sugar, vitamins, organic acids, amino acids, mucilage, phytosiderophores, nucleosides, phenolic compounds and

other signals. This results in enrichment of microorganisms, such as bacteria, fungus, algae and protozoa, among which bacteria influence the plant growth in a most significant manner (Uren 2007).

Plant growth promoting rhizobacteria (PGPR) found in the rhizosphere in association with roots (Glick, 2012) are beneficial bacteria which can heighten the growth of plants either directly by nitrogen fixation, phosphate solubilization, iron chelation and phytohormone production or indirectly by suppression of plant pathogenic organisms, induction of resistance in host plants against plant pathogens and abiotic stresses (Vessey,2003).

Rhizobium is one of the plant growths promoting rhizobacteria (PGPR) which exhibit a variety of characteristics responsible for influencing plant growth and performance. As a symbiotic partner in addition to nitrogen supply, rhizobium also improves nutritional uptake by promoting the growth of plant root system through production of indole acetic acid (IAA) (Etesami, et al.2009) and as a rhizospheric microbe solubilises phosphorus and various mineral nutrients (Khan, *et al*, 2006).

Plant growth could be induced by rhizobia through some of their growth stimulating mechanisms such as mobilization of nutrient, enhancement in stress resistance, solubilization of phosphates, production of phytohormones and siderophores (Ahmad, *et al*.2006).

The plant growth promoting (PGP) rhizobia olso serve as biocontrol agents. The ability of bacterial siderophores and antibiotics to suppress phytopathogens could be the significant agronomic importance. Both mechanisms have essential functions in microbial antagonism but also the mechanisms leads to bring out induced resistance. Resistance-inducing and antagonistic rhizobacteria might be useful in formulating new inoculants, offering an attractive alternate of environmentally friendly biological control of plant disease and improving the cropping systems into which it can be most profitably applied (Beneduzi *et al*., 2012).

Plant growth promoting (PGP) rhizobia contains a useful variation for tolerating environmental stresses like extremes of temperature, pH, salinity and drought; heavy metals, antibiotics and pesticide pollution. Seeking such tolerant PGP rhizobia is expected to offer enhanced plant growth and yield even under a combination of stresses. The multiplicity of beneficial effects of rhizobial inoculants emphasize the need for further strengthening the research and their use in modern agriculture.Therefore genetic modification may accelerate the commercialization of PGP rhizobia as biocontrol agents that could further contribute to sustainable development of agriculture.

In this review we emphasized on the plant Growth-Promoting (PGP) traits of *rhizobial* related researchs and benefits of PGP rhizobia as well as their environmental stress tolerance. Moreover, the potential of PGP rhizobia and the unique properties of plant growth induction, defense pathways and the resistance spectrum available against various abiotic stresses on a variety of agricultural crops was summarized.

2. PHYTOBENEFICIAL TRAITS OF RHIZOBIA

Rhizobia, in addition to nitrogen supply, promote growth of the plants as a symbiotic partner and soil microbes in a number of ways. Plant growth could be induced by rhizobia through some of their growth stimulating mechanisms such as mobilization of nutrient, enhancement in stress resistance, solubilization of phosphates, production of phytohormones and siderophores (Ahmad *et al.,* 2008). Generally, rhizobia can promote plant growth directly by either often due to their ability for nutrient supply (nitrogen, phosphorus, potassium and essential minerals) or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens and ecophysiological Stress on plant growth and development in the forms of biocontrol agents, root colonizers and serve as environmental protectors (Kloepper and Schroth, 1981; Vessey, 2003).

2.1. Nitrogen Fixation

Biological Nitrogen fixation (BNF) is one of the most important biological processes on this planet and continued improvement in the understanding of the legume-rhizobia interaction is necessary to sustain a food supply to its inhabitants. Although 78% of the atmospheric air is N and it is required for synthesis of nucleic acids, enzymes, proteins and chlorophyll, this gaseous form is unavailable for direct assimilation by plants.

Rhizobia are bacterial symbionts of legumes that fix and convert atmospheric nitrogen in a process known as biological nitrogen fixation (BNF) into plant assimilable N such as ammonia through a cascade of reactions between prokaryotes and plants with the use of complex enzyme systems (Wilson and Burris 1947). Inside the symbiotic root nodules, the bacteria reduce nitrogen to ammonia and supply it to the host (Denarie *et al.*, 1996**).** This interaction starts with a signal exchange between both partners. Plant roots secrete specific flavonoids that interact with the bacterial NodD protein, resulting in the activation of rhizobial *nod* genes (nifH gene) and synthesis of Nod factors (Oldroyd, 2013).The nifH gene codes for the dinitrogenase reductase, one of the subunits of the nitrogenase complex, responsible for reducing atmospheric nitrogen into ammonia (Fischer, 1994).

The ability of PGPR to colonize roots in the presence of competing indigenous soil microflora is a major key to success in inoculation with beneficial bacteria.The PGP *R. leguminosarum biovar trifolii* R39, isolated from red clover nodules colonized the rhizospheres of pea, maize, and sugar beet better than the PGPR strain *Pseudomonas fluorescens* PsIA12 isolated from a wheat rhizosphere (Höflich *et al.,* 1995). Yield increases caused by inoculation of nonlegumes with PGPR rhizobia have been reported in pot and field experiments. *R. leguminosarum biovar trifolii* R39 promoted the growth of maize, spring wheat, and spring barley in field trials performed between 1985 and 1993 in a loamy sand soil resulted in yields that were significantly ($P<0.05$) increased by 6 to 8% in Germany (Höflich *et al*., 1994).

The beneficial effects resulting from the use of legumes in crop rotations or in intercropping systems have conventionally been attributed to their ability to form atmospheric nitrogen fixing symbioses with rhizobia and other rotational benefits relating to disease suppression (Graham and Vance, 2000). This symbiotic N contribution is reported to benefit the cereal crops, such as maize, rice, wheat and sorghum with a relative yield increase of 11–35.3 % (Peoples and Cranswell 1992). Moreover Mehboob *et al*. (2012) could have been shown the capacity of rhizobia in inducing the plant growth of non leguminous plants. In this regard, *Azo-rhizobium caulinodans* (endophytic rhizobia) is known to enter the root system of cereals, other non-legume crops and *Arabidopsis*, by intercellular invasion between epidermal cells and to internally colonize the plant intercellularly, including the xylem (Cocking et al., 1994).

Legume crops substantially reduce the N requirement from external sources (Bhattacharyya and Jha 2012). However, N fixation efficiency of legumes varies, and depends on the host genotype, rhizobial efficiency, soil conditions, and climatic factors (Peoples and Cranswell 1992).

Nitrification is an important process in nitrogen cycle in which ammonia is converted to nitrite and nitrate by nitrifying bacteria such as Nitrosomonas and Nitrobacter. The nitrification products, nitrite and nitrate, are lost by denitrification (Parker 1972). It had been demonstrated that nitrification inhibitor produced by B. humidicola as root extracts were seen to inhibit nitrifying bacteria, with no adverse effects on other soil microorganisms such as Azospirillum lipoferum, R. leguminosarum and Azotobacter chroococcum (Gopalakrishnan *et al*. 2009).

Nitrification and denitrification remain to be the only known biological processes that generate nitrous oxide (N_2O), a powerful greenhouse gas contribute to global warming. Therefore, biological nitrification inhibition is seen as the only major mitigation process towards global warming besides improving N recovery and N use efficiency of agricultural systems (Subbarao *et al*. 2012). However BNF ability, Nitrogen self sustainability and protein-rich grains of legumes require high energy and productivity tradeoffs (Hall 2004).

Moreover, various environmental factors limit nitrogen fixation, such as soil moisture deficiency, osmotic stress, extremes of temperature, soil salinity, soil acidity, alkalinity, nutrient deficiency, over doses of fertilizers and pesticides are an important driver for BNF (Zahran 1999).

2.2. Siderophore Formation

Microorganisms also enhance plant growth by scavenging available iron (Fe^{3+}), which involves secretion of high affinity, low molecular weight iron chelating ligands called siderophores (Anitha and Kumudini, 2014).Under aerobic environments, iron exists as insoluble hydroxides and oxyhydroxides which are not accessible to both plants and microbes (Rajkumar *et al*. 2010). It can occur in either as divalent (ferrous or Fe^{2+}) or trivalent (ferric or $Fe^{3+)}$ states which is determined by the pH and Eh (redox potential) of the soil

(Bodek *et al*. 1988). Bacteria have the ability to synthesis low molecular weight compounds termed as siderophores, capable of sequestering Fe^{3+} (ferric or Fe^{3+}).These siderophores are known to have high affinity for $Fe^{3+,}$ and thus makes the iron available for plants. The siderophores are water soluble and are of two types viz. extracellular and intracellular. Ferric (Fe^{3+}) ions are reduced to ferrous (Fe^{2+}) and released into the cells by gram positive and negative rhizobacteria; resulting in destruction or recycling of siderophores (Rajkumar *et al*. 2010). Siderophores can form stable complex compounds with heavy metals such as Al, Cd, Cu etc. and with radionucleides including Uranium, Nitrogen and Phosphorus (Neubauer *et al*. 2000). Thus, the siderophore producing bacteria can relieve plants from heavy metal stress and assist in iron uptake.

Like other PGPR, rhizobia produce siderophores that are strain specific (Reigh and O'Connell, 1993), and they can utilize a large spectrum of these molecules to overcome iron starvation (Carson *et al*., 2000). Groups of *rhizobia* reported to produce siderophores include *R. meliloti, R. tropici, R. leguminosarum biovar viciae, R. leguminosarum biovar trifolii, R.leguminosarum biovar phaseoli, Sinorhizobium meliloti and Bradyrhizobium sp.*(Arora *et al*. 2001).

Plessner *et al*., (1993) was indicated that the future research will elucidate the importance of rhizobial siderophores in the biological control of pathogens and the possible competitive advantage gained by rhizobia through their ability to utilize siderophores of other organisms.

2.3. Phosphate Solubilization

Next to Nitrogen, phosphorus (P) is the most crucial nutrient for plant growth. It exists in both inorganic and organic forms and the concentration depends on the parental material (Gray and Murphy 2002). Although the parent material has a strong control over the soil phosphorus status of terrestrial ecosystems (Buol and Eswaran 2000), the availability of phosphorus to plants is influenced by pH, compaction, aeration, moisture, temperature, texture and organic matter of soils, crop residues, extent of plant root systems and root exudate secretions and available soil microbes. Soil microbes help in phosphorus release to the plants that absorb only the soluble phosphorus like monobasic ($H_2PO_4^-$) and dibasic ($H_2PO_4^{2-}$) forms (Bhattacharyya and Jha 2012). The soil solution remains to be the main source of phosphorus supply to plants. The phosphorus content of agricultural soil solutions are typically in the range of 0.01–3.0mgL^{-1} representing a small portion of plant needs. The rest must be obtained from the solid phase through intervention of biotic and abiotic processes where the phosphate solubilizing activity of the microbes has a role to play (Sharma *et al*. 2013).

Some bacterial strains are found to possess both solubilization and mineralization capacity (Tao *et al.* 2008). Importance of this phosphorus solubilizing capacity in enhancing plant growth by *Mesorhizobium mediterraneum* has been demonstrated in chickpea and barley plants (Peix *et al*. 2001). Rhizobia, including *R. leguminosarum, R. meliloti, M. mediterraneum, Bradyrhizobium sp.* and *B. japonicum* (Afzal and Bano 2008) are the potential phosphorus solubilizers. These rhizobium synthesize low molecular organic acids which acts on inorganic phosphorous. For instance, 2-ketogluconic acid with a phosphate-solubilizing ability has been identified in *R. leguminosarum* (Halder *et al.* 1990) and *R. meliloti* (Halder and Chakrabarty 1993).

2.4. Phytohormone Production

As symbiotic partner, in addition to nitrogen supply, rhizobium also produces phytohormones, mainly including cytokinins, Indole-3-acetic acid (IAA), auxins, abscisic acid, gibberellins, and ethylene to induce some important physiological responses at different stages of plant development at low concentrations (Ma *et al*. 2008).

2.4.1. Cytokinins

Cytokinin is known to stimulates plant cell division and in some instances root development and root hair formation (Frankenberger and Arshad 1995). It is documented that 90% of rhizospheric microorganisms are capable of releasing cytokinins and about 30 growth-promoting compounds of the cytokinin group has been identified from microbial origin (Nieto and Frankenberger, 1991). Rhizobium strains are also reported

as the potent producers of cytokinins (Senthilkumar *et al.* 2009).The prominent producers of Cytokinins *are S. meliloti*, *S. fredii*, *S. medicae*, and *Mesorhizobium loti(*Anna et al.2013).

2.4.2. Indole-3-Acetic Acid (IAA)

Indole-3-acetic acid is considered to be the best categorized auxin found in plants. IAA is known to enhance cell elongation, cell division and differentiation in plants (Singh *et al*. 2013). It has been estimated that 80% of bacteria isolated from the rhizosphere can produce IAA (Patten and Glick 1996). The prominent producers of IAA are Azorhizobium *caulinodans, B.japonicum, B. elkanii, Mesorhizobium loti, R. japonicum, R. leguminosarum, R. lupine, R. meliloti, R. phaseoli, R. trifolii and Sinorhizobium spp.* (Afzal and Bano 2008).

IAA production in rhizobium takes place via indole-3-pyruvic acid and indole-3-acetic aldehyde pathway. On inoculation of *R. leguminosarum biovar Viciae*, 60-fold increase in IAA was observed in the nodules of vetch roots (Camerini *et al*. 2008). One of the highest productions of IAA had been reported with the inoculation with *B. japonicum-SB1* and with *B. thuringiensis—KR1* (Mishra *et al*. 2009). Both environmental stress factors (acidic pH, osmotic and matrix stress and carbon limitation) and genetic factors (auxin biosynthesis genes and the mode of expression) were shown to influence the biosynthesis of IAA (Spaepen and Vanderleyden 2011).

2.4.3 Gibberellins

Gibberellic acid is a plant growth regulator of economic and industrial importance (Bandelier *et al.*1997).Various gibberellins are available and are associated with several plant growth and development processes, such as seed germination, stem elongation, sex expression of flowers and fruit development (Boğa *et al*. 2009). It is also believed that certain types of dwarfness are due to gibberellins deficiency, but it has no effect on roots. Application of gibberellins is known to promote securing of the plants, parthenocarpy in fruits, increase fruit size and number of buds and break down the tuber dormancy. Many PGP microbes are reported to produce gibberellins (Dobbelaere *et al.* 2003) including *Rhizobium* and *Sinorhizobium meliloti* (Boiero *et al.* 2007).

2.4.4. Abscisic Acid

Abscisic acid in plants is synthesized partially in the chloroplasts and the whole biosynthesis primarily takesplace in the leaves. The production of abscisic acid is affeted by stresses such as water deficit and freezing temperatures. It is believed that biosynthesis occurs indirectly through the production of carotenoids. The transport of abscisic acid can occur in both xylem and phloem tissues and can also be translocated through paranchyma cells. The movement of abscisic acid in plants does not exhibit polarity like auxins (Walton and Li 1995).

Abscisic acid was reported to stimulate the stomatal closure, inhibit shoot growth while not affecting or even promoting root growth, induce seeds to store proteins and in dormancy, induce gene transcription for proteinase inhibitors and thereby provide pathogen defense and counteract with gibberellins (Davies 1995). Boiero *et al.* (2007) reported that *Rhizobium sp*. and *B. japonicum* produced abscisic acid.

2.4.5. 1-Aminocyclopropane-1-Aarboxylic Acid (ACC) Deaminase

ACC deaminase is a member of a large group of enzyme that utilizes vitamin B6 and considered to be under tryptophan synthase family. Rhizobia have the ability to uptake ACC and convert it into α-ketobutyrate and NH_3. Hence, on inoculation of rhizobia producing ACC deaminase, the plant ethylene levels are lowered and result in longer roots providing relief from stresses, such as heavy metals, pathogens, drought, radiation, salinity, etc.

Rhizobial strains that express ACC deaminase are up to 40% more efficient at forming nitrogen-fixing nodules than strains that lack this activity (Ma *et al*. 2004). However, strains of rhizobia that express ACC deaminase have only a low level of enzyme activiity compared with free-living plant growth-promoting bacteria, i.e.typically around 2–10%. Thus, free-living bacteria bind relatively non-specifically to plant

tissues (mainly roots) and have a high level of ACC deaminase activity that can protect plants from different abiotic and biotic stresses by lowering ethylene levels throughout the plant. The common mode of acdS transcriptional regulation genes from various strains of *Mesorhizobium loti* have been found to be under the transcriptional control of the *nif*A promoter that is normally responsible for activating the transcription of *nif*, nitrogen fixation genes (Nascimento *et al.* 2012a). Moreover, Nascimento *et al*. (2012a) suggested that in many *Mesorhizobium spp*. acdS genes appear to be horizontally transferred between strains by the exchange of the symbiosis island. This suggestion is based on observing the presence of the acdS gene in the symbiosis islands of *M. loti R7A, M. sp. MAFF303099*, *Mesorhizobium ciceri bv.biserrulae WSM1271*, *Mesorhizobium australicumWSM2073T* and *Mesorhizobium opportunistum WSM2075T*, close to the nitrogen fixation gene cluster.

Strains, such as *R. leguminosarum bv.viciae, R. hedysari, R. japonicum, R. gallicum, B. japonicum, B. elkani, Mesorhizobium loti and S. meliloti* also had been known to produce ACC deaminase (Duan *et al.* 2009).Inoculation with these bacteria had shown to promote root elongation, shoot growth, enhanced rhizobial nodulation and minerals uptake (Glick, 2012).

2.5. Synergistic Effects of Rhizobial Dual Inoculation

A range of PGP microbes can be used with rhizobium that not only improves legume growth and yield but also cost effective and efficient. Certain specific dual inoculation causes synergy by functioning as helper bacteria to improve the performance of the other bacteria (Bashan and de Bashan 2005). Therefore in such co-inoculations, the combination of PGP bacteria, rhizobia and the host genotype has to be selected after extensively careful evaluations (Remans *et al*. 2008).

Azospirillum, the free living diazotroph, *Azotobacter, Bacillus, Psuedomonas, Serretia*, and *Enterobacter* are some of the genera that are successfully used with *rhizobium* as co-inoculants. Azospirillum was found to enhance growth and yield of several leguminous crops upon inoculation (Roseline *et al*. 2008). Improved nodulation was found when *Azospirillum lipoferum* and *R. leguminosarum biovar trifolii* were co-inoculated in white clovers (Tchebotar *et al*. 1998), pigeonpea and chickpea (Deanand *et al.* 2002). It was found that Azospirillum can increase the infection site providing a space for rhizobium resulting in higher nodule formation (Tchebolas *et al.* 1988).Moreover, co-inoculation with Azospirillum and Rhizobium were shown to increase phytohormones, vitamins and siderophore production (Cassan *et al*. 2009). Co-inoculation of common bean with Azospirillum- rhizobium was also shown to increase the fixed nitrogen quantity (Reman *et al.* 2008). Azotobacter was found to be a potential co-inoculant with rhizobium that enhanced the production of phytohormones and vitamins and increase the nodulation (Akhtar *et al*. 2012).

Enhanced nodulation and nitrogen fixation was noticed upon inoculation of Bacillus and Azospirillum sp.along with rhizobial inoculants in pigeonpea (Rajendran *et al*. 2008). Interaction between Streptomyces lydius WYEC108 and rhizobium of pea were shown to promote growth of the plant (Tokala *et al*. 2002) including nodule number and growth, probably by the root and nodule colonization of Streptomyces. Enterobacter is another most abundant PGP bacterium that increased the yield of nodules on green gram when co-inoculated with *Bradyrhizobium sp*. (Gupta *et al.* 1998).

Recently, it was found that nodulation, root and shoot dry weight, grain and straw yield, nitrogen and phosphorus uptake were significantly increased in chickpea upon co-inoculation with Mesorhizobium sp. *and* Psuedomonas aeruginosa *(Verma* et al*. 2013). Similar plant growth effects along with the antagonistic activities against* F. oxysporum *and* R. solani *has been observed on chickpea by co-inoculation of* Mesorhizobium, Azotobacter chroococcum, P. aeruginosa and Trichoderma harzianum *(Verma* et al*. 2014).*

2.6. Bio-Control Abilities of Rhizobia

One of the functions associated with soil microorganisms is disease suppression and protection of plants from disease when pathogens are present. The ability of bacterial siderophores and antibiotics to suppress phytopathogens could be the significant agronomic importance. Both mechanisms have essential functions in microbial antagonism but also the mechanisms leads to elicit induced resistance. Resistance-inducing and antagonistic rhizobacteria might be useful in formulating new inoculants, offering an attractive alternate of environmentally friendly biological control of plant disease and improving the cropping

systems into which it can be most profitably applied (Beneduzi et al. 2012).

Siderophore is one of the biocontrol mechanisms belonging to PGPR groups under iron limiting condition. Rhizobial strains also compete for nutrients by displacing the pathogens. Rhizobia starve the pathogens of available iron by producing high affinity siderophores and thereby limit the growth of the pathogen (Arora *et al*. 2001). Therefore, the low availability of iron in the environment would suppress the growth of pathogenic organisms including plant pathogenic fungi (Whipps, 2001).In addition to siderophore, there are other mechanisms of biocontrol including antibiotic compounds, elicitation of induced systemic resistance (ISR) of plant, and lytic enzyme secretion (Haas and Defago, 2005).

Production of volatiles such as hydrogen cyanide, suppress the growth of fungal pathogens; the ability to successfully compete with pathogens for nutrients or specific niches on the root; and the ability to induce systemic resistance (ISR) (Compant *et al*., 2005). Several rhizobial strains are reported to have the bio-control properties. Hence, usage of these strains against soil borne pathogens can lead to potential control.

The mechanisms of bio-control by rhizobia includes, competition for nutrients (Arora *et al.* 2001), production of antibiotics (Chandra *et al*. 2007), production of enzymes to degrade cell walls (Ozkoc and Deliveli 2001) and production of siderophores (Deshwal *et al*. 2003b). Hydrogen cyanide (HCN) synthesized by some rhizobacteria inhibits diseases in plant and thereby increasing the biocontrol mechanism (Schippers, 1990).The production of metabolites such as HCN, phenazines, pyrrolnitrin, viscoinamide and tensin by rhizobia are also reported as other mechanisms (Bhattacharyya and Jha 2012). For example, the strains including *R. leguminosarum biovar trifolii, R. leguminosarum biovar viciae, R. meliloti, R. trifolii, S. meliloti and B. japonicum* have been reported to secrete antibiotics and cell-wall degrading enzymes that can inhibit the phytopathogens (Ozkoc and Deliveli 2001). For example, a study on colonization behavior of *P. fluorescens* and *S. meliloti* in alfalfa rhizosphere had sufficiently demonstrated the usage of biocontrol agents to suppress pathogens (Villacieros *et al*. 2003). Pathogens that infect okra and sunflower, such as *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium solani* were shown to be controlled with the usage of *B. japonicum, R. meliloti and R. leguminosarum* (Ozkoc and Deliveli, 2001). Some more examples are cyst nematode of potato controlled by *R. etli strain G12* (Reitz *et al.* 2000), Pythium root rot of sugar beet by *R. leguminosarum viciae* (Bardin *et al.* 2004) white rot disease in Brassica campestris by *M. loti* and sheath blight of rice by *R. leguminosarum biovar* (Mishra *et al*. 2006). *Bradyrhizobium sp.* had been shown to control the infection of *M. phaseolina* in peanut, while enhancing seed germination, nodule number and grain yield (Deshwal *et al.* 2003b).

2.7. Induction of Plant Resistance

The plant growth promoting strains have been confirmed to trigger the resistance of plants against pathogens, by process known as induced systemic resistance (ISR). In this process, a signal is generated involving jasmonate or ethylene pathway and thus inducing the host plant's defense response. Various rhizobial species are reported to induce systemic resistance in plants by producing bio-stimulatory agents including *R. etli*, *R. leguminosarum biovar phaseoli* and *R. leguminosarum biovar trifolii* (Mishra *et al*. 2006). Even individual cellular components of the rhizobium had been shown to induce ISR viz. *lipopolysaccharides*, *flagella, cyclic lipopeptides, homoserine lactones, acetoin and butanediol* (Lugtenberg and Kamilova 2009).

3. ADAPTATION TO ECOPHSIOLOGICAL STRESS

3.1. Tolerance to Extremes of Temperature

The growth and survival of rhizobia in soils are adversely affected by high soil temperatures (Meghvansi, 2006).Temperature stress alters the permeability of the membrane and causes denaturation of certain enzymes/proteins leading to the death/poor growth of the rhizobia. High temperatures lead to increased drought intensity, due to enhanced transpirational water loss. This can lead to delay in nodulation or restrict the nodule to the subsurface region, reduction in nodule number, rhizobial growth, rate of colonization and infectious events (Munns *et al*.1979). The optimum temperature for rhizobial growth is 28–31^{0}C, while many of them are unable to grow beyond 37^{0}C. However, rhizobia isolated from hot and

dry environments of the Sahel Savannah are reported to tolerate temperature up to 45^{0}C, but they were found to lose their infectiveness (Karanja and Wood 1988). Similarly, a heat treatment of 35 and 37^{0}C to *R. phaseoli* was found to cause loss of melanin synthesis plasmid DNA and symbiotic properties (Beltra *et al*. 1988). In contrast, at 35 and 38^{0}C, *R. leguminosarum biovar phaseoli* was found to be effective and formed nodules in *Proteus vulgaris*, but these nodules were found to remain ineffective (Hungria and Franco 1993). Upon exposing the wild and heat resistant Rhizobium sp. to 30 and 43^{0}C, changes in the cell surface including extracellular polymeric substances/exo polysaccharides (EPS), lipopolysaccharide (LPS) and proteins had been demonstrated (Nandal *et al.* 2005).

Intra species difference in competitive efficiency was demonstrated by Krasova-Wade *et al*. (2006) in which *Bradyrhizobium ORS 3257* was found to compete their best under favorable water conditions while *Bradyrhizobium ORS 3260* was the best under limited water conditions.

3.2. Salinity Stress

Salinity is known to be the higher concentration of ions (Na^+, Cl^-, SO_4 nutrient supply via photosynthesis products and oxygen consumption) and BNF (by reducing the nodule metabolism, leghemoglobin content and atmospheric nitrogen diffusion). One of the major problems in semi-arid regions is increased salinity levels of the soil. Application of salinity tolerant rhizobia in legume cropping area helps in the formation of effective nodules and efficient nitrogen fixation. Symbiotic effectiveness depends on the specific combination of compatible legume and rhizobium under the saline conditions (Faghire *et al.,* 2013).

Salinity decreases the nutrition uptake of plants, particularly phosphorus, due to their binding with Calcium ions in salt-stressed soils.

Rhizobial species are known to vary in their salt sensitivity. Some of them are categorized as salt tolerant, such as *R. meliloti* (Zhang *et al*. 1991), *R. fredii* (Yelton *et al.* 1983), *Rhizobium sp*. from Acacia senegal, Prosopis chilensis (Zahran et al. 1994) and Vigna unguiculata (Mpepereki and Makoneses 1997), chickpea, soybean (El Sheikh and Wood 1990), and pigeonpea (Subbarao *et al.* 1990) whereas others as salt sensitive such as *R. leguminosarum* (Chein *et al*. 1992). The existence of a high degree of phenotypic and genotypic diversity in *Sinorhizobium* populations sampled from marginal soils of arid and semi-arid regions of Morocco have been demonstrated recently (Thami-Alami *et al.* 2010).

The effect of salt stress on halotolerant rhizobia by their LPS (Lloret *et al*. 1995), protein profiles (Saxena *et al*. 1996) and exopolysaccharide (Lloret *et al*. 1998) have been studied. Large variability in the efficiency of host plant and rhizobial strains on BNF under salinity had been reported (Jebara *et al.* 2001).

Salt tolerance mechanisms involve several gene families which have been reported largely in *S. meliloti* followed by *R. etli, R. tropici, Rhizobium sp., Sinorhizobium fredi and B. japonicum*. Osmoprotectants, the compatible solutes/osmolytes play a dual role as evidenced in *S. meliloti* by proline-betaine which serves as both osmoprotectant (under high osmotic stress) and energy source (under low osmotic stress) (Miller-Williams *et al*. 2006).

3.4. pH Tolerance

Soil pH influences the growth and survival of rhizobia through alteration in the permeability of the membrane and uptake of nutrients. Neutral pH allows the uptake of appropriate amount of nutrients and results in optimum growth of rhizobia (Bhargava *et al*. 2016). Low survival and poor growth of rhizobia and inhibition of initiation and formation of root nodules are the important responses that lead to the failure of rhizobia–legume symbiosis in acid soils (Richardson *et al*. 1988). The addition of lime on acid soils has been followed as a common practice to raise the soil pH creating a favorable condition for the growth and survival of root nodule bacteria (Watkin *et al*. 1997).

Graham *et al*. (1994) proposed some strains of *Rhizobium, Azorhizobium and Bradyrhizobium* to be low pH tolerant. Tolerance to acidity by rhizobia was correlated with the production of extracellular polysaccharide or polyamines glutamate concentration in the cell. Muglia *et al.* (2007) highlighted the role of glutathione, a tripeptide for the growth of *R. tropici* under low pH conditions. Watkin *et al.* (2003)

reported the ability of acid tolerant *R. leguminosarum biovar trifolii* in accumulating higher level of potassium and phosphorous than an acid sensitive strain.

3.4. Tolerance to Heavy Metals

Heavy metals are the key pollutants causing serious illness to plants, ecosystem and humans by their non-degradable nature. For the reclamation and removal of heavy metals, phytoremediation is suggested to be practiced as it preserves natural soil properties and microbial biomass (Gianfreda and Rao 2004). Ma *et al*. (2011) were also proposed the use of microorganisms such as Bacillus sp., Pseudomonas sp., *Azotobacter sp*., *Enterobacter sp*., and *Rhizobium sp.* to speed up the phytoremediation process.

Rhizobia multiply slowly in soil until they infect a compatible host. Rapid growth of rhizobia occurs only after successful infection by a single cell and formation of a nitrogen-fixing nodule on the host-root (Downie, 1997). In heavy metal contaminated sites, after the successful establishment of symbiosis with the host plant, the heavy metals tend to accumulate in the nodules. Effects of heavy metals on growth, abundance, morphology and physiology of various strains of R. leguminosarum have been well documented (Lakzian *et al*. 2002). However, despite demonstrating the extent of benefits through the use of PGPR in remediation of contaminated sites, there had been very few field studies while most of the successful studies are either from greenhouse or growth chambers (Lucy *et al*. 2004). Continuous exposure to heavy metals leads the viable bacterial cells not only to transform into a non-viable form, but also adversely affects the genetic diversity and nodulation of the host plants (Paton *et al*. 1997). Reductions in bacterial counts of rhizobium sp. have been reported with the increasing concentrations of heavy metals such as Cu, Zn and Pb, either sole or in combinations, and variations in the expression of symbiotic genes including nod genes (Stan *et al.* 2011). A great diversity in terms of plasmid types has been observed in isolates of un-polluted soil than the isolates from polluted soils. In addition, the dominant plasmid groups present in un-polluted soils were found to be absent in isolates of polluted soils and vice versa (Castro *et al*. 1997). Changes in physiology were found to lead to the variations in protein profiles that serve as a marker for stress response analysis in *R. leguminosarum biovar viciae* isolated from heavy metal polluted sites (Pereira *et al*. 2006a).

Similar to the non-nodulating bacterial species, rhizobia also has its own features such as EPS and LPS for influencing heavy metal resistance. EPS are biopolymers that possess negatively charged ligands which instantly form complexes with metal ions through electrostatic interactions (Sutherland 2001). Lakzian *et al*. (2002) identified that plasmids are the major contributing factor for this as highly tolerant strains were noticed to have 6–9 plasmids whereas moderately tolerant strains have only three plasmids. EPS from *Rhizobium Etli (strain* M4), isolated from an acid mine drainage, was shown to impact ecosystem near a manganese mine in Northern Australia (Pulsawat *et al*. 2003). However, an alternate view was reported by Pereira *et al.* (2006b) on cadmium (Cd) resistance as they found similar number (a maximum of four) plasmids in all the tolerant, moderately tolerant and sensitive isolates.

Rhizobia, such as *R. fredii, R. meliloti, R. etli, R. leguminosarum biovar viceae, R. leguminosarum biovar trifolii, Bradyrhizobium sp. and B. japonicum* had been evaluated for heavy metal resistance and of which R. fredii and R. meliloti alone were found to exhibit higher metal tolerance against Tellurium (Te) and Selenium (Se) (Kinkle *et al.* 1994). Nonnoi *et al*. (2012) demonstrated differences in the heavy metal resistance spectrum of *S. medicae and R. leguminosarum biovar trifolii* strains isolated from mercury-contaminated soils. Paudyal *et al.* (2007) reported the negative effect of heavy metals such as Al, Fe and Mo on two Rhizobium strains and their symbiotic efficiency on host plants. Chaudri *et al*. (2000) observed greatly reduced symbiosis of *R. leguminosarum biovar viciae* with pea and R. leguminosarum *biovar* trifolii with white clover under Zn toxicity as a consequence of reduced numbers of free living rhizobia in the soil indirectly affecting N fixation and Zn phytotoxicity.

3.5. Pesticide tolerance of Rhizobia

Pesticide affects plant growth by altering plant root's architecture, number of root sites for rhizobial infection, transformation of ammonia into nitrates, transformation of microbial compounds to plants and vice versa. Besides this growth and activity of free living or endophytic nitrogen fixing bacteria has also

been affected (Mathur 1999). Several studies have documented the effects of various pesticides on the reduction of microbial diversity and density on various soil types (Martinez-Toledo *et al.* 1996). Numerous microorganisms have the capacity to degrade the pesticides by the action of degradative genes in plasmids/transposons/chromosomes (Kumar *et al.* 1996). In addition to Nitrogen fixation, rhizobia are also reported to degrade toxic pesticides to non-toxic forms (Ahemad *et al*.2009), synthesise antifungal compounds (Zaidi *et al*. 2009). Therefore, identifying rhizobia possessing multiple growth-promoting activities and exhibiting insecticide tolerance ability is useful in optimising the yields of grain legumes in both conventional and stressed production systems. Among insecticides, the broad-spectrum insecticides fipronil and pyriproxyfen are used to control insects such as locusts, ticks, whiteflies, houseflies and mosquitoes, both at larval and adult stages (Aajoud *et al*. 2003) at low field application rates (Bobe *et al*. 1997) for various crops including legumes. The fipronil and pyriproxyfen-tolerant Rhizobium sp. strain MRL3 produced plant-growth-promoting substances in substantial amounts, both in the presence and in the absence of the insecticides. Interestingly, when applied with any concentration of the two insecticides, *Rhizobium* sp. strain MRL3 significantly increased the measured parameters compared with plants grown in soils treated solely with the same concentration of each insecticide but without inoculants **(**Ahemad and Khan, 2011).

3.6. Tolerance to Antibiotics

Antibiosis is the most studied and widely used mechanism of biocontrol activities (Gupta *et al.,* 2001). It refers to the inhibition of pathogen by the metabolic products released through the antagonist secondary metabolic pathways. These products include volatile compounds, toxic compounds and antibiotics, which are deleterious to the growth or metabolic activities of other microorganism at low concentrations (Fravel, 1988).

Although antibiotic resistance in bacteria is a threat in the health sector (Wright, 2007), it is a desirable trait in both indigenous and introduced rhizobial populations (Anand et al.2012). Resistance to antibiotics increases the rhizobium's chances of survival in the rhizosphere. The antibiotic-resistant rhizobium makes itself competitive in soil environment to occupy high number of nodules in legumes (Belachew 2010). Large differences in degree of tolerance to antibiotics among fast and slow-growing rhizobia have been reported (Frioni *et al.* 2001).

Rhizobial strains should be resistant to concentrations of antibiotics that inhibit the growth of other soil bacteria and they should be able to retain their infectivity and symbiotic effectiveness. While the majority of rhizobial strains in the soil are susceptible to antibiotics, others have developed resistance in response to naturally produced antibiotics (Xavier *et al*.1998). The resistance may be developed towards one or multiple antibiotic classes (Anand *et al*.2012). Cole and Elkan (1973) reported that *R. japonicum* (now *Bradyrhizobium japonicum*) carries extra chromosomal antibiotic resistance genes.

It was demonstrated early by Balassa (1963) that acquisition of resistance to streptomycin by three rhizobial species (*R. japonicum, R. meliloti and R. lupini*) is through transformation and later acquisition of penicillin resistance genes by *R. phaseolus* and *R. leguminosarum* strains through transformation was also reported (Gadre et al.1967). Later on in *R. etli,* the existence of rhizobium multiresistance genes (rmrA and rmrB) against phytoalexin and salicylic acids were identified (Gonzalez-Pasayo and Martinez-Rpmero 2000). In general rhizobial strains reported to develop antibiotic resistance *includes R. leguminosarum, R. trifolii, R. meliloti, R. Japonicum, R. phaseolus, R. lupini , and R. etli, (*Naamala *et al.2016)*

4. CONCLUSION

Rhizosphere is a unique niche that provides habitation and nutrition to PGP microorganisms. In turn, these microorganisms produce multiple benefits of induced plant growth, defense against diseases and survival under stress with many other unknown benefits.

Rhizobia in addition to the nitrogen supply, they promote growth of the plant as a symbiotic partner and soil microbe in a number of ways. Plant growth could be induced by rhizobia through some of their growth stimulating mechanisms such as mobilization of nutrient, enhancement in stress resistance, solubilization of phosphates, production of phytohormones and siderophores (Ahmad *et al.*, 2008).

Symbiotically produced IAA alone or along with other plant hormones involved in several stages of establishment of symbiotic relationship and also transported to the plant for its use. Rhizobial induced local accumulation of auxins stimulates the formation of nodule primordial (Mortier *et al*.2012) and also necessary for growth and maintenance of root nodules. Combinations of beneficial bacterial strains that interact synergistically are currently being devised and numerous recent studies have shown a promising trend in the field of inoculation technology. PGP rhizobia are excellent model systems which can provide biotechnologist with novel genetic constituents and bioactive chemicals having diverse uses in agriculture and environmental sustainability.

Therefore, generation of comprehensive knowledge on screening strategies and intense selection of best rhizobial strain for rhizosphere competence and survival should be the current need to enhance the inoculums usage successes. Thus, additional comprehensive research to exploit the potential of PGP rhizobia would provide for expansion of this research area, commercialization and improve sustainability in agricultural production.

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