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A review on Rhizobia and PGPRs interactions in legumes

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Abstract
In recent Years research on the use of *Rhizobium*, *Mesorhizobium*, *Bradyrhizobium*, *Ensifer*, *Sinorhizobium* etc. along with potential Plant Growth Promoting Rhizobacteria (PGPRs) *viz*. *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Klebsiella*, *Enterobacter* and *Serratia* in leguminous crops has been increased dramatically. Potential benefits in the co-inoculation of *Rhizobium* and PGPRs in various legumes have been studied both under green house as well as field conditions. Rhizobacteria enhance the efficacy of the inoculated *Rhizobium*, increasing nodulation as well as yield in leguminous crop. Thus, the presence of PGPRs has direct influence on competitiveness, survival and nodulating ability of seed inoculated rhizobia. In this review we will discuss about the plant microbe interaction, mechanism of nodulation and nitrogen fixation as well as several direct and indirect mechanism of PGPRs for plant growth promotion and effect of rhizobium and PGPR co-inoculation in legume.

Keywords: Interactions, legume, microbs, PGPR, rhizobia, nodulation

1. Introduction
Pulses are the important segments of Indian agriculture. India being the largest producer (25% of global production), consumer (27% global consumption) and importer (14%) of pulses in the world, accounts for about 20% of the area and 7-10% of the total food grain production in the country. Pulses not only provide food and nutritional security to the vegetarian and weaker section of the people but also add nitrogen to the soil, improving its fertility and nutrient status. Pulses derive 5-83% of their nitrogen (N) requirement by fixing atmospheric N and remaining from the soil nitrogen pool (Dudeja and Duhan, 2005) [16]. Hence, biological nitrogen fixation (BNF) in legumes offers more flexible management than nitrogenous fertilizers. This biologically fixed nitrogen becomes slowly available to non-legume species and hence, provides sustainability to cereal-based cropping systems. It is documented that legume can fix as much as 360 kg N/ha (Pandher *et al*., 2000) [55] which is not only sufficient to meet their own requirement but also enrich soil nitrogen content, making it available to the next crop in rotation. Thus, nitrogen fixing system by legumes offers an economically attractive and ecologically sound mean of reducing chemical fertilizers promoting sustainable crop production.
The composition of rhizospheric micro flora affects growth and development of the crop greatly, through various plant-microbe interactions in an intricate and interdependent manner (Parmar and Dufresnes, 2011) [58]. These rhizospheric microbes play a key role in ecosystem functioning by controlling bio-geochemical nutrient cycles, supplying available nitrogen and phosphorous to plants. Soil-plant-microbe interaction is a complex phenomenon and there are lots of ways in which it can influence the crop vigour and yield.

1.1 Legume-rhizobium interaction
Legume-Rhizobium symbiotic system is the major source of nitrogen in most cropping systems. Rhizobium, inhabiting in legume root nodules said to develop a positive interaction with the host plant. They reduce the atmospheric nitrogen to ammonia thus providing sufficient useable nitrogen source for uptake by host plants (Sessitsch *et al*., 2002) [72].
It is observed that use of Rhizobium fertilizers in lentil, soyabean and mung bean not only reduced nitrogen input up to 12-15 Kg/ha but also enhanced crop yield and soil fertility to a great extent (Pandher *et al*., 2000) [55]. Rhizobium/Bradyrhizobium species, in association with legume roots supplies about 20-40 kg N/ha and can be considered as a complementary or

supplementary source of plant nutrient (Das *et al*., 2014) [13]. Several workers have reported, seed inoculation with Rhizobium which significantly increased growth and yield of legume crops (Pathak *et al*., 2001) [59]. Beside this an increase in plant height, leaf area, photosynthetic rate, leghaemoglobin content, dry matter content as well as nutrient uptake was also achieved when mung bean crop inoculated with Rhizobium species. It is also documented that; Rhizobium inoculation increase grain yield up to 9-76% but on an average 10-30% increase in yield is achieved under farmer's field condition. Etesami *et al*., (2009) [18] also found that, rhizobial bacteria are the best plant growth promoters among rhizobacteria that are able to increase plant growth and yield by various mechanisms.

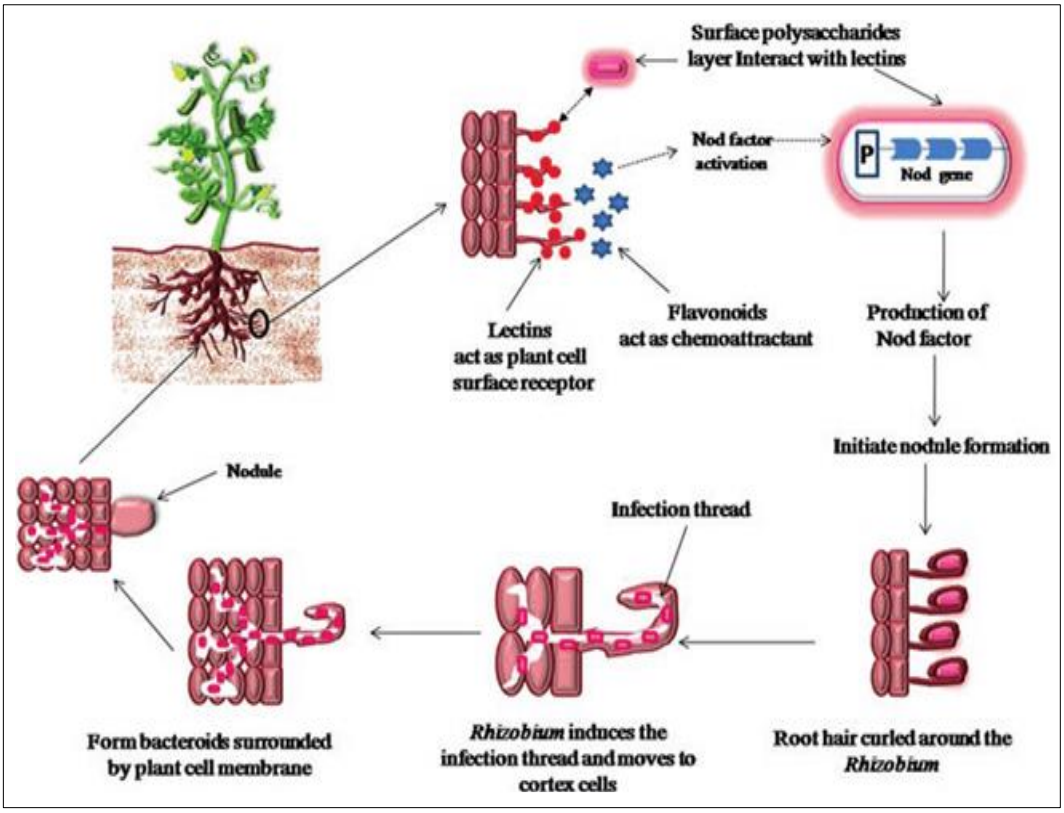
1.1.1 Nodulation and biological nitrogen fixation

Nodulation in legumes is a highly specific interaction between legume roots and the rhizobia which involve quorum sensing mechanism. In low nitrogen conditions legume roots secrete specific flavonoids, in response to which compatible rhizobia undergo chemotaxis towards the plant roots, leading to an increase in cell density and production of specific lipo-chitooligosaccharide a signaling compound. These signaling compounds are called Nod factors. These nod factors are responsible symbiotic signaling and nodulation in legumes.

Nod factors induce cell divisions in the root cortex and successive divisions lead to the formation of the nodule primordium. Simultaneously, the rhizobia enter the host plant via the root hairs through the infection threads. Rhizobia are released from infection threads in to the cytoplasm by endocytosis. This intracellular membrane encapsulated bacteria are called bacteroid. Loss of the ability to produce or perceive either Nod factors or flavonoids prevent nodulation. In addition to their role in nodulation, secreted flavonoids have other roles in the rhizosphere, particularly in P and Fe acquisition, IAA biosynthesis etc.

The bacteria within the nodule cells gain the ability to fix nitrogen gas by means of their nitrogenase enzyme complex and supply the host plant with the reduced nitrogen for plant growth. The plant provides photosynthates to the bacteria and a micro-aerobic niche for the oxygen-sensitive nitrogenase.

1.1.2 Nodule formation



(***Source*:** Prachi Singh)

Fig 1: Process of legume-rhizobial symbiosis and nodulation in roots of legume plant

1.2 Legume-rhizobium-PGPRs interaction

Besides indigenous rhizobia, legume roots are colonized by numerous rhizospheric bacteria that are present in on or around the plant roots. It is observed that, these rhizospheric microorganisms not only help the inoculated rhizobia in survival through synergism but also increase their nodulation ability and N fixing efficiency.

In legumes these PGPRs including *Pseudomonas*, *Bacillus*, *Azotobacter*, *Serratia*, *Flavobacter*, *Agrobacterium*, *Arthrobacter*, *Cellulomonas*, *Erwinia*, *Streptomyces* etc. manage important biological functions by symbiotically interacting with Rhizobium populations within the rhizosphere and help create a beneficiary region where interacting microorganisms benefit from additional nutrient resources. These microbes interact synergistically with the plants as well as with inoculated Rhizobium, affecting nodulation and N fixation (Gaind *et al*., 2007) [20].

Among PGPRs, Pseudomonas and Bacillus are predominant and the most commonly studied genera possessing plant growth promoting traits, they are aggressive colonizers of rhizosphere and they do interact symbiotically with Rhizobium, affecting nodulation and nitrogen fixation (Parmar and Dufresnes, 2011) [58]. Due to the potential benefits observed by these soil microorganisms, researchers

are now focusing on co-inoculation of PGPRs with Rhizobium and it is becoming the most popular approach for improving the growth in legumes (Iqbal *et al*., 2012)

2. Mechanism of PGPRs

Rhizobacteria enhance plant growth by multitudinous mechanisms which include; production of plant growth-regulating substances (PGRs); phytohormones, Biological Nitrogen Fixation (BNF), enhancement of mineral uptake, mineralisation of organic phosphorus and Zn solubilisation, suppression of plant pathogens through antibiosis, bacteriocinogenic action, siderophore production, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, hydrogen cyanide (HCN), and ammonia production and production of phytoalexins/flavonoids-like compounds.

2.1 Production of plant growth regulators or phytohormone like substances

Phytohormones are organic compounds which at extremely low concentration influence the physiological processes in plants. PGPRs produce an identical or nearly identical compound that mimics the action of phytohormones known as phyto-stimulator/plant hormone analogues. Indole-3-acetic acid (IAA), gibberelic acid, cytokinins, and in some cases ethylene are the known phyto-stimulators produced by microorganisms (Ahmad *et al*., 2008) [3]. Production of these substances by inoculated microorganisms has been proved as one of the most plausible mechanisms of action affecting plant growth (Gangwar, 2013) [21]. Strains of PGPRs inducing the production of phytohormone like substances include *Azotobacter* sp, *Azospirillum* sp., *Rhizobium* sp. *Pseudomonas* sp. and *Bacillus* sp. etc.

2.1.1 Production of IAA

Indole-acetic-acid being the major and most abundant auxin in plants plays a key role in plant growth regulation and development. It is believed that approximately 80% of rhizobacteria produce IAA equivalents (Khalid *et al*., 2004) [39]. Many of these PGPRs that produce indole-3-acetic acid (IAA), indole-3- butyric acid (IBA) or their precursors remarkably affect the plant growth by altering the endogenous level of auxin synthesized in plants. Glick, (2012) [23] reported that, secretion of IAA by soil bacteria can alter the endogenous pool of plant IAA that in turn interferes with plant developmental processes. It has also been well documented that the biosynthesis of auxins with their excretion into soil makes a major contribution to the bacterial plant growth-promoting effect (Steenhoudt and Vanderleyden, 2000) [78].

Bacterial IAA producers (BIPs) have the potential to interfere in controlling many important physiological processes including tissue differentiation, cell enlargement and division, flowering as well as responses to light and gravity. Again, it is observed that the production of IAA by rhizobacteria mainly affect the plant root system causing overall development of root system which led to an increase in nutritional uptake by plant (Etesami *et al*., 2009) [18]. IAA also acts as reciprocal signalling molecule that associated with down regulation of plant defense against a number of phytopathogenic bacteria which consequently plays an important role in rhizobacteria-plant interaction (Spaepen and Vanderleyden, 2011) [77].

2.1.2 Production of gibberellins

Gibberellins are a class of phytohormones most commonly associated with modifying plant morphology by stimulating stem elongation and seed germination. GA includes the largest group of plant regulators which can be translocated from root to aerial parts of the plant, affecting its morphology. Evidence for GA production by microorganism is provided by several scientists. Atzorn *et al*., (1988) [5] provided the first evidence on GA production by *Rhizobium* sp. followed by Gutierrez-Manero *et al*., (2001) [27] who showed four different forms of GA produced by *Bacillus pumilus* and *B. Licheniformis*. However, several sp of *Azospirillum*, *Acetobacter*, *Herbaspirillum* and *Pseudomonas* have been claimed to produce gibberellin-like substances.

2.1.3 ACC deaminase activity

Ethylene is an essential metabolite for the normal growth and development of plants (Khalid *et al*., 2006) [38]. It is produced endogenously by approximately all plants and also by different biotic and abiotic processes in soils inducing multiple physiological changes in plants. Besides a plant growth regulator, ethylene has also been established as an important stress hormone (Saleem *et al*., 2007) [4]. Under stress conditions like those generated by salinity, drought, water logging, heavy metals and pathogenicity, the endogenous level of ethylene is significantly increased which negatively affects overall plant growth. For instance, high concentration of ethylene induces defoliation and other cellular processes that may lead to reduced crop performance (Bhattacharyya and Jha, 2012) [8] beside these, various biotic and abiotic stresses also produce ethylene in plants.

Plant growth promoting rhizobacteria which possess the enzyme; 1-aminocyclopropane-1-carboxylate deaminase (ACC-deaminase), facilitate the growth and development of plants by decreasing ethylene levels, increase salt tolerance and reducing drought stress in plants (Zahir *et al*., 2008) [99]. Currently, bacterial strains exhibiting ACC deaminase activity have been identified in a wide range of genera such as *Acinetobacter*, *Alcaligenes*, *Serratia*, *Achromobacter*, *Bacillus*, *Agrobacterium*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Azospirillum*, *Ralstonia*, *Rhizobium* etc. (Kang *et al.*, 2010) [37]. These rhizobacteria take up ACC and convert it into 2-oxobutanate and NH3 (Arshad *et al*., 2007) [4]. The major noticeable effects of seed/ root inoculation with ACC deaminase producing rhizobacteria are the plant root elongation, promotion of shoot growth and enhancement in rhizobial nodulation and N, P and K uptake as well as mycorrhizal colonization in various crops (Nadeem *et al*., 2009) [52].

Baber *et al*., (2007) observed that the rhizobacterial strains possessing ACC-deaminase activity promote plant growth and nodulation in legumes when co-inoculated with Rhizobium. Ma *et al*., (2004) also provided the direct evidence of ACC-deaminase activity on nodulation of alfalfa. Saharoona *et al*., (2006) suggested that, co-inoculation of PGPR showing ACC deaminase activity and competitive rhizobia could be an attractive and sustainable approach to enhance nodulation in legumes. Iqbal *et al*., (2012) reported that, co-inoculation of PGPR having ACC-deaminase activity with Rhizobium positively affect plant biomass and nodulation in mung bean. It was also observed that co-application of Rhizobium with PGPRs containing ACC deaminase activity, further improved the growth of lentil as compared to inoculation with either PGPR or Rhizobium alone. Zahir *et al*., (2011) [100] revealed that, PGPR containing ACC deaminase activity in combination with potential rhizobia, enhanced nodulation in

lentil by regulating the ethylene synthesis. Saini and Khanna, (2013) [68] suggested that, inoculation of PGPR exhibiting ACC deaminase activity along with Rhizobium as expedious biofertilizes can be utilized to increase growth as well as nodulation in legume plants. According to Saravana-Kumar and Samiyappan (2007) [70], Bradyrhizobium in combination with active ACC deaminase containing PGPRs have the potential to promote growth and nodulation in legumes.

Zafer-ul-hye (2007) showed that, inoculation with selected rhizobacterial isolates increased the root-shoot growth and biomass of lentil seedlings over un-inoculated control. Plants that are treated with PGPR having ACC deaminase activity are dramatically more resistant to the injurious effects of ethylene, synthesized as a consequence of stressful condition of flooding (Grichko and Glick, 2001) [25], drought (Zahir *et al*., 2007) [53], high salt concentration (Nadeem *et al*., 2007) [53], and the presence of phytopathogen (Wang *et al*., 2000) [91]. It is highly likely that, presence of ACC deaminase producing PGPRs in legume roots could possibly suppress endogenous synthesis of excess C2H4 during the rhizobial infection and thus may facilitate nodule formation in roots (Zafer-ul-hye, 2007).

2.2 Solubilisation of mineral nutrients

Solubilisation of mineral salts is an imperative feature of PGPR. The bacteria that are found to be present in near vicinity of rhizosphere are more versatile in solubilising, mineralising, transforming and mobilising essential plant nutrients as compared to those from edaphosphere. Therefore, the rhizobacteria are the dominant driving forces in recycling soil nutrients and consequently crucial for soil fertility (Glick, 2012) [23]. These rhizospheric microorganisms are also known to activate and stimulate the root system by secreting several plant growth regulators. PGPRs that secret organic acids are believed to solubilise and mineralise nutrients thus facilitating their easy availability to plants. Solubilisation of essential plant nutrients such as zinc, iron and phosphorus by rhizobacteria makes them more readily available for plant uptake (Biswas *et al*., 2000) [9].

2.2.1 Phosphate solubilisation

Phosphorus is one of the most essential elements for plant growth and development, which is available in both organic and inorganic forms in soils (Khan *et al*., 2009) [42]. It affects various physiological and biochemical plant processes like photosynthesis, sugar to starch transformation and transmission of genetic traits (Mehrvarz, 2008) [50]. Microorganisms are an integral component of the soil phosphorus cycle and are important for the transfer of P between different pools of soil P (Richardson, 2003) [65]. Plant growth promoting rhizobacteria have been shown to solubilise precipitated phosphates and enhance phosphate availability to the plants (Upadhyaya *et al*., 2013). Various soil microorganisms are known to solubilise insoluble phosphorous complexes into solution and make it possible for its use by plant (Tripura *et al*., 2005) [82].

It is observed that most of the applied phosphatic fertilizers are also re-precipitated into insoluble mineral complexes and are not efficiently taken up by the plants. Richardson (2000) [64] suggested that, use of soil microorganisms as bioinoculants for mobilisation of phosphorus is the most efficient and reliable approach, where soils are poor in available phosphorus and application of phosphatic fertilizers represents a high cost to the farmer. Use of these microbial inoculants help in mobilisation of inorganic phosphorus in poor soil and make it available to plants. Microbial solubilisation of inorganic phosphate compounds is of great economic importance in plant nutrition (Gaur, 2002) [22]. Therefore, use of PSM is found to mobilise the soil P for plant uptake and hence act as viable substitute to chemical phosphatic fertilizers (Khan *et al*., 2006) [41]. Goldstein (2001) [24], identified bacteria of genera Achromobacter, Agrobacterium, Bacillus, Enterobacter, Erwinia, Escherichia, Flavobacterium, Mycobacterium, Pseudomonas and Serratia as highly efficient in solubilising unavailable complexed phosphate into available inorganic phosphate ions. Vora and Shelat (1996, 1998) [89, 90], isolated PSM and reported novel strain *Torulopsora globosa*, solubilizing insoluble phosphate.

The organic substrates present in soil act as a source of P for plant growth. This organic form of P must be hydrolized to inorganic form to make it available for plant nutrition. Mineralisation is carried out mostly by enzymes like phosphatase, phytase, phosphonoacetate hydrolase, D-α-glycerophosphatase and C-P lyase (Hayat *et al*., 2010). Zaidi *et al*., (2009) [42] showed that, synthesis of low molecular weight organic acid by various rhizobacteria, consequently affect the solubilisation of inorganic phosphorus. Bhattacharya and Jha (2012) [8] reported that, most potent phosphate solubilising bacteria expressed a significant level of acid phosphatase activity. In this context of organic acid production, Chen *et al*., (2006) [11] for the first time reported four bacterial strains, *Arthrobacter ureafaciens*, *Phyllobacterium myrsinacearum*, *Rhodococcus erythropolis* and *Delftia* sp. as most potent phosphate-solubilising bacteria (PSB) after confirming their capacity to solubilise considerable amounts of tricalcium phosphate in the medium.

Vassilev *et al*., (2006) [85] proved that, microorganism mediated *in vitro* solubilisation of insoluble phosphates is associated with the detachment of organic acids, which are often combined with other metabolites. According to Glick (2012) [23], microbial synthesis of different phosphatases helps in mineralisation of soil organic phosphorus. It is also observed that potential phosphate solubilisers may produce siderophores, lytic enzymes and phytohormones for effective solubilisation of organic phosphates. Tao *et al*., (2008) [79] reported that, phosphate solubilisation and mineralisation may coexist in the same bacterial strain. These PSBs, when inoculated onto plants often result in improved growth and P nutrition with responses being observed under both glasshouse and field conditions (Whitelaw, 2000) [94].

Siddiqui *et al*., (2006) [75] observed, application of PSBs in green gram, increased the nodule numbers and nodule dry biomass. Further, Kumar and Chandra (2008) [45], in a study found that application of PGPR and PSB in lentil enhanced the strain competition of inoculated Rhizobium leguminosarum under field condition. They also reported increase in nodule occupancy of inoculated *Rhizobium* sp when lentil co-inoculated with PSBs and PGPRs along with Rhizobium. Oberson (2001) [54], observed an increased biological activity and microbial uptake as well as release of P can be achieved by incorporation of organic residues through legume rotations. Wani *et al*., (2007) [92], Mittal *et al*., (2008) [51] and Hosseini *et al*., (2014) [32] reported that, interaction of PSMs results in an increase of plant-available soil phosphorus in rhizosphere. Further, Ponmurugan and Gopi (2006) [61] claimed that, besides providing P, the PSBs synthesize important plant growth promoting substances to augment the growth of plant.

Beside these characteristics, PSBs are also responsible for stimulating the efficiency of biological nitrogen fixation (BNF) and boosting the availability of other trace elements such as Fe and Zn. Production of siderophore (Vassilave *et al*., 2006, Wani *et al*., 2008) [93], antibiotics (Fernando *et al*., 2006) and providing protection to plants against soil borne plant pathogens (Ahmed and Khan, 2011) [2] also been observed in potential PSBs.

2.2.2 Zn solubilisation

Zinc is an essential element necessary for plants, humans and microorganisms (Hafeez *et al*., 2013). Most of the Indian soils are either Zn deficient or contain Zn in fixed form i.e. unavailable to plants. Zn is mostly found as *viz*. ZnS (sphalerite), zincite (ZnO), smithsonite ($ZnCO_3$), zinkosite ($ZnSO_4$), franklinite ($ZnFe_2O_4$) etc. About 90% of the Zn in soil exists in insoluble form and is inaccessible for uptake by plants (Barber, 1995) [7]. According to Hacisalihoglu and Kochian (2003), Zn2+ may exist as low as 10-11-10-9 M in soils and is responsible for reduction in crop growth. Zinc being an important micronutrient, affects crop vigour, growth, maturity and yield (Hirschi, 2008) [31]. It is also observed that, many physiological functions in plant such as synthesis of auxin and the photochemical reaction of chlorophyll, stability of biological membranes and activity of various enzymes, e.g. Cu/Zn superoxide dismutase (SOD) and carbonic anhydrase containing structurally bound Zn is affected by plant Zn nutrition (Hafeez *et al*., 2013). Zn also influences the synthesis of nucleic acid, proteins, and lipids which positively affect the quality of grains (Kramer and Clemens, 2006) [43]. According to Khalifa *et al*., (2011) [40], Zn availability enhances the plant growth, leaf and flower number, yield, nutrient content and plant chemical constituents i.e. carbohydrates, pigments and oil concentration.

Several scientists reported that, in addition to mobilisation of phosphates, PGPRs are responsible to play key role in carrying out the bioavailability of soil zinc, potassium, iron and silicate to plant roots (Saravanan *et al*., 2011) [71]. Generally, the mechanism involved includes; acidification, exchange reactions, chelation, and release of organic acids by rhizobacteria (Chung *et al*., 2005) [12]. More possibly, mobilisation mechanism of Fe and Zn involves the production of siderophore (Saravanan *et al*., 2011) [71], gluconate, derivatives of gluconic acids and various other organic acids produced by PGPR (Tariq *et al*., 2007) [80].

Mahdi *et al*., (2010) [48] suggested that, use of Bacillus biofertilizer as an alternative to expensive $ZnSO_4$ in Zn deficient soils or in soils where native zinc is in elevated or in conjugated with insoluble zinc compounds like $ZnCO_3$, ZnO and ZnS could be beneficial. It is evident that, inoculation of potent strains of Zn mobiliser rhizobacteria, increase the yield of field crops such as wheat, rice, maize and barley. Tariq *et al*., (2007) [80] described the effect of Zn mobilising PGPR that had significantly alleviated the Zn deficiency and increased the grain yield (65%), total biomass (23%) harvest index as well as Zn concentration in the grains of rice. Furthermore, the study revealed; inoculation of Zn-mobilising PGPR showed a positive impact on root-shoot growth, panicle emergence index and exhibited the maximum Zn mobilisation efficiency over untreated control.

2.3 Siderophore production

Iron is an important micronutrient essential for bacterial metabolism. The bacteria inhabiting in soil acquire iron by the secretion of low-molecular weight iron chelators referred to as siderophores which have high association constants for complexing iron. In the aerobic environment, iron occurs principally as Fe3+ and is likely to form insoluble hydroxides and oxyhydroxides, thus making it inaccessible to both plants and microorganisms (Rajkumar *et al*., 2010) [62]. In soil, Fe is unavailable for direct assimilation by microorganisms. As the predominant form of iron i.e. ferric iron ($Fe3^+$) is only sparingly soluble and available in very low concentration to support microbial growth. This $Fe3^+$ undergoes reduction to form ferrous ion which is susceptible to oxidation. Alternatively, this reduced ferrous form may be directly transferred into metabolic activities before it gets oxidized.

As iron is essential for survival and growth, bacteria that are better adapted to obtain iron can compete better in rhizosphere. Competition for iron mainly occurs for ferric ion and for the iron siderophore complex. The bacterium that originally synthesizes siderophores takes up iron-siderophore complex by using a receptor that is specific to the complex and is located in the outer cell membrane of the bacterium. Once inside the cell, the iron is released and is then available to support the microbial growth. A myriad of environmental factors like pH, iron level and forms of iron ions, presence of trace elements, and an adequate supply of C, N and P can also modulate the siderophore synthesis (Duffy and Defago, 1999) [17].

Iron also plays a dominant role in N fixation and assimilation process in legumes. The iron enzyme involved in the process include, nitrogenase, leghaemoglobin, ferredoxin and hydrogenase with nitrogenase and leghaemoglobin constituting up to 12 and 30% of total protein in the bacterial and infected plant cells respectively (Verma and Long, 1983) [86]. Siderophore producing rhizobacteria improve plant health at various levels; they improve iron nutrition, inhibit growth of other microorganisms by releasing antibiotic molecules and hinder the growth of pathogens (Jha and Saraf, 2015) [36]. Recently, Sharma *et al*., (2003) [74] assessed the role of siderophore positive Pseudomonas strain GRP3 on iron nutrition of Vigna radiata. In the study, it was observed that, at 45 DAS the plants showed a decline in chlorotic symptoms whereas, chlorophyll a and chlorophyll b content increased significantly in GRP3 inoculated plants over un-inoculated control. According to Mathiyazhagan *et al*., (2004) [49] siderophore producing PGPRs cause growth inhibition, decrease in nucleic acid synthesis inhibition of sporulation of plant pathogens and thus responsible for suppression of the plant diseases. Indirectly rhizobacteria helps plant growth by acting as biocontrol agents for protecting plants against various phytopathogens (Jha and Saraf, 2011) [35].

Study revealed that, seed inoculation of siderophore producing Pseudomonas not only help in root rot disease control but also stimulate plant growth in green gram (Sahu and Sindhu, 2011) [67]. The co-inoculation of siderophore producing *Pseudomonas* sp with *Bradyrhizobium* sp. have been found to cause a significant increase in nodule number, nodule weight and plant dry weight of green gram and chickpea (Sindhu *et al*., 2002) [76]. Seed inoculation of mung bean with siderophore producing microorganism significantly enhanced nodulation, nitrogenase activity, dry matter accumulation and yield as reported by several researchers. Numerous studies of the plant growth promotion vis-a-vis siderophore-mediated Fe-uptake as a result of siderophore producing rhizobacterial inoculations have been reported (Rajkumar *et al*., 2010) [62]. Similarly, the Fe-pyoverdine

complex synthesized by Pseudomonas fluorescens C7 was taken up by Arabidopsis thaliana plants, leading to an increase of iron inside plant tissues and to improved plant growth (Vansuyt *et al*., 2007).

2.4 Volatile compounds production and antagonistic activity

The application of microorganisms to control diseases is a form of biological control, (Lugtenberg and Kamilova, 2009) [46]. Glick (2012) [23] reported that, rhizobacteria acting as biocontrol agents show major indirect mechanism of plant growth promotion. In general, niche exclusion, competition for nutrients, induced systemic resistance and antifungal metabolites production are the chief modes of biocontrol activity exhibited by PGPRs. Many rhizobacteria have been reported to produce antifungal metabolites like, HCN, phenazines, pyrrolnitrin, 2, 4-diacetylphloroglucinol, pyoluteorin, viscosinamide and tensin (Bhattacharya and Jha, 2012) [8]. Interaction of some rhizobacteria with the plant roots can result in plant resistance against some pathogenic bacteria, fungi, and viruses (Ahemad, 2012) [1].

Hydrogen cyanide (HCN) production during the early stationary growth phase is an important feature of rhizobacteria. HCN production for biological control of weeds to minimizing deleterious effects on the growth of desired plants has been reported by Kremer and Souissi, (2001) [44]. HCN is generally considered to be a secondary metabolite that has an ecological role and confers a selective advantage on the producer strains (Vining, 1990) [87]. The production of HCN was a more common trait of Pseudomonas as reported by Ahmad *et al*., (2008) [3]. They further found that, the antifungal activity of the PGPRs was greatly enhanced when both HCN and siderphores were produced. As these two PGP traits work synergistically to inhibit pathogenic fungi reducing the incidence of diseases. Nevertheless, at present applications of HCN producing rhizobacteria, in areas of biocontrol methods are increasing (Devi *et al*., 2007) [14].

Ammonia production by several PGPR strains, affects the rhizospheric plant-microbe interactions. Symbiotic association of nitrogen fixing microorganisms with legumes helps in biochemical reactions of biological nitrogen fixation reducing atmospheric elemental nitrogen (N2) into ammonia (NH3).

2.5 Stimulate flavonoid production by legume roots

Flavonoids are diverse group of secondary plant metabolites which are derived, via phenyl propanoid biosynthetic pathway that play an array of important functions in plants, ranging from auxin transport inhibitors and floral pigments for the attraction of insect pollinators to antioxidants. Flavonoids also act as signal molecules for beneficial microorganisms in the root rhizosphere of many plant species and functions as antimicrobial defense compounds in their interactions with pathogenic microbes. Further, flavonoids also play a critical role in promoting nitrogen-fixing symbiosis with rhizobia in legumes where legume root exuded flavonoids act both as chemo-attractants for symbiotic rhizobia. It also helps in activating the rhizobial nod genes, which are responsible for the synthesis of nod factors (Reddy *et al*., 2007) [63]. Nod factors are proteins, perceived by plant root hairs for initiation of nodules in host plant. This vast group of compounds comprise of chalcones, flavanones, flavones, flavandioles, anthocyanoidines, condensed tannins, aurones and caumarines. In addition, isoflavones are mainly synthesized by legumes which are indispensable for the establishment of legume-Rhizobium symbiosis.

Dieter (2006) [15], showed the de-novo activation of flavonoid biosynthesis during symbiotic interactions as well as a defense response against pathogens. Parmar and Dadarwal (1997) [56], observed the nodule stimulating rhizobacteria can produce enhanced levels of flavonoids in roots, on seed bacterization. Also, ethyl acetate extracts of culture supernatant fluids when applied to seeds resulted in enhancement of flavonoids in roots, suggesting that the rhizobacteria have a direct influence on root flavonoids which might be an additional factor in nodule promotion by these bacteria. Volpin *et al*., (1996) [10] and Burdman *et al*., (1996) [10] observed that A. brasilense increased the exudation of flavonoids, which enhanced the establishment of the Rhizobium-legume symbiosis, in alfalfa and common bean rhizosphere respectively.

2.6 Abiotic stress response

Microbial adaptation to stress is a complex regulatory process in which a number of genes are involved. Certain microbial species live in extreme habitats (thermophiles and halophiles) and they use different mechanisms to reduce stress (Grover *et al*., 2010) [26]. When subjected to stress conditions, most rhizobacteria produce osmoprotectors (K+, glutamate, trehalose, proline, glycine, and polysaccharates), enhance plant tolerance to abiotic stresses such as drought, nutrient stress and salinity (Yang *et al*., 2009) [97]. Specifically, soil microorganisms in root vicinity trigger different mechanism of actions that affect plant tolerance to stress. They produce phytohormones like; IAA and gibberellins to promote the growth of root hairs and increase total root surface area, which in turn facilitate nutrients uptake by plants. These rhizobacteria are known to influence the plant's immunity and productivity. According to Grover *et al*., (2010) [26], production of exopolysaccharides, synthesis of IAA, ACC-deaminase, and proline, induction of resistance genes in plants by certain rhizospheric microorganisms may help in mitigating the impact of soil drought. They induce the defense mechanism of host by inducing specific resistance mechanisms; Induced Systemic Resistance (ISR) and Systemic Acquired Resistance (SAR) and mitigate the stress condition in several pulse crops *viz*. black gram, green gram, horse gram, chick pea, arhar etc.

2.7 Interactive effect of Rhizobium-PGPRs co-inoculation in legume

During past few decades several studies were conducted to show the effectiveness of co-inoculation of Rhizobium with PGPRs on legumes. A variety of PGPRs including Bacillus and Pseudomonas species, are commonly associated in the rhizosphere of legume as well as non-leguminous crops. Combinations of beneficial bacterial strains exhibiting synergistic interactions are currently being devised and numerous recent studies show a promising trend in the field of inoculation technology. In this context PGPRs, in combination with efficient rhizobia increased the nodule occupancy by inoculated rhizobia thus improve the nitrogen fixation and growth of legumes (Tilak *et al*., 2006) [81]. Sindhu *et al*., (2002) [76] observed that, the co-inoculation of some Bacillus strains with effective Bradyrhizobium enhanced nodulation and growth of green gram (*Vigna radiata* L.). Interactive effect of rhizobia and free-living soil bacteria could be more beneficial due to a variety of mechanisms such

as production of additional infection sites by PGPRs for rhizobial infection, antibiosis and siderophore production to chelate insoluble cations or to colonize root surfaces (Plazinski and Rolfe, 1985) [60]. Few studies have shown that, the inoculation of seed with mixed cultures have tremendous positive effects on plant growth compared to the single strain inoculation (Xavier and Germida, 2002) [95].

Parmar and Dadarwal (1999) [57] revealed that, co-inoculation of the rhizobacteria with effective Rhizobium strains of chickpea, significant increase in nodule weight, root and shoot biomass and total plant nitrogen. Another study by Izhar *et al*., (1995) [34], showed a greater number of nodules per plant where Bradyrhizobium was used with strains of *Pseudomonas aeruginosa*. The influence of PGPR for improved nodulation, dry-matter accumulation in roots and shoots, grain yields, biomass and protein content in chickpea (*C. arietinum* L.) under field conditions has been thoroughly studied by Rokhzadi *et al*., (2008) [66]; The influence of PGPR on dry-matter accumulation chickpea *(C. arietinum* L.) yield under field conditions has been thoroughly studied by Rokhzadi *et al*., (2008) [66], they reported an improved nodulation, increased dry matter accumulation in roots and shoots, grain yields, biomass and protein content of chickpea by a significant margin. This can be attributed to the cumulative effects of an enhanced supply of nutrients, mainly nitrogen and phosphorus and the production of growth promoting substances. In addition, *P. fluorescens* has been found to synergistically interact with additional rhizobacteria and responsible for interactions within the rhizosphere, phytohormone production, stimulation of nutrient uptake and the bio-control of deleterious pathogens in soil. Synergistic effects of PGPRs and Rhizobium inoculation on nodulation and nitrogen fixation in pigeonpea (*Cajanus cajan*) were also observed by Tilak *et al*., (2006) [81]. Inoculation of Rhizobium phaseoli and PGPR such as P. fluorescens P-93 and A. lipoferum S-21 yielded promising results in terms of bean yield and plant growth promoting parameters (Yadegari *et al*., 2008) [96]. Similarly, simultaneous inoculation of *Pseudomonas* sp. with *Rhizobium* sp. has been reported to enhance nodulation, nitrogen fixation, plant biomass and grain yield in various leguminous species including green gram, soybean, chickpea and alfalfa. Co-inoculation with *Bacillus* spp. and Rhizobium or *Bradyrhizobium* spp. enhanced the nodulation and plant growth of common bean and soybean respectively (Bai *et al*., 2002) [6]. From these studies, on combined effect of PGPRs with *Rhizobium* sp., it has been documented that, the seed inoculation with dual culture produced more pronounced effect on agronomic parameters such as root-shoot length, root-shoot fresh and dry weight, nodule number, nodule fresh and dry weight and yield response of crop as compared to un-inoculated control. Further an enhancement in chlorophyll, leghaemoglobin, flavonoids as well as proline content has also been reported.

3. Conclusion

Rhizobium-PGPRs interaction is a complex phenomenon in rhizosphere of legume plants, which not only influence the nodulation and nitrogen fixation but also increase the growth and yield of the legume crop. In order to improve the rhizobial interaction with legume roots, it is essential to exploit certain compatible PGPRs which can positively influence the rhizobial colonization and interaction with legume plant. Research is needed to isolate specific Rhizobia and their compatible PGPRs and develop formulations which can be commercially available for seed treatment as well as direct field application to different legume crops.

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