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Impact of Plant Growth Promoting Rhizobacteria on Crop Production

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Abstract: Rhizospheric soils of crop plants have more flora and fauna due to availability of more organic compound, macronutrient and micronutrient. Rhizobacteria that exert beneficial effects on plant growth and development are referred to as Plant Growth Promoting Rhizobacteria (PGPR). Plant growth promoting rhizobacteria is a group of free living soil bacteria, which have ability to promote growth and yield of crop plant by direct and indirect mechanism. PGPR is generally two type, one is colonies inside plant cells that called intracellular PGPR (iPGPR) and other colonies out side plant in rhizosphere that called extracellular PGPR (ePGPR). This review generally focused on direct and indirect mechanism of PGPR. Direct mechanism of plant growth promotion may involve the synthesis of substances by the bacterium or facilitation of the uptake of nutrients from the environment. The indirect mechanism of plant growth occurs when PGPR lessen or prevent the deleterious effects of plant pathogens on plants by production of inhibitory substances or by increasing the natural resistance of the host. The search for PGPR and investigation of their mode of action are increasing at a rapid use as commercial biofertilizers. The mode of action and practical application of PGPR in crop production are the major focus of this review.

Key words: PGPR, rhizosphere, IAA, phosphate solubilization, growth, yield

INTRODUCTION

Bacteria able to colonize plant root systems and promote plant growth are referred to as Plant Growth Promoting Rhizobacteria (PGPR) (Kloepper and Schroth, 1978). PGPR can affect plant growth either indirectly or directly; indirect promotion of plant growth occurs when PGPR lessen or prevent the deleterious effects of one or more phytopathogenic organisms; while direct promotion of plant growth by PGPR involves either providing plants with a compound synthesized by the bacterium or facilitating the uptake of certain nutrients from the environment (Glick, 1995). Thus, in the broadest sense, PGPR include the N_2-fixing rhizobacteria that colonize the rhizosphere, providing N to plants, including the rhizobia of the well-characterized legume-rhizobia symbiosis. Regardless of the mechanisms of plant growth promotion, PGPR must colonize the rhizosphere around the roots, the rhizoplane

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(root surface) or the root itself (within root tissues) (Glick, 1995). Plant growth promoting bacteria (PGPR) associations range in degree of bacterial proximity to the root and intimacy of association. In general, these can be separated into extracellular PGPR (ePGPR), existing in the rhizosphere, on the rhizoplane or in the spaces between cells of the root cortex and intracellular PGPR (iPGPR), which exist inside root cells, generally in specialized nodular structures. The latter includes rhizobia and Frankia species, both of which fix nitrogen in symbiosis with higher plants. There has been considerable development in understanding signaling mechanisms of rhizobia (iPGPR) during the establishment of the rhizobia-legume symbiosis and this may serve as a model of knowledge regarding cross-talk and plant growth promoting mechanisms (Gray and Smith, 2005). The inoculation of seeds with PGPRs is known to increase nodulation, nitrogen uptake and growth and yield response of crop plants (Dorosinsky *et al*., 1975; Herandez *et al*., 1983; Johri *et al*., 2003; Yadav and Verma, 2009; Verma *et al*., 2010).

PGPR and Their Hosts: Rhizospheric and Endophytic Region

For PGPR to have a beneficial effect on plant growth via an enhancement of the nutrient status of their host, there obviously needs to be an intimate relationship between the PGPR and the host plant. However, the degree of intimacy between the PGPR and the host plant can vary depending on where and how the PGPR colonizes the host plant. Relationships between PGPR and their hosts can be categorized into two levels of complexity: (1) Rhizospheric and (2) Endophytic.

Rhizospheric Region

The rhizosphere can be defined as any volume of soil specifically influenced by plant roots and/or in association with roots and hairs and plant-produced material (Andrade *et al*., 1997; Mahafee and Kloepper, 1997; Bringhurst *et al*., 2001). This space includes soil bound by plant roots, often extending a few mm from the root surface (Bringhurst *et al*., 2001) and can include the plant root epidermal layer (Mahafee and Kloepper, 1997). Plant exudates in the rhizosphere, such as amino acids and sugars, provide a rich source of energy and nutrients for bacteria, resulting in bacterial populations greater in this area than outside the rhizosphere. Extracellular PGPR (ePGPR) which is existing in the rhizosphere. ePGPR increase plant growth through a variety of mechanisms; these include genera such as *Bacillus*, *Pseudomonas*, *Burkholderia*, *Erwinia*, *Caulobacter*, *Serratia*, *Arthrobacter*, *Micrococcus*, *Flavobacterium*, *Chromobacterum*, *Agrobacterium*, *Hyphomycrobium* and free-living nitrogen-fixing bacteria (*Azotobacter* and *Azospirillum*) (Foster *et al*., 1983; Prithiviraj *et al*., 2003; Gray and Smith, 2005). Most rhizosphere organisms occur within 50 mm of root surface and populations within 10 mm of root surface may reach 1.2×10^{8} cells cmK^{-3} or 10^{9}-10^{12} microbial cells g k^{-1} soil. Despite large numbers of bacteria in the rhizosphere, only 7-15% of the total root surface is generally occupied by microbial cells (Foster *et al*., 1983; Pinton *et al*., 2001; Gray and Smith, 2005).

Endophytic Region

Rhizobacteria that establish inside plant roots, forming more intimate associations, are endophytes. To aid in this conceptualization, simple terms have been adopted: intracellular PGPR-(iPGPR) bacteria residing inside plant cells, producing nodules and being localized inside those specialized structures. These include a wide range of soil bacteria forming less formal associations than the rhizobia-legume symbiosis; endophytes may stimulate plant growth, directly or indirectly and include the rhizobia. Soil bacteria in the genera *Rhizobium*,

Bradyrhizobium, *Sinorhizobium*, *Azorhizobium*, *Mesorhizobium* and *Allorhizobium*, belonging to the family Rhizobiaceae, invade plant root systems and form root nodules (Martinez-Romero and Wang, 2000). Collectively they are often referred to as rhizobia. These iPGPR are mostly Gram-negative and rod-shaped, with a lower proportion being Gram-positive rods, cocci and pleomorphic forms. The primary mechanism by which rhizobia increase plant growth is N_2-fixation.

Root Colonization by PGPR

Successful colonization and persistence in the plant rhizosphere are required for PGPR to exert their beneficial effect on plants (Elliott and Lynch, 1984). Rhizosphere colonization is also considered to be a crucial step in the application of microorganisms for beneficial purposes such as biofertilization, phytostimulation, bicontrol and phytoremediation (Lugtenberg *et al*., 2001). Root colonization, which is a complex process, is under the influence of various parameters such as bacterial traits, root exudates, biotic and abiotic factors (Benizri *et al*., 2001). The method chosen for studying the traits associated with root colonization depends on the objective and different approaches and techniques have been used to quantify and identify inoculated strains on the host plant (Lugtenberg *et al*., 2001). The major rhizobacterial genera include free living and symbiotic bacterial species belonging to *Acetobacter*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *flavobacter*, *Pseudomonas*, *Proteus*, *Rhizobium*, *Serratia*, *Xanthomonas* and other (Glick, 1995).

MODE OF ACTION OF PGPR AS BIOFERTILIZERS

PGPR inoculant currently commercialized as novel solution for plant growth enhancements through direct, indirect mechanism and combination of mode of action.

The direct mechanisms of plant growth promotion may involve the synthesis of substances by the bacterium or facilitation of the uptake of nutrients from the environment (Glick *et al*., 1999). The direct growth promoting mechanisms are as follows (1) Biological nitrogen fixation (2) Increasing the availability of nutrients in the rhizosphere (solubilization of phosphorus, facilitated absorption of iron by production of siderophores) and (3) inducing of phytohormones production such as auxins, cytokinins, gibberellins (Kloepper *et al*., 1989; Glick, 1995; Glick *et al*., 1999; Vessey, 2003; Patten and Glick, 2002). The indirect mechanism of plant growth occurs when PGPR lessen or prevent the deleterious effects of plant pathogens on plants by production of inhibitory substances or by increasing the natural resistance of the host (Handelsman and Stabb, 1996; Nehl *et al*., 1996; Cartieaux *et al*., 2003). The indirect mechanisms of plant growth promotion by PGPR include (1) antibiotic production (2) depletion of iron from the rhizosphere (3) synthesis of antifungal metabolites (4) production of fungal cell wall lysing enzymes (5) competition for sites on roots and (6) induced systemic resistance (Weller and Cook, 1986; Dunne *et al*., 1993; Kloepper *et al*., 1988; Liu *et al*., 1995; Glick *et al*., 1999).

Direct Mechanism

Biological Nitrogen Fixation

Symbiotic Nitrogen Fixation

Legume and their rhizobia are often introduced to agricultural ecosystems to improve soil fertility and farming systems flexibility (Brockwell and Bottomley, 1995; Sessitsch *et al*., 2002). Nitrogen is known to be an essential nutrient for plant growth and development. Intensive farming practices that achieve high yield require chemical fertilizers, which are not

costly but may also create environmental problems. The extensive use of chemical fertilizers in agriculture is currently under debate due to environmental concern and fear for consumer health. Consequently, there has recently been growing level of interest in environmentally and friendly sustainable agricultural practices and organic farming system (Rigby and Caceres, 2001; Lee and Song, 2007). Increasing and extending the role of biofertilizers such as rhizobium would reduce the need for chemical fertilizers and decrease adverse environmental effects. Therefore, in the development and implementations of sustainable agriculture techniques, biofertilization is great importance in alleviating environmental pollution and the deterioration of nature (Elkoca *et al*., 2008).

Rhizobium symbiosis with legumes species is of special importance, producing 50% of 175 million tons of total biological nitrogen fixation annually worldwide (Sarioglu *et al*., 1993). Chickpea and *Rhizobium leguminosarum* subsp. *ciceri* association annually produce up to 176 kg N ha^{-1} depending on cultivar, bacterial strain and environmental factors (Rupela and Saxena, 1987; Beck *et al*., 1991, Yadav *et al*., 2010). However, Rhizobium species producing nodules in chickpea are specific only to this species and thus inoculation with effective strains is advised in soils with no or weak bacterial presence (Rupela and Saxena, 1987).

Nitrogen is one of the most common nutrients required for plant growth and productivity as it forms an integral part of proteins, nucleic acids and other essential bimolecular (Bøckman, 1997). More than 80% of nitrogen is present in the atmosphere, but is unavailable to plants. It needs to be converted into ammonia, a form available to plants and other eukaryotes. Biological nitrogen fixation involves the conversion of nitrogen to ammonia by microorganisms using a complex enzyme system identified as nitrogenase (Kim and Rees, 1994). Biological nitrogen fixation fixes about 60% of the earth's available nitrogen and represents an economically beneficial and environmentally sound alternative to chemical fertilizers (Ladha and Kundu, 1997). Plant growth-promoting rhizobacteria that fix nitrogen in non-leguminous plants are diazotrophs that form a non-obligate interaction with the host (Glick *et al*., 1999). The nitrogen fixation in chickpea is performed by two species of genus *Mesorhizobium*, *M. ciceri* (Nour *et al*., 1994) and *M. mediterraneum* (Nour *et al*., 1995). The symbiosis between chickpea and its specific rhizobia has been recently studied in several countries-Tunisia (Aouani *et al*., 2001), Morocco (Maatallah *et al*., 2002), Canada (Kyei-Boahen *et al*., 2002), Turkey (Icgen *et al*., 2002) and Portugal (Laranjo *et al*., 2001, 2002) because of the promising agricultural usefulness of this crop as a grain legume for human and animal nutrition and because of the interesting extreme host specificity of its rhizobia. The most studied and longest exploited PGPR are the rhizobia (including the *Allorhizobium, Azorhizobium, Bradyrhizobium, Mesorhizobium*, *Rhizobium* and *Sinorhizobium*) for their ability to fix N_2 in their legume hosts. The root nodules bacteria in the α-*Proteobacteria* are contained in five families: Rhizobiaceae, Phyllobacteriaceae, Bradyrhizobiaceae, Hyphomicrobiaceae and Methylobacteriaceae as defined by their 16S rDNA sequence analysis (Sawada *et al*., 2003). Commercial rhizobia inoculants for use on legume crops were first introduced in the 1890s (Fred *et al*., 1932).

The rhizobial species involved in symbiotic association are *Bradyrhizobium japonicum* (Guerinot and Chelm, 1984), *Rhizobium ciceri* (Nour *et al*., 1994), *Rhizobium etli* (Segovia *et al*., 1993), *Rhizobium galegae* (Lindstrom, 1989), *Rhizobium gallicum* (Amarger *et al*., 1997), *Rhizobium fredii* (Scholla and Elkan, 1984), *Sinorhizobium fredii* (Chen *et al*., 1988), *Sinorhizobium medicae* (Rome *et al*., 1996), *Sinorhizobium arboris* (Nick *et al*., 1999), *Mesorhisobium chacoense* (Velazquez *et al*., 2001), *Mesorhizobium pluriforium* (De Lajudie *et al*., 1998a), *Azorhizobium caulinodans* (Dreyfus *et al*., 1988) and *Allorhizobium undicola* (De Lajudie *et al*., 1998b). Understanding of the *Frankia* sp.

symbioses expanded rapidly in the 1970s and 1980s and their ability to produce nodules on sea buckthorn (Hippophae rhamnoides) (Oremus, 1980) solidified a new line of dynamic research. Jarvis *et al*. (1997) described the genus *Mesorhizobium* (rhizobia phylogenetically intermediate between the genera *Bradyrhizobium* and *Rhizobium*) to include root nodule bacteria that had considerable phenotypic and genotypic differences to the other root nodules bacteria genera. In case of lentil, seed treatment with *Rhizobium leguminosarum*, increased seedling height, root nodules mass and shoot biomass. Similarly, nodulation was found to be improved in peanut by an array of *Rhizobium* species (Dey *et al*., 2004). Similarly data were reported by Yanni (1992), the response of lentil, chickpea and pea upon with *Rhizobium*.

Non-Symbiotic Nitrogen Fixation

Azotobacter species (*Azotobacter chroococcum* and *Azotobacter vinelandii*) and *Azospirillum* species (Mirza *et al*., 2001) are free living, hetrotrophic diazotrophs that fix nitrogen in non leguminous plant such as wheat, rice and others crops. Yield of rice in field trials increased significantly 20% with applications of *Azotobacter* (Yanni and El-Fattah, 1999). One of the best studied diazotrophs for nitrogen fixation is *Azospirillum* sp. isolated from nitrogen-poor soils by Beijernick in 1925 (Holguin *et al*., 1999). Members of this bacterial genus are capable of fixing atmospheric nitrogen and of promoting plant growth. A mixed inoculum of *Staphylococcous* and *Azospirillum* promoted the nitrogen fixing activity of *Azospirillum* (Holguin and Bashan, 1997). Combined inoculation of *A. brasilense* with *Pseudomonas striata* significantly increased grain yield, nitrogen and phosphorus uptake of sorghum (Alagawadi and Gaur, 1992). Oliveira *et al*. (1997) demonstrated that co-inoculation of clover with a mixture of *A. brasilense* Sp7 and *Rhizobium* sp. resulted in increased acetylene reduction, nodulation and shoot dry weight of plants. Endophytic bacteria that colonize the interiors of plant tissues such as roots, stem and leaves and are able to fix nitrogen are also found to be beneficial for plant growth (James *et al*., 1997). Some experiments have involved the colonization of para-nodules on associations involving wheat and various diazotrophs (Chen *et al*., 1993; Gantar and Elhai, 1999; Tchan *et al*., 1991; Youssef *et al*., 1998), *Azorhizobium caulinodans* and rice (Christiansen-Weniger, 1996), *Azotobacter nigricans* and rape (Koval'skaya *et al*., 2001) and *Azospirillum* sp. and maize (Christiansen-Weniger and Vanderleyden, 1994).

Increasing the Availability of Nutrients in the Rhizosphere

There is ample evidence that the mode of action of many PGPR is by increasing the availability of nutrients for the plant in the rhizosphere (Glick, 1995; Vessey, 2003). The method by which these increases take place involve solubilization of unavailable forms of nutrients, siderophore production and ammonia production which helps facilitate the transport of certain nutrients.

Solubilization of Phosphorus

Phosphorus is second only to nitrogen in mineral nutrients most commonly limiting the growth of terrestrial plants. Ironically, soils may have large reserves of total P, but the amounts available to plants is usually a tiny proportion of this total (Stevenson and Cole, 1999). Indian soils are normally deficient in available phosphorus even though the bound component may be sufficient abundant (Johri *et al*., 2003). Therefore, the use of PSM (phosphate solubilizing microorganism) is very common. However, immobilization of added

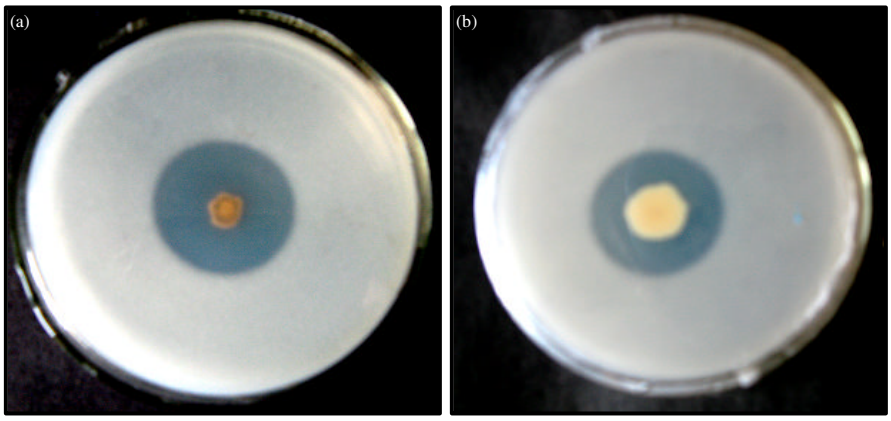

Fig. 1: Phosphate solubilizing (a) bacteria *Pseudomonas aeruginosa* strain BHUPSB01 (accession number GU124822) and (b) *Burkholderia cepacia* strain BHUPSB03 (accession number GU124830) strain showing halo zone around bacterial colony on Pikovskaya agar media

phosphorus in soils is a serious problem (Gaind *et al*., 2000). The low availability of P to plants is because the vast majority of soil P is found in insoluble forms and plants can only absorb P in two soluble forms, the monobasic ($H_2PO_4^-$) and the diabasic (HPO_4^{2-}) ions. *In vitro* phosphate solubilizing bacteria *Pseudomonas aeruginosa* strain BHUPSB01 (accession number GU124822), *P. aeruginosa* strain BHUPSB02 (GU124826) and *Pseudomonas putida* strain BHUPSB04 (accession number GU124834), *Burkholderia cepacia* BHUPSB03 (GU124830), *Paenibacillus polymyxa* BHUPSB16 (GU124833), *P. polymyxa* BHUPSB17 (GU124838) and *Bacillus subtilis* BHUPSB13 (GU124812 showed halo zone around bacterial colony on Pikovskaya agar media (Gaur, 1990). This halo zone showed to solubilization of tricalcium phosphate in soluble form due to production of organic acid (Fig. 1a, b).

The solubilization of P in the rhizosphere is the most common mode of action implicated in PGPR that increase nutrient availability to host plants (Richardson, 2001). Examples of recently studied associations include *Azotobacter chroococcum* and wheat (Kumar and Narula, 1999), *Bacillus circulans* and *Cladosporium herbarum* and wheat (Singh and Kapoor, 1999), *Bacillus* sp. and five crop species (Pal, 1998), *Enterobacter agglomerans* and tomato (Kim and McDonald, 1998), *Pseudomonas chlororaphis* and *P. putida* and soybean (Cattelan *et al*., 1999), *Rhizobium* sp. and *Bradyrhizobium japonicum* and radish (Antoun *et al*., 1998) and *Rhizobium leguminosarum* bv. *Phaseoli* and maize (Chabot *et al*., 1998). Phosphate-solubilizing bacteria are common in rhizospheres (e.g., Nautiyal *et al*., 2000; Vazquez *et al*., 2000). However, the ability to solubilize P by no means indicates that a rhizospheric bacterium will constitute a PGPR. For example, Cattelan *et al*. (1999) found only two of five rhizospheric isolates positive for P solubilization actually had a positive effect on soybean seedling growth. Likewise, not all P-solubilizing PGPR increase plant growth by increasing P availability to the hosts. For example, Freitas *et al*. (1997) found a number of P-solubilizing *Bacillus* sp. isolates and a *Xanthomonas maltophilia* isolate from canola (*Brassica napus* L.) rhizosphere which had positive effects on plant growth, but no effects on P content of the host plants.

Phosphate solubilizing bacteria are also known to increase phosphorus uptake resulting in better growth and higher yield of crop plants (Alagawadi and Gaur, 1988; Gaur *et al*., 2004; Verma *et al*., 2010). These microorganisms are bacteria and fungi that inhabitant the rhizosphere (Azcon *et al*., 1976; Bowen and Rovira, 1999). Most soil bacteria can solubilize insoluble phosphates; particularly active are those that belong to the genera *Pseudomonas, Enterobacter* and *Bacillus* as well as some soil fungi, *Penicillium* and *Aspergillus* (Domey and Lipmann, 1988; Pargiri and Bezbaruah, 1990; Rao, 1992; Rokade and Patil, 1993; Whitelaw, 2000; Yadav *et al*., 2010). Some researchers prefer to use fungal P-solubilizers arguing that bacterial strains can lost their ability to solubilize P after several cycles of *in vitro* culture (Whitelaw, 2000), but this point is quite controversial.

Facilitated Absorption of Iron by Production of Siderophores

Siderophores are low-molecular weight iron-binding molecules that are synthesized by many microorganisms under low-iron conditions (Neilands, 1981). Microbial siderophores may stimulate plant growth directly by increasing the availability of iron in the soil surrounding the roots (Kloepper *et al*., 1980). Marschner and Römheld (1994) reported that plants may also utilize siderophores synthesized by microorganisms colonizing the rhizosphere; this would be a source of soluble iron for the host plant. Plants such as sorghum, oats, peanut, cotton, cucumber and sunflower demonstrated the ability to use radiolabelled microbial siderophores as a sole source of iron (Crowley *et al*., 1988; Bar-Ness *et al*., 1991; Cline *et al*., 1984; Jurkevitch *et al*., 1986; Wang *et al*., 1993). Growth of cucumber in the presence of microbial siderophores resulted in increased plant biomass and chlorophyll content (Ismande, 1998). Uptake of microbial siderophores by plants has been attributed to microorganisms living.

Iron is an essential nutrient of plants, but it is relatively insoluble in soil solutions. Plant roots prefer to absorb iron as the more reduced ferrous (Fe^{2+}) ion, but the ferric (Fe^{3+}) ion is more common in well aerated soil although it is easily precipitated in iron-oxide forms (Salisbury and Ross, 1992). However, there is a controversy as the significance of bacterial Fe^{3+} siderophore uptake to the iron nutrition of plants. While some believe that there contribution of these siderophores to the overall iron requirements of plants is small (Glick, 1995), other suggest an important role (Duijff *et al*., 1994), even a vital role, especially in calcareous soils (Masalha *et al*., 2000). Bar-Ness *et al*. (1992), who had earlier supported the concept of bacterial siderophore uptake by plants (Bar-Ness *et al*., 1991), concluded that two bacterial siderophores (pseudobactin and ferrioxamine B) were inefficient as iron sources for plants and that rhizospheric siderophore-producing bacteria can be in competition with the plant for iron. In fact, the vast majority of research on microbial siderophores in the rhizosphere is associated with their biocontrol activities due to their competitive effects with plant pathogens (Hiifte *et al*., 1994).

Production of Phytohormones

One of the direct mechanisms by which PGPR promote plant growth is by production of plant growth regulators or phytohormones (Glick, 1995). Frankenberger and Arshad (1995) have discussed in detail the role of auxins, cytokinins, gibberellins, ethylene and Absicisic Acids (ABA) which, when applied to plants, help in increasing plant yield and growth. Microbial production of individual phytohormones such as auxins and cytokinins has been reviewed by various authors over the last 20 years (Pilet *et al*., 1979; Hartmann *et al*., 1983; Fallik *et al*., 1988; Barbieri and Galli, 1993; Patten and Glick, 1996, 2002).

Auxins

The production of an active substance by the fungi *Rhizopus suinus* and *Absidia ramosa* was identified to be auxin by Thimann (1935), following induction of curvature in Avena as demonstrated by Nielsen (Frankenberger and Arshad, 1995). Various authors have identified the production of indole-3-acetic acid by microorganisms in the presence of the precursor tryptophan or peptone. Some of the plant responses to auxin are as follows: (1) cell enlargement (2) cell division (3) root initiation (4) root growth inhibition (5) increased growth rate (6) phototropism (7) geotropism and (8) apical dominance (Frankenberger and Arshad, 1995). Eighty percent of microorganisms isolated from the rhizosphere of various crops have the ability to produce auxins as secondary metabolites (Kampert *et al*., 1975; Loper and Scroth, 1986). Bacteria belonging to the genera *Azospirillum*, *Pseudomonas*, *Xanthomonas* and *Rhizobium* as well as *Alcaligenes faecalis*, *Enterobacter cloacae*, *Acetobacter diazotrophicus* and *Bradyrhizobium japonicum* have been shown to produce auxins which help in stimulating plant growth (Patten and Glick, 1996). Various metabolic pathways such as (1) indole-3-acetamide pathway (2) indole-3-pyruvic acid pathway (3) tryptophan side chain pathway (4) tryptamine pathway and (5) indole-3-acetonitrile pathway are involved in the production of IAA. Phytopathogens such as *Agrobacterium tumefaciens*, *A. rhizogenes* and *P. syringae* pv. savastanoi synthesize IAA via the indole-3-acetamide pathway (Liu *et al*., 1982; Offringa *et al*., 1986). Synthesis of IAA by *Rhizobium* sp. in the presence and absence of tryptophan has also been demonstrated (Kittell *et al*., 1989; Verma *et al*., 2010). Bent *et al*. (2001) reported that the production of indole compounds by three different strains, *Paenibacillus polymyxa* L6, *P. polymyxa* Pw-2 and *Pseudomonas fluorescens* M20 increased in concentration with increasing concentrations of tryptophan (0-200 mg mL^{-1}) at different times. Reports by Asghar *et al*. (2002) showed that PGPR strains produced 24.6 μg mL^{-1} of auxins in the presence of the precursor L-tryptophan in the medium, which was 184-fold more than that without L-tryptophan. Example of phytohormones producing bacteria under *in vito* condition (Table 1). The isolates of *Bacillus, Pseudomonas* and *Azotobacter* produced IAA, whereas only 85.7% of *Rhizobium* was able to produce IAA. Production of ammonia was commonly detected in the isolates of *Bacillus* (95.0%) followed by *Pseudomonas* (94.2%), *Rhizobium* (74.2%) and *Azotobacter* (45.0%) (Joseph *et al*., 2007). Higher level of IAA production by *Pseudomonas* was recorded by other workers (Xie *et al*., 1996). Ahmad *et al*. (2005) has reported that bacterial isolates were tested for the production of indole acetic acid (IAA) in a medium with 0, 1, 2 and 5 mg mL^{-1} of tryptophan. Production of IAA in fluorescent *Pseudomonas* isolates increased with an increase in tryptophan concentration from 1 to 5 mg mL^{-1} in the majority of isolates. IAA production in *Azotobacter* isolates has been reported by other researchers (Gonzalez Lopez and Vela, 1981; Nieto and Frankenberger, 1989; Khalid *et al*., 2001). IAA production has reported in *Azospirillum* sp. under in vitro condition (Dobbelaere *et al*., 2001; Lambrecht *et al*., 2000) (Table 1).

Other Phytohormones

Production of other phytohormones by biofertilizing-PGPR has been identified, but not nearly to the same extent as bacteria which produce IAA. Cytokinins are a class of phytohormones which are known to promote cell divisions, cell enlargement and tissue expansion in certain plant parts (Salisbury, 1994). Researchers have recently begun to identify cytokinin production by PGPR. Gibberellins (gibberellic acid; GA) are a class of phytohormones most commonly associated with modifying plant morphology by the extension of plant tissue, particularly stem tissue (Salisbury, 1994). Evidence of GA production by PGPR is rare, however Gutierrez-Manero *et al*. (2001) provide evidence that

Table 1: *In vitro* production of plant growth regulators by rhizobacteria

PGPR	PGR	References
Arthrobatcter mysorens 7, *Flavobacterium* sp. L30, *Klebsiellu* CIAM 880	Indole-3-acelic acid, ethylene	Pishchik *et al.* (2002)
Azotobacter sp.,	Indole-3-acetic acid	Zahir *et al.* (1998a, b 2000), Khalid *et al.* (2001)
Azotobacter chroococcum	Indole-3-acetic acid	Verma *et al.* (2010)
Azospirillum sp.	Gibberellic acid	Lucangeli and Botlini (1997)
Azospirillum sp.	Indole-3-acetic acid	Dobbelaere *et al.* (2001)
Azospirillum sp.	Indole-3-acetic acid	Lambrecht *et al.* (2000)
Bacillus licheniformis	Ethylene	Fukudah *et al.* (1989)
B. licheniformis	Physiologically active gibberellins	Gutierrez-Manero *et al.* (2001)
B. pumilus	Physiologically active gibberellins	Gutierrez-Manero *et al.* (2001)
B. subtilis	Ethylene	Mansouri and Bunch 1989)
Bacillus megaterium BHUPSB14	Indole-3-acetic acid	Verma *et al.* (2010)
Pseudomonas sp.	Auxins.	Pal *et al.* (2000)
Pseudomonas aeruginosa	Ethylene	Mansouri and Buncli (1989)
Pseudomonas fluorescens	Ethylene	Swanson *et al.* (1979)
Pseudomonas fluorescens G20-18	Isopentyl adenosine, *t*-zeatin ribose, dihydrozeatin riboside	Garcia de Salamone *et al.* (2001)
Pseudomonas fluorescens BHUPSB06	Indole-3-acetic acid	Verma *et al.* (2010)
Pseudomonas putida	Ethylene	Pazout *et al.* (1981), Fukudah *et al.* (1989)
Pseudomonas syringae	Ethylene	Sato *et al.* (1997), Swanson *et al.* (1979)
Pseudomonas tabaci	Ethylene	Swanson *et al.* (1979)
Rhizobacterial isolates	Auxins	Asghar *et al.* (2000, 2002)
Rhizohium sp.	Isopentyl adenine, isopenty) adenosine	Sturtevant and Taller (1989)
R. leguminosarum	Indole-3-acetic acid	Badcnoch-jones *et al.* (1982a), Dazzo *et al.* (2000)
R. leguminosarum	Indole-3-acetic acid	Wang *et al.* (1993)
R. leguminosarum	Isopentyj atlenosine, zeatin, methylthiozeatin	Taller and Slunevant (1991)
Rhizobium sp.	Indole-3-acetic acid	Verma *et al.* (2010)

four different forms of GA are produced by *Bacillus pumilus* and *Bacillus licheniformis* (Table 1). Inoculation of alder (*Alnus glutinosa*) with these PGPR could effectively reverse a chemically induced inhibition of stem growth. Although this is not strong evidence of GA production being a common method of growth promotion by PGPR, it does suggest that it may have a role and indicates that more research is this area is warranted. Ethylene is the only gaseous phytohormone. It is also known as the 'wounding hormone' because its production in the plant can be induced by physical or chemical perturbation of plant tissues (Salisbury, 1994). Among its myriad of effects on plant growth and development, ethylene production can cause an inhibition of root growth. Glick *et al.* (1998) put forward the theory that the mode of action of some PGPR was the production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase, an enzyme which could cleave ACC, the immediate precursor to ethylene in the biosynthetic pathway for ethylene in plants. They submitted that ACC deaminase activity would decrease ethylene production in the roots of host plants and result in root lengthening. Subsequently, Glick's research program has provided numerous empirical studies with wild-type and genetically modified PGPR to support their theory (Holguin and Glick, 2001; Li *et al.*, 2000; Mayak *et al.*, 1999; Saleh and Glick, 2001; Shah *et al.*, 1998; Wang *et al.*, 2000). In some cases, the growth promotion effects of ACC deaminase-producing PGPR appear to be best expressed in stressful situations such as flooded (Grichko and Glick, 2001) or heavy metal-contaminated soils (Burd *et al.*, 1998).

The Indirect Mechanism

The indirect mechanism of plant growth occurs when PGPR lessen or prevent the deleterious effects of plant pathogens on plants by production of inhibitory substances or

Table 2: Biological control by PGPR against certain diseases, pathogens and insects in different crops

Crop	Disease/pathogen/insect	PGPR	References
Barley	Powdery mildew	*B. subtilis*	Schobeck *et al.* (1980)
Beans	Halo blight	*Pseudomonas fluorescens* strain 97	Alstrom (199l)
	Sclerotium rolfsii	*Pseudomonas cepacia*	Fridlender *et al.* (1993)
Carnation	*Fusarium* wilt	*Pseudomonas* sp. (WCS 417r)	Van Peer *et al.* (1991)
Cotton	Damping off	*Pseudomonas flourescens*	Howell and Stipanovic (1979, 1980)
	Meloidogyne incognita, M. Arenaria	*B. subtilis*	Sikoraa (l998)
	Rhizoctonia solani	*Pseudomonas cepacia*	Fridlender *et al.* (1993)
	Helicoverpa armigera	*Pseudomonas gladioli*	Quingwen *et al.* (1998)
Cucumber	*Cucumber anthracnose*	*Pseudomonas putida* (89B-27), *Serratia marcescens* (90-166)	Wei *et al.* (1991, 1996)
	Pythium ultimum	*Pseudomonas cepacia*	Fridlender *et al.* (1993)
	Bacterial wilt	*Pseudomonas putida* (89B-27), *S. marcescens* (90-166)	Kloepper *et al.* (1993)
	Bacterial angular leaf spot	*P. putida* (89B-27). *Flavomonas oryzihabitans* INR-5. *S. marcescens* (90-166). *Bacillus pumilus* (INR-7)	Kloepper *et al.* (1993)
	Cucumber mosaic virus	*P. putida* (89B-27). *S. marcescens* (90-166)	Raupach *et al.* (1996)
	Striped Cucumber beetle	*P. putida* (89B-27), *Flavomonas oryzihabitans* INR-5,	Zehnder *et al.* (1997)
Green gram	*Aspergillus* sp., *Curvularia* sp., *Fuxarium oxysporum, Rhizoctonia solani*	*Pseudomonas* sp.	Sindhu *et al.* (1999)
Mung bean	Root rot. Root knot	*P. aeruginosa. B. subtilis*	Siddiqui *et al.* (2001a, b)
Rice	Rice sheath blight	*Streptomyces* spp. and *Bacillus cereus* in combination with *Ps. fluorescens* and *Burkholderia*	Sung and Chung (1997)
	Rice sheath blight	Combination of *P. fluorescens* strains Pf1 and Fp7	Nandakumar (1998)
	Rhizoctonia solani (sheath blight pathogen)	*P. fluorescens* Strains Pf 1 and Fp7	Vidhayasekaran and Muthamilan (1999)
	Blue mold	*S. marcescens* 90-1 16. *B. pumilus* SE 34, *Ps. fluorescens* 898-6 1 . *B. pumilus T4, B. pasteurii* C-9	Zhang *et al.* (2002)
Tomato		*B. subtilis strain* 1N 937b	
	Tomato mottle virus	B. *amyloliquifacians* strain 1N 937a. B. *subtilis* strain 1N 937b	Murphy *et al.* (2000)
Wheat	Take till disease	*Bacillus. pseudomonas, Penicillium. Beauveria, Rhodococcus*	Renwick *et al.* (1991)
		Mixture of *Pseudomonas* sp.	Pierson and Weller (1994)

by increasing the natural resistance of the host. Control of phytopathogenic microorganism by releasing siderophores, B-1,3-glucanase, chitinases, antibiotics, fluorescent pigment and cynide (Scher and Baker, 1982; Voisard *et al*., 1989; Catellan *et al*, 1999; Pal *et al*., 2001).

Many rhizobacteria including fluorescent *Pseudomonas* secrete a variety of antifungal molecule under in vitro and in situ conditions (Cook *et al*., 1995). There are several reports in vitro antagonism of pathogenic fungi and field performance by bacteria recovered from the rhizosphere of plants in India (Johri *et al*., 1997). Some important plant disease controlling bacteria have been depicted in Table 2. *Pseudomonas aeruginosa* strain BHUPSB01 (accession number GU124822), *P. aeruginosa* strain BHUPSB02 (GU124826) and *Pseudomonas putida* strain BHUPSB04 (accession number GU124834), *Burkholderia cepacia* BHUPSB03 (GU124830), *Paenibacillus polymyxa* BHUPSB16 (GU124833), *P. polymyxa* BHUPSB17 (GU124838) and *Bacillus subtilis* BHUPSB13 (GU124812 showed antagonistic against phytopathogenic fungi *Fusarium oxysporum* and *Rhizoctonia solani* (Fig. 2, 3).

The rhizoshere and root zone of tea (Camellia sinensis) is a good habitat for PGPR strains represented by *Bacillus*, *Proteus* and *Pseudomonas*, inhibitory to phytopathogenic fungi *in vitro*, viz., *Fusarium oxysorum* f. sp. *lycospersici*, *Fusarium oxysporum* f. sp.

Fig. 2: Antagonistic activity of *Pseudomonas aeruginosa* strain BHUPSB01 (accession number GU124822) against *Fusarium oxysporum*

Fig. 3: Antagonistic activity of *Pseudomonas putida* strain BHUPSB04 (accession number GU124834) against *Rhizoctonia solani*

ciceri, *F. udum, F. solani, F. moniliformae, F. semitectum, Fomes lamiensis* and *Ustulina zonata* (Kumar-Dileep and Bezbaruah, 1996; Kumar and Bezbaruah, 1997). Suppression of soil borne plant pathogens by siderophore producing pseudomonads was observed (Bakker *et al*., 1986, 1987; Loper, 1988) and the wild type strain was more effective in suppressing disease compared to non-siderophore-producing mutants. *Pseudomonas* sp. strains MRS23 and CRP55b showed varying diameters of inhibition zones of the four pathogenic fungi, *Aspergillus* sp. *F. oxysporum*, *P. aphanidermatum* and *R. solani* in the plating media (Kings B, PDA and NB) (Goel *et al*., 2002). *Pseudomonas* strains MRS23 and CRP55b inhibited the growth of pathogenic fungi, i.e., *Aspergillus* sp. *Fusarium oxysporum* f. sp. *ciceri* and *Rhizoctonia solani* under culture condition (Goel *et al*., 2002). Like this, several species of pseudomonas, viz. *P. fluorescens, P. putida, P. cepacia* (*Burkholderia cepacia*) and *P. aeruginosa* isolated from plant tissue or soil have been reported as potential biocontrol agents of several phytopathogenic fungi (Duffy *et al*., 1996; Goel *et al*., 2000). Saikia *et al.* (2003, 2004, 2005, 2009) reported that *Pseudomonas fluorescens1*-94 (Pf1-94) and

P. fluorescens (Pf4-92) strains that were isolated from rhizosphere soil of chickpea showed Induced Systemic Resistance (ISR) against fusarium wilt of chickpea and charcoal rot (Srivastava *et al*., 2001). Earlier studies indicated that shoot and root lengths were significantly increased in *P. fluorescens* treated chickpea plants and the reduction in disease severity was more pronounced when chemical inducers were applied with *P. fluorescens* (Saikia *et al*., 2003). Root rot disease in mung bean has controlled by *Pseudomonas aeruginosa*, *Bacillus subtilis* (Siddidui *et al*., 2001) (Table 2).

Siderophore production is an important feature for the suppression of plant pathogens and promotion of plant growth. Fluorescent siderophore production was observed as a mechanism of biocontrol of bacterial wilt disease in the fluorescent pseudomonads RBL 101 and RSI 125. Press *et al*. (2001) reported the catechol siderophore biosynthesis gene in *Serratia marcescens* 90-166 and associated with induced resistance in cucumber against anthracnose. Pseudomonads also produce two other siderophores, pyochelin and its precursor salicylic acid and pyochelin is thought to contribute to the protection of tomato plants from *Pythium* by *Pseudomonas aeruginosa* 7NSK2 (Buysens *et al*., 1996). Howell and Stipanovic (1979, 1980) has been reported damping off disease in cotton controlled by *Pseudomonas fluorescens*. Different environmental factors can also influence the quantity of siderophores produced (Duffy and Défago, 1999).

EFFECT OF PGPR ON PLANT GROWTH AND YIELDS

Table 3 lists many of the PGPR which have been implicated as helper bacteria in various legume-rhizobia symbioses and co-inoculation with other plant growth promoting rhizobacteria for inhancesed crop production. There is evidence for a number of modes of action for PGPR stimulation of legume-rhizobia symbioses, but the most commonly implicated mode is phytohormone-induced (usually IAA) stimulations of root growth (e.g., Molla *et al*., 2001; Srinivasan *et al*., 1996; Vessey and Buss, 2002). In this way, the stimulation of nodulation is most commonly an indirect effect; the PGPR stimulates root growth, which provides more sites for infection and nodulation. However, this is not always the case. Cattelan *et al*. (1999) found that a number of putative PGPR had positive effects on shoot and/or root growth in soybean and were positive for production of IAA or ACC deaminase, but these putative PGPR had no positive effects on nodulation. In fact, Cattelan *et al*. (1999) found several rhizospheric isolates which stimulated aspects of the soybean-bradyrhizobia symbiosis and which had β-glucoanase or cyanide production.

Phosphate-solubilizing *Bacillus* sp. stimulates plant growth through enhanced P nutrition (Elkoca *et al*., 2008; Whitelaw *et al*., 1997; Verma *et al*., 2010) and increasing the uptake of N, P, K and Fe (Biswas *et al*., 2000). Some PGPR strains, from a range of genera, enhance legume growth, nodulation and nitrogen fixation, root and shoot biomass, nodule dry matter, nitrogenase activity and grain yield in chickpea (Parmar and Dadarwal, 1999; Hamaoui *et al*., 2001; Sindhu *et al*., 2002a; Zaidi *et al*., 2003; Gull *et al*., 2004). Combined inoculation of *Rhizobium* with *Pseudomonas striata* or *Bacillus* and with *Bacillus megaterium* (Elkoca *et al*., 2008) have shown increased dry matter, grain yield and phosphorus uptake significantly over the uninoculated control in legumes. Interactions between these PGPR with *Rhizobium* may be antagonistic or synergistic and the beneficial effects of such interactions could be exploited for economic gain (Dubey, 1996; Glick, 1995). Many different N_2-fixing bacteria, including symbionts, such as root nodulating *Rhizobium* sp. (Bohlool *et al*., 1992) and different free-living rhizobacteria, such as *Azospirillum*, *Azotobacter, Bacillus, Enterobacter, Pseudomonas, Serratia* and *Streptomyces* (Kloepper

Table 3: Co-inoculation of Legumes with PGPR and *Rhizobium*

Crop	Rhizobium	Coinoculating PGRP	Plant responses to inoculation	Reference
Alfalfa	*R. meliloti*	*Pseudomonas*	Upon co-inoculation, a significant increase in plant growth, nitrogenase activity, nodule total nodule weight and total plant nitrogen was reported	Knight and Langston-Unkefer (1988)
Bean	*R. phaseoli*	*Pseudomonas putida*	Inoculation with *P. putida* markedly increased nodulation compared to a *R. phaxeuli* control. Also, 2-ketogluconic acid, a pflOSpbate-solubilizing compound, was detected in *P. putida*	Grimes and Mount (1984)
Chickpea	Indigenous *Rhizobium*	*A. brasilense*	Inoctilation with *A. brasilensc* has the potential to prevent or at least diminish the effects: caused by salinity stress in greenhouse experiments with chickpeas irrigated with saline water	Hamaoui *et al.* (2001)
	Mesorhizobium	*Pseudomonas*	A significant increase in nodule weight and shoot biomass was observed when co-inoculated with *Masorhizahium* and *Pseudomonax* in sterilized chillum jar conditions. In pot experiment, co-inoculation significantly increased root and shoot biomass	Sindhu *et al.* (2002a, b)
	Rhizobium	*Pseudomonas, Bacillus*	Upon co-inoculation, reduction in wilt incidence and an increase in nodulation were observed	
	Rhizobium	*Pseudomonas, Bacillus*	A significant increase in nodule weight, root and shoot biomass and total plant nitrogen were reported due to co-inoculation	Parmar and Dadarwal (1999)
	Rhizobium sp.	*P. fluorescens* *B. megaterium* *A. chroococcum*	A significant increase in nodule weight, root and shoot biomass and total plant nitrogen were reported due to co-inoculation	Verma *et al.* (2010)
Chickpeas, common beans	*Rhizobium*	*A. brasilense*	Inoculation with *A. brasilense* increased nodule dry weight, various plant growth parameters and seed yield of nodulated chickpeas in the field. In common bean, inoculation with *Rhizobium meliloti* and *R. tropici* increased seed yield while combined inoculation with *Rhizobium* and *A. brasilense* resulted in a further increase. Plants inoculated with *A. brasilense* alone did not differ in yield from the non- inoculated controls, despite a relative increase in shoot dry weight	Burdman *et al.* (1996)
Clover	*R. leguminosarum* bv. *trifolii* 24	*Pseudomonas* sp. Strain 267 and R.	Co-inoculation of clover plants significantly increased shoot weight and nodule weight in comparison with control plants infected only with *R. leguminosarum* bv. *trifolii*	Derylo and Skorupska (1993)
Common bean	*R. phaseoli* *Rhizobium*	*P. putida* *A. brasilense*	Co -inoculation caused a marked increase in nodulation. Co-inoculation promoted root hair formation and an increase in secretion of the nod gene induced flavonoids resulting in greater numbers of nodules	Grimes and Mount (1984), Burdman *et al.* (1996)
Green gram	*Bradyrhizobium* sp. (Vigna) strains Cog 15 and S24	*Bacillus*	Co-inoculants of the PGPR. *Bacillus* and *Bradyrhizobium* strains failed to show any conclusive influence on nodulation and ARA at 50 days of plant growth. *Bacillus* had a direct effect on shoot biomass development, N-content and grain yield of green gram when co-inoculated with *Bradyrhizobium* strain S24 at 50 and 80 days of plant growth. Also, single inoculation of *Bacillus* isolates significantly increased grain yield over the non-inoculated control	Gupta *et al.* (1998)
	Bradyrhizobium	*Bacillus*	Co-inoculation enhanced nodulation and plant growth of green gram.	Sindhu *et al.* (2002b)
	Bradyrhizobium	*Pseudomonas*	Co-inoculation resulted in significant increases in nodule weight, plant dry weight and total plant N compared to inoculation with *Bradyrhizobium* alone	Sindhu *et al.* (1999)
Natural pasture	Indigenous *Rhizobium*	*A. brasilense*	Inoculation with *A brasilense* showed a similar effect to that of phosphate fertilization.	Itzigsohn *et al.* (2000)

Table 3: Continued

Crop	Rhizobium	Coinoculating PGRP	Plant responses to inoculation	Reference
Pea	*R. leguminosarum*	*Pseudomonas*	Numbers of nodules were greater in co-inoculated plants than with *R. leguminosarum* inoculation alone	Bolton *et al.* (1990)
Peas. Chick peas, Wetch	*Rhizobium*	*A. brasilense*	Co-inoculation significantly increased seed yield, but did not affect dry matter yield of garden peas and chickpeas. Vetch co-inoculation significantly increased N_2-fixatinn, nitrogen content and dry matter yield	Sarig *et al.* (1986)
Soybean (1998)	*B. japonicum*	*S. lequefacians*	Nodulation, nitrogen fixation, protein and N yield	Dashti *et al.*
		2-68, *S. Protea maculans* I-102	were increased by co- inoculation	
	B. japonicum	*P. fluorescens*	Co-inoculation increased colonization of *Bradyrhizobium japonicum* on soybean roots, nodule number and the acetylene reduction assay	Chebotar *et al.* (2001)
	B. japonicum	*Azospirillum*	Co-inoculation promoted nodulation. nitrogenase activity and plant growth	Iruthayathas *et al.* (1983), Polonenko *et al.* (1987)
(1997)	*B. japonicum*	*S. liquefaciens*	Co-inoculation increased grain yield, grain protein	Dashti *et al.*
		2-68, *S. protea maculans* I-102	and total plant protein production under short season conditions	
	B. japonicum	*P. putida, P. fluorescens, Aeromonas hydrophia*	Co-inoculation promoted significant increase in the weight and number of nodules. Several strains increased the dry weight of shoots and roots when inoculated with *B. japonicum,* but these effects did not con-elate with changes in number and weight of nodules	Polonenko *et al.* (1987)
White clover	*R. leguminosarum* bv. *trifolii*	*Azospirillum lipoferum*	Co-inoculation enhanced the number of nodules by 2-3 limes, from 5 to 20 days after inoculation. Acetylene reduction activity was also increased by 2.3- to 2.7-fold at 20 days after inoculation	Tchebotar *et al.* (1998)

and Beauchamp, 1992; Hiifte *et al.*, 1994; Cakmakci *et al.*, 1999), have already been described. When used as seed inoculants, some of these free-living N_2-fixing bacteria show beneficial effects on plant growth and hence they are called Plant Growth-Promoting Rhizobacteria (PGPR) (Davison, 1988). *Bacillus* species used as biofertilizers may have direct effects on plant growth through the synthesis of plant growth hormones (Amer and Utkhede, 2000), N_2-fixation and solubilization of phosphate (Sahin *et al.*, 2004). Phosphate-solubilizing *Bacillus* sp. stimulates plant growth through enhanced P-nutrition (Whitelaw *et al.*, 1997), increasing the uptake of N, P, potassium (K) and iron (Fe) (Biswas *et al.*, 2000). Phosphorus biofertilizers could help increase the availability of phosphates accumulated in the soil and could enhance plant growth by increasing the efficiency of biological nitrogen fixation and the availability of Fe and zinc (Zn) through production of plant growth promoting substances (Kucey *et al.*, 1989). Indeed, trials with *Bacillus* species have shown yield increases in many crops (Greenough and Batson, 1989; Ozturk *et al.*, 2003).

Rhizobium or *Bradyrhizobium* are also widely used in agriculture for crop improvement because of their ability to fix atmospheric nitrogen. Some PGPR strains, from a range of genera, enhance legume growth, nodulation and nitrogen fixation when co-inoculated with rhizobia (Grimes and Mount, 1984; Petersen *et al.*, 1996). Co-inoculation studies with PGPR and *Rhizobium/Bradyrhizobium* sp. have been shown to increase root and shoot biomass, nodule dry matter, nitrogenase activity, N_2-fixation and grain yield in chickpea (Parmar and Dadarwal, 1999; Hamaoui *et al.*, 2001; Sindhu *et al.*, 2002a; Zaidi *et al.*, 2003; Gull *et al.*, 2004) and various legumes such as common bean (Grimes and Mount, 1984), green gram

(Sindhu *et al*., 1999) and soybean (Dashti *et al*., 1998). Furthermore, combined inoculations with N_2-fixing and P-solubilizing bacteria were more effective than single inoculation possibly by providing a more balanced nutrition for plants (Belimov *et al*., 1995). Dual inoculations increased yields in many crops compared to single inoculations with N_2-fixing or P-solubilizing bacteria (Alagawadi and Gaur, 1992; Belimov *et al*., 1995; Verma *et al*., 2009).

Anjair *et al*. (2003) has been reported that *Pseudomonas aeruginosa* PNA1, an isolate from chickpea rhizosphere in India, protected pigeonpea and chickpea plants from *fusarium* wilt disease, which is caused by *Fusarium oxysporum* f.sp. *ciceri* and *Fusarium udum*. Inoculation with strain PNA1 significantly reduced the incidence of *fusarium* wilt in pigeonpea and chickpea on both susceptible and moderately tolerant genotypes. Valverde *et al*. (2006) has been reported effects of coinoculations with *Pseudomonas jessenii* PS06 (a) phosphate-solubilizing bacteria) and *Mesorhizobium ciceri* C-2/2 strains enhanced the growth and seed yield of chickpea under greenhouse and field conditions. Co-inoculation studies with PGPR and *Rhizobium*/*Bradyrhizobium* sp. have been shown to increased root and shoot biomass, nodule dry matter, nitrogenase activity, N2-fixation and grain yield in chickpea (Sindhu *et al*., 2002a, b; Zaidi *et al*., 2003; Gull *et al*., 2004; Elkoca *et al*., 2008) and various legume such as green gram (Sindhu *et al*., 1999) and pigeonpea (Tilak *et al*., 2006). Mittal *et al*. (2008) has been reported the effect of six phosphate-solubilizing fungi (PSF, two strains of *Aspergillus awamori* and four of *Penicillium citrinum*) isolated from rhizosphere of various crops, was observed on the growth and seed production of chickpea plants (*Cicer arietinum* L. cv. GPF2) in pot experiments. Akhtar and Siddiqui (2009) has been reported that effects of Phosphate solubilizing microorganisms (*Glomus intraradices*, *Pseudomonas putida*, *P. alcaligenes*, *P. aeruginosa* (Pa28), *A. awamori*) and *Rhizobium* sp. was observed on the growth, nodulation yield and root-rot disease complex of chickpea under field condition. Inoculation of *Rhizobium* sp. caused a greater increase in growth and yield than *P. putida, P. aeruginosa* or *G. intraradices*. Hamaoui *et al*. (2001) also reported that inoculation with PGPR significantly enhanced nodulation by native rhizobia in chickpea and faba bean. Favorable effects of inoculation with N2-fixing and P-solubilizing microorganisms and significant increase in nodulation and N2-fixation, N% and total plant nitrogen of legume crops have been reported by many workers (Manjunatha and Devi, 1990; Petersen *et al*., 1996; Khan *et al*., 1997; Sindhu *et al*., 2002a, b). Zaidi and Khan (2007) reported that the stimulatory effect of rhizotrophic microorganisms on growth, yield and nutrient uptake of chickpea was determined in a pot experiment using sterilised soil deficient in available phosphorus (P). Plant vigour, yield and nutrient uptake were significantly enhanced following inoculation with *Mesorhizobium ciceri* and the phosphate solubilising bacterium *Serratia* (T1) or phosphate solubilising fungus *Penicillium* (WF6). Akhtar and Siddiqui (2008) have been reported that *Glomus intraradices*, *Pseudomonas alcaligenes* and *Bacillus pumilus*: effective agents for the control of root-rot disease complex of chickpea (*Cicer arietinum* L.). Sivaramaiah *et al*. (2007) reported that Rhizobacteria belonging to *Bacillus* sp. were isolated from the rhizosphere of chickpea (*Cicer arietinum*). Two *Bacillus* strains CBS127 and CBS155 inhibited the growth of all the four pathogenic fungi (*Alterneria* sp., *Fusarium oxysporum*, *Pythium aphanidermatum* and *Rhizoctonia solani*) tested on nutrient agar medium plates *in vitro*. Ben-Romdhane *et al*. (2008) reported that selection of high nitrogen-fixing rhizobia (*Mesorhizobium ciceri* strain CMG 6 and *Mesorhizobium ciceri* strain CTM 226) nodulating chickpea (*Cicer arietinum* L.) for semi-arid Tunisia. Siddiqui and Mahmood (2001b) have been reported the effects of rhizobacteria, i.e. *Pseudomonas fluorescens*, *Azotobacter chroococcum* and *Azospirillum brasilense*, alone and in combination with root symbionts; *Rhizobium* sp. and *Glomus mosseae*, on the growth of chickpea, *Cicer arietinum* and reproduction of *Meloidogyne javanica* were studied.

Rhizobium sp. with *P. fluorescens* was better for nodulation than the use of *Rhizobium* sp. alone or its use with *A. brasilense*. Root colonization by *G. mosseae* was better in the presence of *P. fluorescens* or *A. chroococcum* than the use of *G. mosseae* alone or its use with *A. brasilense*. *P. fluorescens* caused greater root colonization followed by *A. chroococcum* and *A. brasilense*. Verma *et al.* (2009) have reported that the maximum significant increase in nodule number, dry weight of nodule, root and shoot were recorded in coinoculation of *Mesorhizobium* sp. and *Pseudomonas fluorescens* followed by co-inoculation of *Mesorhizobium* sp., *Azotobacter chroococcum* and *Bacillus megatrium* over uninoculated control in both year of field study while nitrogen and phosphorus content increase in nodules, grain and straw. Verma *et al.* (2010) have reported that application of *Rhizobium* sp. BHURC01 and plant growth promoting rhizobactria on nodulation, plant biomass and yields of chickpea (*Cicer arietinum* L.).

The higher grain yield 37.26 and 23.64% and straw yield 27.95 and 21.70% in first and second year of field study, respectively was increased significantly in co-inoculation of *Rhizobium* sp. BHURC01 with *P. fluorescens* followed by *B. megaterium* and *A. chroococcum* over uninoculated control.

CONCLUSION

The rhizosphere of a plant is a zone of intense microbial activity. Rhizobacteria that exert beneficial effect on plant growth and development or reffered to as plant growth promoting rhizobacteria (PGPR). PGPR can affect plant growth directly or indirectly. Indirect promotion of plant growth occurs when introduced PGPR lessense or prevents deterious effect of one and more phytopathogenic organisms in the rhizosphere. The direct promotion of plant growth by PGPR may include the production of plant growth regulators or facilitating the uptake of certain nutrient from the root environment. There is increasing evidence that rhizobia can promote the growth of non-legumes via direct and indirect mechanism. Further more, co-inoculation of legumes with rhizobia and PGPR is even more effective for improving nodulation and growth of legumes. Application of PGPR strains can provide an effective, economical and practical way of plant protection via disease suppression. PGPR strains mixture often show synergistic action in plant protection and growth promotion involving many mechanisms. Effective plant growth promoting rhizobacteria may be enhancing plant growth and grain yield in multiple crop production.

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