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Plant Growth Promoting Mesorhizobia as a Potential Inoculant for Chickpea (*Cicer arietinum* L.): A Review

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Abstract

Chickpea establish symbiotic relationship with *Mesorhizobium ciceri* and fixes atmospheric nitrogen, which depends on the efficacy of the *Mesorhizobium* strains, crop management, soil and environmental factors. A multi-trait *Mesorhizobium* isolate having more than one Plant growth promoting (PGP) traits often stimulates plant growth directly by IAA and GA production, enhances nutrient uptake by solubilizing phosphorus and siderophore production while indirect growth promotion is by prevention of some pathogenic microorganisms by producing HCN. Several studies revealed that plant growth attributes and yield was increased with inoculation of multi trait *Rhizobium* isolates. The occurrence of different PGP traits of *Mesorhizobium* and their effects on plant growth will be discussed in this review paper.

Key words: ACC deaminase, Chickpea, GA, IAA, *Mesorhizobium*, Siderophore.

Grain legumes belong to an important group of food crops in Indian agriculture covering an area of about 25.26 m ha. These crops are major source of protein in the vegetarian diet. Chickpea (*Cicer arietinum* L.) is one of the important grain legumes and cultivated mainly in the Indian subcontinent, Mediterranean region the West Asia, North Africa and Eastern Africa. India is the largest producer and consumer of chickpea in the world sharing about 71.0% of the global acreage and 67% production. Chickpea grains contain 29% protein, 59% carbohydrate, 3% fibre, 5% oil and 4% ash. The protein is rich in lysine and arginine but most deficient in sulphur containing amino acids such as methionine and cysteine (Iqbal *et al*., 2006). Chickpea restores and maintains soil fertility through symbiotic nitrogen fixation in association with *Mesorhizobium* species in addition to its valuable nutritional quality (Nour *et al.,* 1994; Jarvis *et al.,* 1997). The deep root system and leaf shading habits of chickpea also contributes significantly in nutritional and physical health of soil. The economic cost and environmental hazards of the heavy use of chemical N fertilizers in agriculture are a global concern. Biological nitrogen fixation (BNF) provides economic, environmental and agronomic benefits to reduce the use of chemical fertilizers in various cropping systems. Biological nitrogen fixation, a free source of nitrogen ensures a healthy plant and leaves residual nitrogen which is further used by cereal production in subsequent crop seasons. Among the known plant-bacteria associations, the legume rhizobia symbiosis is the best known and perhaps deserve first place among present opportunities in increasing productivity and protein yield of grain legumes.

The term plant growth promoting rhizobacteria (PGPR) was first coined by Kloepper and Schroth (1978) to a group of soil bacteria which induces increased plant growth and yield after inoculation into seed or roots. PGPR affect the plant growth either directly by providing nutrients or growth hormones to the host plant or indirectly by positively influencing root growth and morphology or by aiding other beneficial symbiotic relationship. Several *Rhizobia* sp. have been documented for their PGPR properties such as secretion of IAA (Stajkovic *et al.,* 2011), siderophore (Mehboob *et al.,* 2010), gibberellic acid (Afzal *et al*., 2010), HCN and P-solubilization (Chandra *et al.,* 2007) and ACC deaminase production (Tittabutr *et al.,* 2008). The numbers of PGPR are increasing every day as more research are performed for screening rhizobacteria for plant growth promotion. PGPR are reported to influence the growth, yield and nutrient uptake by an array of mechanisms. There has been much research interest in PGPR and a large number of PGPR being commercialized for various crops. Kumar and Bezbaruah (1996) found that PGPR treated seeds of chickpea; pigeonpea and maize grew more rapidly and registered 27.9, 22.2 and 6.9% significant increases in shoot height over untreated control, respectively.

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Biological nitrogen fixation

Biological nitrogen fixation is the process that changes inert N_2 into biologically useful NH_3. This process is mediated in nature only by N_2-fixing microorganisms. Nitrogen fixing organisms are divided in two groups (1) symbiotic N_2-fixing bacteria which belongs to the family rhizobiaceae (*Rhizobium, Sinorhizobium, Bradyrhizobium, Mesorhizobium* and *Azorhizobium*), collectively termed rhizobia and forms symbiosis with leguminous plants and non leguminous trees (*e.g. Frankia*) and (2) non-symbiotic (free living, associative and endophytes) nitrogen fixing forms such as cyanobacteria (*Anabaena, Nostoc*), *Azospirillum, Azotobacter, Gluconaceto bacter diazotrophicus* and *Azocarus, etc*. (Bhattacharyya and Jha, 2012). Thus biological nitrogen fixation is a key to sustain agriculture and to reduce soil fertility decline. Legumes are BNF capable and meet their own N needs. Major part of N fixed by legumes is harvested as grains, while the soil and succeeding crops also get benefitted by N in the form of root and shoot residues. However, N_2 fixation efficiency of legumes varies and depends on the host genotype, rhizobial efficiency, soil conditions and climatic factors. Legume-rhizobia association is reported to fix 200 to 300 kg N ha^{-1} $season^{-1}$ (Peoples *et al.,* 1995). Reported quantum of nitrogen fixation ranged from 126 to 319 kg N ha^{-1} in groundnut, 33 to 643 kg N ha^{-1} in soybean, 77 to 92 kg N ha^{-1} in pigeonpea, 25 to 100 kg N ha^{-1} in cowpea, 71 to 74 kg N ha^{-1} in green gram and 125 to 143 kg N ha^{-1} in black gram (Peoples and Crasswell, 1992). Aslam *et al.* (2003) reported thatchickpea can fix up to 187 kg ha^{-1} of atmospheric nitrogen in a single growing period.

Occurrence of *Rhizobium* in soil

Rhizobia in soil can live on plant residues (saprophytes) or entirely within plants (endophytes) or as close association with the plant roots (rhizobacteria) (Anwer, 2013). Survival of rhizobia in soil have been reported to vary in their ability for saprophytic competitiveness in soil and various factors such as host symbiont compatibility, soil salinity, soil pH, mineral and heavy metal toxicity, temperature extremes, inadequate or extreme soil moisture and nutrient status (Sharma *et al.,* 2012). Rhizobial populations residing in soils are dynamic and continuously evolving communities and their diversity stems from the size and plasticity of rhizobial genomes (Black *et al*., 2012; Palmer and Young, 2000), which enables bacteria to survive in different (soil and endo-symbiotic) habitats. Lowther *et al.* (1987) reported that within 10 vegetation types where alfalfa is a potential legume for farming. *R. meliloti* capable of forming effective nodules on alfalfa were detected in 4 of the vegetation types (juniper-pinyon woodland, mountain mahogany-oak scrub, sage brush steppe and wheat grass-needle grass shrub steppe). These rhizobia were detected in only 30% or less of those sites having a natural vegetative cover. Rhizobia were detected in the majority of sites where the sagebrush steppe vegetation type had been converted to wheat or alfalfa-grass mixtures (81 and low respectively). Populations of *R. Meliloti* within the individual vegetation types ranged from 6 to greater than 1.7×10^5 g^{-1} of soil. Karmakar and Chandra (2012) studied the effect of soil type and moisture content on survival, mobility, nodule occupancy of inoculated *Rhizobium leguminosarum* bv. *Viciae* and lentil growth. Results showed that soil moisture affects the movement and survival of inoculated *R. leguminosarum* in soil greatly and optimum soil moisture management is necessary for obtaining the benefits of inoculation in pulse crops. Symbiotic attributes were found better in sandy loam soil, irrespective of moisture levels suggesting relatively more response of *R. leguminosarum* inoculation in lentil in clay loam soil. They also observed that soil moisture level of 50% of water holding capacity supported better survival and movement of the inoculum in both the soils than 30% moisture level. The number of rhizobia in the soil declines drastically as soil dries. Ansari and Rao (2014) reported that rhizobial population in the rhizosphere of soybean ranged from 0.5 to 3.3×10^3 cells g^{-1} soil in central India during post-summer rainy season. The population improved in *Rabi* season 3.6-9.6×10^3 cells g^{-1} in the rhizosphere of chickpea and was three fold higher than in soybean.

Root nodule bacteria and diversity

Legumes are the third largest flowering plant family, consisting of 700 genera and approximately 20,000 species. Nodulation is an important characteristic of majority of legumes. The root nodules in legumes are produced by soil rhizobia. Within these nodules, rhizobia reduce the atmospheric nitrogen to ammonium ion, which is taken up by the plant. This bacterium was first isolated by Beijerinck (1888) from the root nodules of leguminous plant and was named as *Bacillus radicicola*. The root nodulation in legumes are produced by rhizobia after a specific interaction with their host plant *via* chemicals secreted by host legumes and rhizobia and thus it has been assumed that rhizobia have coevolved with their host plants. Chickpea (*Cicer arietinum* L.) 2n=16, is one of the most important staple legume crops widely grown across many semi-arid regions of the world including the Indian subcontinent and has the capacity to fix large quantities of atmospheric nitrogen by forming a symbiotic association with rhizobia. Chickpea root nodule bacteria has recently been named as *Mesorhizobium ciceri* on the basis of 16S rDNA sequence data (Nour *et al.,* 1994). The genus *Mesorhizobium* has intermediate growth rate between fast and slow growing rhizobia and was initially proposed for five rhizobial species (*R. loti, R. ciceri, R. mediterraneum* and *R. tianshanense*) that are phylogenetically related and distinct from the large phylogenetic grouping of *Rhizobium, Agrobacterium* and *Sinorhizobium* (Jarvis *et al.,* 1997).

Occurrence of PGP traits in *Rhizobium*

The mechanisms by which multi-trait *Rhizobium* can have a positive effect on plant growth may be of two types: direct and indirect. Indirect growth promotion is the prevention of deleterious effect of pathogenic microorganisms by synthesis of antibiotics or HCN by the bacteria. Direct growth promotion can be through the synthesis of phytohormones, N_2 fixation and synthesis of some enzymes (such as ACC deaminase) that regulate the level of plant hormones, as well as the solubilization of inorganic phosphate and mineralization of organic phosphate. The occurrence of all these traits in rhizobia and their involvement in the overall effects on plant growth promotion has been discussed below:

Siderophore production

Iron is essential for almost all life processes such as respiration and DNA synthesis. It plays an important role in microorganism and plants (Neilands, 1981) as a component of cell and its deficiency can cause growth inhibition, decrease in RNA and DNA synthesis and can change the cell morphology. The bioavailability of iron in the soil is limited due to very low solubility of the Fe^{3+} ion. Microbes (bacteria and fungi) have, therefore, evolved specific and high affinity strategy to acquire iron from habitat by releasing siderophores. Siderophores scavenge iron from the mineral phases by forming soluble Fe^{3+} complexes and serve as vehicles for the transport of Fe^{3+} into a microbial cell varying greatly in chemical structure. Siderophore also play important role in inducing systemic resistance of plants and suppress the growth of certain pathogenic microorganisms in iron limiting conditions (Carson *et al.,* 1994; Storey *et al.,* 2006). Siderophore production by the rhizobia has been of particular interest in view of the prominent role of iron enzymes in the fixation and assimilation of nitrogen. Raychaudhuri *et al*. (2005) also reported siderophore production by *Mesorhizobium* sp. isolated from chickpea. Bhagat *et al.* (2014) reported that out of 17 isolates of *Mesorhizobium* sp., 88% were able to produce siderophores. Out of these isolates of *Mesorhizobium* sp. LGR 14, LGR 15 and LGR 16 were able to produce maximum siderophore.

IAA production

Several bacterial species can produce indolic compounds (ICs) such as the auxin phytohormone indole-3-acetic acid (IAA), which impart great physiological relevance for bacteria-plant interactions, varying from pathogenesis to phytostimulation (Spaepen *et al*., 2007). The ability to produce ICs is widely distributed among plant-associated bacteria. It is reported that 80% of microorganisms isolated from the rhizosphere of various crops possess the ability to synthesize and release auxins as secondary metabolites (Souza *et al*., 2013). Verma *et al.* (2013) found that the production of phytohormone (IAA) by *Mesorhizobium* strains stimulated the growth of plants. IAA production in bacterial cultures varied from 35.1% to 114.2% at 100 µg ml^{-1} tryptophan after 72 h incubation and was more significant in *P. fluorescens* (114.2%), *Mesorhizobium* sp. (70.4%) and *A. chroococcum* (35.1%) as compared with *B. megaterium*.

HCN production

Cyanide is a secondary metabolite produced during the early stationary growth phase by several rhizobacteria, including *Rhizobium* sp. (Wani *et al.,* 2008) by oxidative decarboxy lation pathway using glycine, methionine or glutamate as precursors (Curl and Truelove, 1986). Although cyanide is a phytotoxic agent which disrupts enzyme activity involved in major metabolic processes, it plays important role as a biocontrol substance (Devi *et al.,* 2007). Among cyanogenic compounds, hydrogen cyanide (HCN) effectively blocks the cytochrome oxidase pathway and is highly toxic to all aerobic microorganisms; however producer microbes, mainly pseudomonads, are reported to be resistant (Bashan and de-Bashan, 2005). HCN affects the respiratory system of pathogenic fungi and results in their growth inhibition. Bhagat *et al*. (2014) reported that out of 17 *Mesorhizobium* sp. 64% isolates were found positive for HCN production. Wani and Khan (2013) reported that production of HCN by *Mesorhizobium* sp. isolates in chickpea and it is known to play an important role in biological control.

Phosphate solubilization

Soil microorganisms showed capability to convert insoluble forms of Phosphorus (P) such as tricalcium phosphate $(Ca_3PO_4)_3$, iron phosphate (Fe_3PO_4), aluminium phosphate (Al_2PO_4), *etc.* into soluble P in soil in several studies (Sharma *et al.,* 2013). Among the various strategies adopted by microbes, for transformation of insoluble P into soluble P, the role of low molecular weight organic acids secreted by microorganisms has been well-recognized and widely accepted theory. Various studies have been done for identification and quantification of organic acids for their involvement in the P-solubilization process (Marra *et al.,* 2012). The efficiency of Psolubilization depends upon the type of organic acids released and their concentration. Among the phosphate solubilizing bacteria, *Rhizobium* is of particular interest because of its dual function, *i.e.* its ability to fix N_2 and to solubilize P. Verma *et al.* (2012) reported that phosphate solubilization was most frequently encountered by *P. fluorescens*, followed by *B. megaterium* and least by *Mesorhizobium* sp. and *A. chroococcum.*

ACC-deaminase activity

The phytohormone ethylene plays an important role in many physiological processes, such as seed germination, root elongation and root hair development, leaf and organ senescence, leaf and petal abscission and fruit ripening under normal conditions (Siddikee *et al.,* 2011). When produced in higher amount ethylene has a negative effect on plant growth and development as it acts as a stress hormone. Plants are known to respond to various kinds of stresses by producing ethylene, which leads to abnormal growth and development. To regulate the ethylene levels in the root region, several rhizobacteria produce enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, which breaks down ACC, the immediate precursor of ethylene, when hydrolyzed by ACC deaminase and results in α-ketobutyrate and NH_3 formation (Safronova *et al.,* 2006). Thus, the decreased levels of ethylene in turn enable the plants to grow better in stress condition. The PGPR containing ACC deaminase are present in various soils and ensures a bacterial inoculum for improvement of plant growth, particularly under unfavourable environmental conditions such as flooding, heavy metals, phytopathogens, drought and high salt. Inoculation of crops with rhizobacteria having ACC-deaminase may encourage plant growth by alleviating deleterious effects of salt stress. Chaudhary and Sindhu (2015) reported that out of 50 isolates of *Mesorhizobium* screened for ACC only two *Mesorhizobium* isolates, namely MHD1 and MHD12 showed significant growth on ACC supplemented plate. Eight isolates, that is, MHD2, MHD4, MHD8, MHD11, MBD25, MBD26, MSD28 and MSD29 showed moderate growth whereas twelve isolates showed little growth on ACC plates. Twenty eight *Mesorhizobium* isolates did not grow on ACC supplemented plates.

PGP traits of rhizobium and their effect on plant growth

Rhizobia have been shown to promote the plant growth through PGP mechanisms that are different from the atmospheric N_2 fixation. PGP traits such as secretion of phyto-hormones like indole acetic acid that enhances various stages of plant growth, synthesis of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which can lower plant ethylene levels, solubilization of minerals such as phosphorus making it more readily available for plant growth, siderophore production that can sequester iron from the soil and provide it to plant cells and by conferring increased resistance against plant pathogens have been reported (Deshwal *et al*., 2003). Inoculation of plants with rhizobia having multiple PGP traits often stimulates plant growth by IAA production, siderophore production, HCN- production and by increasing phosphorus uptake. Rhizobia starve the pathogens of available iron by producing high affinity siderophores and thereby limit the growth of the pathogen. It has been reported that the rhizobia producing ACC deaminase are also efficient nitrogen fixers. The structural gene of ACC deaminase (*acds*) in *Mesorhizobium* sp. is under the control of *nif* promoter (Nascimento *et al*., 2012) which generally regulates the *nif* gene responsible for nitrogen fixation. There are many indirect ways such as production of antibiotics, secretion of siderophores, production of HCN, production of lytic enzymes and competition for nutrient through which rhizobia having PGP traits act as plant growth promoters with their biocontrol properties and induction of systemic resistance against phytopathogens and insect pests (Reddy, 2013). Thus, this ability makes them more important as multi-purpose inoculants for the crop production. Jida and Assefa, (2012) found that out of 36 isolates 44.4% of the *Mesorhizobium* isolates were phosphate solubilizer while 27.8% of them were found to be indole-3-acetic acid (IAA) producer. Furthermore, 19.4% tested isolates showed antagonistic activity against *Fusarium oxysporum* in dual culture. Application of highly competitive and efficient N_2 fixing *Mesorhizobium* isolates having PGP traits in chickpea seems to have better potential in improving the yield of chickpea by providing the benefits of both N_2 fixation and PGP traits. Thus, various aspects of the PGP traits and N economy of chickpea have been identified but not in the same study.In another study Ahmed and Khan, (2012) founded that *Mesorhizobium* sp. strain MRC4 produced indole acetic acid (44 µg ml^{-1}), siderophores, salicylic acid (35 µg ml^{-1}), 2,3-dihydroxy benzoic acid (19 µg ml^{-1}), exo-polysaccharides (21 µg ml^{-1}), HCN and ammonia. Such kind of rhizobial strains may also be useful as multipurpose inoculants for chickpea and non-legume crops grown in rotation or as succeeding crops (Jida and Assefa, 2011).

CONCLUSION

It has been investigated and established that *Rhizobium* with PGP traits can be effective and potential microbe for sustenance of soil health and crop production. Since, the benefit of rhizobia inoculation varies with strains and locations; it necessitated an immense need for isolation and characterization of *Mesorhizobium* isolates having multiple PGP traits and efficient N_2 fixation ability in chickpea.

Conflict of interest: None.

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