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Role of *Rhizobium* in chickpea (*Cicer arietinum*) production - A review

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ABSTRACT

Chickpea (*Cicer arietinum* L.) is the major food legume. It belongs to family Fabaceae (Leguminosae) and is widely grown for its nutritional benefits. In the present scenario, sustainability of agriculture has become a major issue of global concern as the intensive use of chemical inputs has an adverse impact on the environment. Use of biofertilizers such as *Rhizobium* can reduce the need for chemical fertilizers and decrease adverse environmental effects. The studies have shown positive effect of *Rhizobium* (*Mesorhizobium*) inoculation on growth attributes, symbiotic parameters, yield and yield components, nutrient uptake and quality in chickpea. Fluctuations in various environmental factors *viz.* pH, nutrient availability, temperature, moisture, salinity and herbicides greatly influence the growth, survival, and metabolic activity of nitrogen fixing bacteria and plants, and their ability to enter into symbiotic relationship.

Key words: Biological nitrogen fixation, Chickpea, Environment, Growth attributes, Nodules, *Rhizobium*, Salinity, Yield.

Chickpea (*Cicer arietinum* L.), also called Bengal gram, is an important winter season pulse crop. In India, it was grown on area of 8.25 million ha during 2014-15, contributing 7.33 million tonnes to the national pulse basket with productivity of 889 kg/ha (Anonymous, 2016). Nutritionally, it is an important source of protein (16-20%) in vegetarian diet and has become more important to mitigate the problem of protein energy malnutrition (Prasad, 2012). It is also a rich source of carbohydrates, fat, total dietary fibre, vitamins and minerals (Hirdyani, 2014).

Soil is the habitat for prodigious number of living organisms which include the living forms both visible to the naked eye and on the other hand ones with microscopic dimensions. Among these, bacteria play a vital role in a various biochemical processes taking place in the soil and are very much beneficial as far as the plant growth and soil properties are concerned (Anonymous, 2014). Bacteria are able to promote plant growth by establishing symbiotic association with plant or as free living entities. Rhizobia are a unique group of bacterial symbionts of legumes that fix inert elemental atmospheric nitrogen (Araujo *et al,* 2008). Among all the N_2 fixing micro-organisms, symbiotic relationships between legumes and rhizobia are responsible for the largest contributions of fixed N to farming systems (Unkovich *et al,* 2008). Furthermore, it has been reported that rhizobial strains promote plant growth through other mechanisms such as phosphate solubilisation ability in some legumes (Sridevi and Mallaiah, 2009) and lowering of plant ethylene levels due to presence of ACC deaminase activity (Glick, 2012).

During the green revolution, the nutrient demand was substantially increased due to the introduction of high yielding varieties of wheat and rice which resulted in increased use of synthetic fertilizers. Till date, nitrogen (N) fertilizers are crucial part of crop production and rank first among the external inputs to maximize agricultural production. However, N fertilizers contribute significantly to environmental pollution and have capability to disrupt every form of life to a great extent (Verma *et al*, 2014). Thus, emphasis should be laid on developing new production techniques that are sustainable both agronomically and economically. Biological nitrogen fixation (BNF) carried out by rhizobia-legume symbiosis can act as a renewable and environmentally sustainable source of N and can complement fertilizer inputs in crop production. It accounts for 65% of N currently used in agriculture (Gopalakrishnan *et al,* 2014). Under favourable conditions, the symbiotic N_2 fixation can produce as high as 176 kg N/ha and provide up to 85% of its N requirements in legumes.

Biological nitrogen fixation (BNF): Plants absorb most of nitrogen (N) in the NH_4^+ and NO_3^- forms. The crop plants cannot utilize N in its elemental form (N_2) for their growth and development and hence need conversion to a usable form. The conversion from unavailable to available form takes place through one of the following pathways:

- Fixation by symbiotic micro-organisms, especially in the roots of legumes
- Fixation by free-living micro-organisms in the soil
- Fixation through various industrial processes such as Haber-Bosch Process

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- Reaching soil as one of the oxides of N produced by atmospheric electric discharge.

BNF is the biochemical mechanism where rhizobia bacterial symbiont of legumes fix inert atmospheric nitrogen into a plant usable form under the presence of enzyme nitrogenase (Mohammadi and Sohrabi, 2012). The nitrogenase enzyme is a biological catalyst which is present in the bacteriod and mediates the reaction. BNF is an economically attractive and ecologically sound source of nitrogen and helps in decreasing the dependence on external inputs.

Mechanism of biological nitrogen fixation: Binding of rhizobia to plant surface (either root or shoot) is essential for establishing a symbiotic relationship between the host plant and bacterium. In early stages of symbiosis, a complex "molecular dialogue" takes place, which involves production of Nod factors by the bacterium and flavonoids by the roots of legume plants. This helps in the host recognition and initiation of the nodulation process. When bacteria colonize the root surface, it induces curling of root hair tips. Rhizobia caught in the root hairs, locally degrade the plant cell walls and form an invagination called infection thread through which bacterium reaches the cortical cells and within a week after infection, small sac like structures called nodules are visible on the roots of legume plants which enclose the bacteriod and are actual site for nitrogen fixation (Garg and Geetanjali, 2007; Laranjo *et al,* 2014). Rhizobia are able to metabolize atmospheric nitrogen and convert it into available form of nitrogen that plant can take up. In exchange, rhizobia take advantage of carbon substrates derived from the plant photosynthesis.

Effect of *Rhizobium* inoculation on plant growth: Nitrogen fixers benefit the plant by providing them atmospheric nitrogen, which contributes to the development of plant growth and biomass production. Rudresh *et al* (2005) studied the effect of inoculation with *Rhizobium* on growth attributes and observed that chickpea gave higher plant height (3.3%), number of branches per plant (23.3%) and biomass per plant (144%) as compared to uninoculated control. In similar findings, Elkoca *et al* (2008) revealed that *Rhizobium* inoculation increased plant height, shoot dry weight and chlorophyll content in chickpea. These findings are in agreement with that of Giri and Joshi (2010) in chickpea. The increment in the root length was also observed in the inoculated treatments (Solaiman *et al,* 2010) which in return resulted in increased root surface area. The increase in root surface area enhances the nutrient acquisition by plant from the soil (Yadav and Verma, 2014).

Branches are subsidiary parameter of plant biometery which are important to realise higher grain yields. Singh *et al* (2011) found that number of primary and secondary branches were higher when chickpea was inoculated with *Rhizobium*. Similarly, Shahzad *et al* (2014) reported greater plant height and shoot biomass per plant in *Rhizobium* inoculated chickpea in both irrigated and rainfed conditions. Beneficial effects on various growth attributes due to *Rhizobium* inoculation have also been reported (Togay *et al,* 2008; Singh *et al,* 2015) which may be attributed to improved nutrient acquisition (Solaiman *et al,* 2012), enhanced growth and development of photosynthetic organs and the rate of photosynthates accumulation (Moinuddin *et al,* 2014) as well as production of various phytohormones such as indole acetic acid (IAA) (Verma *et al,* 2013).

Symbiotic parameters: The number and dry weight of root nodules are index of degree of infection leading to nodule development (Bhattacharjya *et al,* 2009). The positive effect of *Rhizobium* inoculation on number and dry weight of nodules per plant in chickpea is very well documented by various researchers (Eusuf Zai *et al* 1999; Bhuiyaan *et al*, 2008a). *Rhizobium* inoculation resulted in excellent nodulation in contrast to poor nodulation in control (Khattak *et al*, 2006). Suryawanshi *et al* (2007) observed significant effect of inoculation on nodule number and nodule dry weight. Inoculation studies have been shown to increase nodule number and nodule dry and fresh weight per plant in chickpea (Verma *et al,* 2010; Sahai and Chandra, 2010). Similarly, Singh *et al* (2014) observed higher number (27.6%) and dry weight (22.2%) of nodules per plant as compared to uninoculated control in chickpea.

Leghaemoglobin, red iron-containing protein, occurs in the root nodules of leguminous plants where it facilitates the diffusion of oxygen to the symbiotic bacteriods in order to promote nitrogen fixation. Moreover, leghaemoglobin is synthesized by the symbiotic partners viz. the Rhizobia and the host plant. *Rhizobium* synthesizes the "haem" portion and plant synthesizes the "globine" portion. Lakshmanarao *et al* (1983) evaluated positive relationship between leghaemoglobin content and nitrogen fixation. Tagore *et al* (2013) observed higher leghaemoglobin content in the nodular tissue of chickpea inoculated treatment. Further, there was a positive correlation between nitrogenase activity and both number and dry weight of nodules (Miller *et al,* 1986). Seed inoculation showed significant increase in the nitrogenase activity in contrast to uninoculated control (Dutta and Bandhyopadhyay, 2009). Malik and Sindhu (2011) also reported similar findings in chickpea. Such positive benefits have also been reported by Das *et al* (2013) which may be attributed to presence of either low or ineffective population of native rhizobia nodulating chickpea in the soil allowing the inoculant strain to form greater portion of effective nodules on plant roots (Bhattacharjya and Chandra 2013).

Yield components and yield: The pathways by which the morphological, developmental and reproductive features of plants in a crop stand contribute to yield are called yield components (Reddy, 2012). Many researchers around the globe have reported positive effect of *Rhizobium* inoculation on various yield components viz. number of pods per plant, seeds per pod and 1000-seed weight in chickpea.

Number of pods per plant and number of seeds per plant were reported to be 21.8% and 10.5% higher, respectively in chickpea inoculated with *Rhizobium* over uninoculated control (Sharar *et al,* 2000). Further, Ali *et al* (2004) revealed that 1000-seed weight was significantly better with inoculation. Khan *et al* (2003) in a similar study observed significantly higher number of pods per plant, number of seeds per plant and 1000-seed weight in chickpea. These findings are in agreement with that of Akhtar and Siddiqui (2009) and Meena *et al* (2013).

El Hadi and Elsheikh (1999) studied the effect of *Rhizobium* inoculation on the grain yield of chickpea and reported that inoculation with *Rhizobium* increased yield by 72 and 70% in the first and second year, respectively, in comparison to uninoculated control. Similarly, Maleki *et al* (2009) conducted a pot experiment in Iran and reported higher yield where rhizobial inoculation was done as compared to uninoculated control. Furthur, Ogola (2015) from South Africa reported increase in the grain yield to the tune of 7.9% with *Rhizobium* inoculation in a clayey and slightly acidic soil.

The increase in the yield components through seed inoculation might be due to higher nodulation and more nutrient availability, resulting in vigorous plant growth and dry matter accumulation, which in turn resulted in higher seed yield (Namwar *et al,* 2013; Uddin *et al,* 2014). More examples of the beneficial effects of *Rhizobium* inoculation in chickpea are given in Table 1.

Nutrient content, uptake and protein: Chickpea is valued for its high protein content and has about 16-20% protein in the grain. Since, protein content in grain is directly associated with the nitrogen content, which can be expressed as follows:

Protein content (%) = Nitrogen content (%) × 6.25

Hence, increase in the nitrogen content will result in increase in the protein content. Qureshi *et al* (2009) reported *Rhizobium* inoculation produced higher grain N and P content in comparison to uninoculated control. Similarly, Erman *et al* (2011) found that grain as well as shoot N and P content was higher where the chickpea was inoculated with *Rhizobium*. These findings are in line with those of Yagmur and Kaydan (2011) and Tagore *et al* (2014) in chickpea. Further, Kaur *et al* (2015) reported 61.1% and 11.4% greater grain N and P content, respectively in *Mesorhizobium* inoculated chickpea. Kumar *et al* (2014) also found significantly higher protein content in *Rhizobium* inoculated plots in comparison to uninoculated control.

Similarly, remarkable improvements in uptake of N and P by both grain and shoot have been observed with *Rhizobium* inoculation in chickpea. In a field experiment, Sahai and Chandra (2011) found higher N and P uptake in both grain and shoot when chickpea was inoculated with *Mesorhizobium* sp. in contrast to uninoculated control. This might be due to increased nitrogen fixation (Rokhzadi and Toashih, 2011) as well as improvement in root growth and root behaviour which favourably influence nutrient acquisition (Das *et al,* 2012).

Residual soil nutrient status: Use of *Rhizobium* inoculants helps in improving available N and P in the soil after crop harvest which can be utilized by the next crop (Abdalla *et al,* 2013). Similarly, Chandra and Pareek (2015) recorded 0.6%, 6.5% and 4.3% higher organic carbon, available N and available P, respectively, in a *Rhizobium* inoculated chickpea. These findings are in line with those of Zaidi *et al* (2003) and Tagore *et al* (2013). Moreover, higher soil microbial biomass carbon was found in *Mesorhizobium* inoculated chickpea (Bhattacharjya and Chandra, 2013). These results may be attributed to production of more crop biomass due to inoculation and consequently higher return of organic residues and exudates into the soil enhancing microbial biomass and activities (Babu *et al,* 2015). The pulse crops residues have lower C:N ratio, as a result of which immobilization after incorporation in the soil is low,

Table 1: Increase in grain yield of chickpea due to inoculation with different *Rhizobium* strains.

Species	Strain	Yield (q/ha) (Uninoculated)	Yield (q/ha) (Inoculated)	Percent increase	Reference
Rhizobium sp.	Thal-8	15.2	18.6	22.4	Aslam *et al* (2000)
Rhizobium sp.	TAL-620	15.2	18.1	19.1	Aslam *et al* (2000)
Rhizobium sp.	CC-1192	13.8	17.3	25.4	Khattak *et al* (2006)
Rhizobium leguminosarum bv. *Ciceri*	-	11.6	12.6	8.3	Namwar *et al* (2011)
Mesorhizobium ciceri	LN 7007	19.0	22.1	11.8	Sahai and Chandra (2011)
Sinorhizobium ciceri	EAL 001	7.90	8.7	11.2	Messele and Pant (2012)
Mesorhizobium ciceri	CH-1233	19.4	21.7	11.8	Bhattacharjya and Chandra (2013)
Rhizobium sp.	BHURC03	20.1	23.8	22.8	Verma *et al* (2010)
Mesorhizobium ciceri	LGR33	21.4	23.1	7.9	Mansotra *et al* (2015)
Mesorhizobium sp.	Cp41	21.7	25.9	19.3	Tena *et al* (2016)

which consequently enhances N availability to subsequent crops.

Environmental factors affecting legume-rhizobia symbiosis: Suboptimal levels of several environmental factors can limit both legume plant growth as well as N_2-fixing activities. Fluctuations in pH, nutrient availability, salinity, temperature, herbicides and moisture greatly influence the growth, survival, and metabolic activity of nitrogen fixing bacteria and plants, and their ability to enter into symbiotic relationship (Mohammadi *et al,* 2012).

Soil salinity: Salinization is the accumulation of water-soluble salts in the soil profile to a level that have detrimental impacts on agricultural production, environmental health, and economic welfare. Salinity is an increasing agricultural problem in many regions worldwide. The total global area of salt-affected soils including saline and sodic soils is 831 million hectares, extending over all the continents including Asia, Africa, America, Australia and Europe. It is a serious threat to the production of grain legumes in the semi-arid and arid regions of the world (Rengasamy, 2006).

Over 40 years ago, Bhardwaj (1975) reported that in legume-*Rhizobium* symbiosis legumes are more sensitive to salinity as compared to their microbial symbionts. Salinity does not affect colonization of root by rhizobia (Flowers *et al,* 2010) but it causes significant reduction in nodulation, nodule weight and efficiency of fully formed nodules in chickpea (Singleton and Bohlool, 1984) and hence decreases the proportion of those nodules that are initiated in saline conditions that are able to develop into active nitrogen fixing nodules. Elsheikh and Wood (1990) reported that nodulation was completely inhibited at 7 dS m^{-1} and the chickpea plants died at 8 dS m^{-1}. Rao *et al* (2002) revealed that plants at pH 8.9 showed the least effect of stress and nodules were healthy and pink. But with increase in pH to 9.0 and 9.2 the nodules became black in colour and were damaged. Reduced nodule formation by the chickpea could have been due to the adverse effects of salinity on the nodule initiation.

Soil acidity: Plant growth and most soil processes, including nutrient availability and microbial activity, are favoured by a soil pH range of 5.5 – 8. When pH of the soil declines, aluminium solubility in soil solution increases which is toxic to plants as a result of which root growth is retarded. In India, nearly 25 million hectares of cultivated lands with pH less than 5.5 are critically degraded. The productivity of these soils is very low (one tonne/ha) due to deficiencies of P, Ca, Mg, Mo and B and toxicities of Al and Fe (IISS Vision 2030).

Low pH in top layer of the soil may affect microbial activity, significantly decreasing legume nodulation. Most leguminous plants require a neutral or slightly acidic soil for growth. Soil acidity limits symbiotic nitrogen fixation by reducing the chances of *Rhizobium* survival and its persistence in soils, and ultimately reducing nodulation in legumes (Taylor *et al,* 1991). In most of the cases, a pH sensitive stage in nodulation occurs early in the infection process and that *Rhizobium* attachment to root hairs is one of the stages affected by acidic conditions in soils. At or below pH 4.8, aluminium will reduce root growth while manganese disrupts photosynthesis and other functions of growth, resulting in the reduction of nitrogen fixation by rhizobia (Zahran, 1999)

Temperature: High soil temperature is one of the most critical factors which can prevent the development of a symbiotic association between the host plant and micro-symbiont especially in arid and semi-arid regions. For most of rhizobia, optimum temperature range for growth is 28 to 31°C and majority of them are unable to grow at 38°C (Graham, 1992). Temperature influences the survival of free rhizobia as well as the molecular dialogue between host and rhizobia. Elevated temperature can have inhibiting effect on microsymbiont adherence to root nodules, on nodule structure and on legume root nodule functioning (Zahran, 1999).

Rupela and Rao (1984) reported that with increase in the temperature to 35°C there was significant decrease in nodule number as well as nodule dry mass which may be attributed to drastic decrease in photosynthesis at high temperature and hence reduced supply of photosynthates to *Rhizobium*. These findings are in line with that of Jain *et al* (2014) who also revealed the negative effects of high temperature on nitrogen fixation in chickpea. However, it has been reported that when plant is subjected to high temperature conditions specific set of proteins are over produced, termed as heat shock protein, which are important for survival during temperature stress conditions (Rodrigues, 2006).

Moisture: Soil-water content have direct effect on the growth of rhizobia present in the rhizosphere of the plant by decreasing water activity below critical tolerance limits or on the other hand by creating negative impact on the plant growth, vigour, root architecture, and exudations from host plants (Mohammadi *et al,* 2012). Population levels of rhizobia increases with increase in moisture level until waterlogging whereas poor nodulation is reported in arid soils with low moisture level resulting in lower rhizobial population.

In waterlogged conditions, there is inhibition of root hair development as well as nodulation sites. Moreover, normal diffusion of O_2 in the root system of plants is also negatively affected. This lack of O_2 is a major problem for root respiration and results in inhibition of nitrogenase activity and nodule oxygen permeability (Serraj and Sinclair, 1996). Gan *et al* (2008) reported that chickpea grown under different moisture stresses significantly influences the N_2-

fixing efficiency of the nodules. Furthur, they reported that N_2 fixation is more sensitive to the effect of waterlogging than plant growth. Low soil moisture conditions at early stages affects the rhizosphere colonization and nodule formation by *Rhizobium* whereas water stress at the late vegetative stage decreases the nitrogen fixation efficiency of nodules. Lower dry weight of nodules was reported where irrigation was given 15 days before sowing as compared to 7 days before sowing due to moisture stress during crop growth (Singh *et al,* 2011) in chickpea.

Mineral Nutrients

1. Nitrogen: Nitrogen available to the legumes is mostly in the form of NO_3^- ions which are produced by oxidation of NH_4^+. However, the capacity of nitrogen fixation of a nodulating as well as nodulated legumes are influenced in a number of ways by the mineral nitrogen in the soil in which it is grown:

i. Higher concentration of nitrate or ammonia always depresses the nodulation process in legumes in the early stages of growth.

ii. The nitrogen fixation efficiency of well-nodulated legume is always suppressed by nitrate ions.

Research findings of Abbasi *et al* (2013) revealed that the number of nodules and nodule dry weight increased with incremental dose of nitrogen upto 50 kg N/ha, however these parameters significantly decreased with higher doses of nitrogen. In similar findings, Namwar *et al* (2011) observed decrease in nodule number and dry weight with higher dose of nitrogen (100 kg urea/ha) as compared to 75 kg/ha. In a pot experiment, the application of nitrate to nodulated plants gave rise to structural degradation of the nodule in chickpea (Sheokand *et al,* 1998). In greenhouse experiment, the control treatment where no nitrogen was added showed the maximum number of nodules per plant that were significantly better than all other treatments *viz.* 50, 100 and 100 kg N/ha (David and Khan, 2001).

2. Phosphorus: The process of symbiotic nitrogen fixation is significantly influenced by the application of phosphorus (P) to the legumes. In presence of adequate P, the bacterial cells became motile and flagellate which is the pre-requisite for bacterial migration, but in the absence of P or with inadequate supply the infection remains latent leading to the poor development of nodules (Dutta and Bandyopadhyay, 2009). It promotes early root formation and the formation of lateral, fibrous and healthy roots, which is very important for nodule formation and ultimately the nitrogen fixation (Madzivhandila *et al,* 2012; Dotaniya *et al,* 2014).

Tripathi *et al* (2013) reported that incremental doses of phosphorus along with *Rhizobium* increased nodules per plant by 31.2% over *Rhizobium* alone. In a similar study, Das *et al* (2013) revealed that 45 kg P_2O_5/ha gave 24.2% more plant root nodules per plant over control in chickpea but was found to be statistically at par with 30 kg P_2O_5/ha. Higher nitrate reductase activity as well as leghaemoglobin content was observed when *Rhizobium* inoculation was combined with phosphorus in contrast to *Rhizobium* alone (Moinuddin *et al,* 2014). The improvement in leghaemoglobin content and nitrogenase activity of nodules by P application may be attributed to profuse nodulation leading to increased nitrogen fixation

3. Molybedenum: Micronutrients play an important role in increasing the yield of pulse and oilseed legumes through their effects on plant itself and on the nitrogen fixing symbiotic process. Molybdenum is one of the key micronutrients in the legume production since it is an essential component of nitrate reductase and nitrogenase enzyme which mediates the ion exchange and control the reduction of inorganic nitrate and helps in fixation of inert elemental nitrogen into ammonia. Bhuiyan *et al* (2008b) reported that inoculation with *Rhizobium* along with molybdenum application significantly improved the root length as well as number of nodules per plant. These findings are in corroboration with those of Chatterjee and Bandyopadhyay (2015) in cowpea. Similarly, Poonia and Pithia (2014) reported higher number of root nodules when chickpea seeds were inoculated with *Rhizobium* and seed treated with molybdenum.

Herbicides: Herbicide application is one of the most promising methods of weed control in field crops. The application of herbicides results in the accumulation of these herbicidal chemicals in the upper layer of soil (0-15 cm) which is the seat for various microbiological activities and biochemical reactions. Various researchers have reported the adverse effect of herbicides on nodule formation, nodule growth, nodule efficiency and activities of beneficial microorganisms such as *Rhizobium* in soil (Bai and Nanjappa, 2003; Ahemad and Khan, 2013).

Khan *et al* (2004) reported that application of isoproturon at 10 times higher rate (20 μg a.i./g) decreased the nodule number by 32.3% in contrast with control (no herbicide application) while 10 times higher dose of bentazone (11 μg a.i./g) and 2,4-D (17.5 μg a.i./g) completely inhibited the nodule formation suggesting that higher concentration of these herbicides is highly toxic for *Rhizobium*-legume symbiosis in chickpea. Isoproturon, bentazone and 2,4-D at 2.0, 1.1 and 1.75 μg a.i./g recorded 39.5, 42.8 and 33.7 number of nodules/plant as compared to 40.5 in untreated control. Further, decrease in the nitrogenase activity was also observed due to the application of these herbicides which may be attributed to damage in the photosynthetic apparatus as well as alteration in nodule cortex structure. The effects of herbicides on the legume-rhizobia symbiosis summarized by Parsa *et al* (2014) are as follows:

(i) Reduction of root biomass which leads to limit the number of available sites for rhizobia to attach to root hairs
(ii) Alteration in the carbohydrate preparation of the root nodules
(iii) Alteration in the nitrogenase enzyme activity which is critical for nitrogen fixation
(iv) Inhibiting or inactivating the biochemical dialogue which takes place between host plant and microbial symbiont (*Rhizobium*) which is essential for nodulation process.

CONCLUSION

It is evident from the studies that *Rhizobium* inoculation has positive impact on the growth, symbiotic parameters, yield and yield attributes, nutrient uptake and quality in chickpea. This can be attributed to improved nutrient acquisition (viz. biological nitrogen fixation and phosphorus solubilisation), production of phytohormones and various other mechanisms. For reducing the cost of cultivation there is a need of integrated use of *Rhizobium* and inorganic fertilizers. Furthermore, environmental conditions such as pH, nutrient availability, temperature, herbicides and moisture have a significant effect on legume-*Rhizobium* symbiosis and its nitrogen fixation efficiency

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