INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 10, No. 3, p. 46-53, 2017

RESEARCH PAPER OPEN ACCESS

Yield and quality response of cotton to a consortium of PGPR at graded fertilizer levels

M. Amjad Qureshi*[1], M. Asif Ali[1], F. Mujeeb[1], M. J. Ahmad[2], S. Rashid[3], Sana Ullah[4], M. A. Anjum[5]

[1]*Soil Bacteriology Section, Agri. Biotech. Research Institute, AARI Faisalabad, Punjab, Pakistan*

[2]*Pesticide Quality Control Lab, ISC&ES, Faisalabad, Pakistan*

[3]*Rice Research Station, Bahawalnagar, Pakistan*

[4]*Soil and Environmental Sciences Division, Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan*

[5]*Institute of Soil Chemistry & Environmental Sciences, AARI, Faisalabad, Punjab, Pakistan*

Key words: PGPR, consortium, *Azotobacter*, *Azospirillum*, Cotton

http://dx.doi.org/10.12692/ijb/10.3.46-53 Article published on March 12, 2017

Abstract

Biofertilizers are formulations of rhizobacteria are eco-friendly, cost effective and have potential to sustain the yields in the intensive cropping system. Plant growth promoting rhizobacteria (PGPR) have proved their worth for promoting the yield and quality traits of various crops by producing phytohormones, siderophores, antibiotics, mobilizing nutrients and inducing systemic resistance. In a series of experiment, the consortium of PGPR of *Azotobacter* and *Azospirillum* sp were tested on the yield parameters of cotton with graded levels of nitrogen (N) at Cotton Research Station, Sahiwal. Three levels of N i.e. 60, 90 and 120 kg ha^{-1} were applied while P was applied at 60 kg ha^{-1} to all the treatments. Results revealed that the bacterial consortium affected the cotton growth and yield at all N levels as compared to un-inoculated control. The maximum seed cotton yield i.e. 2478 was observed at 120 kg Nha^{-1} as compared to its respective control i.e. 2238 kg ha^{-1}. The highest number of bolls $plant^{-1}$ (25), boll weight (3.34 g) and plant height (126.9) were also observed at the same treatment. Results also showed that consortium of PGPR had reduced cotton leaf curl virus (CLCV) incidence up to 36.0% as compared to 41.0% without inoculation. The highest ginning out turn (GOT) and staple length was observed with PGPR consortium inoculation i.e. 38.6% and 27.8 mm as compared to un-inoculated control i.e. 37.9 % and 27.5 mm, respectively at 120 kg N ha^{-1}. Present study clearly demonstrated that consortium of PGPR had more assenting effect on the yield components of cotton. More combination of PGPR should be used in different ecologies to validate this approach and to compensate the mineral fertilizer for sustainable agriculture.

* **Corresponding Author:** M. Amjad Qureshi ✉ qureshifsd@gmail.com

Introduction

Rhizosphere, the root soil interface, is relatively rich in nutrients has as much as 40% of the root exudates and supports diversified microbial population. Themicrobial diversity and functioning in the rhizosphere is responsible for healthy root, nutrients acquisition for plants and potential to combat numerous stresses (Cook, 2002).

The root colonizing bacteria, exhibiting beneficial effects on the plants through direct and indirect mechanisms, are termed as plant growth promoting rhizobacteria (PGPR)(Kennedy, 2005; Wu *et al.,* 2005). The mechanisms adopted by PGPR for the plant growth promotion are not well established but literature inveterate the evidences of their utilitydirectly and indirectly (Khalid *et al.,* 2006; Nadeem *et al.,* 2009; Ahmad *et al.,* 2011).

The direct mechanisms by PGPR, for plant growth promotion are, production of phytohormones and siderophores production, solubilization of insoluble phosphates, asymbiotic N fixation, synthesize of antibiotics and enzymes and improvement in ion fluxes at root surface for better nutrient uptake (Mrkovacki and Milic, 2001; Bharathi *et al.,* 2004; Ahmad *et al.,* 2006; Salantur *et al.,* 2006; Nadeem *et al.*, 2009; Ahmad *et al.*, 2011). Indirectly the PGPR suppress the plant pathogens (Jeun *et al.,* 2004). The effectiveness of PGPR depends upon the root colonization potential. The versatile qualities of effective PGP Rare chemotaxis, motility, protein secretion and potential to use components of root exudates (Lugtenberg *et al.,* 2001; Persello-Cartieaux *et al.,* 2003; Gholami *et al.,* 2009).

The main genera involved in plant growth promotion and frequently reported by number of workers, are *Azotobacter*, *Azospirillum*, *Bacillus*, *Pseudomonas*, *Enterobacter*, *Arthrobacter*, *Alcaligenes*, *Burkholderia*, *Klebsiella, Serratia* and *Rhizobium* sp (Joseph *et al.,* 2007).

Azotobacter, a free-living, non-symbiotic, aerobic N_2-fixer, is well known PGPR and boosts up the legumes (Mrkovacki and Milic, 2001) and non-legumes (Gholami *et al.,* 2009). Species of *Azotobacter* evidently affected the seed germination and seedling growth and thus enhanced the crop yields (Gholami *et al.,* 2009)by the production of phytohormones, nutrient mobilization and suppression of plant pathogens (Murphy *et al.,* 2000; Paul *et al.,* 2011).

Azospirillum, the most commonly studied organisms among the PGPR, a fine root colonizer, associative in nature and potential growth hormone producer, enhanced the yield of crops by improving the root architecture and consequently enhanced the nutrient uptake (Steenhoudt and Vanderleyden, 2000; Kennedy and Islam, 2001). Studies reported that free living diazotrophs applied in combination increased the yield of crops. Compared to single inoculants combination of PGPR resulted in todynamic rhizosphere of plants as that provide better root colonization, biostimulants, disease suppression and rhizoremediators (Ramamoorthy *et al.,* 2001; Mahale *et al.,* 2003; Kuiper *et al.,* 2004; Khan *et al.,* 2010; Saharan and Nehra, 2011). Present study was designed to assess the consortium of PGPR inoculation i.e. *Azotobacter* and *Azospirillum,* to the yield components of cotton under graded N levels.

Materials and methods

Isolation of Azotobacter and Azospirillum

Rhizosphere soil samples of cotton, growing at various locations at Ayub Agri. Research Institute (AARI), Faisalabad, were collected. Their serial dilutions in sterilized distilled water were prepared and then inoculated into selective media of *Azotobacter* i.e. Jensen Agar medium (JAM) (Jensen, 1953) and *Azospirillum* N-free biotin medium (Nfb) (Dobereiner and Pedrosa, 1987). Isolates of *Azotobacter* and *Azospirillum* sp were purified and screened out ontheir selective medium. Petri plates carrying Jensen agar medium (JAM) and Nfb were incubated at 28 ± 2 ^{o}C for 72 hours. Typical brownish colonies of *Azotobacter* sp were appeared on the Jensen's medium while veil like pellicles were observed below the Nfb medium surface indicated the *Azospirillum* sp. The growth of *Azotobacter* and *Azospirillum* species were frequently purified on their respective medium to get pure cultures.

For further identification, presumptive/qualitative tests [indole, gram reaction, oxidase, bromothymol blue (BTB) and urease tests] were carried out as outlined in Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984).

Determination of auxin biosynthesis

Three isolates ofeach Azotobacter and *Azospirillum* species were selected and characterized for their auxin biosynthesis potential. Isolates of *Azotobacter* and *Azospirillum* were incubated for one week in the tubes containing general purpose medium (GPM).Auxin biosynthesis potential was determined as Indole-3-acetic acid (IAA) equivalents using Salkowski's reagent as reported by Sarwar *et al.* (1992). On the basis of tests and auxin biosynthesis potential, isolates of *Azotobacter* (AZ_3) and *Azospirillum*sp (AS_3) were selected for the experimentation.

Inoculum preparation

Isolates of *Azotobacter* and *Azospirillum* sp were multiplied on the broth of their selective medium and incubated at 28 ± 2 °C under shaking at 100 rpm for three days to give an optical density of 0.5 recorded at 535 nm. Peat based carrier was sterilized at 121 °C and 15 psi pressure for half an hour. Inocula of each isolate were made with 15% sugar solution, 25 mL broth in250 g of sterilized peat and incubated for three days. Inocula of both isolates were mixed in 1:1 ratio with 15% sugar solution to cotton seed and were left for half an hour.

Field experiments

Field studies were conducted to assess PGPR inoculation (*Azotobacter +Azospirillum*) on the yield parameters of cotton at Cotton Research Station, Sahiwal. The pre-sowing soil sample was collected, air dried, thoroughly mixed, passed through 2 mm sieve and analyzed for various physico-chemical characteristics. Studies were conducted on the soil having pH, 8.13; electrical conductivity, 0.89 dS m^{-1}; organic matter, 0.60%; total N, 0.034; available phosphorus, 7.43 mg kg^{-1}soil. Standard agronomic and plant protection practices were followed. Data regarding seed cotton yield, number of bolls $plant^{-1}$, plant population ha^{-1}, boll weight, plant height, CLCV %, GOT and staple length were recorded. Data were subjected to statistical analysis fallowing randomized complete block design using Statistix v. 8.1 (Steel *et al.*, 1997). The differences among the treatment means were checked by applying the Duncan's multiple range tests (Duncan, 1955).

Results

Results regarding screening of *Azotobacter* and *Azospirillum* isolates were presented in Table 1.

Table 1. Screening of isolates for different traits and biochemical, qualitative tests under study.

Isolates	*IAA equivalents (µg mL^{-1})*	*Gram reaction*	*Oxidase test*	*BTB test*	*Urease test*
		Azotobacter sp			
AZ_1	3.46	-ve	++*	+ve	+++*
AZ_2	3.62	-ve	++	+ve	+
AZ_3	3.79	-ve	+++	+ve	+++
		Azospirillum sp			
AS_1	3.57	-ve	+	+ve	+
AS_2	3.46	-ve	++	+ve	+
AS_3	3.73	-ve	++	+ve	++

*Qualitative tests: + sign shows the extent of color / mentioned activity of given tests compared to others.

*+: light; ++: medium; +++: high;

Figure 1. Inoculation effect on seed cotton yield with PGPR inoculation at graded N levels.

Results showed that three isolates of *Azotobacter* and *Azospirillum* sp produced IAA equivalents. The highest IAA equivalents i.e. 3.79 and 3.73 µg mL^{-1} by *Azotobacter* isolate AZ_3 and *Azospirillum* sp AS_3, respectively. Other biochemical tests like oxidase, BTB and urease tests were also performed and showed that isolates have variable degreeof above mentioned isolates.

Results revealed that consortium of PGPR i.e. *Azotobacter* and *Azospirillum* sp positively influenced the yield components of cotton at all the three levels of N (Figure 1). Increase in seed cotton yield with PGPR inoculation was 15.08, 14.08, and 10.72 % over their respective controls viz; 60, 90 and 120 kg N ha^{-1}, respectively. The maximum seed cotton yield (2478 kg ha^{-1}) was observed at highest N level with PGPR inoculation.

Table 2. Inoculation effect on number of bolls $plant^{-1}$, boll weight and plant height of cotton.

Treatments	*No. of bolls plant^{-1}*		*Boll weight (g)*		*Plant height (cm)*	
kg N ha^{-1}	*Un-inoculated*	*Inoculated*	*Un-inoculated*	*Inoculated*	*Un-inoculated*	*Inoculated*
60	17 e*	19 d	2.84 d	3.01 c	101.5 e*	106.5 d
90	21 c	24 b	3.04 c	3.21 b	110.9 c	117.8 b
120	23 b	25 a	3.20 b	3.34 a	119.3 b	126.9 a
LSD	1.17		0.07		3.76	

Data regarding number of bolls $plant^{-1}$, boll weight and plant height (Table 2) clearly demonstrated that inoculation of PGPR consortium increased the number of bolls $plant^{-1}$ and boll weight significantly. The highest number of bolls i.e. 25 $plant^{-1}$ and average boll weight i.e. 3.34 g $boll^{-1}$were observed at highest N level with application of PGPR consortium. Application of graded N levels also affected the number of bolls and boll weight significantly. Increase in plant height (Table 2) with consortium of PGPR inoculation was 4.93, 6.22, and 6.37 % over their respective controls viz; 60, 90 and 120 kg N ha^{-1}, respectively. The maximum plant height (126.9 cm) was observed with inoculation @ 120 kg N ha^{-1}.

Table 3. Inoculation effect on CLCV, GOT and Staple length of cotton.

Treatments	*CLCV Infestation (%)*		*GOT (%)*		*Staple length (mm)*	
kg N ha^{-1}	*Un-inoculated*	*Inoculated*	*Un-inoculated*	*Inoculated*	*Un-inoculated*	*Inoculated*
60	46.8 a	41.3 c	37.1 e*	37.9 c	27.0 e	27.4 c
90	43.1 b	38.9 d	37.6 d	38.4 b	27.2 d	27.7 b
120	41.0 c	36.0 e	37.9 c	38.8 a	27.5 c	27.8 a
LSD	1.10		0.29		0.13	

*Means sharing the same letter(s) in a column do not differ significantly at $p<0.05$ according to Duncan's Multiple Range Test.

Data regarding CLCV infestation, GOT and staple length is presented in Table 3. Results showed that consortium of PGPR affected the plant height and CLCV % positively. Higher CLCV % was observed at lower level of N but it was reduced with inoculation. The least CLCV % i.e.36 % was observed with inoculation at highest N level compared to the respective 41 % of control, and it was 12 % less than the un-inoculated treatment. The GOT and staple length was significantly increased with inoculation.

The graded levels of N enhanced GOT and staple length and the effect was more pronounced with microbial inoculation. The highest GOT and staple length was observed with inoculation i.e. 38.8 % and 27.8 mm at 120 kg N ha^{-1}, respectively.

Discussion

Isolations of *Azotobacter* and *Azospirillum* sp were carried out by serial dilutions from the cotton rhizosp here. Three isolates of each *Azotobacter* and *Azospirillum* were screened out for the auxin biosynthesis as IAA equivalents (Sarwar *et al.,* 1992; El-Komy, 2005). The tested isolates of *Azotobacter* and *Azospirillum* sp produced variable degrees of IAA equivalents. Microbial production of auxins in the solution culture and in soil was reported by many researchers affecting the plant growth (Sarwar *et al.,* 1992; Martins *et al.,* 2004; Khalid *et al.,* 2006).

Inoculation of PGPR consortium comprising of *Azotobacter* and *Azospirillum* sp in 1:1ratio was checked for the growth and yield promotion of cotton. Different rates of N viz. 60, 90 and 120 kg $ha^{-1}$and uniform dose of P i.e. 60 kg ha^{-1} was applied in RCBD-factorial arrangements.

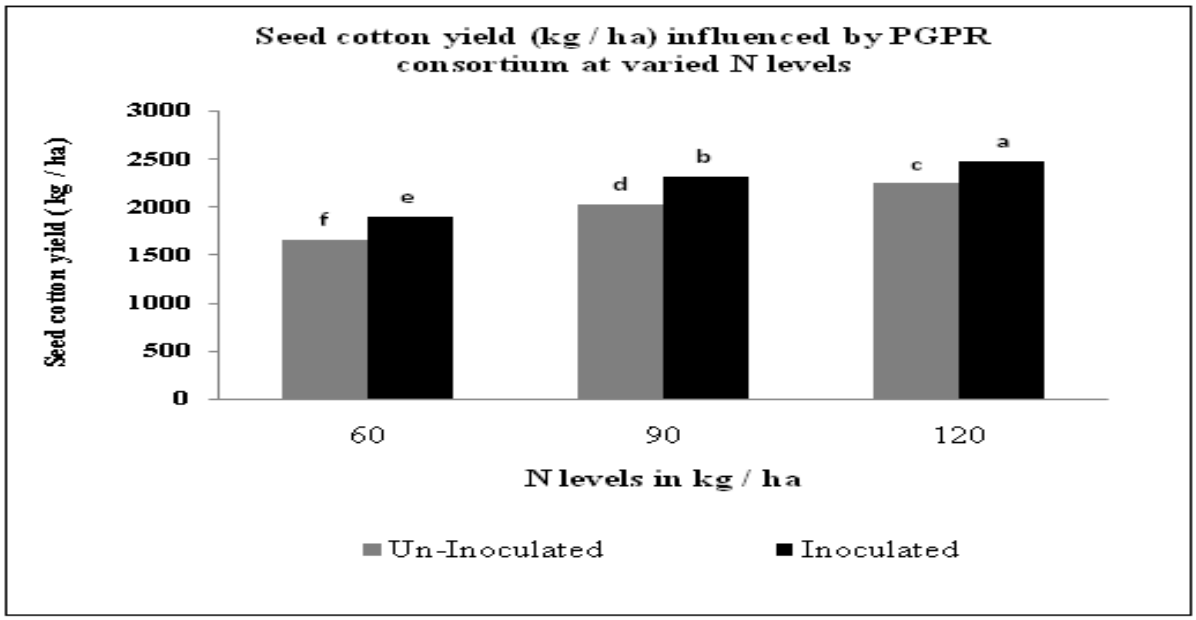

Fig. 1. Inoculation effect on seed cotton yield with PGPR inoculation at graded N levels.

Results demonstrated that inoculation of *Azotobacter* and *Azospirillum* sp enhanced the yield components of cotton at all N levels compared to respective un-inoculated controls (Pandey and Kumar, 1998; Mahale *et al.,* 2003). *Azotobacter* and *Azospirillum* sp having the potential of phytohormone production and root colonizing ability increased the root surface area, nutrient availability and root shoot growth (Anjum *et al.,* 2005; 2007; Paul *et al.,* 2011). Inoculation of PGPR consortium improved the seed cotton yield, planting density, number of bolls $plant^{-1}$, boll weight, plant height, CLCV, GOT and staple length of cotton. Our results corroborated with the work of many researchers who verified the PGPR influence on growth of crops (Egamberdiyeva *et al.,* 2004; Anjum *et al.,* 2005; El-Komy, 2005; Anjum *et al.,*2007; Sridevi and Ramakrishnan, 2010; ; Ahmad *et al.,* 2011; Paul *et al.,* 2011).

Anjum *et al.* (2007) observed an increase in GOT and staple length in the inoculated treatments yet the increase was statistically non-significant while Akhtar *et al.* (2010) reported significant increase in GOT and staple length. Sridevi and Ramakrishnan (2010) reported that dual inoculation of PGPR had a synergistic effect on plant height, boll weight and seed cotton yield. Combined application of *Azotobacter* and *Azospirillum* sp affected the yield components like number of bolls $plant^{-1}$, boll weight and plant height of cotton at all N levels might be attributed to biosynthesis of phytohormones, rhizosphere colonization and more nutrient availability as reported by many researchers (Egamberdiyeva, 2007; Khan *et al.,* 2010). *Azotobacter* and *Azospirillum* sp having the potential of plant hormone production might be responsible for better root architecture and ultimate increase in nutrient uptake. (Zahran, 2001; Mahale *et al.,* 2003).

The presence of high population in the inoculated treatments than un-inoculated controls might be attributed to the induced systemic resistance (Ramamoorthy *et al.,* 2001). Plant growth promoting rhizobia (PGPR) mediated structural modifications, biochemical and physiological changes in plant cell wall due to the synthesis of salicylic acid, lipopolysaccharides and siderophores (Ramamoorthy *et al.,* 2001; Nadeem *et al.,* 2009; Ahmad *et al.,* 2011).

Cotton leaf curl virus (CLCV) not only affects the yield but also deteriorates the fiber quality and its attack may be diluted by PGPR inoculation (Khan *et al.,* 2010; Farooq *et al.,* 2011). Reduced incidence of CLCV in the inoculated treatments at all N levels might be attributed to microbial induced systemic resistance, root colonization resulting in better nutrient uptake, vigor and acquired systemic resistance in cotton (Ramamoorthy *et al.,* 2001; Mahale *et al.,* 2003; Khan *et al.,* 2010).

Many mechanisms were reported by numerous scientists like production of antibiotics, siderophores and hydrogen cyanide having detrimental effect on the pathogens (Ramamoorthy *et al.,* 2001; Zehnder *et al.,* 2001; Vessey, 2003; Ahmad *et al.,* 2011). Number of evidences confirmed that above mentioned mechanisms involved in the suppression of fungal and viral diseases like cucumber mosaic virus, cotton leaf curl virus, tomato viruses, blue mold of tobacco and control of bacterial angular leaf spot and anthracnose (Murphy *et al.,* 2000; Zehnder*et al.,*2000; Zehnder *et al.,* 2001; Zhang *et al.,* 2002; Anjum *et al.,* 2005; Sridevi and Ramakrishnan, 2010; Paul *et al.,* 2011). Similarly, Saharan and Nehra (2011) reported that PGPR inoculation enhanced the yield parameters of cotton and decreased the pathogen influence. Results demonstrated that stimulation in plant growth by improvement in plant height, number of bolls and boll weight, yield and fiber quality like GOT and staple length might be owed to balanced acquisition of nutrients and hormonal balance (Anjum *et al.,* 2007; Akhtar *et al.,* 2010; Paul *et al.,* 2011).

Study clearly demonstrated that PGPR consortium can play a vital role in growth and yield of cotton and further field studies should be carried out to test the technology at large and validate this approach.

References

Ahmad F, Ahmad I, Khan MS. 2006. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiological Research **36,** 1-9.

Ahmad M, Zahir ZA, Asghar HN, Asghar M. 2011. Inducing salt tolerance in mung bean through co-inoculation with rhizobia and plant-growth-promoting rhizobacteria containing 1-aminocyclopropane-1-carboxylate-deaminase. Canadian Journal of Microbiology **57,** 578-589.

Akhtar N, Iqbal A, Qureshi MA, KhanKH. 2010. Effect of phosphate solubilizing bacteria on the Phosphorus availability and yield of cotton (*Gossypium hirsutum*). Journal of Scientific Research. **40,** 15-24.

Anjum MA, Sajjad MR, Qureshi MA, Hussain SI, Rashid S, Jami AR. 2005. Response of cotton to diazotrophic bacterial inoculation under different nitrogen levels. Journal Agricultural Research **43,** 39-52.

Anjum MA, Sajjad MR, Akhtar N, Qureshi MA, Iqbal A, Jami AR, Mahmud-ul-Hasan. 2007. Response of cotton to plant growth promoting Rhizobacteria (PGPR) inoculation under different levels of nitrogen. Journal of Agricultural Research **45,** 135-143.

Bharathi R, Vivekananthan R, Harish S, Ramanathan A, Samiyappan R. 2004. Rhizobacteria-based bio-formulations for the management of fruit rot infection in chillies. Crop Protection. **23,** 835–843.

Cook RJ. 2002. Advances in plant health management in the twentieth century. Annual Review in Phytopathology **38,** 95-116.

Dobereiner J, Pedrosa FO. 1987. Nitrogen fixing bacteria in non-leguminous crop plants. Science Tech. Madison, 155 P.

Duncan DB. 1955. Multiple Range and Multiple F-Test. Biometrics 11, 1-42.

Egamberdiyeva D. 2007. The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. Applied Soil Ecology **36,** 184-189.

Egamberdiyeva D, Juraeva D, Poberejskaya S, Myachina O, Teryuhova Seydalieva L, Aliev A. 2004. Improvement of wheat and cotton growth and nutrient uptake by phosphate solubilizing bacteria. p. 58-66. *In:* D. Jordan and D. Caldwell (eds.) 26th Southern Conservation Tillage Conference for Sustainable Agriculture, June 8-9, 2004. Raleigh, North Carolina. North Carolina Agricultural Research Service.

El-Komy HMA. 2005. Coimmobilization of *Azospirillum lipoferum* and *Bacillus megaterium* for Successful Phosphorus and Nitrogen Nutrition of Wheat Plants. Food Technology and Biotechnology **43,** 19–27.

Farooq A, Farooq J, Mahmood A, Shakeel A, Rehman A, Batool A, Riaz M, Shahid MTH, Mehboob S. 2011. An overview of cotton leaf curl virus disease (CLCuD) a serious threat to cotton productivity. Australian journal of Crop Science **5,** 1823-1831.

Gholami A, Shahsavani S, Nezarat S. 2009. The effect of Plant Growth Promoting Rhizobacteria (PGPR) on germination, seedling growth and yield of maize. International Journal of Biology and Life Sciences **1,** 35-40.

Jensen HL. 1953. *Azotobacter* as a crop inoculant. 245-251 P. *In*: Proceeding 5th International Congress of Microbiology, Rome, Italy. 8-13 August.

Jeun YC, Park KS, Kim CH, Fowler WD, Kloepper JW. 2004. Cytological observations of cucumber plants during induced resistance elicited by rhizobacteria. Biological Control **29,** 34–42.

Joseph B, Patra RR, Lawrence R. 2007. Characterization of plant growth promoting Rhizobacteria associated with chickpea (*Cicer arietinum* L). International Journal of Plant Production **2,** 141-152.

Kennedy AC. 2005. Rhizosphere, *In*: Principles and Applications of Soil Microbiology, D.M., Sylvia, J.J., Fuhrmann, P.G., Hartel, and D.A., Zuberer, eds., 2nd Ed. Pearson, Prentice Hall, New Jersey, 242-262 P.

Kennedy IR, Islam N. 2001. The current and potential contribution of asymbiotic nitrogen fixation to nitrogen requirements on farms: a review. Australian Journal of Expermental Agriculture **41,** 447–457.

Khalid A, Arshad M, Zahir ZA. 2006. Phytohormones: microbial production and applications. p. 207-220. *In:* Biological Approaches to Sustainable Soil Systems. N. Uphoff , A.S. Ball, E. Fernandes, H. Herren, O. Husson, M. Laing, C. Palm, J. Pretty, P. Sanchez, N. Sanginga and J. Thies (Eds.), Taylor & Francis/CRC, Boca Raton, Florida.

Khan I, Masood A, Ahmad A. 2010. Effect of nitrogen fixing bacteria on plant growth and yield of *Brassica juncea*. Journal of Phytology **2,** 25-27.

Krieg NR, Holt JG. 1984. Bergey's Manual of Systematic Bacteriology, Vol. 1, Williams and Wilkins, Baltimore, MD, USA. 694.

Kuiper I, Lagendijk EL, Bloemberg GV, Lugtenberg BJJ. 2004. Rhizoremediation: A Beneficial Plant-Microbe Interaction. Molecular Plant-microbe Interaction **17,** 6–15.

Lugtenberg BJJ, Dekkers L, Bloemberg GV. 2001. Molecular determinants of rhizosphere colonization by Pseudomonas. Annual Review in Phytopathology **38,** 461-490.

Mahale BB, Nevase VB, Thorate ST, Bhekale JS. 2003. Effect on non-symbiotic nitrogen fixers on the forage yield of oat (*Avena sativa* L.). Annuals of Agricultural Research New Series **24,** 21-23.

Martins A, Kimura O, Goi SR, Baldani JI. 2004. Effect of co-inoculation of plant growth promoting rhizobacteria and rhizobia on development of common bean plants (*Phaseolus vulgaris*, L.). Floresta e Ambiente **11,** 33-39.

Mrkovacki N, Milic V. 2001. Use of *Azotobacter chroococcum* as potentially useful in agricultural application. Annals of Microbiology **51,** 145–158.

Murphy JF, Zehnder GW, Schuster DJ, Sikora EJ, Polston JE, Kloepper JW. 2000. Plant growth-promoting rhizobacterial mediated protection in tomato against Tomato mottle virus. Plant Disease **84,** 779-784.

Nadeem SM, Zahir ZA, Naveed M, Arshad M.2009. Rhizobacteria containing ACC deaminase confer salt tolerance in maize grown on salt affected soils. Canadian Journal Microbiology**55,** 1302-1309.

Paul S, Rathi MS, Tyagi SP. 2011. Interactive effect with AM fungi and *Azotobacter* inoculated seed on germination, plant growth and yield in cotton (*Gossypium hirsutum*). Indian Journal of Agricultural Sciences **81,** 1041–5.

Persello-Cartieaux F, Nussaume L, Robaglia C. 2003. Tales from the underground: Molecular plant-rhizobacteria interactions. Plant Cell Environment **26,** 189-199.

Ramamoorthy V, Viswanathan R, Raguchander T, Prakasam V, Samiyappan R. 2001. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. Crop Protection **2,** 1–11.

Saharan BS, Nehra V. 2011. Plant Growth Promoting Rhizobacteria: A Critical Review. Life Sciences and Medicine Research, LSMR-**21,** 1-30.

Salantur A, Ozturk A, Akten S. 2006. Growth and yield response of spring wheat (*Triticum aestivum* L.) to inoculation with rhizobacteria. Plant Soil Environment **52,**111–118.

Sarwar M, Arshad DA, Martens JR, Frankenberger Jr WT. 1992. Tryptophan dependent biosynthesis of auxins in soil. Plant and Soil **147,** 207-215.

Sridevi S, Ramakrishnan K. 2010. The effect of NPK fertilizer and AM fungi on the growth and yield of cotton (*Gossypium hirsutum* L.) var. LRA 5166. Recent Research in Science and Technology **2,** 39–41.

Steel RGD, Torrie JH, Dicky DA. 1997. Principles and Procedures of Statistics- A Biometrical Approach. 3rd Edition, Mc Graw-Hill Book International Co., Singapore. 204-227 P.

Steenhoudt O, Vanderleyden J. 2000. *Azospirillum*, a free-living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. FEMS Microbiology Reviews **24,** 487–506.

Vessey JK. 2003. Plant growth promoting rhizobacteria as biofertilizers, Plant Soil **255,** 571-586.

Wu SC, Cao ZH, Li ZG, Cheung KC, Wong MH. 2005. Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma **125,** 155-166.

Zahran HH. 2001. Rhizobia from wild legumes: diversity, taxonomy, ecology, nitrogen fixation and biotechnology. J. Biotech. **91,** 143-153.

Zehnder GW, Yao C, Murphy JF, Sikora EJ,Kloepper JW. 2000. Induction of resistance in tomato against cucumber mosaic cucumovirus by plant growth-promoting rhizobacteria. Biocontrol **45,** 127–137.

Zhang S, Reddy MS,Kloepper JW. 2002. Development of assays for assessing induced systemic resistance by plant growth promoting rhizobacteria against blue mold of tobacco. Biological Control **23,** 79-86.