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Rhizobial inoculation improves seedling emergence, nutrient uptake and growth of cotton

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Abstract. Experiments were conducted to determine the growth promoting activities of various rhizobia in cotton (*Gossypium hirsutum* L.) under growth room conditions. Seeds of 4 cotton cultivars were inoculated with 4-indole-3-acetic acid producing selected (*Brady*) *rhizobium* strains and *Azotobacter* plant growth promoting rhizobacteria strains, included as a positive control. Growth responses to inoculation exhibited bacterial strain-cotton cultivar specificity and also included increase in rate of seedling emergence by 3–9%. Shoot dry weight, biomass and N uptake were increased by 48, 75 and 57%, respectively, due to inoculation with both the *Rhizobium leguminosarum* bv. *trifolii* E11 and *Azotobacter* sp. S8, whereas, strain E11 also increased root dry weight, root length and area by 248, 332 and 283%, respectively. K^+ and Ca^{2+} uptake was also increased by 2–21% and 9–14%, respectively, due to rhizobial inoculation. The results also showed that (*Brady*) *rhizobium* strains promoted cotton growth through efficient nutrient uptake, which was mainly related to increased root growth due to the effect of IAA produced by these strains. However, growth promotion by *Azotobacter* sp. S8, in addition to 4-indole-3-acetic acid production, might also involve biological N_2 fixation by this rhizobacterial strain at some stage during its growth.

Additional keywords: *Rhizobium*, PGPR, *(Brady)rhizobium*, BNF, biomass, root growth.

Introduction

The minimum standard for germination percentage of certified seed in cotton is 70%, which is lower than that in other crops (Anon. 1984). Seedling vigor is an important attribute that determines the overall performance of a crop. Seedlings with a vigorous growth pattern can compete successfully under stress; influencing stand establishment and ultimately yield. The vigor parameters of a crop cultivar can be influenced by genetic manipulations and cultural manipulations: the former time consuming and costly whereas cultural manipulations can provide quicker, short-term boosts in crop yield by changing the physiological status of young plants that persists throughout their life cycle (Teng 1990).

Cultural manipulations can be achieved by delivery of a balanced fertilisation, optimum water management, seed treatment, etc. Treatment of seeds with beneficial microbes can help to control disease incidence and severity (O'Sullivan and O'Gara 1992), improve nutrient uptake efficiency (Bashan *et al.* 1990), and promote growth leading to enhanced yield (deFreitas and Germida 1990).

Nutrient uptake and nutrient use efficiency in crop plants can be manipulated by varying the time of fertilisation, the source and amount of fertilisers, by adding organic materials and by inoculating with plant growth promoting rhizobacteria (PGPR). Most inoculation studies have focused on free-living diazotrophs, although a few reports indicate that rhizobia can act as PGPR (Hoflich *et al.* 1995; Noel *et al.* 1996; Yanni *et al.* 1997). The PGPR influence crop growth and development by changing the physiological status (Glick and Bashan 1997; Volpin and Phillips 1998) and morphological characteristics of inoculated roots (Noel *et al.* 1996; Yanni *et al.* 1997; Biswas 1998) that favour improved nutrient uptake (Okon and Kapulnik 1986). The growth-promoting effects of rhizobacteria may include phytohormone production (Tien *et al.* 1979; Hussain *et al.* 1987; Chabot *et al.* 1996*a*; Sardar 2000), fungal growth inhibition (Nautiyal 1997), N_2 fixation (Urquiaga *et al.* 1992), more efficient use of the nitrogen (N) source (Yanni *et al.* 1997) and other nutrients (Chabot *et al.* 1996*a*), antibiotics against phytopathogens (Handlesman and Staab 1996), production and secretion of siderphores (Neilands and Leong 1986), and induction of systemic disease resistance (Tuzun and Kloepper 1994). Associative and endophytic N_2 fixation have been reported in graminaceous plants with free-living diazotrophs (Urquiaga *et al.* 1992; Lee *et al.* 1994; Shrestha and Ladha 1996). Growth promotion by PGPR inoculation has been reported in wheat (deFreitas 2000) and maize (Zahir *et al.* 2000; Rai and Hunt 1993).

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deFreitas JR, Grmida JJ (1990) Plant growth-promoting rhizobacteria for winter wheat. *Canadian Journal of Microbiology* **36**, 265–272.

deFreitas JR (2000) Yield and N assimilation of winter wheat (*Triticum aestivum* L. var. Norstar) inoculated with rhizobacteria. *Pedobiologia* **44**, 97–104.

Glick BR, Bashan Y (1997) Genetic manipulation of plant growth-promoting bacteria to enhance biocontrol of phytopathogens. *Biotechnology Advances* **15**, 353–378. doi:10.1016/S0734-9750(97)00004-9

Hafeez FY, Hameed S, Zaidi AH, Malik KA (2002) Biofertilizers for sustainable agriculture. In 'Proceedings of a national workshop on techniques for sustainable agriculture 2001'. (Eds F Azam, MM Iqbal, C Inayatullah, KA Malik) pp. 67–73. (NIAB: Faisalabad, Pakistan)

Handlesman J, Staab E (1996) Biocontrol of soilborne plant pathogens. *The Plant Cell* **8**, 1855–1869. doi:10.1105/TPC.8.10.1855

Hardy RWF, Holsten RD, Jackson EK, Burns RE (1968) The acetylene–ethylene assay for nitrogen fixation: laboratory and field evaluation. *Plant Physiology* **43**, 1185–1207.

Hoagland DR, Arnon DI (1950) 'The water culture method of growing plants without soil.' California Berkeley College of Agriculture Cirucular No. 347.

Hoflich G, Wiche W, Buchholz CH (1995) Rhizosphere colonization of different crop with growth promoting *Pseudomonas* and *Rhizobium* bacteria. *Microbiological Research* **150**, 139–147.

Hussain A, Arshad M, Hussain A, Hussain F (1987) Response of maize (*Zea mays*) to *Azotobacter* inoculation under fertilized and unfertilized conditions. *Biology and Fertility of Soils* **4**, 73–77.

Ladha JK, Garcia M, Miyan S, Padre AT, Watanabe I (1989) Survival of *Azorhizobium caulinodans* in the soil and rhizosphere of wetland rice under *Sesbania rostrata*-rice rotation. *Applied and Environmental Microbiology* **55**, 454–460.

Lee KK, Wani SP, Yoneyama T, Trimurtula N, Harikrishnan R (1994) Associative N2-fixation in pearl millet and sorghum, levels and response to inoculation. *Soil Science and Plant Nutrition* **40**, 477–484.

Muller M, Deigele C, Ziegler H (1989) Hormonal interaction in the rhizosphere of maize and their effects on plant development. *Z. Pflanzen. Bodenk.* **152**, 247–254.

Nautiyal CS (1997) Rhizosphere competence of *Pseudomonas* sp. NBR 19926 and *Rhizobium* sp. NBR 19513 involved in the suppression of chickpea (*Cicer arietinum* L.) pathogenic fungi. *FEMS Microbiology Ecology* **23**, 145–158. doi:10.1016/S0168-6496(97)00022-6

Neilands JB, Leong SA (1986) Siderophores in relating to plant growth and disease. *Annual Review of Plant Physiology* **37**, 187–208. doi:10.1146/ANNUREV.PP.37.060186.001155

Noel TC, Sheng C, Yost CK, Pharis RP, Hynes MF (1996) *Rhizobium leguminosarum* as a plant growth promoting rhizobacterium: direct growth promotion of canola and lettuce. *Canadian Journal of Microbiology* **42**, 279–283.

Okon Y (1985) Azospirillum as a potential inoculant for agriculture. *Trends in Biotechnology* **3**, 223–228.

Okon Y, Kapulnik Y (1986) Development and function of *Azospirillum*-inoculated roots. *Plant and Soil* **90**, 3–16.

O'Sullivan DJ, O'Gara F (1992) Traits of fluorescent *Psendomonas* spp. involved in suppression of plant root pathogens. *Microbiological Reviews* **56**, 662–676.

Rai R, Hunt PG (1993) Inoculation of maize varieties with salt tolerant mutants of *Azospirillum brasilense* and VAM fungi in saline calcareous soil. *Microbial Releases* **1**, 243–251.

Sardar R (2000) Growth hormone production by *Rhizobium* spp. and their effect on plant growth and nitrogen fixation. M. Phil Thesis, University of Agriculture, Faisalabad, Pakistan.

Shan XQ, Jing YX (1998) Cell structure of pseudonodules formed on the roots of barley and rice. In 'Nitrogen fixation with non-legumes'. (Eds KA Malik, MS Mirza, JK Ladha) pp. 133–142. (Kluwer Academic: London, UK)

Shrestha RK, Ladha JK (1996) Genotypic variation in promotion of rice dinitrogen fixation as determined by nitrogen-15 dilution. *Soil Science Society of America Journal* **60**, 1815–1821.

Somasegaran P, Hoben HJ (1985) 'Methods in legume–*Rhizobium* technology.' Niftal Project, University of Hawaii, Paia, Hawaii.

Steel RGD, Torrie JH (1984) 'Principles and procedures of statistics (2nd edn).' (McGraw Hill Book Co.: New York)

Teng S (1990) Grain characteristics and seedling vigor in rice (*Oryza sativa* L.). MSc Thesis, University of the Philippines, Los Banos, Philippines.

Tien T, Gaskins MH, Hubbell DH (1979) Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). *Applied and Environmental Microbiology* **37**, 1016–1024.

Tuzun S, Kloepper JW (1994) Induced systemic resistance by plant growth promoting rhizobacteria. In 'Improving plant productivity with rhizosphere bacteria'. (Ed. MH Ryder, PM Stephens, GD Bowen) pp. 104–109. (CSIRO: Adelaide, Australia)

Urquiaga S, Cruz KHS, Boddey RM (1992) Contribution of nitrogen fixation to sugarcane: nitrogen-15 and nitrogen balance estimates. *Soil Science Society of America Journal* **56**, 105–114.

Vincent JM (1970) 'A manual for the practical study of root-nodule bacteria.' IBP Handbook No. 15. (Blackwell: Oxford, UK)

Volpin H, Phillips DA (1998) Respiratory elicitors from *Rhizobium meliloti* affect intact alfalfa roots. *Plant Physiology* **116**, 777–783. doi:10.1104/PP.116.2.777

Yanni YG, Rizk RY, Corich V, Squartii A, Ninke K, *et al.* (1997) Natural endophytic association between *Rhizobium leguminosarum* bv. *trifolii* and rice roots and assessment of its potential to promote rice growth. *Plant and Soil* **194**, 99–114. doi:10.1023/A:1004269902246

Zahir ZA, Abbas SA, Khalid M, Arshad M (2000) Substrate dependent microbially derived plant hormones for improving growth of maize seedlings. *Pakistan Journal of Biological Sciences* **3**, 289–291.

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Table 5. Nutrient uptake in shoot of cotton cultivars as influenced by seed inoculation

Any two means not sharing same letter differ significantly by Duncan's multiple range test

Bacterial strains	K^+ concentration (%)				Ca^{2+} concentration (%)				Na^+ concentration (%)			
	NIBGE-1	K-99	NIAB-98	FH-901	NIBGE-1	K-99	NIAB-98	FH-901	NIBGE-1	K-99	NIAB-98	FH-901
Control	4.13k	4.97h	6.24b	3.32m	1.03ab	0.77hi	0.90def	0.55k	1.20l	1.07j	1.92a	1.69b
Azotobacter sp. S8	4.21k	5.51fg	4.56j	4.75hij	0.68j	0.81gh	0.66j	0.76hi	1.52d	1.35gh	0.92lm	1.22i
Rhizobium leguminosarum bv. *trifolii* E11	3.64l	4.30k	5.37g	3.83l	0.52k	0.57k	1.01abc	0.83fgh	0.96kl	1.01k	1.47e	0.91m
Rhizobium leguminosarum bv. *viciae* PS1	5.79de	5.36g	6.50a	4.95hi	0.98bcd	1.07a	0.81gh	0.67j	1.37g	1.53d	1.61c	0.96lm
B. japonicum MnS	6.12bc	4.33k	5.80de	5.75e	0.94cde	1.02ab	0.88efg	0.71ij	1.54d	1.60c	1.31h	1.31h
TAL-102	4.73 ij	6.18bc	6.01cd	5.60ef	0.70ij	1.06a	1.04ab	0.91de	1.45ef	1.40fg	1.44ef	1.22i
F-values												
Inoculation (I)	237.8395**				41.3969**				201.6441**			
Variety (V)	238.5205**				41.4249**				131.2434**			
I × V	89.6544**				40.8964**				180.6101**			

**$P<0.01$.

bacterial derived plant growth regulators in these processes (Muller *et al.* 1989).

Improvement in shoot and root growth as a result of rhizobial inoculation has also been found by Chabot *et al.* (1996*a*) in lettuce; Noel *et al.* (1996) in canola and lettuce; and Yanni *et al.* (1997) in rice. Zahir *et al.* (2000) demonstrated an increase in shoot weight of maize by inoculation with the PGPR *Azotobacter*. Induction of longer roots with increased number of root hairs and root laterals is a growth response attributed to IAA production by rhizobacteria, which improve their nutrient uptake efficiency (Okon 1985).

Biswas *et al.* (2000*a*) reported an increase in uptake of N and K^+ in rice seedlings inoculated with rhizobia, and an increase in N uptake due to PGPR inoculation has also been reported by deFreitas (2000). Acetylene reduction Assay (ARA) of cotton roots was found to be negative for all the inoculation treatments at 45 days after planting. Stimulation of rice growth by rhizobial inoculation without nitrogenase activity has also been reported by Shan and Jing (1998). The plant growth promotion as a result of rhizobial association with rice roots involved the efficient uptake of soil nutrients rather than biological N_2 fixation (BNF) (Yanni *et al.* 1997). Increase in the uptake of nitrogen and resultant increase in growth and yield of rice plants inoculated with rhizobia was not due to BNF but due to changes in growth physiology and root morphology induced by IAA, which produced and accumulated rhizobia in the external root environment of rice plants (Biswas *et al.* 2000*a*).

In conclusion, our results indicate that stimulation of root growth and thereby enhanced nutrient uptake by inoculation with (*Brady*) *rhizobium* strains is consistent with growth promoting substances produced by these strains, rather than BNF. However, increased nitrogen uptake and shoot growth, without a concomitant increase in root growth by *Azotobacter* sp. S8, supports the conclusion that this PGPR strain, besides producing some IAA, might also fix nitrogen at some stage during its growth. Keeping in view the overall growth stimulation performance, rhizobial strains E11, MnS and PGPR strain S8, were found to be best and it is advisable that these strains may be used in field trials to ascertain whether the production of biofertiliser for enhanced growth and yield of cotton would be feasible.

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References

Anon. (1984) Regulations and procedures for seed certification system in Pakistan. pp. 19–30. Federal Seed Certification Department, Ministry of Food, Agriculture and Cooperatives. Food and Agriculture Division, Government of Pakistan, Islamabad.

Bashan Y, Harrison SK, Whitmoyer RE (1990) Enhanced growth of wheat and soybean plants inoculated with *Azospirillum brasilense* is not necessarily due to general enhancement of mineral uptake. *Applied and Environmental Microbiology* **56**, 769–775.

Biswas JC (1998) Effect of nitrogen fixing bacteria on growth promotion of lowland rice (*Oryza sativa* L.). PhD Thesis, University of the Philippines, Los Banos.

Biswas JC, Ladha JK, Dazzo FB (2000*a*) Rhizobial inoculation improves nutrient uptake and growth of lowland rice. *Soil Science Society of America Journal* **64**, 1644–1650.

Biswas JC, Ladha JK, Dazzo FB, Yanni YG, Rolfe BG (2000*b*) Rhizobial inoculation influences seedling vigor and yield of rice. *Agronomy Journal* **92**, 880–886.

Bremner JM (1965) Total nitrogen and inorganic forms of nitrogen. In 'Methods of soil analysis'. (Eds CA Black, DD Evans, JL White, LE Ensminger, FE Clark) pp. 1149–1237. (American Society of Agronomy: Madison, WI, USA)

Chabot R, Antoun H, Cescas MC (1996*a*) Growth promotion of maize and lettuce by phosphate solubilizing *Rhizobium leguminosarum* bv. *phaseoli*. *Plant and Soil* **184**, 311–321.

Chabot R, Antoun H, Kloepper JW, Beauchamp CJ (1996*b*) Root colonization of maize and lettuce by bioluminescent *Rhizobium leguminosarum* biovar *phaseoli*. *Applied and Environmental Microbiology* **62**, 2767–2772.

Rhizobial inoculation of legume seed is well studied, and exploitation of this beneficial N_2-fixing root nodule symbiosis represents a hallmark of successfully applied agricultural microbiology. However, much less information is available regarding the association and growth promoting activities of rhizobia with non-legumes. In nature, rhizobia do associate with roots of non-legumes without forming nodules (Ladha *et al.* 1989; Yanni *et al.* 1997), but their populations decrease in number in the absence of legume-host plants (Ladha *et al.* 1989; Chabot *et al.* 1996*b*). Direct growth promotion of some non-leguminous crops such as rice (Yanni *et al.* 1997; Biswas *et al.* 2000*a*; Biswas *et al.* 2000*b*), maize, canola and lettuce (Chabot *et al.* 1996*a*; Noel *et al.* 1996) by rhizobial inoculation has also been reported. Yanni *et al.* (1997) and Biswas (1998) found increased N uptake by rice plants inoculated with rhizobia. Use of diazotrophs as microbial inoculants has resulted in 20, 15 and 60–80% increase in yield of paddy, wheat and legumes, respectively and a saving of 50–100% of chemical fertiliser (Hafeez *et al.* 2002).

Cultural manipulation of plant growth and nutrient uptake by rhizobial inoculation would be a potentially useful technology for sustainable agriculture without compromising other natural resources. This study was undertaken to investigate the ability of rhizobia to promote seedling emergence, growth, and uptake of mineral N, K^+, Ca^{2+} and Na^+ in cotton.

Materials and methods

Bacterial cultures

Rhizobium leguminosarum bv. *trifolii* strain E11, *Rhizobium leguminosarum* bv. *viciae* strain PS1, *Bradyrhizobium japonicum* MnS and *Bradyrhizobium japonicum* TAL-102 were obtained from BIRCEN culture collection, Biofertiliser Division, NIBGE, Faisalabad. All these strains produce indole-3-acetic acid (IAA) (Sardar 2000). In addition, *Azotobacter* sp. S8, a PGPR isolated from cotton root zone was also included as a positive control.

Inocula preparation

(*Brady*) *rhizobium* and *Azotobacter* strains were maintained by streaking on Yeast Mannitol (YM) Congo red and Luria-Bertani (LB) agar plates, respectively, from the culture collection. Pure culture of each bacterial strain was obtained by sub-culturing on YM and LB agar plates (Vincent 1970). After sufficient growth, a single colony of the (*Brady*) *rhizobium* strain and the *Azotobacter* strain were transferred to YM broth and LB broth, respectively, in 250 mL flasks, under aseptic conditions. Growth of bacterial strains was obtained by shaking the culture flasks at 100 rpm at 28 ± 2°C on an orbital shaker. Gram staining was used to study the morphological features of each strain under a microscope for their identification (Vincent 1970).

Plant material and growth conditions

Acid-delinted cotton seeds were surface sterilised by immersion of seeds in 0.1% $HgCl_2$ solution for 3 min followed by repeated washings with sterilised water (Vincent 1970). Seeds of 4 cotton cultivars, NIBGE-1, Karishma-99, NIAB-98 and FH-901, were grown in sterilised sand which had been previously washed with concentrated H_2SO_4 and maintained at a pH of 6 by washing with water.

Evaluation of plant growth-promotion responses to inoculation

Two growth room experiments were performed. Seeds of each of the 4 cotton cultivars were subjected to 6 inoculation treatments, including uninoculated control. Both the experiments were laid out in completely randomised design with 2 factor factorial arrangement and each treatment was replicated 3 times.

Experiment 1

The experiment was conducted to determine the effect of bacterial inoculation on seedling emergence. Similar-sized seeds of each cotton cultivar were sorted, dipped in the inocula of each bacterial strain, and 10 seeds were sown in each petri dish (9 cm diameter) filled with sterilised sand. In case of control, the seeds were sown without dipping in inoculum. Then petri dishes were kept in a growth room, maintaining a day/night temperature of 30 ± 2°C/25 ± 2°C and day length of 16 h. Light intensity during the day was 20000 Lux. Both control and inoculated petri dishes were watered with equal volume of distilled water whenever needed. Germination percentage was calculated on the basis of number of normal seedlings per plate at final count, whereas, cumulative emergence rate (CER) was determined by counting emerged seedlings daily for a period of 12 days using following formula:

$$\text{CER} = \frac{\text{No. of seedlings at first count}}{\text{Days to first count}} + \ldots + \frac{\text{No. of seedlings at last count}}{\text{Days to last count}}$$

Experiment 2

A second study was performed to examine the effect of inoculation on various parameters of seedling vigor and growth in a pot experiment. Three similar-sized seeds of each cultivar were sown in each plastic pot (7 cm diameter × 10 cm height) having sterilised sand and then inoculated with suspension of each bacterial strain in a proportion of 0.5 mL inoculum/seed containing a pre-determined number of bacterial cells counted by plate count (Somasegaran and Hoben 1985). In the case of the control, no inoculum was given. These pots were then kept in a growth room maintaining a day/night temperature of 30 ± 2°C/25 ± 2°C and a daylength of 16 h. Light intensity during the day was 20000 Lux. The seedlings were thinned to 1 plant per pot after 5 days of germination. The plants were watered with equal volume of half-strength nutrient solution with nitrogen source (Hoagland and Arnon 1950) with an interval of 3 days. The observations on various parameters of seedling vigor and growth were recorded after 45 days of sowing.

Cotton roots from both inoculated and uninoculated plants were assayed for nitrogenase activity by the acetylene reduction technique on a gas chromatograph (Hardy *et al.* 1968). The shoot and root of each plant were oven-dried at 85°C for 24 h to constant weight for determining their dry weights, and the total root length and root area were measured by scanning on a personal IBM computable computer desktop scanner using root image analysis programme. Shoot nitrogen content was determined by the micro kjeldahl method (Bremner 1965) and the shoot K^+, Ca^{2+} and Na^+ concentration by the flame photometer.

The data were analysed using Fisher's analysis of variance technique and the treatment means were compared relative to control following Duncan's multiple range test (DMRT) (Steel and Torrie 1984). The differences were only considered when significant at $P<0.05$.

Results

Inoculation response of cotton cultivars on seedling emergence

Cotton cultivar FH-901 showed maximum value of cumulative emergence rate (CER) when inoculated with Bradyrhizobial strain TAL-102 with an increase of 15% over the corresponding uninoculated control. However, inoculation

Table 1. Effect of seed inoculation on seedling emergence of four cotton cultivars

Any two means not sharing the same letter differ significantly by Duncan's multiple range test

Bacterial strains	Germination (%)				CER			
	NIBGE-1	Krishma-99	NIAB-98	FH-901	NIBGE-1	Krishma-99	NIAB-98	FH-901
Control	86.7	93.3	86.7	96.7	16.1efgh	16.1efgh	14.8h	17.6bcde
Azotobacter sp. S8	100.0	76.7	83.3	96.7	19.0ab	16.2efgh	16.3efgh	18.6abc
Rhizobium leguminosarum bv. *trifolii* E11	90.0	83.3	80.0	96.7	17.6bcde	16.0efgh	15.6fgh	18.7abc
R.leguminosarum bv. *viciae* PS1	83.3	86.7	56.7	93.3	15.3gh	16.9cdefg	16.7defgh	17.5bcde
B. japonicum MnS	93.3	86.7	80.0	86.7	19.0ab	16.2efgh	16.7defgh	17.4bcdef
TAL-102	80.0	93.3	80.0	96.7	15.2gh	18.4abcd	16.4efgh	20.2a
Inoculation (I)	1.4716n.s.	4.0337**						
Variety (V)	7.6872**	17.8234**						
I × V	1.4848n.s.	4.6040**						

**$P<0.01$; n.s., not significant.

did not significantly change the final germination percentage relative to uninoculated control. It was also not significantly different from the combinations of cotton cultivar NIBGE-1 with strains S8 and MnS; FH-901 with strains E11 and S8; and Karishma-99 with strain TAL-102 (Table 1).

Nitrogen-fixing activity

Nitrogenase activity of cotton roots at harvest (45 days after planting) was found to be negative for all the inoculation treatments. It means that cotton roots showed no nitrogen-fixing activity in association with all the bacterial strains used.

Effect of rhizobial inoculation on shoot and root growth

Shoot dry weight was found to be higher in cotton cultivar NIBGE-1 when inoculated with strain S8 by 115.5% compared with the corresponding uninoculated control (Table 2). NIBGE-1 also gave maximum root dry weight in response to inoculation with strain E11, which remained statistically similar with that shown by the combination of cotton cultivars NIAB-98, Karishma-99 and FH-901 with strains S8, E11 and MnS, respectively. Means of 4 cotton cultivars in all the inoculation treatments were significantly higher by 93–248% compared with that of the uninoculated control (Table 2).

In general, average biomass of 4 cotton cultivars increased in all the inoculated treatments by 25–78% relative to uninoculated control; however, cotton cultivar NIBGE-1 produced maximum biomass when inoculated with strain E11. This was statistically similar with that recorded for the combination of cotton cultivars NIAB-98, Karishma-99 and FH-901 with strains S8, E11 and MnS, respectively (Table 3).

Maximum root area and total root length were recorded in cotton cultivar Karishma-99 when inoculated with strain E11. Inoculation significantly increased total root length and root area by 62–332% and 84–283%, respectively, compared with the uninoculated control (Table 4).

N, K^+, Ca^{2+} and Na^+ concentration

Total accumulation of plant N was significantly higher in the cotton cultivar NIBGE-1 in response to inoculation with

Table 2. Shoot and root dry weight of four cotton cultivars as influenced by rhizobial inoculation

Any two means not sharing the same letter differ significantly by Duncan's multiple range test

Bacterial strains	Shoot dry weight (g)				Root dry weight (g)			
	NIBGE-1	Krishma-99	NIAB-98	FH-901	NIBGE-1	Krishma-99	NIAB-98	FH-901
Control	0.110m	0.130k	0.147hi	0.143ij	0.030j	0.023j	0.023k	0.020k
Azotobacter sp. S8	0.237a	0.170f	0.210c	0.170f	0.047gh	0.033j	0.110a	0.090c
Rhizobium leguminosarum bv. *trifolii* E11	0.227b	0.230b	0.163g	0.160g	0.113a	0.110a	0.070e	0.043hi
Rhizobium leguminosarum bv. *viciae* PS1	0.117l	0.140j	0.130k	0.150h	0.070e	0.033j	0.070e	0.067e
B. japonicum MnS	0.140j	0.143ij	0.140j	0.183d	0.100b	0.040i	0.080 days	0.110a
TAL-102	0.150h	0.177e	0.113lm	0.143ij	0.043hi	0.033j	0.050g	0.060f
F-values								
Inoculation (I)	24.6214**	65.2372**						
Variety (V)	1.8227n.s.	20.4714**						
I × V	5.6408**	19.6371**						

**$P<0.01$; n.s., not significant.

Table 3. Response of inoculation on biomass and N uptake of four cotton cultivars

Any two means not sharing the same letter differ significantly by Duncan's multiple range test

Bacterial strains	Biomass (g/plant)				N uptake (mg/plant)			
	NIBGE-1	Krishma-99	NIAB-98	FH-901	NIBGE-1	Krishma-99	NIAB-98	FH-901
Control	0.130g	0.153fg	0.170efg	0.163efg	3.67lm	4.07jkl	4.03jkl	5.07h
Azotobacter sp. S8	0.283ab	0.230cdef	0.320a	0.260bc	8.73a	5.70g	5.87fg	6.17ef
Rhizobium leguminosarum bv. *trifolii* E11	0.340a	0.340a	0.213cdef	0.203cdef	7.03c	8.10b	6.30de	5.07h
Rhizobium leguminosarum bv. *viciae* PS1	0.187defg	0.173efg	0.200cdef	0.217cde	3.87kl	4.43ij	2.43o	5.23h
B. japonicum MnS	0.240 bcd	0.183defg	0.220cde	0.293ab	4.57I	3.17n	3.37mn	6.63d
TAL-102	0.193def	0.210cdef	0.163efg	0.203cdef	4.23ijk	5.73g	3.73lm	4.27ijk
F-values								
Inoculation (I)	46.6444**	313.4288**						
Variety (V)	2.1943n.s.	85.9497**						
I × V	11.5407**	74.1074**						

**$P<0.01$; n.s., not significant.

strain S8 (Table 3). Inoculation with all the bacterial strains used significantly increased N uptake by 5–57% relative to uninoculated control.

Inoculation with *R. leguminosarum* bv. *viciae* rhizobial strain PSI significantly improved K^+ and Ca^{2+} uptake in cotton cultivar NIAB-98 and Karishma-99, respectively (Table 5). The average K^+ concentration of the 4 cotton cultivars was increased by inoculation for strains S8, PS1, MnS and TAL-102 compared with the control, whereas it was reduced by inoculation for strain E11. Ca^{2+} concentration was higher in plants inoculated in strains PS1, MnS and TAL-102, whereas lower in strains S8 and E11 than in the uninoculated control plants.

Shoot Na^+ concentration was found to be lower in all the inoculated treatments than in the uninoculated control (Table 5).

Discussion

The overall performance of all the inoculated strains was found to be better than the uninoculated control, including increased rates of emergence, shoot and root growth, biomass and plant N uptake. However, inoculation elicited a mixed response for K^+ and Ca^{2+} concentrations (i.e. some strains increased K^+ and Ca^{2+} uptake and the others decreased their uptake). Although, certain strain combinations of cotton cultivars resulted in a greater improvement in all these parameters than shown by other combinations. Thus, earlier reports on the ability of certain rhizobial strains to promote emergence and growth of non-legumes rice, maize, wheat, canola and lettuce (Chabot *et al.* 1996*a*; Noel *et al.* 1996; Yanni *et al.* 1997) have been supported by this study. The increase in the rate of emergence due to rhizobial inoculation has also been reported by Biswas *et al.* (2000*b*) in rice.

Different ratios of plant hormones produced by plant roots as well as rhizosphere bacteria result in the morphogenetic effects in plants. Bacterial-induced morphological changes, such as early germination and emergence and root elongation, indicated modifications of developmental pathways with the possible involvement of

Table 4. Influence of seed bacterization on root length and area of four cotton cultivars

Any two means not sharing the same letter differ significantly by Duncan's multiple range test

Bacterial strains	Total root length (mm)				Root area (mm^2)			
	NIBGE-1	Krishma-99	NIAB-98	FH-901	NIBGE-1	Krishma-99	NIAB-98	FH-901
Control	50.6ij	41.6j	35.6j	36.0j	8.53q	7.08r	7.10r	6.71s
Azotobacter sp. S8	68.7fghij	42.9j	62.9ghij	91.3defgh	14.69j	10.17n	17.90e	17.94e
Rhizobium leguminosarum bv. *trifolii* E11	85.5defg	311.4a	207.7b	92.2defgh	24.14c	40.97a	32.39b	15.26i
Rhizobium leguminosarum bv. *viciae* PS1	85.5efgh	183.6b	118.8cde	134.2c	12.07m	12.99l	17.16f	19.53d
B. japonicum MnS	114.0cde	83.2efghi	104.4cdef	98.5defg	17.92e	9.37o	16.64g	15.26i
TAL-102	101.8cdef	58.6hij	92.7defgh	123.4cd	16.14h	8.98p	13.94k	14.97ij
F-values								
Inoculation (I)	80.3233**	11981.9221**						
Variety (V)	10.8452**	581.2362**						
I × V	22.1286**	2109.8775**						

**$P<0.01$.