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Nutrient uptake and yield of chickpea (*Cicer arietinum* L.) inoculated with plant growth-promoting rhizobacteria

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Abstract

This research was carried out to evaluate the effects of single and combined inoculation with plant growth-promoting rhizobacteria from four genera including *Azospirillum*, *Azotobacter*, *Mesorhizobium* and *Pseudomonas* on nutrient uptake, growth and yield of chickpea plants under field conditions. Nodulation and nutrient concentration in shoots were significantly affected by the treatments at the beginning of flowering stage. The maximum dry weight of root nodules was recorded by applying the combined inoculation with *Azospirillum* spp. + *Azotobacter chroococcum* 5 + *Mesorhizobium ciceri* SWRI7 + *Pseudomonas fluorescens* P21. All inoculants were statistically superior over uninoculated control with respect to nitrogen concentration of shoots. The treatments containing *Azospirillum* + *Azotobacter* significantly improved phosphorus concentration in shoots. Grain yield, biomass dry weight and nitrogen & phosphorus uptake of grains were statistically improved by applying every inoculation treatment in comparison with control plants. Group comparisons between treatments showed that the occurance of *Azospirillum* or *Azotobacter* inoculants in the treatment composition caused an expressive improvement in grain yield and plant biomass. In conclusion, application of every inoculation treatment studied here, especially treatments which contained *Azospirillum* or *Azotobacter* may stimulate growth and yield of chickpea as compared with uninoculated plants.

Keywords: *Azospirillum*; *Azotobacter*; *Cicer arietinum* L.; *Mesorhizobium*; Plant growth-promoting rhizobacteria; *Pseudomonas*.
Abbreviations: CFU- colony forming units; DAS- days after sowing; PGPR- plant growth promoting rhizobacteria.

Introduction

Plant growth promoting rhizobacteria (PGPR) represent a wide variety of soil bacteria which, when grown in association with a host plant, result in stimulation of growth of their host plant (Vessey, 2003). Several mechanisms have been suggested by which PGPR can promote plant growth, including phytohormone production, N_2 fixation, stimulation of nutrient uptake and biocontrol of pathogenic microorganisms (Kloepper et al., 1981; Rodriguez and Fraga, 1999; Sindhu et al., 1999; Benizri et al., 2001; Persello-Cartieaux et al., 2003; Somers et al., 2004). Many different genera of plant growth promoting rhizobacteria such as *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter* and *Pseudomonas* have been used as biofertilizers for economically important crops. Seed inoculation with a combination of beneficial microorganisms including rhizobia, PGPR and PSB (Phosphate Solubilizing Bacteria) have been shown to increase crop growth and productivity (Dashti et al., 1998; Rodelas et al., 1999; Chebotar et al., 2001; Sindhu et al., 2002; Zaidi et al., 2003; Rudresh et al., 2005). However little is known about the response of chickpea to combined inoculation with rhizobium and plant growth promoting rhizobacteria under field conditions. Chickpea (*Cicer arietinum* L.) is one of the major pulse crops in the world and provides high quality protein for the people in South, West and East Asia and North Africa. It is also used as feed for livestock and has a significant role in farming systems (Singh, 1997). In Iran chickpea is the most important grain legume and improving it's productivity is a necessity. Hence the present study was conducted to evaluate the effects of single and combined inoculations with strains of bacteria from genera *Mesorhizobium*, *Azospirillum*, *Azotobacter* and *Pseudomonas* on nutrient uptake, growth and yield of chickpea under field conditions.

Materials and methods

Experimental site

This experiment was carried out at the agricultural research station of Saral (35˚ 43′ N and 47˚ 8′ E with an altitude of 2100 m) in Kurdistan, Iran, during the cropping season of 2005-2006 in rainfed conditions. The long-term rates of average temperature and annual precipitation in the region are 7.9˚C and 393.6 mm respectively. The total precipitation during 2005-2006 was 305.8 mm. Some of the soil properties were: sand 35.3%, silt 38.7%, clay 26%, pH 7.5, OC 0.89%, total N 0.076%, available P and K, 8.7 & 409.3 ppm respectively. All plots of experimental field treated with 30 kg nitrogen ha^{-1} in urea form according to soil tests before sowing.

Bacterial strains

The bacterial cultures used in this study were obtained from the soil biology department, Soil & Water Research Institute (SWRI), ministry of agriculture , Tehran, Iran. The strains

Table 1. Root nodules dry weight and concentration of nitrogen and phosphorus in shoots of chickpea in response to inoculation with plant growth-promoting rhizobacteria at the flowering stage

Treatments	Nodule dry weight (mg plant^{-1})	N concentration (%)	P concentration (%)
1. Uninoculated	33.37 d	2.96 f	0.280 e
2. *Azos.*	40.02 b-d	3.18 e	0.287 e
3. *Azot.*	44.62 b-d	3.51 ab	0.303 de
4. *M.*	53.35 b-d	3.47 a-c	0.297 de
5. *P.*	53.38 b-d	3.32 b-e	0.293 de
6. *Azos.+Azot.*	35.77 cd	3.46 a-c	0.390 ab
7. *Azos.+M.*	45.78 b-d	3.43 a-d	0.320 c-e
8. *Azos.+P.*	52.28 b-d	3.45 a-c	0.353 b-d
9. *Azot.+M.*	52.70 b-d	3.31 c-e	0.300 de
10. *Azot.+P.*	32.64 d	3.40 b-d	0.280 e
11. *M.+P.*	34.41 cd	3.33 b-e	0.283 e
12. *Azos.+Azot.+M.*	60.54 b	3.30 c-e	0.417 a
13. *Azos.+Azot.+P.*	40.77 b-d	3.63 a	0.370 a-c
14. *Azos.+M.+P.*	54.49 bc	3.46 a-c	0.310 de
15. *Azot.+M.+P.*	50.16 b-d	3.23 de	0.280 e
16. *Azos.+Azot.+M.+P.*	83.29 a	3.37 b-e	0.283 e
Selected group comparisons			
Comparison 1			**
Comparison 2			NS
Comparison 3			NS
Comparison 4			NS
Comparison 5			**

Azos: *Azospirillum, Azot*: *Azotobacter, M*: *Mesorhizobium, P*: *Pseudomonas,* Values followed by the same letters in a column are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test. Comparison 1: *Azospirillum*-cotaining versus non*Azospirillum*-containing treatments . Comparison 2: *Azotobacter*-containing versus non*Azotobacter*-containing treatments. Comparison 3: *Mesorhizobium*-containing versus non*Mesorhizobium*-containing treatments. Comparison 4: *Pseudomonas*-containing versus non*Pseudomonas*-containing treatments. Comparison 5: *Azospirillum*+*Azotobacter*-containing versus non*A Azospirillum*+*Azotobacter*-containing treatments.

** Significant at the 0.01 probability level, NS: not significant

included: *Mesorhizobium ciceri* strain SWRI7 (1×10^7 CFU mL^{-1} carrier), *Pseudomonas fluorescens* strain P21 (5×10^7 CFU mL^{-1} carrier), *Azotobacter chroococcum* strain 5 (2.5×10^9 CFU mL^{-1} carrier) and *Azospirillum* spp. (5×10^8 CFU mL^{-1} carrier). *Azospirillum* culture contained a combination of *Azospirillum brasilense* (strain OF) and *Azospirillum lipoferum* (strain 21) at an equal ratio.

Experimental design and treatments

The layout of the trial was a randomized complete block design (RCBD) with 3 replications and 16 treatments included: (1) Uninoculated control (2) *Azospirillum* (3) *Azotobacter* (4) *Mesorhizobium* (5) *Pseudomonas* (6) *Azospirillum* + *Azotobacter* (7) *Azospirillum* + *Mesorhizobium* (8) *Azospirillum* + *Pseudomonas* (9) *Azotobacter* + *Mesorhizobium* (10) *Azotobacter* + *Pseudomonas* (11) *Mesorhizobium* + *Pseudomonas* (12) *Azospirillum* + *Azotobacter* + *Mesorhizobium* (13) *Azospirillum* + *Azotobacter* + *Pseudomonas* (14) *Azospirillum* + *Mesorhizobium* + *Pseudomonas* (15) *Azotobacter* + *Mesorhizobium* + *Pseudomonas* (16) *Azospirillum* + *Azotobacter* + *Mesorhizobium* + *Pseudomonas.* Each plot contained 6 rows of 5 m length with 30 cm inter-row spacing and 10 cm between plants in each row.

Seed inoculation and sowing

Seeds of chickpea (*Cicer arietinum* L.) cv. Pirooz (a desi type cultivar) were mixed with 1% gum arabic as adhesive agent and then inoculation was performed at the rate of 2 mL bacterial inoculant suspension per 100 g seeds. Then the inoculated seeds were dried under shed (to avoid direct sunshine) and sowing was immediately performed by hand. In order to prevent cross infection between treatments, the uninoculated control plots were sown beforehand and about other plots new sterile medical gloves were used for sowing each plot.

Sampling and data collection

At the beginning of flowering stage (74 days after sowing) the whole plants located in an area of 1.2 m^2 from the central four rows of each plot were carefully uprooted. Roots were washed through slow running tap water to remove adhering soil particles. Nodules were precisely separated from roots, dried and weighed. Then the shoots were oven dried at 65°C for 48 h and nitrogen and phosphorus concentration of shoots was determined in the laboratory of Soil and Water Research Institute, Sanandaj, Iran. At maturity, a 2.4 m^2 area of unsampled four central rows of each plot was hand-harvested. The plants were air dried and biomass dry weight, grain yield, nitrogen and phosphorus contents in grain samples were determined.

Statistical analysis

The data were subjected to analysis of variance (ANOVA), and comparison among treatment means was performed by Duncan's multiple range test (at $P \leq 0.05$) using MSTAT-C software (Version 2.10). About some of the recorded parameters, group comparisons between treatments were made.

Table 2. Inoculation effects of plant growth-promoting rhizobacteria on grain yield, biomass dry weight and nitrogen and phosphorus uptake by grains

Treatments	Grain yield (kg ha^{-1})	Biomass (kg ha^{-1})	N yield (kg ha^{-1})	P yield (kg ha^{-1})
1. Uninoculated	543.9 i	1082.3 f	14.49 e	2.088 d
2. *Azos.*	826.8 b-f	1609.3 b-d	27.85 ab	3.252 a-c
3. *Azot.*	797.1 b-g	1549.4 b-d	28.60 a	3.168 a-c
4. *M.*	772.5 d-h	1514.4 b-e	27.05 a-d	2.890 bc
5. *P.*	739.3 gh	1445.8 de	22.17 cd	2.747 c
6. *Azos.+Azot.*	877.2 ab	1695.8 a-c	27.05 a-c	3.459 ab
7. *Azos.+M.*	751.8 e-h	1493.1 c-e	24.53 a-d	2.869 bc
8. *Azos.+P.*	781.1 c-g	1509.6 b-e	20.32 d	3.132 a-c
9. *Azot.+M.*	835.3 a-e	1629.4 b-d	27.53 a-c	3.142 a-c
10. *Azot.+P.*	697.5 h	1427.8 de	21.97 cd	2.963 bc
11. *M.+P.*	689.9 h	1340.8 e	22.96 b-d	3.017 a-c
12. *Azos.+Azot.+M.*	818.6 b-g	1621.8 b-d	25.40 a-d	3.147 a-c
13. *Azos.+Azot.+P.*	910.6 a	1845.8 a	29.78 a	3.606 a
14. *Azos.+M.+P.*	864.0 a-c	1705.6 ab	26.60 a-c	3.277 a-c
15. *Azot.+M.+P.*	847.5 a-d	1665.3 abc	25.28 a-d	3.151 a-c
16. *Azos.+Azot.+M.+P.*	743.3 f-h	1442.8 de	22.10 cd	3.016 a-c
Selected group comparisons				
Comparison 1	**	**		
Comparison 2	**	**		
Comparison 3	NS	NS		
Comparison 4	NS	NS		

Azos: *Azospirillum, Azot*: *Azotobacter, M*: *Mesorhizobium, P*: *Pseudomonas,* Values followed by the same letters in a column are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test. Comparison 1: *Azospirillum*-cotaining versus non*Azospirillum*-containing treatments . Comparison 2: *Azotobacter*-containing versus non*Azotobacter*-containing treatments. Comparison 3: *Mesorhizobium*-containing versus non*Mesorhizobium*-containing treatments. Comparison 4: *Pseudomonas*-containing versus non*Pseudomonas*-containing treatments
** Significant at the 0.01 probability level, NS: not significant

Results

Data analysis at the beginning of flowering stage (74 DAS) showed that inoculation treatments significantly affected nodule dry weight and the concentration of nitrogen and phosphorus in shoots (Table 1). Maximum dry weight of root nodules per plant was recorded by applying the combined inoculation of *Azospirillum* spp. + *Azotobacter chroococcum* 5 + *Mesorhizobium ciceri* SWRI7 + *Pseudomonas fluorescens* P21 (Table 1). All inoculants were statistically superior over uninoculated control with respect to nitrogen concentration of shoots. The highest rates of phosphorus concentration in shoots were recorded by the treatments of number 12 (*Azospirillum* spp. + *Azotobacter chroococcum* 5 + *Mesorhizobium ciceri* SWRI 7), number 6 (*Azospirillum* spp. + *Azotobacter chroococcum* 5) and number 13 (*Azospirillum* spp. + *Azotobacter chroococcum* 5 + *Pseudomonas fluorescens* P 21) respectively (Table 1). These inoculation treatments contain *Azospirillum* and *Azotobacter* strains in their combinations, suggesting that *Azospirillum* and *Azotobacter* jointly may have a role in promoting phosphorus uptake by plant. Therefore a group comparison between *Azospirillum* + *Azotobacter*-containing treatments vs. non *Azospirillum* + *Azotobacter*-containing treatments was performed with respect to phosphorus concentration of shoots, as a result this comparison was significant ($P \leq 0.01$), demonstrating that treatments containing *Azospirillum* + *Azotobacter* significantly improved phosphorus concentration in shoot (Table 1). Furthermore group comparison analyses showed that *Azospirillum*-containing treatments statistically enhanced phosphorus concentration of shoots in comparison with other treatments, even though the comparison of *Azotobacter*-containing treatments vs. other treatments was not significant (Table 1).

The mentioned contrast analyses revealed that *Azospirillum* had the main role in improving phosphorus uptake in plant, besides *Azotobacter* had an auxiliary role in this respect. Grain yield and biomass dry weight of chickpea plants were significantly affected by inoculation treatments. Grain yield ranged from 543.9 kg ha^{-1} in uninoculated control to 910.6 kg ha^{-1} in triple inoculation with *Azospirillum* spp., *Azotobacter chroococcum* 5 and *Pseudomonas fluorescens* P21. Plant biomass ranged from 1082.3 kg ha^{-1} in control to 1845.8 kg ha^{-1} in combined inoculation with *Azospirillum* spp., *Azotobacter chroococcum* 5 and *Pseudomonas fluorescens* P21 (Table 2).

The uninoculated control treatment was statistically alone in a class with respect to grain yield and biomass. Therefore application of any bacterial treatments resulted a significant improvement in grain yield and plant biomass as compared with control (Table 2). Group comparisons between treatments showed that the occurance of *Azospirillum* or *Azotobacter* inoculants in the treatment composition caused an expressive improvement in grain yield and plant biomass (Table 2). The total nitrogen and phosphorus yield of grains followed a similar trend to grain yield. The lowest rates of total N and P uptake by grain were recorded in control plants which significantly differed from all inoculation treatments, showing that treating the plant with any inoculation treatments caused the elevation of N & P uptake in grains.

Discussion

The observed promotion in root nodulation of plant in this study could be attributed to the cumulative effects of these rhizobacteria. Similar results were obtained by Wani et al. (2007). They showed that multiple inoculation with *Mesorhizobium ciceri* and phosphate-solubilizing rhizobacteria increased the nodule number and biomass per plant. The lowest rate of N concentration in shoots at the flowering stage was shown in control plants that was in a class alone, in other words the application of all bacterial inoculants studied in this experiment resulted in significant promotion of N concentration in shoots as compared with uninoculated control. This is in agreement with the results of Wani et al. (2007). In this study the presence of *Azospirillum* in treatment composition played an important role in improving P concentration in shoots at the flowering stage that was similar to the findings of other authors (Lin et al., 1983; Dobbelaere et al., 2001). Grain yield, biomass and N & P uptake by grains were significantly improved by applying all inoculant compositions in comparison with control plants. Moreover, the presence of *Azospirillum* or *Azotobacter* in the composition of inoculant stimulated the growth and yield of chickpea in this study. *Azospirillum* is one of the best characterized genera among associative plant growth-promoting rhizobacteria and the bacterial strains of this genus are able to exert beneficial effects on plant growth and yield of many agronomic crops (Okon and Vanderleyden, 1997; Steenhoudt and Vanderleyden, 2000). *Azotobacter* spp. are free-living and nitrogen fixing bacteria which under appropriate conditions can enhance plant development and promote the crop yield (Rodelas et al., 1999). Stimulation of crop performance by *Azotobacter* inoculation has also been reported by other workers. For example Narula et al. (2005a, b) declared that inoculation of wheat and cotton by various *Azotobacter* strains resulted in significant improvement in crop yield and growth parameters under field conditions. The enhancement of nutrient uptake by plant, following the inoculation with rhizobacteria has been illustrated in many experiments (Zaidi et al., 2003; Rudresh et al., 2005; Wu et al., 2005; Wani et al., 2007). Under conditions similar to present experiment the application of every inoculation treatment studied here, especially treatments which contained *Azospirillum* or *Azotobacter* may stimulate growth and yield of chickpea in comparison with uninoculated plants.

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