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Influence of Plant Growth-Promoting Rhizobacteria on Dry Matter Accumulation and Yield of Chickpea (*Cicer arietinum* L.) under Field Conditions

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Abstract: The effects of a symbiotic bacterium (*Mesorhizobium ciceri*) and some strains of nonsymbiotic rhizobacteria from three genera including *Azospirillum*, *Azotobacter* and *Pseudomonas* on growth and yield of chickpea *(Cicer arietinum L.)* were studied under field conditions, using a randomized comlete block design with 3 replications and 16 treatments. The treatments consisted of uninoculated control and all cases of single, dual, triple and quadruple inoculants of these four bacteria. The maximum rates of dry matter accumulation in root nodules, roots and shoots were recorded by applying the combined inoculation of *Azospirillum* + *Azotobacter* + *Mesorhizobium* + *Pseudomonas*. Combined inoculation of these four microorganisms resulted in promotion of pods number per plant, grain yield, biomass and protein yield as compared with other inoculation treatments and uninoculated control. Furthermore group comparison analyses showed that the presence of *Pseudomonas fluorescens* inoculant in the combination of treatments played an effective role in stimulating yield and growth traits of chickpea.

Key words: *Azospirillum* · *Azotobacter* · *Mesorhizobium* · *Pseudomonas* · Plant Growth-promoting Rhizobacteria (PGPR) · chickpea

INTRODUCTION

Biofertilizer is defined as a substance which contains living microorganisms which, when applied to seed, plant surface, or soil, colonize the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant [1]. Biofertilizers are well recognized as an important component of integrated plant nutrient management for sustainable agriculture and hold a great promise to improve crop yield [2, 3]. A group of biofertilizers contain beneficial rhizobacteria. Many of these bacteria have been termed plant growth-promoting rhizobacteria (PGPR) [4] and among them are strains from genera such as *Pseudomonas, Azospirillum, Azotobacter, Bacillus, Burkholderia, Enterobacter, Rhizobium, Erwinia* and *Flavobacterium* [5]. 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 [5-7].

Chickpea (*Cicer arietinum* L.) is one of the major pulse crops throughout the world. It has considerable importance as food, feed and fodder [8]. In Iran, chickpea is the most important grain legume and improving it's yield and quality is a necessity. The objective of this study was to evaluate the effects of a symbiotic bacterium (*Mesorhizobium ciceri*) and some strains of nonsymbiotic rhizobacteria from three genera, including *Azospirillum, Azotobacter* and *Pseudomonas* on growth and yield of chickpea (*Cicer arietinum* L.) under field conditions.

MATERIALS AND METHODS

This experiment was conducted at the agricultural research station of Saral, Kurdistan, Iran, during the growing season of 2004-2005 in rainfed conditions. The area is located at latitude of 35°43′ N and longitude of 47°8′ E at an altitude of 2100 m above the mean sea level. The total precipitation during 2004-2005 was 306.5 mm. Some of the soil properties were: sand 19%, silt 31%, clay 50%, pH 7.6, OC 1.08%, total N 0.082%, available P and K, 20.2

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Table 1: Strains and cell densities of microorganisms used in this study

Strain	Cell density (CFU g^{-1})*
Mesorhizobium ciceri *SWRI 7*	2.05×10^{9}
Azospirillum spp.**	2.00×10^{7}
Azotobacter chroococcum *5*	1.70×10^{8}
Pseudomonas fluorescens *P21*	1.70×10^{9}

*CFU g^{-1}: Colony Forming Units per 1 g of peat-based carrier, **_Azospirillum_ spp. comprised a combination of _Azospirillum brasilense_ OF and _Azospirillum lipoferum_ 21 in a 1:1 ratio(w/w)

and 427 ppm, respectively. All plots received 30 kg N ha^{-1} in urea form according to soil tests before sowing.

The strains and cell densities of microorganisms used as biofertilizers in this experiment are shown in Table 1. The experimental design was randomized complete block 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.

Sowing was performed by hand after mixing seeds of chickpea (*Cicer arietinum* L. cv. Pirooz) with 20% sugar solution and bacterial inoculants at the rate of 2 g peat-based inoculant per each 100 g seeds, on 4 April 2005. The uninoculated control plots were sown beforehand and in order to prevent cross-inoculation between other treatments, new sterile medical gloves were used for sowing each plot.

At the beginning of flowering stage the whole plants located in an area of 1.2 m^2 from the central four rows of each plot were carefully uprooted, then root nodules, roots and shoots were separately dried at 65°C for 48 h and weighed. At maturity, 2.4 m^2 area of unsampled four central rows of each plot was hand-harvested and the following parameters were determined: seed yield and it's components, total shoot weight and nitrogen concentration of seeds (by Kjeldahl analysis). The protein concentration was calculated by multiplying the nitrogen concentration by 6.25.

The data were subjected to analysis of variance (ANOVA) using MSTATC statistical package (Version 2.1) and comparison of treatment means were performed by the LSD test at $P\leq0.05$. About some of the recorded parameters, group comparisons between treatment means were made.

RESULTS

Dry matter accumulation at the flowering stage: The results indicated that dry matter accumulation in plant organs at the flowering stage was significantly affected by the treatments. The maximum dry weight of root nodules per plant was recorded by applying the treatment of number 16 viz. combined inoculation of *Azospirillum* spp.+ *Azotobacter chroococcum* 5+ *Mesorhizobium ciceri* SWRI7+*Pseudomonas fluorescens* P21 (Table 2). The highest rates of dry matter accumulation in roots were recorded by the treatments of number 16 and number 5 (*Pseudomonas fluorescens* P21) respectively (Table 2). The dry weight of shoots in the quadruple inoculation treatment (number 16) and in the treatment of coinoculation with *Mesorhizobium ciceri* SWRI7 and *Pseudomonas fluorescens* P21 (number 11) were 769.2 and 749.2 mg $plant^{-1}$,respectively, which were significantly superior over other treatments (Table 2).

Yield components: The number of pods per plant was affected by treatments ($P\leq0.01$), so that the highest

Table 2: Effects of bacterial inoculants on dry matter accumulation in root nodules, roots and shoots at the flowering stage

	Dry weight (mg $plant^{-1}$)		
Treatment	Root nodules	Roots	Shoots
Uninoculated	14.98g-i	89.40g	413.2i
Azos.	13.75hi	95.57fg	454.2h
Azot.	25.72d-f	122.5cd	515.6ef
M.	19.88f-i	105.2ef	531.4de
P.	54.43b	157.7ab	739.0b
Azos.+Azot.	11.51i	89.07g	491.2g
Azos.+M.	24.16e-g	108.1d-f	507.3fg
Azos.+P.	33.51c-e	120.3c-e	541.2d
Azot.+M.	50.95 b	151.0b	643.2c
Azot.+P.	40.63c	150.2b	742.4b
M.+P.	41.18c	154.2b	749.2ab
Azos.+Azot.+M.	41.24c	131.0c	503.1fg
Azos.+Azot.+P.	22.06f-h	93.50fg	393.4i
Azos.+M.+P.	34.65cd	116.1c-e	500.2fg
Azot.+M.+P.	40.29c	113.1de	400.8i
Azos.+Azot.+M.+P.	64.52a	170.0a	769.2a
LSD (0.05)	9.43	15.35	20.51

Azos: *Azospirillum*, *Azot*: *Azotobacter*, *M*: *Mesorhizobium*, *P*: *Pseudomonas*, Values followed by the same letters in a column are not significantly different at $P\leq0.05$

Table 3: Effects of bacterial inoculants on the number of pods per plant, grain yield, biomass and grain protein yield

Treatment	Number of pods (plant^{-1})	Grain yield (kg ha^{-1})	Biomass (kg ha^{-1})	Grain protein (kg ha^{-1})
Uninoculated	12.2g	622.4 g	1418.6ef	125.1f
Azos.	17.1b-e	822.0 c-f	1582.0c-f	164.2c-f
Azot.	15.4c-f	819.6 c-f	1578.8c-f	163.1c-f
M.	13.3fg	685.2 fg	1358.4f	129.4ef
P.	19.0ab	969.0 a-c	1868.4a-c	195.7a-d
Azos.+Azot.	13.7fg	762.1 d-g	1472.6d-f	159.8d-f
Azos.+M.	14.3d-g	796.2 c-g	1549.3c-f	164.6c-f
Azos.+P.	17.4b-d	938.6 b-d	1803.9b-d	206.1a-c
Azot.+M.	15.3c-g	927.2 b-e	1788.7b-d	194.7a-d
Azot.+P.	19.6ab	1066.0 ab	2069.1ab	220.5ab
M.+P.	17.9bc	920.4 b-e	1803.3b-d	182.9b-d
Azos.+Azot.+M.	15.3c-g	869.7 c-e	1680.3c-f	178.0b-d
Azos.+Azot.+P.	14.1e-g	749.8 e-g	1467.1d-f	160.4d-f
Azos.+M.+P.	15.2c-g	822.7 c-f	1574.1c-f	173.5c-e
Azot.+M.+P.	15.7c-f	904.8 b-e	1741.0b-e	177.0b-d
Azos.+Azot.+M.+P.	21.5a	1142.0 a	2192.4a	231.3a
LSD (0.05)	3.17	178.7	342.1	44.35
Selected group comparisons				
Comparison 1	NS	NS	NS	NS
Comparison 2	NS	NS	NS	NS
Comparison 3	NS	NS	NS	NS
Comparison 4	**	**	**	**

Azos: *Azospirillum, Azot*: *Azotobacter, M*: *Mesorhizobium, P*: *Pseudomonas*, Values followed by the same letters in a column are not significantly different at $P \leq 0.05$, 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

number of pods per plant (21.5) was produced by the treatment number 16 and the lowest rate (12.2 pods plant^{-1}) was recorded by number 1 (uninoculated control treatment) (Table 3). The number of seeds per pod and 100-seed weight were not significantly influenced by the treatments and were same in all the treatments (data not shown).

Grain yield and biomass: The effects of treatments on grain yield and biomass were significant ($P \leq 0.01$). Grain yield ranged from 622.4 kg ha^{-1} in uninoculated control to 1142 kg ha^{-1} in combined inoculation with *Azospirillum*, *Azotobacter*, *Mesorhizobium* and *Pseudomonas* strains (Table 3). The highest rates of biomass and grain yield were obtained by the treatment numbers of 16, 10, 5 and 8. Presence of *Pseudomonas fluorescens* P21 in the combination of these treatments is their joint trait, suggesting that *Pseudomonas* may has a role in improving biomass and grain yield. Therefore a group comparison between *Pseudomonas*-containing treatments vs. non*Pseudomonas*-containing treatments was performed. Analysis of contrasts revealed that treatments containing *Pseudomonas fluorescens* P21 improved the number of pods per plant, grain yield and biomass (Table 3).

Grain protein content: The protein content of grain followed the trends similar to yield. Protein yield of grains ranged from 125.1 kg ha^{-1} in uninoculated control to 231.3 kg ha^{-1} in quadruple treatment. Group contrast analysis indicated that *Pseudomonas*-containing inoculants were superior to other inoculants, with respect to protein yield of chickpea grain.

DISCUSSION

Desirable effects of combined inoculation with plant growth-promoting rhizobacteria (PGPR) on growth parameters, grain yield and nutrient uptake by plants have been reported in many experiments [7, 9-15].

In the present study combined inoculation of *Azospirillum* spp.+ *Azotobacter chroococcum* 5+ *Mesorhizobium ciceri* SWRI7+*Pseudomonas fluorescens* P21, caused a considerable improvement in nodulation, dry matter accumulation in roots and shoots, grain yield, biomass and protein yield. The observed promotion in yield and growth parameters of chickpea by combined inoculation of these rhizobacteria could be attributed to cumulative effects including enhanced supply of nutrients such as N and P to the crop and production of growth promoting substances by these microorganisms [14, 16]. Similar results were obtained by Wani et al. [15]. They showed that multiple inoculation with *Mesorhizobium ciceri* and phosphate-solubilizing rhizobacteria promoted plant growth, grain yield and nutrient uptake by field-grown chickpea.

Analyses of group contrasts between treatment means showed that *Pseudomonas fluorescens* P21 had a key role in improving the parameters such as number of pods per plant, grain yield, biomass and protein yield of chickpea. *Pseudomonas fluorescens* is a plant growth-promoting rhizobacterium which exerts beneficial influences on crop growth through several mechanisms such as phytohormone production, stimulation of nutrient uptake and biocontrol of deleterious soil bacteria and phytopathogenic fungi [7, 17, 18].

According to the results it can be concluded that, applying the combined inoculation of *Azospirillum*, *Azotobacter*, *Mesorhizobium* and *Pseudomonas* strains used in this experiment, as biofertilizer, can affect beneficially the growth and yield of chickpea in field conditions. Furthermore considering the effective role of *Pseudomonas fluorescens* in crop productivity, the presence of this rhizobacteria in the combination of microbial biofertilizer will be useful in chickpea production.

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