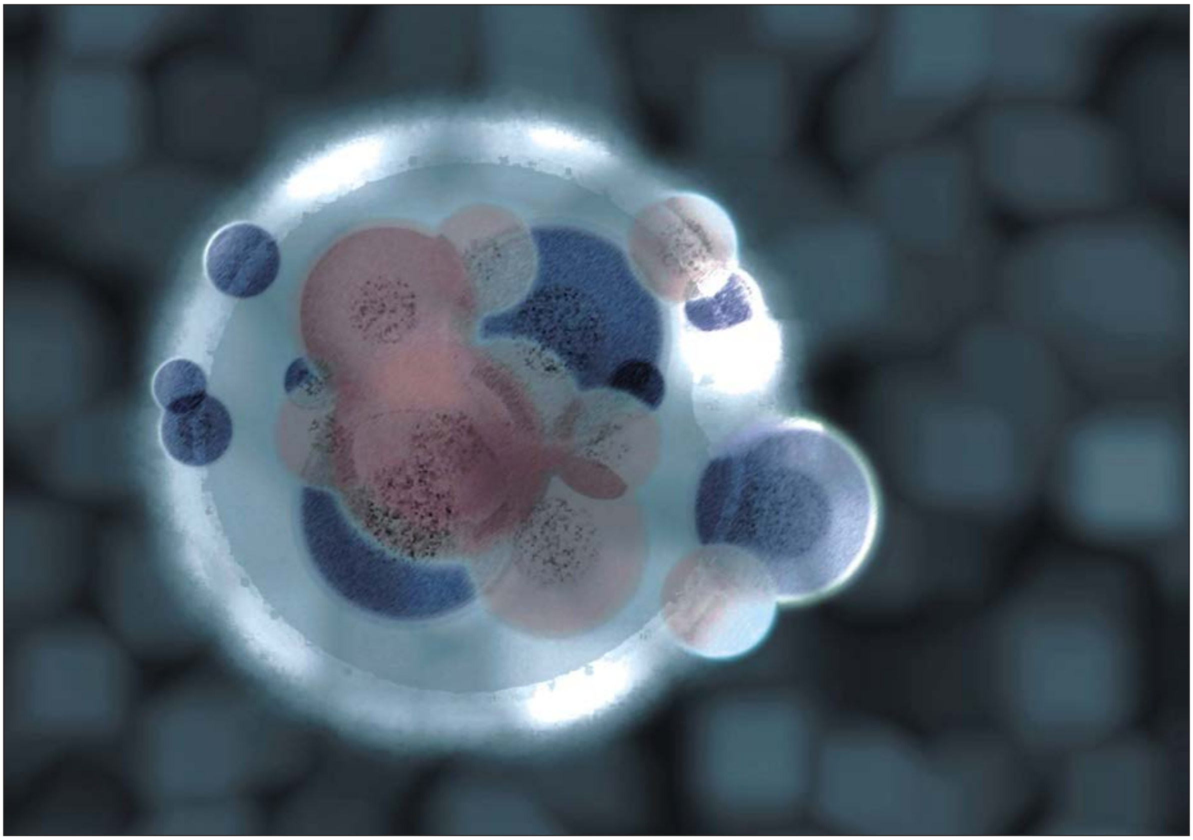

Journal of Biological Sciences

ISSN 1727-3048

Journal of Biological Sciences 8 (6): 1015-1020, 2008
ISSN 1727-3048

Growth Promotion and Enhanced Nutrient Uptake of Maize (*Zea mays* L.) by Application of Plant Growth Promoting Rhizobacteria in Arid Region of Iran

[1]A. Biari, [1]A. Gholami and [2]H.A. Rahmani
[1]Department of Agronomy, Shahrood University of Technology, Shahrood
[2]Department of Soil Microbiology, Soil and Water Research, Tehran Institute, Tehran, 14155-6185, Iran

Abstract: The effect of plant growth-promoting rhizobacteria (PGPR) belonging to the genera *Azospirillum* and *Azotobacter* on the growth and nutrient uptake of maize (*Zea mays*) was investigated in the field as factorial experiment. *Azospirillum* strains were: Z_0 = no inoculation, Z_1 = *Azospirillum* sp. Strain 21, Z_2 = *Azospirillum lipoferum* DSM 1691, Z_3 = *Azospirillum brasilense* DSM 1690 and *Azotobacter* strains were: A_0 = no inoculation, A_1 = *Azotobacter* sp. Strain 5, A_2 = *Azotobacter chroococcum* DSM2286. Treatment with PGPR(s) significantly increased plant height, shoot and seed dry weight, ear dry weight and length and number of seeds per row. Plants nutrient uptake of N, P, K, Fe, Zn, Mn and Cu were also significantly influenced by application of PGPR(s). These results indicate some PGPR inoculants have the potential to increase maize growth yield and nutrients uptake.

Key words: Maize, PGPR, *Azotobacter*, *Azospirillum*, nutrient uptake

INTRODUCTION

Increasing demand for food and livestock feed caused maize to be introduced as an important crop in temperate and semi-arid regions. Intensive farming practices that aim to produce higher yield, require extensive use of agrochemicals which are costly and create environmental pollutions (Kozdro *et al*., 2004; Tate, 1995). Inappropriate application of chemical fertilizers and water irrigation systems in maize production in semi-arid regions of Iran has resulted in pollution and salinization of agricultural land and water resources. Colonization of plant roots by bacteria has been observed for a long time, but only lately its importance for plant growth and development become clear (Glick, 1995). Plant growth-promoting rhizobacteria (PGPR) are beneficial soil bacteria that colonize plant roots and increased plant growth (Glick, 1995). Many rhizobacteria have the capacity to fix atmospheric nitrogen (Dobbelaere *et al*., 2003), although it has been reported that in the most cases this amount of nitrogen is negligible for plant demand and they can promote plant growth through the production of plant growth regulators (Dobbelaere *et al*., 2003; Verma *et al*., 2001). Roots of *Azospirillum*-inoculated maize seedlings were found to have higher amounts of both free and bound IAA as compared to control. The amount of free IAA significantly increased in the inoculated roots 2 weeks after sowing (Falik and Okon, 1989). In other cases these bacteria increased water and nutrient uptake (Jacoud *et al*., 1999; Okon and Labandera-Gonzalez, 1984). PGPR can also enhance the plant competitiveness and responses to external stress factors as well as inhibiting soil-borne plant pathogens through antifungal activity (Sharma and Chahal, 1987) and also siderophore production (Neiland, 1981). Different plant growth-promoting rhizobacteria, including free-living and associative bacteria such as *Azospirillum*, *Azotobacter*, *Bacillus* and *Pseudomonas* have been used in agricultural systems as biofertilizer for their beneficial effects on plant growth (Tilak *et al*., 1982). Inoculation of maize and wheat with *Azotobacter* and *Azospirillum* increased plant growth, nutrient uptake and yield (Abbass and Okon, 1993; Dobbelaere *et al*., 2001; Okon and Labandera- Gonzalez, 1994; Tilak *et al*., 1982). It was found that *Triticum aesriuum* cv. Miriam inoculated with *Azospirillum* accumulated 20% more N at the booting stage than did the uninoculated control (Kapulnik *et al*., 1985). Also, co-inoculation with *Giomus mosseae* and *G. fasciculatum* in the presence of *Azospirillum brasilense* produced significantly higher dry matter production and grain yield of barley than their corresponding controls (Suba Rao *et al*., 1985).

The aim of the present study was to evaluate the effects of seed inoculation with PGPR on growth and nutrient uptake of corn grown in a semi-arid environment of Iran.

Corresponding Author: A. Gholami, Department of Agronomy, Shahrood University of Technology, Shahrood, Iran
Tel: 0098-273-3350570 Fax: 0098-273-3350570

MATERIALS AND METHODS

Microorganisms: The bacterial strains studied were *Azospirillum lipoferum* s-21 and *Azotobacter chroococcum* s-5 with known positive effects on wheat (Mahour, 2005) and canola (Khalilian, 2006). *Azospirillum lipoferum* DSM 1691, *Azospirillum brasilense* DSM 1690 and *Azotobacter chroococcum* DSM 2286 were used as reference strains.

Experimental conditions and plant material: Field experiment was carried out at research farm of Shahrood University of Technology in 2006. The area is located at latitude of 36° 25'N and longitude of 54° 57' E at an elevation of 1345 m. The annual mean precipitation, temperature and relative humidity are 156.6 mm, 14.4°C and 48%, respectively. The soil was clay loam characterized by a pH, 7.8; EC, 3.9 dS m^{-1} and organic carbon, 0.75% (Table 1). The experiment was conducted using randomized complete block design as factorial with two factors (*Azospirillum* and *Azotobacter* strains) and three replications. Four-row plots were prepared with row width and intra-row space of 70 and 20 cm, respectively. There was a space of 70 cm between plots and 3 m between replications. Seeds of maize (*Zea mays*, hybrid 647) were washed with distilled water and then moistened with 20% solution of sugar before inoculation with bacteria at concentration of 10^8 cfu mL^{-1}. Before sowing, the soil was irrigated and 300 kg of urea, 150 kg of single super phosphate and 50 kg of potassium sulphate per hectare were applied according to the results of soil analysis. Nitrogen application was at the rate of 50:50 at sowing time and the starting of reproductive stage. For planting, two seeds were placed at 5 cm depth and at three-leaf stage plants were thinned to one plant per hill and the final plants were considered as population. Plants were harvested at November and the following data were collected: shoot dry weight, ear, grain and 100-seeds weight, number of rows per ear, number of seeds per row, ear length and plant height. Dry weights of the separate organs were measured after oven dried at 75°C for 72 h.

Seed nutrient analysis: For nutrient analysis, the following procedure was applied: 1 g seed was digested in an acid mixture of HNO_3 and $HClO_4$ (9:4). The resulting ash was analyzed for nutrient content. The concentration of K was determined by flame photometer. The concentration of organic C was determined by the Walkley and Black method (Walkley and Black, 1934). Total nitrogen was determined by Kjeldhal's method (Nadeem *et al*., 2006). Phosphorus content was determined with the molybdenum-ascorbic acid colorimetric method (Hanson, 1950). Zn, Fe, Mn and Cu were determined by atomic absorption.

Statistical analysis: Analysis of variance (ANOVA) was performed on all experimental data and means were compared using the Duncan's multiple range test with SAS software (SAS Institute, 1998). The significance level was p>0.05 unless otherwise stated.

RESULTS

Growth and yield: Responses of field-grown maize to PGPR inoculation depend on strain and plant growth parameter. In all cases, apart from the strain, inoculation significantly increased plant growth parameters except for the number of rows per ear (Table 2). The inoculation of maize increased shoot dry weight from 63 to 115%. The most effective treatment was A_1Z_1, A_0Z_1 followed by A_1Z_0 with a small difference. Ear dry weight was significantly affected by bacterial inoculants (p<0.01), A_0Z_1 increased ear dry weight by 141% as compared to the control plants. This increase in ear dry weight resulted in significantly higher ear length and grain dry weight. Inoculation promoted number of seed per row from 32 to 64%. Co-inoculation of maize with A_1Z_1 enhanced 100-seed weight. The results of the study showed, when plants inoculated with *A. lipoferum* s-21 alone or in combination with *Azotobacter* sp. strain 5, growth parameters increased compared to all other treatments. The exception was strain *A. chroococcum* DSM 2286 which produced highest number of seed per row.

The maize yield and growth enhancement due to bacterial inoculation used in this study could be explained with N_2-fixing and phosphate solubilizing capacity of bacteria. Another researches also showed the positive effects of PGPR on the yield and growth of plants such as apricot, tomatoes, sugar beet and barley were explained by N_2 fixation ability, phosphate solubilizing capacity and production of antimicrobial substances by PGPR (Abbasi *et al*., 2002; Cakmakci *et al*., 2001; Esitken *et al*., 2003).

Plant Nutrient Element (PNE) contents of seeds: The PNE content in corn seeds treated by PGPR strains may

Table 1: Soil chemical properties of the top soil layer (0-30 cm)

Texture	OC (%)	pH	EC (dS m^{-1})	N (%)	P	K	Fe (mg kg^{-1})	Mn	Zn	Cu
Loamy clay	0.75	7.78	3.9	0.04	6.4	230	2.72	6.44	0.54	0.78

Table 2: The effects of inoculation with *Azospirillum* and *Azotobacter* on yield and yield component of corn

Treatments	Shoot dry weight (g plant^{-1})	Ear dry weight (g plant^{-1})	Grain dry weight (g plant^{-1})	100-seed weight (g)	No. of rows/ear	No. of seed/row	Ear length (cm)	Plant height (cm)
A_0Z_0	166.49c	107.64c	87.97c	21.20c	13.83	30.75c	13.13c	157.83d
A_0Z_1	356.23a	260.06a	204.72a	29.07ab	15.83	46.75ab	19.87a	175.08abc
A_0Z_2	271.46b	192.60b	153.19b	26.02b	15.16	42.16ab	17.64ab	163.83cd
A0Z_3	339.12ab	246.39ab	199.10ab	27.53ab	15.50	48.50ab	19.13ab	172.25abc
A_1Z_0	353.93a	248.24a	193.90ab	27.65ab	15.50	47.58ab	19.58ab	177.75abc
A_2Z_0	315.30ab	233.33ab	189.30ab	28.57ab	14.33	50.50a	18.98ab	165.25bcd
A_1Z_1	359.78a	253.13a	202.70ab	29.83a	14.83	46.33ab	18.32ab	182.58a
A_1Z_2	345.72a	251.61a	199.80ab	28.91ab	15.33	48.41ab	19.40ab	168.08bcd
A_1Z_3	314.60ab	227.27ab	182.00ab	28.84ab	15.16	47.41ab	18.65ab	168.91abcd
A_2Z_1	328.58ab	228.12ab	176.40ab	26.66ab	15.83	44.41ab	18.41ab	173.83abc
A_2Z_2	321.43ab	231.93ab	189.10ab	26.83ab	14.83	48.91ab	19.37ab	178.25ab
A_2Z_3	298.58ab	210.81ab	163.50ab	27.18ab	15.83	40.50b	17.09b	175.25ab

A_0: Control, A_1: *Azotobacter* sp. Strain 5, A_2: *Azotobacter chroococcum* DSM2286, Z_0: Control, Z_1: *Azospirillum* sp. Strain 21, Z_2: *Azospirillum lipoferum* DSM 1691, Z_3: *Azospirillum brasilense* DSM1690, The means with same letter(s) in column have no differences at the 0.05 probability level

Table 3: The effects of biofertilization with co-inoculated *Azospirillum* and *Azotobacter* on plant nutrient element (PNE) in seed of corn

Treatments	N	P	K	Fe	Mn	Zn	Cu
	(g kg^{-1})						
A_0Z_0	2.26c	0.31c	0.50b	5.05b	0.86b	2.73b	0.65c
A_0Z_1	5.19a	0.66a	1.00a	12.78a	2.13a	5.66a	1.48a
A_0Z_2	3.38bc	0.50ab	0.81a	9.92a	1.63a	4.34a	1.00bc
A_0Z_3	4.73ab	0.57ab	0.95a	12.55a	1.87a	5.05a	1.29ab
A_1Z_0	5.03a	0.60ab	0.95a	12.09a	1.96a	5.32a	1.34ab
A_2Z_0	4.76ab	0.63ab	0.90a	11.44a	1.86a	5.66a	1.31ab
A_1Z_1	5.17a	0.61ab	0.89a	11.13a	1.77a	5.42a	1.39ab
A_1Z_2	5.02a	0.57ab	1.01a	12.12a	2.08a	5.80a	1.24ab
A_1Z_3	3.92ab	0.53ab	0.89a	11.49a	1.70a	5.20a	1.35ab
A_2Z_1	4.87a	0.60ab	0.88a	12.03a	2.09a	5.65a	1.20ab
A_2Z_2	5.11a	0.57ab	0.91a	12.38a	1.95a	5.32a	1.36ab
A_2Z_3	5.04a	0.49b	0.82a	9.33a	1.47a	4.62a	1.05ab

A_0: Control, A_1: *Azotobacter* sp. Strain 5, A_2: *Azotobacter chroococcum* DSM2286, Z_0: Control, Z_1: *Azospirillum* sp. Strain21, Z_2: *Azospirillum lipoferum* DSM 1691, Z_3: *Azospirillum brasilense* DSM1690, The means with same letter(s) in column have no differences at the 0.05 probability level

provide important information about the effect of bacterial inoculation in crop growth. The results showed a clear and significant increase in total nutrient content of N, P, K, Fe, Mn, Zn and Cu in seeds due to bacterial inoculation (Table 3). Nearly in all treatments, plants in A_0Z_1 plot showed the best performance and increased seed content of N (130%), P (113%), K (100%), Fe (153%), Mn (147%), Zn (107%) and Cu (127%) which was significantly different from non-inoculated control plants.

DISCUSSION

Plant growth promoting rhizobacteria (*Azospirillum* and *Azotobacter*) used in this study had positive effects on yield and growth parameters of corn grown under field conditions. All bacterial inoculations caused significant increase on growth parameters, such as shoot dry weight, ear and seed dry weight with respect to the control, although differences between various bacterial strains almost were insignificant. The results showed the *Azospirillum lipoferum* s-21 had the most effect on ear and seed dry weight. Similar findings reported by Dobbelaere *et al.* (2002), who assessed the inoculation effect of *Azospirillum brasilense* on growth of spring wheat. They observed that inoculated plants resulted in better germination, early development and flowering and also increase in dry weight of both root and shoot.

The potential use of *Azotobacter* spp. as biofertilizer has been reviewed by Brown (1982), who concluded that inoculation with these microorganisms occasionally promoted yields, probably by mechanisms other than biological N fixation. Also similar results have been reported in *Azospirillum* spp. (Okon, 1984; Zimmer *et al.*, 1988).

Woodard and Bly (2000) reported that the corn inoculated with *A. brasilense* increased shoot dry weight and grain yield. In other study, Nandakumar *et al.* (2001) reported that application of PGPR strains increased the yield of rice. The seed treatment with PGPR resulted in increased plant growth and yield of potato under field conditions (Kloepper *et al.*, 1980). The possible reason might be associated with the initial increase in root growth by the application of PGPR strains, which could promote better absorption of essential nutrients. PGPR synthesize phytohormones that promote plant growth at various stages (Kloepper *et al.*, 1986).

The present experiment revealed that seed co-inoculation with *Azospirillum* s-21 and *Azotobacter* sp.-5 resulted in an increased 100-seed weight compared to control. This finding in the present study was supported by Sharaan and El-Samie (1999) finding. They showed that co-inoculated *Azospirillum* and *Azotobacter* and some of other PGPR, increased 1000-seed weight, number and weight of seeds per spike in wheat under field condition.

Bacterial inoculation did not influence number of row per ear. Similarly, Zaied *et al.* (2007) reported that biofertilization of corn with *Azospirillum* strains did not any significant effect on number of row per ear.

Results have also showed that the number of seed per row increased by inoculation. Naidu *et al.* (2003) reported that *Azospirillum* sp. increased the number of tillers, dry matter, number of panicles, number of filled grains and 1000-grain weight in rice under field experiment. Results have also showed that, all of PGPR strains affected the ear length and plant height significantly (Table 2). *Azospirillum* s-21 was the most effective strain on increase the ear length. Although, Co-inoculation with *Azospirillum* s-21 and *Azotobacter* sp-5 had highest plant height than control and also the other strains tested.

Azospirillum inoculation increased the rice yield significantly by 1.6-10.5 g plant^{-1} (32-81% increase) in greenhouse conditions (Malik *et al.*, 2002). However, in field conditions, the estimated yield increase was around 1.8 t ha^{-1} (22% increase) as reported by Balandreau (2002). This species can also increase the height and tiller number of rice plants (Nayak *et al.*, 1986). They mentioned that the reason may be due to the increase in photosynthetic ability of the plants with high chlorophyll content due to PGPR treatment. Similarly, the efficacy of PGPR formulations on yield attributes of various crops was reported by Weller and Cook (1983). The other possible reason might be associated with the initial increase in root growth by the application of PGPR strains, which could help in promoting better absorption of essential nutrients that are responsible for high rate of photosynthesis. Better root growth may be also responsible for better synthesis of hormones like auxin and cytokinin which could help in better partitioning efficiency which resulted in increased economic yield. (Dobbelaere *et al.*, 2003; Glick, 1995; Kloepper *et al.*, 1986). *A. brasilense* alter pH of the rhizosphere (Carrillo *et al.*, 2002) and inoculation with *Azospirillum* may change root physiology and patterns of root exudation (Heulin *et al.*, 1987).

The present study indicates that, Inoculation with the *Azotobacter* and *Azospirillum* significantly increased content of N and P in seed of corn. The higher total N and P uptake of corn indicated that *Azotobacter* and *Azospirillum* were able to fix N and solubilize P and consequently promotion of plant growth (Dobbelaere *et al.*, 2001; Lynch, 1990; Marschner, 1995).

Plants inoculated with the PGPR generally have higher N content than the uninoculated plants (Puente *et al.*, 2004). However *Azospirillum brasilense* and *Azospirillum irakense* strains stimulated overall plant growth, including root development and grain yield of spring wheat and maize, but both rhizobacteria did not change the N concentration in plants or grains (Dobbelaere *et al.*, 2002).

Azospirillum inoculation can increase PO_4^{3-} and NH_4^+ uptake by rice plants (Murty and Ladha, 1988).

Results showed that all bacterial inoculations caused significant differences on the K, Fe, Mn and Zn contents in seed corn, although differences between various bacterial strains were insignificant. In general, PGPR may affect the initiation and development of lateral roots (Rolfe *et al.*, 1997), increase root weight and nutrient-uptake (Canbolat *et al.*, 2006). Also phosphate and potassium K-solubilizing bacteria may enhance mineral uptake by plants through solubilizing insoluble P and releasing K from silicate in soil (Goldstein and Liu, 1987). This evidence confirms that the percentage of P, K, Ca, Mg, Fe, Mn and Zn and Cu was significantly and/or relatively increased in the bacteria-treated plants. Amending soil with beneficial bacteria able to compensate for nutrient deficiency and maintain at least partly a normal plant development. This biofertilizer therefore may have a potential to decrease the input cost of agricultural production and be applied to the revegetation of low commercial value sites, such as metal tailings ponds (Carlot *et al.*, 2002).

ACKNOWLEDGMENT

We thank Shahrood university of technology and the Soil Microbiology Department, Soil and Water Research, Tehran for helping to carry out this research.

REFERENCES

Abbasi, P.A., J. Al-Dahmani, F. Sahin, H.A. Hoitink and S.A. Miller, 2002. Effect of compost amendments on disease severity and yield of tomato in conventional and organic production systems. Plant Dis., 86: 156-161.

Abbass, Z. and Y. Okon, 1993. Plant growth promotion by *Azotobacter paspali* in the rhizosphere. Soil Biol. Biochem., 25: 1075-1083.

Balandreau, J., 2002. The Spermosphere Model to Select for Plant Growth Promoting Rhizobacteria. In: Biofertilisers in Action. Kennedy, I.R. and A.T.M.A. Choudhury (Eds.). Rural Industries Research and Development Corporation, Canberra, pp: 53-63.

Brown, M.E., 1982. Nitrogen Fixation by Free-Living Bacteria Associated with Plants-Fact or Fiction?. In: Bacteria and Plants. Rhodes-Roberts, M.E. and F.A. Skinner (Eds.). Academic Press, London, pp: 25-41.

Cakmakci, R., F. Kantar and F. Sahin, 2001. Effect of N_2-fixing bacterial inoculations on yield of sugar beet and barley. J. Plant Nutr. Soil Sci., 164: 527-531.

Canbolat, M.Y., S. Bilen, R. Çakmakçi, S. Ahin and F. Aydin, 2006. Effect of plant growth promoting bacteria and soil compaction on barley seedling growth, nutrient uptake, soil properties and rhizosphere microflora. Biol. Fertil. Soils, 42: 350-357.

Carlot, M., A. Giacomini and S. Casella, 2002. Aspects of plant-microbe interactions in heavy metal polluted soil. Acta Biotechnol., 22: 13-20.

Carrillo, A.E., C.Y. Li and Y. Bashan, 2002. Increased acidification in the rhizosphere of cactus seedlings induced by *Azospirillum brasilense*. Natur. Wissenschaften, 89: 428-432.

Dobbelaere, S., A. Croonenborghs, A. Thys, D. Ptacek and J. Vanderleyden *et al*., 2001. Response of agronomically important crops to inoculation with *Azospirillum*. Aust. J. Plant Physiol., 28: 871-879.

Dobbelaere, S., A. Croonenborghs, A. Thys, D. Ptacek, Y. Okon and J. Vanderleyden, 2002. Effect of inoculation with wild type *Azospirillum brasilense* and A. irakense strains on development and nitrogen uptake of spring wheat and grain maize. Biol. Fertil. Soils, 36: 284-297.

Dobbelaere, S., J. Vanderleyden and Y. Okon, 2003. Plant growth-promoting effects of diazotrophs in the rhizosphere. Crit. Rev. Plant Sci., 22: 107-149.

Esitken, A., H. Karlidag, S. Ercisli, M. Turan and F. Sahin, 2003. The effect of spraying a growth promoting bacterium on the yield, growth and nutrient element composition of leaves of apricot (*Prunus armeniaca* L. cv. Hacihaliloglu). Aust. J. Agric. Res., 54: 377-380.

Falik, F. and Y. Okon, 1989. Identification and quantification of IAA and IBA in *Azospirillum brasilense* inoculated maize roots. Soil Biol. Biochem., 21: 147-133.

Glick, B.R., 1995. The enhancement of plant growth by free living bacteria. Can. J. Microbiol., 41: 109-114.

Goldstein, A.H. and S.T. Liu, 1987. Molecular cloning and regulation of a mineral phosphate solubilizing gene from *Erwinia herbicola*. Biotechnology, 5: 72-74.

Hanson, W.C, 1950. The photometric determination of phosphorus in fertilizers using the phosphovanado-molybdate complex. J. Sci. Food Agric., 1: 172-173.

Heulin, T., A. Guckert and J. Balandreau, 1987. Stimulation of root exudation of rice seedlings by *Azospirillum* strains: Carbon budget under gnotobiotic conditions. Biol. Fertil. Soils, 4: 9-17.

Jacoud, C., D. Job, P. Wadoux and R. Bally, 1999. Initiation of root growth stimulation by *Azospirillum lipoferum* CRT1 during maize seed germination. Can. J. Microb., 45: 339-342.

Kapulnik, Y., M. Feldman and Y. Okon, 1985. Contribution of nitrogen fixed by *Azospirillum* to the N nutrition of spring wheat in Israel. Soil Biol. Biochem., 17: 509-515.

Khalilian, H., 2006. Evaluating the effects of PGPR and *Thiobacillus* on yield and oil content of canola. 1st Edn., Tabriz Azad University, Tabriz, Iran .

Kloepper, J.W., F.M. Scher, M. Laliberte and B. Tipping, 1986. Emergence Promoting Rhizobacteria: Description and Implications for Agriculture. In: Iron, Siderophore and Plant Diseases, Swinburne, T.R. (Ed.). Pelnum Publishing Company, New York, pp: 155-164.

Kloepper, J.W., N.M. Schrogh and T.D. Miller, 1980. Effects of rhizosphere colonization by plant growth promoting rhizobacteria on potato plant development and yield. Phytopathology, 70: 1078-1082.

Kozdro, J., J.T. Trevors and J.D. Van Elsas, 2004. Influence of introduced potential biocontrol agents on maize seedling growth and bacterial community structure in the rhizosphere. Soil Biol. Biochem., 36: 1775-1784.

Lynch, J.M., 1990. Microbial Metabolites. In: The Rhizosphere. Lynch, J.M. (Ed.). Wiley, Chichester, pp: 177-206.

Mahour, A., 2005. Effect of plant growth-promoting rhizobacteria (PGPR) on yield and yield components of wheat. M.Sc. Thesis Varamin Azad University, Iran

Malik, K.A., M.S. Mirza, U. Hassan, S. Mehnaz, G. Rasul, J. Haurat, R. Bally and P. Normand, 2002. The Role of Plant-associated Beneficial Bacteria in Rice-wheat Cropping System. In: Biofertilisers in Action, Kennedy, I.R. and A.T.M.A. Choudhury (Eds.). Rural Industries Research and Development Corporation Canberra, pp: 73-83.

Marschner, H., 1995. Mineral Nutrition of Higher Plant. 2nd Edn., Academic Press Ltd., London, UK .

Murty, M.G. and J.K. Ladha, 1988. Influence of *Azospirillum* inoculation on the mineral uptake and growth of rice under hydroponic conditions. Plant Soil, 108: 281-285.

Nadeem, S.M., Z.A. Zahir, M. Naveed, M. Arshad and S.M. Shahzad, 2006. Variation in growth and ion uptake of maize due to inoculation with plant growth promoting rhizobacteria under salt stress. Soil Environ., 25: 78-84.

Naidu, V.S.G.R., J.D.S. Panwar and K. Annapurna, 2003. Yield response in rice to auxin application and inoculation with *Azospirillum brasilense*. Indian J. Plant Physiol., 8: 96-98.

Nandakumar, R., R. Viswanathan, S. Babu, J. Sheela, T. Raghuchander and R. Samiyappan, 2001. A new bio-formulation containing plant growth promoting rhizobacterial mixture for the management of sheath blight and enhanced grain yield in rice. Biocontrol, 46: 493-510.

Nayak, D.N., J.K. Ladha and I. Watanabe, 1986. The fate of marker *Azospirillum lipoferum* inoculated into rice and its effect on growth, yield and N_2 fixation of plants studied by acetylene reduction, $15N_2$ feeding and 15N dilution techniques. Biol. Fertil. Soils, 2: 7-14.

Neiland, J.B., 1981. Microbial iron compounds. Annu. Rev. Biochem., 50: 715-731.

Okon, Y., 1984. The physiology of *Azospirillum* in relation to its utilization as inoculum for promoting growth in plants. Proceedings of the 14th Steinbock Symposium at the University of Wisconsin, (PSSUW'1984) Elsevier Science Publishing, New York, pp: 165-174.

Okon, Y. and C.A. Labandera-Gonzalez, 1984. Agronomic applications of *Azospirillum*: An evaluation of 20 years worldwide field inoculation. Soil Biol. Biochem., 26: 1591-1601.

Page, W.J., 1987. Iron dependent production of hydroxamate by sodium dependent *A. chroococcum*. Applied Environ. Microbiol., 53: 1418-1424.

Puente, M.E., C.Y. Li and Y. Bashan, 2004. Microbial populations and activities in the rhizoplane of rock-weathering desert plants. II. Growth promotion of cactus seedlings. Plant Biol., 6: 643-650.

Rolfe, B.G., M.A. Djordjevic, J.J. Weinman, U. Mathesius, C. Pittock, E. Gärtner, K.M. Ride, Z. Dong, M. McCully and J. Mclver, 1997. Root morphogenesis in legumes and cereals and the effect of bacterial inoculation on root development. Plant Soil, 194: 131-144.

Sharaan, A.N. and F.S.A. El-Samie, 1999. Response of wheat varieties to some environmental influences. 1. Effect of seeding rates and N fertilization levels on growth and yield of two wheat varieties (*Triticum aestivum* L.). Ann. Agric. Sci., 44: 589-601.

Sharma, P.K. and V.P.S. Chahal, 1987. Antagonistic effect of *Azotobacter* on some plant pathogenic fungi. J. Res. Punjab Agric. Univ., 24: 638-640.

Suba Rao, N.S., K.V.B.R. Tilak and C.S. Singh, 1985. Synergistic effects of vesicular arbuscular mycorrhizas and *Azospirillum brasilense* on the growth of barley in pots. Soil Biol. Biochem., 17: 119-121.

Tate, R.L., 1995. Soil Microbiology. 1st Edn. Wiley, New York, pp: 398.

Tilak, K.V.B.R., C.S. Singh, N.K. Roy and N.S. Subba Rao, 1982. *Azosprillum brasilense* and *Azotobacter chroococcum* inoculum: Effect on yield of maize (*Zea mays*) and sorghum (*Sorghum bicolor*). Soil Biol. Biochem., 14: 417-418.

Verma, S., V. Kumar, N. Narula and W. Merbach, 2001. Studies on *in vitro* production of antimicrobial substances by *Azotobacter chroococcum* isolates/mutants. J. Plant Dis. Protect., 108: 152-165.

Walkley, A. and I.A. Black, 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed chronic acid titration method. Soil Sci., 37: 29-38.

Weller, D.M. and R.J. Cook, 1983. Suppression of take all of wheat by seed treatment with *Fluorescent pseudomonads*. Phytopathology, 73: 463-469.

Woodard, H.J. and A. Bly, 2000. Maize growth and yield responses to seed-inoculated N_2-fixing bacteria under dryland production conditions. J. Plant Nutr., 23: 55-65.

Zaied, K.A., A.H. Abd El-Hady, A.E. Sharief, E.H. Ashour and M.A. Nassef, 2007. Effect of horizontal DNA transfer in *Azospirillum* and *Azotobacter* strains on biological and biochemical traits of non-legume plants. J. Applied Sci. Res., 3: 73-86.

Zimmer, W., K. Roeben and H. Bothe, 1988. An alternative explanation for plant growth promotion by bacteria of the genus *Azospirillum*. Planta, 176: 333-342.