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RESPONSE OF WHEAT TO INOCULATION WITH PLANT-GROWTH PROMOTING RHIZOBACTERIA AT DIFFERENT P FERTILIZATION LEVELS

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ABSTRACT

Effect of seed inoculation with the rhizobacteria *Azospirillum brasilense* AC1 and *Micrococcus roseus* SW1 in combination with P fertilization at levels of 0, 50 and 100% of the recommended dose on yield and nutrient contents of wheat plants grown on a sandy soil was studied in a greenhouse pot experiment. The results showed that grain, straw and biological yields of wheat were significantly improved with raising P-level. Also, the seed inoculation with the two bacterial strains caused significant increases in both grain and straw yields as compared to the uninoculated control. In addition, both yield parameters were significantly higher with the strain AC1 than with SW1.The highest grain and straw yields were obtained with 100% P dose plus *A. brasilense* at which the increments over the corresponding uninoculated control were 44.2 and 50.9%, respectively. The uptake of N, P, Fe and Mn by grains as well as the total uptake of N, Zn and Cu by plant were significantly increased at 50 and 100% P doses as compared to the control, but the differences between the two P fertilizer doses were not significant. The uptake of K by grains and straw and uptake of P, Fe, Mn and Zn by straw as well as the total uptake of P, K, Fe and Mn by plant were also significantly increased with increasing P fertilization level up to 100% dose. The uptake of all measured nutrients (N, P, K, Fe, Mn, Zn, Cu) by grains and straw were significantly increased by seed inoculation with the two tested strains relative to the uninoculated controls. The foregoing results revealed that the effectiveness of the tested strains individually for improving yield and nutrient uptake by wheat decreased with increasing the applied levels of P. The results indicated also that inoculation of wheat with the two mentioned rhizobacterial strains improved yield and nutrient contents in plant. The dosage of P mineral fertilizer currently applied for wheat in the Egyptian sandy soils might be reduced by combination of *A. brasilense* AC1 inoculation plus 50% of the recommended superphosphate dose. These results are recommended for field evaluation under different soils and environmental conditions before generalization of the two tested strains as biofertilizers.

Keywords: Pot experiment, wheat, seed inoculation, nutrient uptake

INTRODUCTION

Microorganisms are important components of soil living biomass. They play a vital role in agriculture through promoting the circulation of plant nutrients and reducing the need for mineral fertilizers. Beneficial free – living rhizospheric bacteria are usually referred to as plant growth promoting rhizobacteria (PGPR). They affect plant nutrition, growth and yield through several mechanisms including: (1) solubilization of sparingly soluble minerals, (2) nitrogen fixation, (3) decomposition of organic matter, (4) production of siderophores, phytohormones, vitamins and enzymes and (5) increase efficiency of root system in nutrient uptake (Glick *et al*., 1995; Lucy *et al*. 2004; Rosas *et al*., 2008; Altomare and Tringovska, 2011). Several published studies under greenhouse and field conditions proved the importance of PGPR in increasing growth and yield of field crops (Ozturk *et al*. 2003; Abd El-Azeem *et al*. 2008; Zorita and Conigia, 2009; Zabihi *et al*. 2011; Shabayev, 2012). Among the most used PGPR are bacteria belonging to the genera *Azospirillum* and *Micrococcus*. Species of these genera were reported by many of investigators to enhance growth and yield of wheat (Abd El-Azeem *et al*. 2008; Naiman *et al*. 2009; Hassen and Labuschagne, 2010).

In Egypt, the farmers use excessive amounts of mineral phosphorus fertilizers to improve soil fertility and plant production particularly in sandy and calcareous soils which are poor in organic matter and plant nutrients and have alkaline pH. Under such conditions considerable amounts of available forms of fertilizer P transform rapidly to less available or unavailable forms (Mehana and Abdul Wahid 2002). On the other hand, the excessive use of mineral fertilizers is costly and creates environmental problems. Therefore, the present study was conducted to evaluate the suitability of the above – named rhizobacterial strains as biofertilizers for wheat at different doses of P mineral fertilizers in a sandy soil. Biofertilizers are expected to be less expensive and more environmentally –safely than mineral fertilizers.

MATERIALS AND METHODS

Rhizobacterial strains

The rhizobacterial strains *Azospirillum brasilense* AC1 and *Micrococcus roseus* SW1 were isolated by Abd El-Azeem *et al*. (2007, 2008) from the rhizospheric soils of clover and wheat, respectively, Ismailia, Egypt. They were identified and selected based on a previous knowledge of their ability to solubilize inorganic phosphate, produce indole acetic acid and promote growth and yield of wheat and faba bean plants under greenhouse conditions.

Preparation of inoculants and seed inoculation

The PGPR strains were cultured in 100 ml flasks containing 40 ml of sterilized tryptic soy agar (TSA) medium. Inoculum of each isolate was prepared by taking a loopful from its stock culture and incubated at 30 ºC for 3 days. At this time, the viable cell counts ranged from 27.6×10^8 - 35.6×10^8 colony forming unit (CFU) ml^{-1} in the cell suspensions for the two strains. For inoculation, wheat seeds (*Triticum aestivum* cv. Sakha 93) were surface sterilized by dipping in 95% ethanol solution for 5 min and then washed thoroughly with sterilized water (Jacobson *et al*., 1994). The sterilized seeds were soaked in 40 ml of the cell suspension for 1 h for each PGPR strain before cultivation. For the uninoculated control, sterilized wheat seeds were soaked in 40 ml of sterilized TSA medium.

Pot experiment

A greenhouse pot experiment was conducted in the farm of the Faculty of Agriculture, Ismailia, Egypt using a sandy soil sample (0 – 15 cm depth). Cattle manure (CM) was added at a rate of 10 g Kg^{-1} soil. The soil and CM were air-dried, crushed and sieved through a 2-mm sieve. The selected properties of the soil and CM were determined according to Gee and Bauder (1986) and Sparks *et al*., (1996) and presented in Table 1.

Table 1: Selected properties of the investigated soil and cattle manure (CM)

Properties	Soil	CM
Particle size distribution, (%)		
Sand	97.65	-
Silt	1.51	-
Clay	0.84	-
Textural class	Sandy	-
Field Capacity, %	18.0	-
pH	7.88*	7.47**
EC_e (dS m^{-1})***	1.23	13.8
Organic C (g kg^{-1})	1.39	138
Total N (g kg^{-1})	0.13	11.2
Available N (mg kg^{-1})	4.10	124
Available P (mg Kg^{-1})	10.3	118
Av. micronutrients (mg kg^{-1})****		
Fe	0.78	0.19
Mn	1.07	27.4
Zn	0.58	4.75
Cu	0.26	0.34

*** In soil-water suspension (1:2.5)** **** In CM-water suspension (1:5)**
***** In soil and CM saturated extracts** ****** In DTPA extract**

The soil was uniformly packed in plastic pots each of 17 cm height and 18.6 cm mean diameter at a rate of 5.0 kg pot^{-1}. A drainage hole of about 1 cm in diameter was made in the bottom of each pot. The soil in each pot was thoroughly mixed with 50 g air-dried CM. The experimental design was a randomized complete block (two-factorial) with three replications for each treatment.

The experimental design included nine treatments which were the combinations of three levels of P_2O_5 viz. 0.0% (no P fertilizer), 50% (15.5 mg P_2O_5 Kg^{-1} soil) and 100% (31 mg P_2O_5 Kg^{-1} soil) of the recommended dose for wheat in Ismailia sandy soil (equivalent to 0, 36.9 and 73.8 Kg P_2O_5 ha^{-1}) in the form of ordinary superphosphate (15.5% P_2O_5) and three rhizobacterial inoculants i.e. without inoculation (control), *Azospirillum brasilense* AC1 and *Micrococcus roseus* SW1. The nitrogen and potassium fertilizers were applied at rates of 120 mg N Kg^{-1} soil (equivalent to 286 Kg N ha^{-1}) and 50 mg K_2O Kg^{-1} soil (equivalent to 119 Kg K_2O ha^{-1}) in the forms of ammonium sulfate (21.6% N) and potassium sulfate (50% K_2O). Superphosphate was mixed with the soil in each pot before sowing, while potassium sulfate was applied to all pots at two equal splits after 45 and 70 days from sowing. Ammonium sulfate was applied in three split dressing (20, 30, 50% of the total amounts) after 21, 45 and 70 days from sowing. Ten inoculated wheat seeds were sown in each pot and irrigated to the almost field capacity with Ismailia canal water (0.30 dSm^{-1}). The seedlings were thinned to be three uniform plants pot^{-1} after two weeks from sowing .The plants were harvested after 130 days from sowing, dried at 65°C and the dry weights of the straw and grains were recorded.

Plant analysis

Total N was determined by the micro-Kjeldahl method and P was determined according to Jackson (1973). K was determined flamephotometrically. Fe, Mn, Zn and Cu were determined using an atomic absorption spectrophotometer as described by Jackson (1973).

Statistical analysis

All the obtained data were subjected to analysis of variance (ANOVA) using Costat statistical software, Version 6.311 (CoHort Program, 1998). The least significant difference test (LSD) was applied to make comparison between the means (P < 0.05).

RESULTS AND DISCUSSION

Yield parameters

Wheat response to combination of P fertilization and PGPR inoculants was evaluated by measuring grain and straw yields of wheat plants harvested after 130 days from sowing date. Concerning the main effects of P levels and seed inoculatin with PGPR *brasilense* AC1 and *M. roseus* SW1 on grain and straw yields, Table 2 shows that grain and biological yields were significantly improved with raising P- level from 0 to 100% P dose. Straw yield was increased significantly at 50 and 100% P doses as compared to the control, but the difference between the two doses was not significant. This stimulatory effect of P on wheat yield may be attributed to its vital role in enhancing metabolic activities of plant.

Table 2: Effect of plant growth promoting rhizobacteria (PGPR) on grain and straw yields (g pot^{-1}) of wheat plant at different levels of P fertilization

Treatments		Grain yield	Straw yield	Biological yield
P –level (mg P_2O_5 Kg^{-1} soil)	PGPR strains			
P (0%)*	Uninoculated	2.14f	2.49e	4.62f (0.463)**
	A.brasilense AC1	4.22e	5.13cd	9.35d (0.451)
	M.roseus SW1	3.50e	4.38d	7.87e (0.444)
Mean		3.28c	4.00b	7.28c (0.453)
15.5 (50%)*	Uninoculated	4.27de	4.96cd	9.23d (0.463)
	A.brasilense AC1	6.46b	7.88ab	14.3b (0.452)
	M.roseus SW1	5.83b	7.29b	13.1b (0.445)
Mean		5.52b	6.71a	12.2b (0.452)
31(100%)*	Uninoculated	5.05cd	5.87c	10.9c (0.463)
	A.brasilense AC1	7.28a	8.86a	16.1a (0.452)
	M.roseus SW1	6.01b	7.53b	13.5b (0.445)
Mean		6.11a	7.42a	13.5a (0.453)
Overall of means	Uninoculated	3.82c	4.44c	8.26c (0.463)
	A.brasilense AC1	5.99a	7.29a	13.3a (0.450)
	M.roseus SW1	5.11b	6.40b	11.5b (0.444)

*** Percent of the recommended P levels in the form of superphosphate.**

**** Numbers in parentheses indicate the harvest index (HI)**

-Values followed by different letters in a column were significantly different (P<0.05) using LSD test.

Such activities may include photosynthesis, starch synthesis, glycolysis and protein synthesis (Shalaby and Ahmed ,1993). Similar results were obtained by Mehana and Abdul Wahid (2002) with faba bean under field conditions. In the meantime, the seed inoculation with the two bacterial strains caused significant increases in both grain and straw yields as compared to the uninoculated control. However, both yield parameters were significantly higher with AC1 than with SW1. The positive response of wheat yield due to seed inoculation with the two bacterial strains could be partially explained on the basis that these strains possess a number of plant growth promoting traits including solubilization of insoluble phosphates, root colonization and production of indole acetic acid (IAA) and siderophores (Abd El- Azeem *et al*. 2007) . Furthermore, strains of the two species were reported as PGPR having several mechanisms for promoting plant growth and yield by many investigators. For instance, Amir *et al*. (2003) found that inoculation of oil palm seedlings with *A. brasilense* could contribute up to 20-50% of the total nitrogen requirement of the host plant through N_2 fixation process and increase plant growth and nutrient content relative to the full inorganic N.

The beneficial effects of rhizobacteria strains belonging to *A. brasilens* and *Micrococcus spp.* on wheat growth and yield were also confirmed those obtained by many investigators. For example, the results of numerous greenhouse and field experiments extensively reviewed by Lucy *et al*. (2004) supported the significant increases in wheat yield in response to inoculation with *A. brasilense*. *Micrococcus spp.* have a wide distribution and extensively studied as PGPR (Abd El-Azeem, 2006). The highest grain and straw yields were obtained when the soil was treated with 100% P dose and seed inoculated with *A. brasilense* at which the increments over the corresponding uninoculated control were 44.2 and 50.9%, respectively. Table 2 also shows that the increases in both grain and straw yields caused by PGPR inoculants were reduced with increasing P fertilization level. For instance, the increases in grain yield over the corresponding control with *A. brasilense* were 73.0, 51.3 and 44.2% at 0, 50 and 100% P doses, respectively. Shaharoona *et al*. (2008) found that the efficacy of the PGPR *Pseudomonas. fluorescens* and *P. fluorescens* biotype F for improving growth and yield of wheat reduced with increasing the applied levels (0, 25, 50, 75, 100% of the recommended doses) of NPK fertilizers in pot and field experiments. Also Zabihi *et al*. (2011) found similar trend in a pot trial with wheat inoculated with *P. putida* 108 at different levels (0, 50, 100% of the recommended doses) of P fertilization.

Table 2 also indicates that the increases in grain and straw yields due to inoculation with the two rhizobacteria plus 50% P fertilization were significantly higher than those recorded with 100% dose of P fertilization without inoculation. This result ensured the beneficial effect of the two strains as biofertilizers for wheat grown in a sandy soil. In this respect, Mehana and Abdul Wahid (2002) found that the ordinary used quantity of superphosphate fertilizer could be reduced by 50%, in the presence of the phosphate dissolving fungus *Aspergillus fumigatus*, without any significant reduction in the yield of *Vicia faba* L. under field conditions. Carlier *et al*. (2008) found that the increases in growth and yield parameters of wheat due to inoculation with the rhizobacterium *P.chlororaphis* subsp. *aurantiaca* strain SR1 plus 50% NP fertilization under field conditions were almost equivalent to those recorded with 100% dose fertilization without inoculation. They concluded that the dosage of mineral fertilizer currently applied in wheat field in Argentia could be reduced through proper combination of SR1 inoculation plus fertilization.

Nutrient content in plant

Concerning the main effects of P levels and PGPR application on N, P, K contents in plant, results in Tables 3 and 4 indicate that the concentration of N in grains and straw was not significantly affected by P fertilization. Though the uptake of N by both yield components were observed to be significantly increased at 50 and 100% P doses as compared to the control, but no significant differences were observed between the two P levels. The concentration and uptake of P in grains were also significantly increased at the two levels of P as compared to the control, but without significant differences between them. The concentration and uptake of P in straw and uptake of K in grains and straw were significantly observed to be increased with raising P-level. As shown in Tables 3 and 4, except for the concentration of N in grains and straw and K in grains, the concentration and uptake of the three nutrients by grains and straw were found to be significantly increased by seed inoculation with the two tested strains as compared with the uninoculated control.

However, the differences in the concentrations of the three nutrients in plant as affected by the two bacterial strains were not significant in most cases. The values of N, P and K uptake by grains and total N uptake by plant as well as uptake of P and K by straw, all were observed to be significantly higher with *A. brasilense* than those with *M. roseus.* This could be attributed to the higher grain and straw yields with *A. brasilense* (Table 2). The highest NPK uptake values by grains and straw were obtained under the treatment *A. brasilense* plus 100% P fertilization. The uptake of N, P and K by grains under this treatment over 100% P alone were 73.2, 59.3 and 69.5%, respectively. The corresponding increases by straw were 57.2, 65.2 and 48.8%, respectively. The above promoting effects obtained with PGPR were previously reported by several investigators (Bashan *et al*., 1990 ; Mantelin and Touraine 2004; N aveed *et al*., 2008 ; Turan *et al*.,2010).

Concerning the main effect of P-level on the Fe, Mn, Zn and Cu contents in plant, Tables 5 and 6 show that their concentrations were increased mostly due to P fertilization but the differences between the treatments were not always significant. However, the uptake of these elements by grains and straw were significantly higher at 50 and 100% doses of P fertilization compared to the control, but no significant differences were mostly observed between the two treatments. Tables 5 and 6 also indicate that the micronutrient concentrations in grains and straw significantly increased mostly due to inoculation with the two PGPR strains as compared to the uninoculated control. Nevertheless, the nutrient uptake by both yield components were observed to be significantly enhanced by inoculation with both strains relative to the control. Moreover, the micronutrient uptake in the presence of *A. brasilense* was always significantly higher than that with *M. roseus* giving similar trend to those found for the above mentioned macronutrients (Tables 3, 4) which reflected the trend of the grain and straw yields (Table 2).

T3-4

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Like the above named macronutrients the concentrations and uptake of Fe, Mn, Zn and Cu for both crop components reached their highest values under the treatment of 100% P fertilization plus *A. brasilense* where the increases in uptake by grains were 90.4, 102.2, 66.8 and 61.9%, compared to 100% P alone, respectively. The corresponding increments by straw were 71.2, 119.5, 77.0 and 75.4%. Similar results were obtained by Turan *et al*. (2010) who found that seed inoculation with *Azospirillum sp*. 245 under field conditions significantly increased uptake of N, P, K, Fe, Mn, Zn and Cu by grains and straw of wheat over the control.

Results of this investigation indicated that inoculation of wheat with two rhizobacterial strains significantly improved yield and nutrient uptake by plant. On the other hand, the effectiveness of the tested strains found to be decreased with raising the applied level of P. Under the conditions of this experiment the dosage of P mineral fertilizer currently applied for wheat in the Egyptian sandy soils might be reduced by combining *A. brasilense* AC1 inoculum with 50% of the recommended superphosphate dose. These results are recommended to be evaluated under different field conditions before offering the two tested strains as biofertilizers.

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استجابة نبات القمح للتلقيح ببكتريا الجذور المنشطة لنمو النبات تحت مستويات مختلفة من التسميد الفوسفاتى

أسامة محمد غانم ، رأفت خلف الله ربيع ، سامى عبدالملك محمد عبدالعظيم و طه عبدالحميد مهنا
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- أقيمت تجربة أصص فى الصوبة بمزرعة كلية الزراعة ك 4.5 ، جامعة قناة السويس بهدف دراسة تأثير تلقيح تقاوى القمح بسلالتين من بكتريا الجذور المنشطة لنمو النبات وهما *Micrococcus roseus* SW1 ، *Azospirillum brasilense* AC1 مع مستويات ثلاثة من التسميد الفوسفاتى هى صفر ، 50 ، 100% من الجرعة الموصى بها ، على المحصول والمحتوى المعدنى لنبات القمح المنزرع فى تربة رملية.
- أوضحت النتائج أن محصول الحبوب والمحصول البيولوجى ازدادا زيادة معنوية مع زيادة مستوى التسميد الفوسفاتى من صفر حتى 100% ، كما أن محصول القش تحسن أيضاً معنوياً عند المستويين 50 ، 100% بالمقارنة بالكنترول ، إلا أنه لم تلاحظ فروق معنوية بين المعدلين السابقين، وكذلك حدثت زيادة معنوية فى محصولى الحبوب والقش بالمقارنة بالكنترول نتيجة التلقيح بهاتين السلالتين ، وكلا المحصولين كان أعلى معنوياً عند التلقيح بالسلالة AC1 .
- لوحظ أن أعلى القيم لمحصولى الحبوب والقش كانت عند المعدل 100% من التسميد الفوسفاتى وفى وجود السلالة AC1 حيث كانت تزيد عن الكنترول المقابل بـ 44,2 ، 50,9% على الترتيب ، أما امتصاص النيتروجين والفوسفور والحديد والمنجنيز فى الحبوب وكذلك الامتصاص الكلى للنيتروجين والزنك والنحاس فى النبات فقد ازدادا معنوياً عند المعدلين 50 ، 100% لكن لم تلاحظ فروق معنوية بين المعدلين.
- تبين أن امتصاص البوتاسيوم فى الحبوب والقش وكذلك امتصاص الفوسفور والحديد والمنجنيز والزنك فى القش والامتصاص الكلى للفوسفور والبوتاسيوم والحديد والمنجنيز فى النبات تحسن أيضاً معنوياً مع زيادة مستوى التسميد الفوسفاتى حتى 100% .

- تشير نتائج هذه الدراسة أن امتصاص العناصر الكبرى (N, P, K) والصغرى (Fe, Mn, Zn, Cu) قد ازداد معنوياً نتيجة التلقيح الفردى بهاتين السلالتين بالمقارنة بالكنترول. وتوضح أن التلقيح الفردى بهاتين السلالتين يزيد من المحصول والمحتوى المعدنى لنبات القمح عند زيادة مستوى التسميد الفوسفاتى المضاف ، وكذلك عند نفس المستوى من السماد الفوسفاتى.
- وتشير هذه الدراسة إلى أن جرعات التسميد الفوسفاتى التى تضاف حالياً للأراضى المصرية عند زراعة القمح يمكن خفضها إلى 50% مع استخدام السلالة *A. brasilense* AC1 .
- نوصى بإجراء هذه الدراسة فى الحقل تحت ظروف مختلفة من التربة والظروف البيئية قبل تعميم هاتين السلالتين كملقحات بكتيرية.

قام بتحكيم البحث

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Table 3. Effect of inoculation with plant growth promoting rhizobacteria (PGPR) on the concentration (g Kg^{-1}) and uptake (mg pot^{-1}) of N and P by wheat plant at different levels of P fertilization

Treatments		N					P				
P –level (mg P_2O_5 Kg^{-1} soil)	PGPR strains	Grain		Straw		Total	Grain		Straw		Total
		conc.	uptake	conc.	uptake	uptake	conc.	uptake	conc.	uptake	uptake
No P (0%)*	Uninoculated	29.4bcde	65.8e	12.9a	31.7c	97.5e	3.04e	6.79e	1.72f	4.23f	11.0g
	A.brasilense AC1	28.8cde	126cd	12.1a	68.0b	194cd	3.24cd	14.2cd	1.87cd	10.6d	24.8e
	M.roseus SW1	32.2a	116d	12.1a	55.8b	172d	3.20d	11.5d	1.77ef	8.15e	19.7f
mean		30.13a	103b	12.3a	51.8b	154b	3.16b	10.8b	1.79c	7.64c	18.5c
15.5 (50%)*	Uninoculated	27.7e	123cd	13.2a	59.7b	182cd	3.23cd	14.3cd	1.86de	8.41e	22.7ef
	A.brasilense AC1	31.4abc	207b	12.6a	90.4a	298b	3.34bc	22.0b	1.97b	14.0c	36.1c
	M.roseus SW1	31.7ab	186b	12.3a	91.0a	277b	3.36ab	19.8b	1.93bcd	14.2c	34.0c
mean		30.23a	172a	12.7a	80.3a	253a	3.31a	18.7a	1.92b	12.2b	30.9b
31(100%)*	Uninoculated	28.3de	142c	11.8a	68.7b	211c	3.33bc	16.7c	1.96bc	11.5d	28.2d
	A.brasilense AC1	32.0ab	246a	12.3a	108a	354a	3.46a	26.6a	2.16a	19.0a	45.6a
	M.roseus SW1	30.8abcd	201b	12.6a	103a	304b	3.42ab	22.3b	2.10a	17.1b	39.5b
mean		30.35a	196a	12.2a	93.2a	289a	3.40a	21.9a	2.07a	15.8a	37.7a
Overall of means	Uninoculated	28.47b	110c	12.6a	53.4b	164c	3.20b	12.6c	1.84c	8.03c	20.6c
	A.brasilense AC1	30.7a	193a	12.3a	88.7a	282a	3.35a	21.0a	2.00a	14.5a	35.5a
	M.roseus SW1	31.55a	168b	12.3a	83.3a	251b	3.33a	17.9b	1.93b	13.2b	31.0b

*** Percent of the recommended P levels in the form of superphosphate.**
-Values followed by different letters in a column were significantly different ($P < 0.05$) using LSD test

Table 4. Effect of inoculation with plant growth promoting rhizobacteria (PGPR) on the concentration (g Kg^{-1}) and uptake (mg pot^{-1}) of K by wheat plant at different levels of P fertilization

Treatments		K				
P –level(mg P_2O_5 Kg^{-1} soil)	PGPR strains	Grain		Straw		Total uptake
		conc.	uptake	conc.	uptake	
No P (0%)*	Uninoculated	12.1a	27.0e	26.5b	65.2e	92.1f
	A.brasilense AC1	12.1a	52.7cd	28.5a	161c	213d
	M.roseus SW1	11.7a	42.1de	27.9a	128d	170e
Mean		11.93a	40.6c	27.6b	118c	159c
15.5 (50%)*	Uninoculated	11.7a	52.0cd	27.0b	122d	175e
	A.brasilense AC1	12.5a	82.2b	28.1a	201b	283c
	M.roseus SW1	13.1a	77.1b	27.9a	206b	283c
Mean		12.42a	70.5b	27.7b	176b	247b
31(100%)*	Uninoculated	12.8a	64.3bc	28.3a	166c	230d
	A.brasilense AC1	14.3a	109a	28.3a	247a	357a
	M.roseus SW1	12.75a	83.6b	28.3a	231a	315b
Mean		13.27a	85.8a	28.3a	215a	301a
Overall of means	Uninoculated	12.18a	47.8c	27.3b	118c	165c
	A.brasilense AC1	12.92a	81.5a	28.3a	203a	284a
	M.roseus SW1	12.52a	67.6b	28.0a	189b	256b

*** Percent of the recommended P levels in the form of superphosphate.**
-Values followed by different letters in a column were significantly different ($P<0.05$) using LSD test.

Table 5. Effect of plant growth promoting rhizobacteria (PGPR) on the concentration (mg Kg^{-1}) and uptake (µg pot^{1}) of Fe and Mn by wheat plant at different levels of P fertilization

Treatments		Fe					Mn				
P –level (mg P_2O_5 Kg^{-1} soil)	PGPR strains	Grain		Straw		Total uptake	Grain		Straw		Total uptake
		conc.	uptake	conc.	uptake		conc.	uptake	conc.	uptake	
No P (0%)*	Uninoculated	67.4e	151h	160d	395f	545f	15.6d	34.8g	8.83c	21.7f	56.6e
	A.brasilense AC1	88.4a	388de	172bc	973d	1360d	21.9b	59.8d	10.5b	59.2cd	155c
	M.roseus SW1	72.8cd	262g	172bc	793e	1055e	18.8c	67.5f	8.35c	38.4e	106d
Mean		76.2b	267b	168a	720c	987c	18.7c	66.0b	9.22a	40.0c	106c
15.5 (50%)*	Uninoculated	71.2d	315fg	160d	725e	1041e	17.5c	77.6ef	8.59c	39.0e	117d
	A.brasilense AC1	88.2a	583b	177ab	1266c	1849b	21.7b	143b	9.61bc	68.9bc	212b
	M.roseus SW1	74.6bc	439cd	173b	1280c	1719c	20.9b	123c	9.42bc	69.5b	193b
Mean		78.0ab	446a	170a	1090b	1536b	20.0b	115a	9.21a	59.1b	174b
31(100%)*	Uninoculated	72.3d	363ef	163cd	953d	1316b	17.7c	89.0de	8.96c	52.4d	141c
	A.brasilense AC1	89.7a	691a	186a	1632a	2322a	23.4a	180a	13.1a	115a	295a
	M.roseus SW1	75.2b	490c	179ab	1468b	1958b	21.1b	138bc	9.29bc	76.0b	214b
Mean		79.0a	515a	176a	1351a	1866a	20.8a	136a	10.5a	81.2a	217a
Overall of means	Uninoculated	70.3c	276c	161b	690c	967c	16.9c	67.2c	8.79b	37.7c	105c
	A.brasilense AC1	88.8a	55.4a	179a	1290a	1844a	22.3a	140a	11.1a	81.1a	221a
	M.roseus SW1	74.2b	397b	175a	1180b	1578b	20.3b	109b	9.02b	61.3b	171b

*** Percent of the recommended P levels in the form of superphosphate.**
-Values followed by different letters in a column were significantly different ($P<0.05$) using LSD test.

Table 6. Effect of plant growth promoting rhizobacteria (PGPR) on the concentration (mg Kg^{-1}) and uptake (µg pot^{-1}) of Z and Cu by wheat plant at different levels of P fertilization

Treatments		Zn					Cu				
P –level (mg P_2O_5 Kg^{-1} soil)	PGPR strains	Grain		Straw		Total	Grain		Straw		Total
		conc.	uptake	conc.	uptake	uptake	conc.	uptake	conc.	uptake	uptake
No P (0%)*	Uninoculated	47.7f	107f	9.62e	23.7e	130g	8.83ab	19.7f	5.27cd	13.0e	32.7e
	A.brasilense AC1	50.6c	222d	11.8ab	66.4c	288de	9.06a	39.8d	6.27abc	35.4c	75.2c
	M.roseus SW1	48.4ef	174e	10.0de	46.2d	221f	8.34ab	30.0e	5.08d	23.4d	53.4d
Mean		48.9b	168b	10.5a	45.4c	213b	8.74a	29.8b	5.54a	23.9b	53.8b
15.5 (50%)*	Uninoculated	47.8f	212d	10.1de	45.9d	258e	8.9ab	39.5de	5.31cd	24.0d	63.6cd
	A.brasilense AC1	51.9b	343b	12.4a	88.6b	431b	8.70ab	57.5b	6.81a	48.6b	106b
	M.roseus SW1	48.9de	288c	11.4bc	84.4b	372c	8.15b	48.0bcd	5.91abcd	43.6b	91.6b
Mean		49.5ab	281a	11.3a	73.0b	354a	8.58a	48.4a	6.01a	38.8a	87.1a
31(100%)*	Uninoculated	48.6de	244d	10.4de	61.0c	305d	8.69ab	43.6cd	5.49bcd	32.1c	75.7c
	A.brasilense AC1	52.9a	407a	12.4a	108a	516a	9.17a	70.6a	6.42ab	56.3a	127a
	M.roseus SW1	49.4d	322bc	10.7cd	88.0b	410b	8.13b	53.0bc	6.11abc	50.0ab	103b
Mean		50.3a	325a	11.2a	85.7a	410a	8.66a	55.7a	6.00a	46.1a	102a
Overall of means	Uninoculated	48.0c	188c	10.1c	43.5c	231c	8.80a	34.3c	5.35b	23.0c	57.3c
	A.brasilense AC1	51.8a	324a	12.2a	87.8a	412a	8.97a	56.0a	6.50a	46.8a	103a
	M.roseus SW1	48.9b	261b	10.7b	72.8b	334b	8.21b	43.6b	5.70b	39.0b	82.6b

*** Percent of the recommended P levels in the form of superphosphate.**

-Values followed by different letters in a column were significantly different ($P < 0.05$) using LSD test.