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Effect of Seed Inoculation with Plant Growth-Promoting Rhizobacteria on the Growth and Yield of Wheat (*Triticum aestivum* L.) Cultivated in a Sandy Soil

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ABSTRACT

A pot experiment was conducted in the greenhouse to evaluate the response of wheat (*Triticum aestivum* var. Giza 168) to inoculation with 46 strains of free-living plant growth-promoting rhizobacteria (PGPR) isolated from the rhizospheric soils of six field crops in Suez Canal region, Egypt. The results showed that growth and yield of the wheat were enhanced by seed inoculation with PGPR. Significant increases in straw, grain and total (straw plus grain) yields were recorded with 35, 33 and 37 strains, respectively, out of the tested isolates. These increases over the control ranged from 33.9 to 70.6% for straw yield, from 26.5 to 57.3% for grain yield and from 27.6 to 64.5% for the total yield. The highest straw and grain yields were obtained with the strain *Micrococcus roseus* SW1 which was isolated from the rhizospheric soil of wheat. From the obtained results, it could be concluded that the use of the tested PGPR as biofertilizers in a sandy soil increased the growth and yield of wheat under greenhouse conditions. These PGPR are recommended for field evaluation before being generalized as biofertilizers.
Keywords: pot experiment, wheat, rhizobacteria, seed inoculation, sandy soil.

INTRODUCTION

The term PGPR is used to describe specific strains of bacteria in the rhizosphere that have the capability to stimulate plant growth (Vessey, 2003; Gray and Smith, 2005; Pallai, 2005). New solutions for plant growth enhancement are required to ease the burden imposed on our environment and other resources. Thus, use of biological application of free-living plant growth promoting rhizobacteria (PGPR) may help to minimize the amounts of chemical fertilizers to be added, to improve plant growth to decrease the production cost and environmental risks (Lucy *et al.,* 2004).

Plant growth stimulation by PGPR involves diverse mechanisms not entirely elucidated. They may release key hormones for plant development such as indole acetic acid (IAA) (Khalid *et al.,* 2004), iron sequestration (Kloepper, 1993), solubilization of mineral phosphate (De Freitas *et al.,* 1997) and enzymatic lowering of plant ethylene level (Glick, 1995).

Wheat is the most important cereal crop in Egypt. Because of increasing human demand for food, attempts are made to cultivate more area with high productivity using the recommended cultural practices. Therefore, the objective of this study was to determine the effect of 46 rhizobacterial strains isolated from Suez Canal region on growth and yield of wheat plants grown under greenhouse conditions as a prelude for selection of some of those strains to be used as a biofertilizer for production of wheat under field conditions.

MATERIALS AND METHODS

Rhizobacterial strains

The tested rhizobacterial strains were isolated from the rhizospheric soils of six field-grown crops (clover, maize, kidney bean, mango, melon and wheat) grown in Port Said, Ismailia and Suez Governorates, Egypt. The rhizobacteria were identified and tested for some plant growth-promoting traits [siderophores, auxin (IAA like substances) production and phosphate solubilization capacity] by Abd El-Azeem (2006) and Abd El-Azeem *et al.* (2007).

PGPR inoculum preparation and seed inoculation

A total of 46 of rhizobacterial strains belonging to 7 genera (Table 1) were used as inocula. The PGPR strains were cultured in 100 ml flasks containing 40 ml nutrient broth. The flasks were incubated at 30^{o}C for 4 days. At the time of inoculation, the viable cell counts ranged from 28 x 10^8 - 36 x 10^8 colony forming unit (CFU) ml^{-1} in the cell suspensions. For inoculation, 15 grams of wheat seeds were soaked in 40 ml of the cell suspension for 1 h for each PGPR strain.

Greenhouse pot experiment

A greenhouse pot experiment was conducted in the Experimental Farm of the Faculty of Agriculture, Ismailia, Egypt using a virgin sandy soil sample (0-15 cm depth). Biogas manure (BM) was used as an organic fertilizer. The soil and BM were air-dried, crushed and sieved through 2-mm screen and analyzed for the selected properties according to Gee and Bauder (1986) and Sparks *et al.* (1996). Pertinent soil properties were sand, 97.9%; $CaCO_3$, 16 g Kg^{-1}; pH in soil water suspension (1:2.5), 8.27; EC_e (in soil saturated extract), 1.72 dSm^{-1}; organic C, 0.48 g Kg^{-1}; total N, 0.05 g Kg^{-1}; available N, 8.0 mg Kg^{-1} and available P, 4.40 mg Kg^{-1}. The selected BM properties were pH in BM-water suspension (1:5), 7.30; EC_e (in BM saturated extract), 3.80 dSm^{-1}; organic C, 140 g Kg^{-1}; total N, 14.8 g Kg^{-1}; available N, 140 mg Kg^{-1} and available P, 171 mg Kg^{-1}. The soil was uniformly packed in plastic pots each of 17 cm height and 18.6 cm mean diameter at a rate of 4 Kg pot^{-1} (11cm depth). A drainage hole of about 1 cm

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Table (1): Effect of seed inoculation with plant growth-promoting rhizobacteria (PGPR) on plant height and shoot dry weight of wheat plants harvested after 31 days from sowing.

PGPR Strains	Plant height		Shoot dry weight	
	(cm)	%*	(g pot^{-1})	%*
Control	22.8		0.45	
Azospirillum brasilense AC1	23.7	3.95	0.47	4.44
A. brasilense SM3	22.4		0.45	0.00
A. brasilense TC1	21.3		0.55	22.22
A. lipoferum FK1	23.9	4.82	0.49	8.89
A. lipoferum SM1	24.7	8.33	0.50	11.11
Cellvibrio mixtus KMe5	23.3	2.19	0.51	13.33
C. mixtus SM4	25.2	10.53	0.63	40.0
C. mixtus SW3	24.8	8.77	0.49	8.89
Enterobacter aerogenes AM1	21.5		0.45	0.00
E. aerogenes AMa2	22.5		0.43	
E. aerogenes BM5	22.9	0.44	0.45	0.00
E. aerogenes BM6	23.8	4.39	0.45	0.00
E. aerogenes BM7	23.3	2.19	0.51	13.33
E. aerogenes BM8	27.2	19.30	0.60	33.33
E. aerogenes BM10	22.3		0.47	4.44
E. aerogenes BM14	23.8	4.39	0.43	
E. aerogenes BM15	24.7	8.33	0.51	13.33
E. aerogenes BM16	24.0	5.26	0.46	2.22
E. aerogenes GM3	25.0	9.65	0.54	20.00
E. aerogenes KMe3	23.2	1.75	0.47	4.44
Micrococcus agilis KMe6	25.1	10.09	0.53	17.78
M. agilis KMe7	21.9		0.47	4.44
M. luteus AMa1	21.9		0.45	0.00
M. luteus KM2	21.7		0.43	
M. luteus SW2	23.6	3.51	0.45	0.00
M. roseus AMa3	21.3		0.40	
M. roseus SW1	23.4	2.63	0.45	0.00
M. roseus TW1	26.3	15.35	0.59	31.11
Pseudomonas fluorescens TC3	23.5	3.07	0.43	
P. fluorescens TW2	24.0	5.26	0.48	6.67
P. putida GM2	23.8	4.39	0.51	13.33
P. putida KM5	24.1	5.70	0.49	8.89
P. putida KMe2	23.3	2.19	0.49	8.89
P. putida TK3	25.4	11.40	0.55	22.22
Serratia liquefaciens BM4	24.9	9.21	0.50	11.11
S. liquefaciens GM5	23.2	1.75	0.47	4.44
S. liquefaciens GM6	24.8	8.77	0.50	11.11
S. liquefaciens KM4	25.8	13.16	0.49	8.89
S. liquefaciens SM2	21.9		0.47	4.44
S. liquefaciens TC4	25.0	9.65	0.52	15.56
S. marcescens BM1	22.1		0.41	
Xanthobacter autotrophicus AM2	24.9	9.21	0.51	13.33
X. autotrophicus BM3	21.7		0.40	
X. autotrophicus BM17	22.7		0.46	2.22
X. autotrophicus GM4	24.1	5.70	0.49	8.89
X. autotrophicus TC2	23.5	3.07	0.50	11.11
$LSD_{0.05}$	**2.54**		**0.09**	

* The percentage increase over control

in diameter was made in the bottom of each pot. The soil in each pot was thoroughly mixed with 40 g air-dried BM. The experimental design was randomized complete blocks with three replications for each treatment.

After inoculation, five grams of wheat seeds (*Triticum aestivum* var. Giza 168) were immediately sown in each pot and irrigated to approximately field capacity using Ismailia Canal water, which is characterized, by 0.40 dSm^{-1} and 1.12 sodium adsorption ratio (SAR) value. The seedling were thinned to be 5 uniform plants pot^{-1} after 12 days from sowing date. All pots were additionally fertilized with 0.30 g N and 0.30 g K_2O pot^{-1} in the forms of $(NH_4)_2SO_4$ and K_2SO_4, respectively, (equivalent to 179 Kg ha^{-1} for both N and K_2O). These fertilizers were dressed in two splits of 60.0 and 119.0 Kg ha^{-1}, for both N and K_2O, after 32 and 54 days from sowing, respectively. The plants were harvested after 31 and 131 days from sowing date and growth parameters were recorded. These parameters were plant height, shoot dry weight, straw and grain yields.

Statistical analysis

All the obtained results were subjected to analysis of variance (ANOVA) using CoHort Program (CoStat Statistical Software, 1990). Arithmetic means were compared by the least significant difference (LSD) test with confidence levels of 0.95.

RESULTS

As shown in Table (1), the plant height and shoot dry weight of the 31-day old wheat plants increased as result of seed inoculation with the most of the tested PGPR relative to the uninoculated control. After 31 days from sowing, 33 out of the tested 46 strains increased plant height by 0.44% - 19.3%, whereas 32 strains increased shoot dry weight by 2.22 - 40.0%, as compared to the uninoculated control. Significant increases in plant height were found with four strains to be in the range of 11.4% with *Pseudomonas putida* TK3 to 19.3% with *Enterobacter aerogenes* BM8. Likewise, significant increases in shoot dry weight were recorded with six strains ranging from 20.0% with *Enterobacter aerogenes* GM3 to 40.0% with *Cellvibiro mixtus* SM4.

Concerning the 131-day old plants, Table (2) indicates that seed inoculation with 15 out of the tested 46 strains resulted in significant increases in plant height ranging from 10.2% with the strain *Serratia liquefaciens* GM6 to 21.3% with the strain *Serratia liquefaciens* KM4. Table (2) also indicates that straw, grain and total (straw plus grains) yields were increased for all strains, but the increases were not all statistically significant compared to the control. That is, the increases in straw, grain and total yields were significant with only 35, 33 and 37 strains out of the tested isolates, respectively. The increases ranged from 33.9 to 70.6% for straw yield, from 26.5 to 57.3% for grain yield and from 27.6 to 64.5% for the total yield. The highest straw, grain and total yields were obtained with the strain *Micrococcus roseus* SW1.

Table (2) also reveals that 43 out of the tested strains decreased grain/straw ratio relative to the uninoculated control by 1 to 17%. The decreases were significant with only 6 out of the tested strains and ranged from 14 to 17% relative to the control.

Table (2): Effect of seed inoculation with plant growth-promoting rhizobacteria (PGPR) on growth and yield of wheat plants harvested after 131 days from sowing.

PGPR strains	Plant height		Straw yield		Grain yield		Total yield		Grain/straw ratio
	(cm)	%*	g pot^{-1}	%*	g pot^{-1}	%*	g pot^{-1}	%*	
Control	*64.7*		*10.9*		*9.41*		*20.3*		*0.86*
Azospirillum brasilense AC1	69.7	7.73	15.3	40.4	12.5	32.8	27.8	36.9	0.82
A. brasilense SM3	71.5	10.51	18.3	67.9	13.3	41.3	31.6	55.7	0.73
A. brasilense TC1	73.0	12.83	15.3	40.4	11.3	20.1	26.6	31.0	0.74
A. lipoferum FK1	73.5	13.60	16.1	47.7	12.8	36.0	28.9	42.4	0.80
A. lipoferum SM1	70.0	8.19	15.6	43.1	12.7	35.0	28.3	39.4	0.81
Cellvibrio mixtus KMe5	68.5	5.87	13.6	24.8	11.1	18.0	24.7	21.7	0.82
C. mixtus SM4	70.0	8.19	15.0	37.6	12.3	30.7	27.3	34.5	0.82
C. mixtus SW3	68.0	5.10	13.7	25.7	10.9	15.8	24.6	21.2	0.80
Enterobacter aerogenes AM1	69.0	6.65	16.8	54.1	14.0	48.8	30.8	51.7	0.83
E. aerogenes AMa2	71.0	9.74	16.2	48.6	12.9	37.1	29.1	43.3	0.80
E. aerogenes BM5	70.0	8.19	14.1	29.4	11.6	23.3	25.7	26.6	0.82
E. aerogenes BM6	71.0	9.74	15.7	44.0	11.9	26.5	27.6	36.0	0.76
E. aerogenes BM7	67.3	4.02	15.0	37.6	12.0	27.5	27.0	33.0	0.80
E. aerogenes BM8	71.0	9.74	13.8	26.6	11.5	22.2	25.3	24.6	0.83
E. aerogenes BM10	71.0	9.74	16.5	51.4	11.7	24.3	28.2	38.9	0.71
E. aerogenes BM14	74.0	14.37	17.1	56.9	12.7	35.0	29.8	46.8	0.74
E. aerogenes BM15	70.0	8.19	14.1	29.4	11.8	25.4	25.9	27.6	0.84
E. aerogenes BM16	70.5	8.96	16.8	54.1	13.5	43.5	30.3	49.3	0.80
E. aerogenes GM3	71.4	10.36	15.2	39.4	11.6	23.3	26.8	32.0	0.76
E. aerogenes KMe3	70.0	8.19	15.1	38.5	12.5	32.8	27.6	36.0	0.83
Micrococcus agilis KMe6	73.0	12.83	14.8	35.8	12.3	30.7	27.1	33.5	0.83
M. agilis KMe7	68.7	6.18	16.4	50.5	11.9	26.5	28.3	39.4	0.73
M. luteus AMa1	72.7	12.36	16.6	52.3	12.8	36.0	29.4	44.8	0.77
M. luteus KM2	69.0	6.65	17.4	59.6	13.7	45.6	31.1	53.2	0.79
M. luteus SW2	65.3	0.93	13.2	21.1	10.1	7.33	23.3	14.8	0.77
M. roseus AMa3	69.0	6.65	17.9	64.2	14.0	48.8	31.9	57.1	0.78
M. roseus SW1	73.0	12.83	18.6	70.6	14.8	57.3	33.4	64.5	0.80
M. roseus TW1	72.3	11.75	15.3	40.4	12.4	31.8	27.7	36.5	0.81
Pseudomonas fluorescens TC3	67.5	4.33	16.3	49.5	13.6	44.5	29.9	47.3	0.83
P. fluorescens TW2	67.7	4.64	13.3	22.0	11.2	19.0	24.5	20.7	0.84
P. putida GM2	70.0	8.19	14.7	34.9	12.3	30.7	27.0	33.0	0.84
P. putida KM5	71.0	9.74	13.1	20.2	11.1	18.0	24.2	19.2	0.85
P. putida KMe2	68.7	6.18	15.5	42.2	12.7	35.0	28.2	38.9	0.82
P. putida TK3	70.3	8.66	15.0	37.6	13.3	41.3	28.3	39.4	0.89
Serratia liquefaciens BM4	70.7	9.27	14.0	28.4	12.0	27.5	26.0	28.1	0.86
S. liquefaciens GM5	70.3	8.66	14.2	30.3	10.9	15.8	25.1	23.6	0.77
S. liquefaciens GM6	71.3	10.20	14.6	33.9	12.1	28.6	26.7	31.5	0.83
S. liquefaciens KM4	78.5	21.33	18.1	66.1	12.8	36.0	30.9	52.2	0.71
S. liquefaciens SM2	73.0	12.83	16.2	48.6	13.2	40.3	29.4	44.8	0.81
S. liquefaciens TC4	68.3	5.56	13.7	25.7	11.7	24.3	25.4	25.1	0.85
S. marcescens BM1	70.0	8.19	18.3	67.9	13.8	46.7	32.1	58.1	0.75
Xanthobacter autotrophicus AM2	68.0	5.10	15.2	39.4	12.7	35.0	27.9	37.4	0.84
X. autotrophicus BM3	72.7	12.36	15.1	38.5	11.9	26.5	27.0	33.0	0.79
X. autotrophicus BM17	71.5	10.51	15.6	43.1	12.8	36.0	28.4	39.9	0.82
X. autotrophicus GM4	70.3	8.66	15.3	40.4	13.1	39.2	28.4	39.9	0.86
X. autotrophicus TC2	73.0	12.83	15.7	44.0	13.3	41.3	29.0	42.9	0.85
$LSD_{0.05}$	***6.54***		***3.34***		***2.44***		***5.52***		***0.11***

* The percentage increase over control.

DISCUSSION

The rhizosphere of cultivated plants is usually occupied by both PGPR and deleterious rhizobacteria, which may inhibit plant growth. In this study, 46 PGPR strains were evaluated for their effects on growth and yield of wheat under greenhouse conditions. Forty-one out of the tested strains were reported to produce siderophores and all strains were found to be able to produce IAA and solubilize inorganic phosphate (Abd El-Azeem *et al.,* 2007).These plant growth promoting traits could partially explain the positive effects of these bacteria on wheat growth and yield at different growth stages. In this respect, Cattelan *et al.* (1999) reported that the mechanisms by which PGPR promote plant growth and yields of many crops are not fully understood, but are thought to include production of phytohormones, solubilization of mineral phosphates and mineralization of other nutrients, production of siderophores, and fixation of N_2. Several investigators reported significant correlations between auxin production by PGPR *in vitro* and some plant growth and yield parameters. For instance, Khalid *et al.* (2004) reported that seed inoculation with 30 bacterial strains isolated from rhizospheric soils of wheat plants cultivated at different sites significantly increased length and weight of wheat roots and shoots. Linear positive

correlation between *in vitro* auxin production by these bacteria and increases in the measured growth parameters was observed. Abd El-Azeem (2006) reported a highly significant positive linear correlation between the *in vitro* auxin production by the tested PGPR strains and each of grain yield, straw and total yield (grain plus straw) as well as the number of tillers of wheat plants. Auxin production is considered a way in which microbes promote plant growth by stimulating enzymological reactions. IAA influences plant processes, such as initiation of cell division and promotes vascular differentiation (Gaspar *et al.,* 1996). Besides its hormonal functions, IAA is involved in the stimulation of ethylene synthesis, which is produced, by higher plants and microorganisms (Glick, 1995). Ethylene plays several active roles in plants including germination of root and shoot and the response of plants to stress (Davies, 1995).

Bacteria that solubilize phosphate in soil and promote its uptake by plants are referred as phosphate solubilizing bacteria (PSB) or phosphobacteria and are included within PGPR. Their counts in the rhizosphere comprise a considerable share of the rhizospheric microorganisms and vary depending on the soil location and type as well as the cultivated plants (Gaind and Gaur, 1991; Abd El-Azeem, 1998). Inoculating the soil or seeds with PSB individually or in combination with other microorganisms, especially the nitrogen-fixing bacteria increased the availability of P, Fe, Mn, Zn and Cu for plants and consequently increased crop yield (Ibrahim *et al.,* 1995; Mehana and Farag, 2000).

Iron is an essential element for the growth, metabolism, and survival of the majority of plants and soil microorganisms. Plant roots prefer to absorb iron as the more reduced ferrous (Fe^{2+}) ion. Fortunately, PGPR play an important role in producing siderophores, which enhance the availability of soil Fe to higher plants. Siderophores are iron-transporting compounds with high Fe^{+3} affinity. These compounds solubilize Fe^{+3} for uptake by the organisms. The iron is released from the siderophore *via* reduction of Fe^{+3} to Fe^{+2}. The Fe^{+2} is then transported into the cell and the siderophore is released to complex more Fe^{+3}. Many investigators confirm the vital role of siderophores compounds produced by PGPR in increasing plant growth and yield through increasing availability of iron for plants and suppression of plant disease. For example, Kumar and Dube (1992) reported that inoculation of chickpea (*Cicer arietinum* L.) and soybean seeds with a siderophore-producing fluorescens pseudomonad resulted in increased seed germination, growth, and yield of the plants. Scher and Baker (1982) reported that the addition of a siderophore-producing *Pseudomonas putida* converted a *Fusarium*-conductive soil into a *Fusarium*-suppressive soil for the growth of different plants. De Freitas and Germida (1991) found that the three PGPR strains *Pseudomonas cepacia* R55 and R85 and *Pseudomonas putida* R104 produced fluorescent siderophores and significantly increased biomass at 50-day old wheat plant in the soil infested with *Rhizocotina solani* AG-1. They concluded that biocontrol of phytopathogens is a key mechanism by which these pseudomonads stimulate wheat growth. In this case, suppression of plant disease by these bacteria may involve secretion of siderophores or antibiotics and/or aggressive root colonization by organisms that displace or exclude deleterious rhizosphere microorganisms.

Inoculation of wheat seeds with most of the tested PGPR enhanced growth parameters of the 31-day old plants. This early positive response of wheat to inoculation with PGPR was also previously reported by several investigators. For example, Egamberdiyeva and Höflich (2003) reported that inoculation of wheat seeds with the strains *Pseudomonas fluorescens* Ps IA12 and *Pantoea agglomerans* 050309 caused significant increases in shoot dry mass of plants harvested after 28 days from seed emergence. The strain *Micrococcus roseus* SW1, which was isolated from the rhizospheric soil of wheat, proved to be the most efficient strain on straw, grain and total yields of wheat. This result may reflect the specificity observed in some crops. In some instances, specific strains of bacteria may promote plant growth only in certain crops. In this regard, Fages and Arsac (1991) reported that the maximum increases in germination and yield often occur in crops inoculated with PGPR strains isolated from the plants native rhizosphere.

The seed inoculation with most of the tested PGPR decreased grain/straw ratio of wheat as compared to the control. This may be attributed to the high nitrogen supply to the soil by the different free-living nitrogen fixing strains, which encourages the vegetative growth and delays flowering of the crop and consequently decreases the grain/straw ratio. It was reported that the soil rich in nitrogen over the satisfactory range will tend to keep down the carbon/nitrogen ratio which delays flowering in nitronegative crops such as wheat (Martin *et al.,* 1976). Similar result and conclusion were reported by Ali (1999) and Ahmed (2008).

The foregoing results confirm the beneficial effects of the tested rhizobacteria on the growth and yield of wheat under greenhouse conditions. These PGPR strains are recommended for field evaluation under different soils and environmental conditions before being practicable for agriculture as biofertilizers.

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تأثير تلقيح التقاوى ببكتيريا منطقة الجذور المنشطة لنمو النبات على نمو ومحصول القمح المنزرع فى تربة رملية

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الملخص العربـــى

أجريت تجربة أصص تحت ظروف الصوبة لتقييم تأثير إستجابة نبات القمح (صنف جيزة 168) للتلقيح بعدد 46 سلالة بكتيرية يُطلق عليها "بكتيريا منطقة الجذور المنشطة لنمو النبات" والتى تعيش بالتربة معيشة حره، وقد تم عزلها من منطقة جذور ستة محاصيل حقلية بمنطقة قناة السويس، مصر.

أوضحت النتائج التأثير الإيجابى والمنشط للتلقيح بالسلالات البكتيرية المختبره على نمو وإنتاح محصول القمح، وقد بلغ عدد السلالات التى أعطت زيادات معنوية فى محصول القش والحبوب والمحصول الكلى (القش + الحبوب) 35 و 33 و 37 سلالة بكتيرية من السلالات المختبره على التوالى. وقد تراوحت نسبة هذه الزيادة من 33.9 إلى 70.6% لمحصول القش و من 26.5 إلى 57.3% لمحصول الحبوب ومن 27.6 إلى 64.5% للمحصول الكلى وذلك بالمقارنة بالنباتات غير الملقحة (التجربة الضابطة). كما أوضحت النتائج أن أقصى محصول من القش والحبوب تم الحصول عليه نتيجة التلقيح بالسلالة البكتيرية *Micrococcus roseus* SW1 والتى تم عزلها من منطقة جذور نبات القمح.

من النتائج السابقة يمكن إستنتاج أن إستخدام بكتيريا منطقة الجذور المنشطة لنمو النبات "PGPR" المختبره فى هذه الدراسة كتسميد حيوى للتربة الرملية يزيد من نمو ومحصول القمح تحت ظروف الصوب. وتوصى الدراسة بإعادة تقييم هذه السلالات البكتيرية فى الحقل فى أراضى مختلفة قبل تعميم إستخدامها كأسمدة حيوية.