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Response of Faba Bean (*Vicia faba* L.) to Inoculation with Plant Growth-Promoting Rhizobacteria

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ABSTRACT

In the greenhouse, pot experiment was carried out to evaluate the response of faba bean (*Vicia faba* var. Giza 429) to inoculation with 56 strains of plant growth-promoting rhizobacteria (PGPR). The used PGPR strains were isolated from the rhizospheric soils of different field-grown crops in Suez Canal region. The results showed that growth and yield of faba bean were stimulated by application of PGPR. Average increases of biomass over the uninoculated control were 105.2, 31.9 and 56.8% for straw, seeds and total yields, respectively. With 54 of the tested PGPR strains, an increase in the number of nodules formed on the faba bean roots by indigenous rhizobia was observed. These increases were significant for 22 strains, ranging from 46.7% with *Micrococcus luteus* TK1 and *Xanthobacter autotrophicus* AM2 to 121.7% with *Azospirillum brasilense* AC1. All the tested isolates increased nodule dry weight, but only 23 isolates caused significant increase over the control ranging from 35.3% with *Azospirillum brasilense* GO1 to 84.4% with *Serratia liquefaciens* GO2. The present study clearly showed that the tested PGPR might have highly beneficial effects on growth and yield of faba bean under greenhouse conditions. Field evaluation under different soils and environmental conditions should be undertaken before generalizations of these PGPR for agricultural application.
Key words: Pot experiment, *Vicia faba*, rhizobacteria, root nodules, seed inoculation.

INTRODUCTION

Environmental protection and the need to enhance agricultural output have made research in new sustainable technologies necessary. In recent years, interest in soil microorganisms that can promote plant growth or help to prevent the attack of soil-borne plant pathogens has increased (*e.g.* Bashan, 1998). These beneficial bacteria are usually referred to as plant growth-promoting rhizobacteria (PGPR). PGPR are naturally occurring soil bacteria that are able to aggressively colonize plant roots and stimulate plant growth when applied to roots, tubers or seeds (Kloepper *et al.,* 1989). Plant growth benefits due to the addition of PGPR include increase in germination rate, root growth, yield (including grain), leaf area, chlorophyll content, magnesium content, nitrogen content, protein content, hydraulic activity, tolerance to drought, shoot and root weight, and delayed leaf senescence. Another major benefit of PGPR application is disease resistance conferred to the plant, sometimes known as 'biocontrol' (Lucy *et al.,* 2004).

The PGPR may promote plant growth directly or indirectly. Direct mechanisms include the provision of bioavailable phosphorus for plant uptake, nitrogen fixation for plant use, sequestration of iron for plants by siderophores, production of plant hormones like auxins, cytokinins and gibberellins, and lowering of plant ethylene levels (Glick, 1995; Glick *et al.*, 1999). Indirect mechanisms include antibiotic protection against pathogenic bacteria, reduction of iron available to phytopathogens in the rhizosphere, synthesis of fungal cell wall-lysing enzymes, and competition with detrimental microorganisms for sites on plant roots (Kloepper, 1993).

In conventional Egyptian farming, agrochemicals are excessively used to improve soil fertility, crop production and for pest control. These chemicals may contaminate deep-water reservoirs; produce significant direct hazards to the rural population, disrupt the local environment and decrease the quality of the products. These negative effects could partially be eliminated by using highly efficient strains of PGPR as one of the most promising biotechnological practices to improve soil fertility, crop production and crop quality with low input of chemical fertilizers, energy and costs. Faba bean (*Vicia faba*) is one of the most important legumes in Egypt, for domestic use as well as for export. Therefore, the objective of this study was to determine the effect of seed inoculation with selected PGPR strains on growth and yield of this crop.

MATERIALS AND METHODS

Rhizobacterial strains

Rhizobacteria were isolated from rhizospheric soils of nine field crops (faba bean, clover, kidney bean, cucumber, okra, melon maize, wheat and mango) from seven locations of the Suez Canal region, Egypt (Table 1). The rhizobacteria were identified and tested for siderophores, auxin (IAA like substances) production and phosphate solubilization capacity by Abd El-Azeem (2006).

PGPR inoculum preparation and seeds inoculation

A total of 56 strains belonging to 14 rhizobacterial species, were isolated. 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 this time, the viable cell counts ranged from 27.6×10^8 - 35.6×10^8colony forming units ml^{-1} in the cell suspensions. For inoculation, 18 faba bean seeds were soaked in 40ml of the cell suspension for 1h for each PGPR strain.

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Table (1): Strains and symbols of the tested plant growth promoting rhizobacteria (PGPR).

PGPR species	Symbol* of isolates
Azospirillum brasilense	AC1, GO1, SM3, TC1
Azospirillum lipoferum	FK1, SM1, TK2
Bacillus subtilis	AC2
Cellvibrio mixtus	KMe5, SM4, SW3
Enterobacter aerogenes	AM1, AMa2, BM5-BM8, BM10 to BM12, BM14 to BM16, GM3, KMe3
Micrococcus agilis	KMe6, KMe7
Micrococcus luteus	AMa1, KM1, KM2, SW2, TK1
Micrococcus roseus	AMa3, SW1, TW1
Pseudomonas fluorescens	KMe4, TC3, TW2
Pseudomonas putida	GM2, KM5, KMe2, TK3
Serratia liquefaciens	BM4, GM5, GM6, GO2, KM4,SM2, TC4
Serratia marcescens	BM1
Xanthobacter autotrophicus	AM2, BM3, BM17, GM4, TC2
Xanthomonas fragariae	KM3

* Bacterial isolates were designated based on the location of samples (A, El- Abtal Village; B, Bahr El-Baker; F, Fanara Village; G, El-Ganayn; K, El-Kassasin ; S, Sarabium and T, El-Tal El-Kabeer) and host plants (C, clover; F, faba bean; K, kidney bean; M, maize; Ma, mango; Me, melon; O, okra; and W, wheat).

Bean cultivation

For cultivating the beans, a mixture of sandy soil (0-15cm) and biogas manure (BM) from the Experimental Farm of the Faculty of Agriculture, Suez Canal University, Ismailia, was prepared. The soil and BM were air-dried, crushed and sieved through a 2mm sieve. Selected properties of soil and BM were determined (Table 2) according to Gee and Bauder (1986) and Sparks *et al.* (1996).

The air-dried soil (4Kg pot^{-1}, 11cm depth) was uniformly packed in plastic pots of 17cm height and 18.6cm mean diameter. A drainage hole of about 1cm in diameter was made in the bottom of each pot. The soil in each pot was thoroughly mixed with 400g air dry BM (1% w/w) as an organic fertilizer. The experimental design was a randomized complete block with three replications for each treatment.

Table (2): Some properties of the soil and biogas manure (BM) used in this study.

Properties	Soil	BM
Particle size distribution (%)		
Coarse sand	79.2	-
Fine sand	18.7	-
Silt + clay	2.1	-
Textural class	Sand	-
$CaCO_3$ (g Kg^{-1})	16.00	12.0
pH	8.27**	7.3*
EC_e (dSm^{-1})***	1.72	3.8
Soluble cations (meq l^{-1})* **		
Ca^{2+}	8.18	5.4
Mg^{2+}	3.33	2.7
Na^{+}	5.14	13.3
K^{+}	0.55	16.6
Soluble anions (meq l^{-1})* **		
CO_3^{2-}	0.00	0.0
HCO_3^{-}	5.50	14.0
Cl^{-}	9.25	20.0
SO_4^{2-}	2.45	4.0
Organic C (g Kg^{-1})	0.48	140.1
Total N (g Kg^{-1})	0.05	14.8
Total P (g Kg^{-1})	0.14	8.4
Available N (mg kg^{-1})	8.00	140.0
Available P (mg Kg^{-1})	4.40	171.3

* In BM-water suspension (1:5), ** In soil-water suspension (1:2.5), *** In BM and soil saturated extract

After inoculation, six faba bean seeds (*Vicia faba* var. Giza 429) were immediately sown in each pot and irrigated to approximately field capacity using Ismailia Canal water. After seedling emergence, the faba bean plants were thinned to two plants pot^{-1}. The plants were harvested after 39 and 112 days from sowing date and growth parameters were recorded. These parameters were plant height, shoot and root dry mass, number of pods plant^{-1}, number and dry mass of nodules, straw and seeds.

Statistical analysis

All growth and yield parameters were analyzed by analysis of variance (ANOVA) using CoStat Statistical Software (CoHort Program, 1990). Arithmetic means were compared by Duncan's multiple range test with confidence levels of 0.95.

RESULTS

Growth and yield of faba bean increased when seeds were inoculated with most of the tested bacteria relative to the uninoculated control (Table 3). After 39-days, 52 of the 56 bacterial strains increased plant height by 0.47-27.2% whereas 55 strains increased shoot dry weight by 3.87-41.9%, as compared to the uninoculated control. For 15 strains, the differences in plant height are statistically significant ranging from 13.6% with the strains *Azospirillum lipoferum* SM1 and *Serratia marcescens* BM1, to 27.2% with the strain *Enterobacter aerogenes* BM11. Likewise, significant increases in shoot dry weight were recorded for 36 strains to be in the range of 15.5% to 41.9% (*Pseudomonas putida* TK3, *Serratia liquefaciens* BM4, respectively).

Concerning the 112-day old faba bean plants, seed bacterization with 55 of the tested 56 isolates caused significant increase in plant height ranging from 29.6 to 81.2% relative to the control. For 51 strains, number of pods was significantly increased over the control by 46.2 to 115.4%. The highest values of plant height and number of pods per plant were obtained when the seeds

Table (3): Effect of inoculation with plant growth promoting rhizobacteria on growth and yield of faba bean plants.

Strain name	39 days old		112 days old							
	Plant height (cm)	Shoot dry mass ($g\ pot^{-1}$)	Plant height (cm)	No. of pods $plant^{-1}$	Straw yield		Seed yield		Total yield	
					$g\ pot^{-1}$	%*	$g\ pot^{-1}$	%*	$g\ pot^{-1}$	%*
Control	21.3	1.55	44.2	3.25	7.98	-	15.3	-	23.3	-
AC2	22.6	1.67	60.8	5.00	11.9	49.1	18.4	20.3	30.3	30.0
KMe3	23.7	1.84	74.1	5.75	16.0	100.5	23.0	50.3	29.0	67.4
SM3	22.2	1.76	74.0	4.75	16.7	109.3	20.7	35.3	37.4	50.5
AMa3	22.1	1.80	69.5	3.83	14.0	75.4	19.4	26.8	33.4	43.3
BM3	23.2	1.82	80.1	7.00	18.9	136.8	22.2	45.1	41.1	76.4
SW1	22.5	1.96	66.3	5.50	12.8	60.4	18.9	23.5	31.7	36.1
KMe7	25.8	1.95	77.2	6.50	20.2	153.1	18.9	23.5	39.1	67.8
BM7	22.8	1.89	73.2	6.50	16.2	103.0	22.1	44.4	38.3	64.3
KMe2	22.8	1.83	57.3	5.00	12.5	56.6	19.5	27.5	32.0	36.9
BM10	22.4	1.77	75.4	4.50	18.0	125.6	20.8	35.9	38.8	66.5
TK2	24.1	1.89	72.8	6.00	19.9	149.4	21.3	39.2	41.2	76.8
TC1	26.2	2.06	58.5	6.50	12.8	60.4	19.1	24.8	31.9	36.9
AMa1	24.4	1.99	70.0	5.25	15.5	94.2	23.1	51.0	38.6	65.7
SM2	23.0	1.71	74.5	6.00	21.3	166.9	19.0	24.2	40.3	73.0
BM1	24.2	1.80	76.3	6.00	19.6	145.6	19.1	24.8	38.7	66.1
KMe6	23.1	1.74	66.3	6.50	18.9	136.8	21.8	42.5	40.7	74.7
KM2	23.0	1.77	70.7	6.50	16.2	103.0	18.8	22.9	35.0	50.2
BM14	20.5	1.84	77.2	6.00	18.7	134.3	21.7	41.8	40.4	73.4
KM3	22.0	1.89	73.2	4.83	16.4	105.5	17.6	15.0	34.0	45.9
GO2	21.1	1.61	68.2	4.75	18.6	133.1			40.0	71.7
AMa2	22.8	1.88	62.7	5.50	13.3	66.7	16.3	6.53	29.6	27.0
AC1	21.5	1.73	61.3	5.00	13.3	66.7	20.5	34.0	33.8	45.1
TK1	23.3	1.78	75.4	4.50	16.0	100.5	22.8	49.0	38.8	66.5
BM17	24.4	2.10	68.7	5.75	16.1	101.8	17.7	15.7	33.8	45.1
AM1	22.1	1.81	66.4	5.50	16.4	105.5	18.1	18.3	34.5	48.1
KM4	25.0	1.89	67.9	5.42	16.9	111.8	21.0	37.3	37.9	62.7
FK1	23.3	1.69	62.8	5.75	17.0	113.0	22.3	45.8	39.3	68.7
KMe4	25.2	2.16	57.9	6.00	14.4	80.5	20.5	34.0	34.9	49.8
BM12	23.3	1.73	63.2	4.75	13.5	69.2	20.6	34.6	34.1	46.4
GM5	22.9	2.11	70.7	5.25	15.0	88.0	18.6	21.6	33.6	44.2
SM4	21.8	1.71	79.2	6.75	19.7	146.9	19.6	28.1	39.3	68.7
GM2	20.8	1.71	69.4	4.25	17.1	114.3	20.6	34.6	37.7	61.8
GM6	18.2	1.42	50.8	5.25	13.7	71.7	18.4	20.3	32.1	37.8
TK3	23.3	1.79	72.1	5.50	16.4	105.5	21.4	39.9	37.8	62.2
BM8	22.5	1.96	64.0	5.67	14.5	81.7	15.6	1.96	30.1	28.8
BM15	22.9	1.92	71.7	6.17	17.7	121.8	24.1	57.5	41.8	79.4
TC4	24.7	1.89	79.8	6.25	19.7	146.9	20.4	33.3	40.1	72.1
KM5	25.8	2.07	78.1	6.25	19.4	143.1	22.1	44.4	41.5	78.1
TC2	22.5	1.65	70.4	5.67	16.5	106.8	19.8	29.4	36.3	55.8
SW2	22.9	1.87	72.2	6.50	19.0	138.1	20.3	32.6	39.3	68.7
SM1	24.2	1.84	71.2	6.00	17.9	124.3	20.5	34.0	38.4	64.8
BM4	26.2	2.20	70.4	6.25	15.3	91.7	19.3	26.1	34.6	48.5
BM5	22.6	1.81	71.7	5.83	16.8	105.3	19.0	24.2	35.8	53.6
KMe5	23.7	1.70	66.8	6.25	17.0	103.3	18.5	20.9	35.5	52.4
GM3	23.7	2.04	69.4	6.17	16.0	100.5	21.3	39.2	37.3	60.1
BM16	22.4	1.70	66.9	5.33	14.0	75.4	19.2	25.5	33.2	42.5
BM6	24.5	1.77	67.8	6.00	15.3	91.7	20.7	35.3	36.0	54.5
TW2	22.7	1.82	71.8	6.33	18.1	126.8	19.4	26.8	37.5	60.9
SW3	26.6	1.96	72.2	5.50	15.5	94.2	19.7	28.8	35.2	51.1
GO1	22.9	1.63	69.0	4.25	18.7	134.3	18.8	22.9	37.5	60.9
KM1	22.7	1.82	70.9	5.25	15.8	98.0	19.3	26.1	35.1	50.6
BM11	27.1	2.07	66.2	6.50	16.1	101.8	20.6	34.6	36.7	57.5
TC3	25.3	2.11	77.4	6.83	17.7	121.8	22.3	45.8	40.0	71.7
GM4	22.8	1.83	59.0	6.00	12.0	50.4	24.0	56.9	36.0	54.5
TW1	21.4	1.95	73.1	5.25	17.4	118.5	20.0	30.7	37.4	60.5
AM2	22.7	1.76	65.7	6.00	13.7	71.7	20.3	32.7	34.0	45.9
$LSD_{0.05}$	2.83	0.24	8.12	1.40	3.45	-	3.27	-	4.80	-

The percentage increase over control

were inoculated with *Xanthobacter autotrophicus* BM3, which was isolated from maize rhizosphere soil, where the increase over the control was 81.2 and 115.4%, respectively.

Table 3 also reveals that straw, seed and total yields (straw plus seeds) were increased for all strains and the increases for straw and total yields were all statistically significant relative to the control. However, the increases in seed yield were significant with only 48 strains of the 56 isolates. The increases ranged from 49.1 to 166.9% for straw yield, from 1.96 to 57.5% for seed yield and from 27.0 to 79.4% for the total yield. The highest straw yield was obtained with the strain *Serratia liquefaciens* SM2, where the increase over the control was 166.9%. On the other hand, the maximum seed and total yields (seeds plus straw) were recorded with the strain *Enterobacter aerogenes* BM15 where the increases over the control were 57.5 and 79.4%, respectively.

Table 4 indicates that after 112 d the root dry mass of inoculated treatments is higher compared to the control for all rhizobacterial strains. Forty-two of the 56 isolates caused significant increases over the control by 46.9 to 160.8%. The bacterial strain *Enterobacter aerogenes* BM14 from maize rhizosphere soil was the most efficient bacterial stimulator causing an increase in root dry mass over the control by 160.8%.

Visual observation on faba bean roots indicated that they were very well nodulated by indigenous rhizobia with active pink well developed nodules. As shown in

Table (4): Effect of inoculation with rhizobacterial strains on root dry weight and nodulation of faba bean plant grown under greenhouse conditions

Strain name	Root dry weight (g pot^{-1})	Nodule number plant^{-1}	Nodule dry weight (mg)	
			per plant	per nodule
Control	5.33	60.0	152.8	2.55
AC2	8.15	82.0	180.2	2.20
KMe3	11.80	65.0	169.7	2.61
SM3	9.12	67.0	238.2	3.56
AMa3	8.97	79.0	178.5	2.26
BM3	13.04	124.0	219.5	1.77
SW1	6.18	65.0	165.9	2.55
KMe7	9.76	82.0	186.7	2.28
BM7	10.10	63.0	164.8	2.62
KMe2	11.20	56.0	157.2	2.81
BM10	7.20	93.0	167.1	1.80
TK2	7.22	92.0	245.9	2.67
TC1	6.83	55.0	153.8	2.80
AMa1	11.70	73.0	180.7	2.48
SM2	8.97	108.0	199.1	1.84
BM1	9.77	90.0	214.1	2.38
KMe6	8.70	129.0	217.1	1.68
KM2	8.02	80.0	176.8	2.21
BM14	13.90	73.0	221.8	3.04
KM3	9.66	105.0	166.6	1.59
GO2	9.91	81.0	281.7	3.48
AMa2	7.52	64.0	175.5	2.74
AC1	10.10	133.0	211.3	1.59
TK1	9.55	88.0	182.5	2.07
BM17	10.30	63.0	159.5	2.53
AM1	6.30	68.0	173.9	2.56
KM4	8.48	64.0	155.4	2.43
FK1	8.02	84.0	250.7	2.98
KMe4	6.81	95.0	188.8	1.99
BM12	7.08	129.0	217.8	1.69
GM5	7.97	122.0	220.1	1.80
SM4	8.64	131.0	235.8	1.80
GM2	7.57	90.0	178.3	1.98
GM6	7.83	67.0	175.7	2.62
TK3	9.78	107.0	212.0	1.98
BM8	11.60	120.0	225.3	1.88
BM15	9.04	119.0	265.6	2.16
TC4	9.82	97.0	238.0	2.45
KM5	8.29	98.0	261.9	2.67
TC2	9.42	85.0	201.6	2.37
SW2	10.00	120.0	215.2	1.79
SM1	10.20	97.0	211.8	2.18
BM4	9.80	82.0	186.9	2.28
BM5	11.40	67.0	172.9	2.58
KMe5	12.40	73.0	166.1	2.28
GM3	9.82	67.0	184.3	2.75
BM16	11.10	76.0	170.5	2.24
BM6	14.10	73.0	208.7	2.86
TW2	7.10	104.0	196.9	1.89
SW3	9.85	66.0	203.3	3.08
GO1	7.37	78.0	206.8	2.65
KM1	7.31	80.0	176.3	2.20
BM11	8.69	80.0	184.7	2.31
TC3	13.90	69.0	253.1	3.67
GM4	6.88	60.0	212.2	3.54
TW1	9.02	68.0	145.8	2.14
AM2	7.51	88.0	176.0	2.00
LSD $_{0.05}$	2.29	25.5	53.6	0.71

Table 4, 54 out of 56 isolates increased the number of nodules on the faba bean roots as compared to the control. However, these increases were significant only with 23 isolates. The significant increases over the control ranged from 46.7% (*Micrococcus luteus* TK1, *Xanthobacter autotrophicus* AM2) to 121.7% (*Azospirillum brasilense* AC1).

Table 4 also indicates that inoculation with PGPR increase nodule dry weight. However, the increases were not always statistically significant. Only 23 of the tested 56 strains caused significant increases over the control in the range from 35.3% (*Azospirillum brasilense* GO1) to 84.4% (*Serratia liquefaciens* GO2). In addition to the enhanced nodulation, 20 of the tested 56 isolates increased the average weight of the individual nodules as compared to the control (Table 4).

DISCUSSION

The plant rhizosphere is a dynamic ecological environment of intense microbe-plant interactions, *e.g.* for harnessing micro- and macro-nutrients from a limited nutrient pool. In the present investigation, 56 PGPR strains were evaluated for their effects on growth and yield of faba bean under greenhouse conditions. Among these 56 strains, 50 produced siderophores and all strains were able to produce IAA and solubilize inorganic phosphate (Abd El-Azeem, 2006). These plant growth-promoting (PGP) traits could partially explain the positive effects of these bacteria on faba bean growth and yield at different growth stages. Beneficial effects of these bacterial species on several crops are reported in the literature. For example, numerous greenhouse and field experiments reviewed by Lucy *et al.* (2004) confirm significant increases for wheat in response to application of *Azospirillum brasilense*, *Pseudomonas fluorescens*, *Pseudomonas putida* and *Bacillus spp.* Alam *et al.* (2001) reported that the inoculation of rice with *Xanthobacter sp.* increased total dry matter yield, grain yield and nitrogen accumulation over two years of field study. They attributed these positive effects of bacteria on rice plant to increases in root length, leaf area and chlorophyll content. In addition to the above-measured PGP traits, other characters of these species were reported to explain their positive effects on plant growth and yield. For example, Glick *et al.* (1997) reported that the free-living PGPR *Pseudomonas putida* GR12-2 has a number of biological attributes that contribute to its ability to stimulate plant growth. These include the synthesis of siderophores that can solubilize iron in the soil and make it available to the plant, the production of phytohormones, especially IAA, and the presence of the enzyme 1-aminocyclopropane-1-cabooxylic acid (ACC) deaminase, which hydrolyzes ACC, the immediate precursor of the phytohormone ethylene in plant. Similarly, the diazotrophic growth promoting bacteria *Azospirillum*, *Pseudomonas*, *Bacillus*, *Enterobacter*, *Herbaspirillum* and *Pseudomonas* have been isolated from the rhizosphere of various crops (Barraquio *et al.,* 2000; James *et al.,* 2000). Their beneficial effects on plants have been attributed to the production of phytohormones (Tien *et al.,* 1979; Haahtela *et al.,* 1990) and competitive suppression of pathogens (Glick, 1995). The positive response of leguminous crops including faba bean to inoculation by rhizobacteria was also reported by many investigators (Rodelas *et al.,* 1999; Dey *et al.,* 2004; Lucas Garcia *et al.,* 2004, Raza *et al.* 2004; Kishore *et al.,* 2005).

Yield-increasing bacteria (YIB) which is a commercial name for a microbial agent made up of different strains of *Bacillus* spp., mainly *B. subtilis* and *B. cereus* as well as *B. firmus*, *B. licheniformis*, *B. coagulans*, *B. brevis* and *B. sphaericus* (Shen, 1997) was applied to more than 50 types of crops in China. It was reported that the average yield of crops had increased by 10% or even more following the application of YIB to the field crops. The spore-forming *Bacillus* spp. have metabolically predominance for long periods and occupy ecological niches. They compete with pathogens for space and nutritional resources and have the ability to suppress pathogen activity, produce of hormones, enzyme and antifungals (Shen, 1997).

The inoculation with the tested PGPR caused significant increases in root dry weight of 112-day old faba bean plants over control. These increases could be attributed to the positive effects of the tested bacteria on plant growth, which are always correlated with remarkable changes in root morphology namely increased lateral root length and root hair number and length. The effect of plant growth promoting bacteria (PGPB) on root morphogenesis is usually attributed to the release of phytohormones including auxins, cytokinins and gibberellins by the bacteria (Steenhoudt and Vanderleyden, 2000; Bloemberg and Lugtenberg, 2001; Persello-Cartieaux *et al*., 2003). One of the more characteristic effects of PGPB is an increased elongation rate, and perhaps initiation rate, of lateral roots resulting in more branched root system architecture (Lifshitz *et al.,* 1987). Enhancement of the root surface area and the volume of soil foraged by the root are expected to enhance nutrient uptake and plant growth. Consequently, increase of plant root dry matter due to enhancement of plant root growth is the most commonly proposed explanation for the beneficial effects of the tested rhizobacteria on plant growth and yield. The favorable effects of the inoculation treatment on plant root growth are known to improve the efficiency of mineral and water uptake in inoculated plants (Okon *et al.,* 1977; Sarig *et al.,* 1988).The enhancement of nodulation observed in this work could be attributed to the promoting effects of the tested rhizobacterial strains on root hair formation as indicated by a significant increase in the root dry mass. Similar results were reported by Volpin *et al.,* (1996) and

Hamaoui *et al.,* (2001). Promotion of nodulation and N_2-fixation in legumes is of economic importance, particularly for faba bean, which is one of the most important seed legumes in Egypt and for export. The greater number of active nodules following inoculation with rhizobacteria is expected to contribute to higher amounts of plant available nitrogen, resulting in higher yields. Therefore, the visual observations on faba bean plants indicated that their growth was normal and no N deficiency symptoms could be diagnosed, although the plants were cultivated on a sandy soil without application of any chemical fertilizers throughout the growing season. As was expected, the increase in nodulation was reflected in increased faba bean growth and yield. This result was in accordance with that of Hamaoui *et al.* (2001). Promotion of plant growth and nodulation of different legumes by PGPR inoculation were also reported by many of investigates (Dashti *et al.,* 1998; Bai *et al.,* 2002; Rao and Pal, 2003). De Freitas *et al.* (1993) reported that the strain *Pseudomonas putida* R105, not only promoted pea plant development, but also enhanced nodule formation by indigenous *R. leguminosarum*.

PGPR have been shown to promote nodulation of soybean (Zhang *et al.,* 1996; Dashti *et al.,* 1998; Molla *et al.,* 2001) and pigeonpea (Tilak *et al.,* 2006). Shaharoona *et al.* (2006) found that inoculation of mung bean with *Pseudomonas putida* biotype A and *P. fluorescens* had significant effect on number of nodules. Rodelas *et al.* (1999) found that mixed inoculation of the faba bean seeds with *Rhizobium leguminosarum* bv. *viceae* strain Z 25 and five different *Azotobacter chroococcum* or *A. vinelandii* strains resulted in significant effects on nodulation, plant growth and nitrogenase activity. Similarly, Hamaoui *et al.* (2001) reported that inoculation of chickpeas and faba beans with *Azospirillum brasilense* Cd caused significant increases in root nodulation by native rhizobia and improved root and shoot development under greenhouse conditions and significantly increased crop yield under field conditions.

Sometimes, rhizobacteria isolated from a particular legume crop show some variations in plant growth-promoting activity with other legume crops. This may be due to different colonization potentials (Anderson *et al.,* 1988), their response to amounts and compositions of root exudates (Miller *et al.,* 1989; Phillips and Streit, 1997), variation in ambient temperature (summer-grown crop versus winter crop) (Seong *et al.,* 1991) and the different kinds of interactions with rhizobial/bradyrhizobial strains predominant in the particular legume rhizosphere (Parmar and Dadarwal, 1999; Sindu *et al.,* 1999).

The results discussed suggest that, although all the tested rhizobacteria were able to exhibit predominately beneficial growth effects on the tested plants, the growth promoting ability of the strains may not always be absolute but may be dependent on the plant species tested. This is shown in several studies that the growth promoting ability of some bacteria may be highly specific to certain plant species, cv. and genotype (Nowak, 1998; Lucy *et al.,* 2004).

The foregoing results display the beneficial effects of the tested rhizobacterial strains on the growth and yield of faba bean. The favorable effects of these strains on plant yield could be attributed to their efficiency in producing auxins and siderophores as well as in solubilizing phosphate. In addition to these three mechanisms of action through which PGPR influenced plant growth, some other plant growth-promoting effects may be involved, *e.g.* asymbiotic N_2 fixation.

The present study clearly shows that the tested rhizobacteria were effective PGPR as indicated by highly beneficial effects on faba bean plant under greenhouse conditions. Field evaluation under different soils and environmental conditions should be undertaken before generalizations of these PGPR for agricultural application.

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إستجابة نبات الفول البلدى للتلقيح ببكتيريا منطقة الجذور المنشطة لنمو النبات

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الملخص العربـــى

يهدف هذا البحث إلى دراسة تأثير التلقيح بـ 56 سلالة من بكتيريا منطقة الجذور المنشطة لنمو النبات كأسمدة حيوية على نمو وإنتاج نبات الفول البلدي في تجربة أصص تحت ظروف الصوبة ، وتم عزل وتعريف هذه السلالات فى دراسة سابقة من منطقة جذور نباتات مختلفة نامية بمنطقة قناة السويس.

أوضحت النتائج أن التلقيح بالسلالات البكتيرية المختبره وعددها 56 سلالة ينشط من نمو وإنتاج الفول البلدى حيث كانت متوسطات الزيادات فى محصولى التبن والبذور والمحصول الكلى (التبن + البذور) هى 105.2 ، 31.9 ، 56.8 ٪ على الترتيب، و أن تلقيح التقاوى بـ54 سلالة من السلالات المختبره وعددها 56 سلالة أدى إلى زيادات فى أعداد العقد الجذرية المتكونة على جذور نباتات الفول البلدى بواسطة الـ rhizobia المتوطنة فى التربة ، إلا أن الزيادات المعنوية قد تم الحصول عليها مع 22 سلالة فقط وقد تراوحت بين 46.7٪ مع السلالتين (TK1) *Micrococcus luteus* ، (AM2) *Xanthobacter autotrophicus* إلى 121.7٪ مع السلالة (AC1) *Azospirillum brasilense* ، كذلك حدثت زيادات فى الوزن الجاف للعقد الجذرية نتيجة التلقيح بكل البكتريا المختبره كل على حده إلا أن الزيادات كانت معنوية مع 23 سلالة فقط وتراوحت بين 35.3٪ مع *Azospirillum brasilense* (GO1) إلى 84.4٪ مع (GO2) *Serratia liquefaciens*.

هذا وقد تبين من نتائج تلك الدراسة ان بكتيريا الجذور المختبره بكتيريا منشطة لنمو النبات PGPR وهذا تم اثباته من خلال تأثيراتها الكبيرة والمفيدة على نمو ومحصول الفول البلدى تحت ظروف الصوبة. وتوصى هذه الدراسة بإعادة تقييم هذه السلالات البكتيرية فى الحقل فى أراضى وبيئات مختلفة قبل أن تعمم كملقحات بكتيرية.