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Araştırma Makalesi/Research Article

Effect of Some Plant Growth Promoting Bacteria on Yield, Yield Components of Dry Bean (*Phaseolus vulgaris* L. cv. Aras 98)

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ABSTRACT : The common bean (*Phaseolus vulgaris* L.) belongs to the family Leguminosae, and is a diverse food resource of high nutritional value. Plant growth promoting bacteria (PGPB) are a group of bacteria that actively colonize plants, increase plant growth and yield, and suppres plant disease. In this study, the effectiveness of ten PGPBs (*Alcaligenes piechaudii* strain RK-136, *Bacillus megaterium* strain M-3, *Bacillus pumilus* strain M-13, *Bacillus subtilis* strain BA-142, *Erwinia rhapontici* strain RK-135, *Burkholderia cepacia* strain RK-277, *Pantoea agglomerans* strain RK-84, RK-123, RK-92, *Pseudomonas putida* strain BA-8, *Serratia liquefaciens* strain RK-102) was evaluated on growth and yield parameters of dry bean. Also, they were investigated for their ability to suppress diseases caused by natural plant pathogenic bacteria and/or fungal infections in field conditions. As a result of all experiments, some of the applications of PGPBs increased growth and yield parameters of dry bean. In addition, some of the PGPBs suppressed the diseases of bean caused by natural bacterial and/or fungal infections. Consequently, our results indicated that some of tested bacteria including *Bacillus megaterium* strain M-3, *Erwinia rhapontici* strain RK-135 and *Pantoea agglomerans* strain RK-92 can be used as biofertilizer for bean production in sustainable and ecological agricultural systems.

Keywords: Bacteria, *Phaseolus vulgaris,* plant growth promoting bacteria, PGPBs, yield, yield component

Bitki Büyümesini Teşvik Eden Bazı Bakterilerin Kuru Fasulyenin (*Phaseolus vulgaris* L. cv. Aras 98) Verim ve Verim Unsurlarına Etkisi

ÖZET : Leguminosae familyasına ait olan kuru fasulye (*Phaseolus vulgaris* L.) bitkisi besin değeri yüksek bir gıda kaynağıdır. Bitki büyümesini teşvik eden bakteriler (PGPBs) aktif olarak bitkilerde kolonize olan ve bitki büyümesini ve verimini artıran ve hastalıkları baskılayan bir bakteri grubudur. Bu çalışmada on adet PGPB'nin (*Alcaligenes piechaudii* strain RK-136, *Bacillus megaterium* strain M-3, *Bacillus pumilus* strain M-13, *Bacillus subtilis* strain BA-142, *Erwinia rhapontici* strain RK-135, *Burkholderia cepacia* strain RK-277, *Pantoea agglomerans* strain RK-84, RK-123, RK-92, *Pseudomonas putida* strain BA-8, *Serratia liquefaciens* strain RK-102) kuru fasulyenin büyümesi ve verimi üzerine etkililiği değerlendirilmiştir. Ayrıca bakteryel ve fungal bitki patojenlerinin arazi koşullarındaki doğal enfeksiyonlarından kaynaklanan hastalıkların önlenmesinde de PGPR bakterilerinin etkinlikleri araştırılmıştır. Çalışmanın sonunda uygulanan bazı PGPB'lerin fasulyede büyümeyi ve verimi arttırdığı tespit edilmiştir. Ek olarak, bu PGPB'lerin bazıları bakteriyel ya da fungal patojen enfeksiyonlarından kaynaklanan hastalıkları baskılamışlardır. Sonuç olarak, *Bacillus megaterium* strain M-3, *Erwinia rhapontici* strain RK-135 ve *Pantoea agglomerans* strain RK-92'in de dahil olduğu test edilen bazı bakterilerin sürdürülebilir ve ekolojik tarım sistemlerinde kuru fasulye üretiminde biyogübre olarak kullanılabilir.

Anahtar Kelimeler: Bakteri, *Phaseolus vulgaris,* bitki büyümesini teşvik eden bakteriler, PGPB, verim, verim unsurları

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) belongs to the family Leguminosae and is a diverse food resource of high nutritional value (protein, energy, fiber and vitamins and minerals) with broad social acceptance. In Turkey, dry bean's sowing area is 949 280 ha, output is 181 205 ton and yield is 191 kg/da [Anonymous, 2008]. Plant diseases, pests and abiotic stress conditions caused significant yield and quality losses in fresh fruit and vegetable production are one of the major problems of crop loss. The total crop lost by diseases and pest is estimated at about 36% or one third of the potential production of the world (Agrios, 2005).

Intensive farming practices that warrant high yield and quality require extensive use of chemical pesticides and fertilizers, which are costly and create environmental problems. Therefore, more recently there has been a resurgence of interest in environmentally friendly, sustainable and organic agricultural practices (Eşitken et al., 2005). Alternative strategies for disease management include the use of bacteria that show benefic effects on plants and these bacteria are known as plant growth-promoting rhizobacteria (PGPR). PGPRs are free-living soil bacteria that are actually divided into three functional groups: plant growth promoting bacteria (PGPB), biocontrol-PGPB and plant stress homeoregulating bacteria (PSHB), that can either directly or indirectly facilitate the plant growth in optimal, biotic, or abiotic stress conditions (Sgroy et al., 2009). Beneficial effects of PGPRs on plant growth have been attributed to mechanisms such as

production of phytohormones, solubilization of phosphates, suppression of pathogens by producing antibiotics and siderophores or bacterial and fungal antagonistic activity. Bacterial species called PGPR are found in several genera including *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillium*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Rhizobium* and *Serratia* (Rodriguez and Fraga, 1999; Sturz and Nowark, 2000; Niranjiyan et al., 2006).

Uses of bio-fertilizers and bio-pesticides containing beneficial microorganisms instead of synthetic chemicals are known to improve plant growth through the supply of plant nutrients and to suppress diseases caused by plant pathogenic bacteria and/or fungal infections. This may help to sustain environmental health and soil productivity (Egamberdieva, 2009). The positive effects of PGPR are normally divided into two categories: growth promotion and biological control (Kloepper, 1997). Also, certain root-colonizing bacteria can protect plants from soil-borne pathogens when used as inoculants (Keel et al., 1989; Slininger et al., 1996).

The objectives of this research were to determine effects of ten bacterial strains (*Alcaligenes piechaudii* strain RK-136, *Bacillus megaterium* strain M-3, *Bacillus pumilus* strain M-13, *Bacillus subtilis* strain BA-142, *Erwinia rhapontici* strain RK-135, *Burkholderia cepacia* strain RK-277, *Pantoea agglomerans* strain RK-84, RK-123, RK-92, *Pseudomonas putida* strain BA-8, *Serratia liquefaciens* strain RK-102 on growth and yield parameters of *Phaseolus vulgaris* L.. Also, they were investigated for their ability to suppress diseases caused by natural plant pathogenic bacteria and/or fungal infections in field conditions.

MATERIALS AND METHODS

Bacterial strains, culture conditions and media

All of the bacterial strains (*Alcaligenes piechaudii* strain RK-136, *Bacillus megaterium* strain M-3, *Bacillus pumilus* strain M-13, *Bacillus subtilis* strain BA-142, *Erwinia rhapontici* strain RK-135, *Burkholderia cepacia* strain RK-277, *Pantoea agglomerans* strain RK-84, RK-123, RK-92, *Pseudomonas putida* strain BA-8, *Serratia liquefaciens* strain RK-102) were obtained from the culture collection unit in the Department of Plant Protection, Faculty of Agriculture at Atatürk University. These bacterial strains had been isolated from the rot of vegetables (from tomato and lettuce fields) and foliage of pome fruits (from apple and pear orchards) growing in the eastern Anatolia region of Turkey (Kotan et al., 2005). The identity of all bacterial strains used in this study was confirmed according to fatty acid methyl esters (FAME) analysis by using Sherlock Microbial Identification System (Microbial ID, Newark, DE, USA) and BIOLOG System (Kotan et al., 2005). The bacterial cultures were grown on nutrient agar (NA) for routine use, and maintained in Luria Broth (LB) with 15% glycerol at -80 ˚C for long-term storage at the Department of Plant Protection, Faculty of Agriculture in Atatürk University.

Identification of the bacterial strains by microbial identification system (MIS)

Identification of the tested bacterial strains was confirmed by using MIS systems. Preparation and analysis of FAMEs from whole cell fatty acids of bacterial strains were performed according to the method described by the manufacturer`s manual (Sherlock Microbial Identification System version 4.0, MIDI, Inc., Newark, DE, USA). FAMEs were separated by gas chromatography (HP-6890, Hewlett Packard, Palo Alto, CA, USA) with a fused-silica capillary column (25 m x 0.2 mm, with cross-linked 5% phenyl methyl silicone). FAME profiles of each bacterial strain were identified by comparing the commercial databases (TSBA 40) with the MIS software package (Miller, 1982).

Identification of the bacterial strains by BIOLOG system

Identification of the tested bacterial strains was confirmed by using BIOLOG systems. One or two days before the inoculation of Biolog GN2 and GP2 plates, bacterial strains were streaked on TSA or BUG agar plates. Each well of Biolog GN2 or GP2 micro-titer plates was inoculated with 125 µl of the Gram-negative or positive bacterial suspension, respectively, adjusted to the appropriate density (10^8 cfu/ml) and incubated at 27 °C for 24 and 48 h. The development of color was automatically recorded using a microplate reader with a 590-nm wavelength filter. Identification (Biolog Microlog 34.20 database) and ASCII file output of test results, applying the automatic threshold option, were performed using BIOLOG420/Databases/ GN601 and GP601 KID software (Holmes et al., 1994). Carbon source utilization rates of the strains were estimated as percentages.

Media and growth condition for bacteria

Tryptic Soy Agar (TSA, Oxoid) and Tryptic Soy Broth (TSB, Oxoid) medium were used in the experiments. All bacterial isolates were incubated in TSA at 27 °C for 24 h. After incubation period, a single colony was transferred to 500-ml flasks containing TSB, and grown aerobically in the flasks on a rotating shaker (150 rpm) for 48 h at 27 °C (Merck KGaA, Germany). The bacterial suspension was then diluted in sterile distilled water (sdH_2O) to a

final concentration of $1x10^8$ cfu/ml with a turbidimeter.

Nitrogen fixation, phosphate solubilization and siderophore production

Each isolated strain was inoculated in plates containing NFb medium with or without addition of NH4Cl as a unique nitrogen source (Döbereiner et al., 1995). Plates were incubated at 28°C for 7 days, and bacterial growth was observed as qualitative evidence of the atmospheric nitrogen fixation. Phosphate solubilization was measured by the methods of (Döbereiner et al., 1995). Plates containing trypticase soya agar medium supplemented with $Ca_5(PO_4)_3OH$ were inoculated with 1 µl LB pure bacterial culture. Plates were incubated at 30°C and observed daily for 7 days formation of transparent "halos" around each colony. Experiments were performed in triplicate. Siderophore production was determined by the method of (Katznelson and Bose, 1995). For this, 1 µl pure bacterial culture grown in LB was inoculated in plates containing agar Chrome Azurol S (CAS). Plates were incubated at 30°C and observed daily for orange color formation around each colony for up to 4 days. Experiments were performed in triplicate.

Field studies

The field trials were conducted on the farms of the eastern Anatolia Agricultural Research Institute at Erzurum in Turkey during 2010. The experimental area was located 39^o 55^1 N and 41^o 61^1 E at an altitude of 1800 m. Average temperature and total precipitation at the study site are 5.7 ^{o}C and 425 mm, respectively. PGPBs were tested for their effectiveness on yield and yield components of dry beans. Also, they were investigated for their ability to suppress diseases caused by natural plant pathogenic bacteria and/or fungal infections in field conditions. Aras 98 dry bean variety of common bean (*Phaseolus vulgaris* L.) was used in this study. In this study, a total of eleven plots (one control and ten treatments plots) were formed and plants were handed tinning to be 25 dry bean plants in each plot. The beans were planted in May 10. Planted seeds in the bacterial treatments plots were not applied any pesticides or fertilizers. However, 3 kg N/da and 6 kg P_2O_5/da were applied at planting in the control treatments. Bacterial suspension containing $1x10^8$ cfu/ml by pipetting (5 ml) was injected into each plant root zone when plant heights were 15 cm. Each plot was divided with dike each other. Two days after bacterial applications, each plot was immediately irrigated, and irrigation was repeated in period when plants need to water. Hand-weeding was done in the treatments plots after irrigation. The beans were harvested in October 1. In vegetation period of 2010 when experiment was conducted, average temperature was 18.27 ^{o}C and relative humidity was 56.88% in the growth period of dry bean during the months May and October. Observations were taken from 5 plants randomly selected in each treatments plots and done statistical analysis.

Any artificial inoculation wasn't done in this study, plant observed symptoms in each plot were counted as percent of diseases plant 7 weeks after planting.

Statistical analysis

In order to determine significant differences in activities among the bacterial treatments, analysis of variance (ANOVA) was carried out using the JUMP statistical software package. The results showed significant differences at the $P<0.01$ level.

RESULTS

The MIS and BIOLOG identification results of the bacterial strains, their similarity index (SIM), carbon source utilization rates (%), their nitrogen fixation, phosphate solubilization and siderophore production results are shown in Table 1. According to the MIS/BIOLOG results, bacterial strains were identified as *Bacillus megaterium*/*Bacillus megaterium* (strain M3), *Pantoea agglomerans*/*Pantoea agglomerans* (strain RK-84), *Alcaligenes piechaudii*/*Pantoea agglomerans* (strain RK-136), *Erwinia rhapontici/Raoultella terrigena* (strain RK-135), *Pantoea agglomerans*/*Raoultella terrigena* (strain RK-123), *Bacillus pumilus*/*Bacillus pumilus* (strain M-13), *Pseudomonas putida*/*Pseudomonas putida* (strain BA8), *Bacillus subtilis*/ *Bacillus subtilis* (strain BA-142), *Pantoea agglomerans*/*Pantoea agglomerans* (strain RK-92) and *Serratia liquefaciens*/*Pantoea agglomerans* (strain RK-102). Carbon source utilization rates of the tested bacterial strains changed from 8.42 to 38.90%. All isolates showed capacity to grow in nitrogen-free conditions and to solubilize phosphate. However, they were not able to produce siderophores.

The effects of bacteria on growth and yield components of dry bean were given in Table 2. The investigated characteristics of dry bean were grouped by LSD Multiple Comparison. There were some significant differences ($P < 0.01$) among the treatments in terms of plant height and first pod height, and seed number per pod was important in $P<0.05$. But, branch number and pod number were not important. It was determined that the plot in area where the research was made was homogenous in every respect. Hence statistical analyses were not done for 100-seed weight, yield and diseases rate.

Table 1: MIS and BIOLOG identification results of bacterial strains, their similarity index (SIM), carbon source utilization rates (%), nitrogen fixation, phosphate solubilization and siderophore production

Strain	MIS results	SIM	BIOLOG results	SIM	Isolated from	CSUR (%)	SP	NF	PS
M-3	*Bacillus megaterium*	0.741	*Bacillus megaterium*	0.57	rice	70.67	-	K+	+
RK-84	*Pantoea agglomerans*	0.718	*Pantoea agglomerans*	0.56	apple	58.94	-	+	+
RK-136	*Alcaligenes piechaudii*	0.409	*Pantoea agglomerans*	0.42	apple	51.57	-	+	+
RK-135	*Erwinia rhapontici*	0.867	*Raoultella terrigena*	0.55	apple	70.52	-	+	+
RK-123	*Pantoea agglomerans*	0.471	*Raoultella terrigena*	0.45	apple	73.68	-	+	-
M-13	*Bacillus pumilus*	0.820	*Bacillus pumilus*	0.42	pepper	68.78	-	+	+
BA-8	*Pseudomonas putida*	0.320	*Pseudomonas putida*	0.54	soil	70.42	-	Z+	+
BA-142	*Bacillus subtilis*	0.724	*Bacillus subtilis*	0.56	tomato	75.78	-	K+	K+
RK-92	*Pantoea agglomerans*	0.889	*Pantoea agglomerans*	0.58	pear	50.52	-	+	K+
RK-102	*Serratia liquefaciens*	0.572	*Pantoea agglomerans*	0.44	apple	55.78	-	+	K+

CSUR: Carbon source utilization rates; SP: Siderophore production; NF: Nitrogen Fixation; PS: Phosphate solubilization. - : negative reaction, +: positive reaction

Table 2: The effects of PGPBs applications on growth and yield components of dry bean

Applications	Plant height (cm)	Branch number (unit)	Pod number (unit)	Seed number per plant (unit)	First pod height (cm)	100-seed weight (g)	Yield (g/25 plant)	Percent of diseases plant (%)
B. megaterium strain M-3	59.0 a	2.80	27.6	71.4 a	12.2 cd	35.0	625.8	0
P. agglomerans strain RK-84	48.0 c	2.80	32.2	65.0 ab	15.6 a	42.7	696.9	3.45
A. piechaudii strain RK-136	51.8 bc	2.80	22.6	49.0 b-d	12.6 b-d	41.7	513.8	3.85
E. rhapontici strain RK-135	48.0 c	2.80	25.2	51.8 a-d	12.0 cd	41.7	541.0	0
P. agglomerans strain RK-123	50.4 bc	2.80	24.0	47.0 b-d	12.2 cd	44.0	515.0	0
B. pumilus strain M-13	47.4 c	3.20	33.2	61.6 a-c	11.8 d	42.3	655.4	7.69
P. putida strain BA-8	52.6 bc	3.20	27.0	55.0 a-c	14.0 a-c	41.7	576.4	3.85
B. subtilis strain BA-142	54.6 ab	2.60	22.0	43.4 cd	13.6 a-d	41.7	454.4	3.45
P. agglomerans strain RK-92	55.6 ab	3.20	28.6	48.4 b-d	13.2 b-d	38.7	466.3	3.57
S. liquefaciens strain RK-102	47.6 c	2.00	20.6	34.4 d	11.8 d	41.0	356.6	3.70
Control treatment	50.8 bc	3.00	29.2	57.0 a-c	14.4 ab	42.7	606.5	10.0
Statistical analyses	** X: 51.4 CV:0.09 LSD:6.03	Ns X: 2.8	Ns X: 26.6	* X: 53.1 CV: 0.3 LSD: 20.3	** X: 13.0 CV: 0.13 LSD:2.09			

*, ** Within a row, treatment values followed by different letters are statistically different from each other with significant of ($P < 0.01$) for plant height and first pod height, and ($P < 0.05$) for seed number per pod.

B. megaterium strain M-3 was more effective on plant height (59.0 cm) than other bacteria, and this increase was statistically significant. *P. agglomerans* strain RK-92 (55.6 cm), *B. subtilis* strain BA-142 (54.6 cm), *P. putida* strain BA-8 (52.6 cm) and *A. piechaudii* strain RK-136 (51.8 cm) increased plant height compared to the control. The effect of applied bacteria on branch number was not statistically significant. But *B. pumilus* strain M-13, *P. putida* strain BA-8 and *P. agglomerans* strain RK-92 with 3.20 unit caused more branch number than the control treatment. *B. pumilus* strain M-13 (33.2 unit) and *P. agglomerans* strain RK-84 (32.2 unit) increased pod number according to the control treatment (29.2 unit), although these increases were not statistically significant.

In this study, when examined the effect of the bacteria used on seed number per plant, *B. megaterium* strain M-3 (71.4 unit) was the most effective on seed number plant, *P. agglomerans* strain RK-84 (65 unit) and *B. pumilus* strain M-13 (61.6 unit) were more effective on seed number per plant than control treatment (57.0 unit). *S. liquefaciens* strain RK-102 (34.4 unit) among the

bacteria applied was the less effect on seed number plant. Control treatment (57.0 unit) and *P. putida* strain BA-8 (55.0 unit) showed seed number per plant over the average of the seed number per plant (53.1 unit).

P. agglomerans strain RK-84 (15.6 cm) was the most effective on first pod height which is an important criterion for machine-harvested and only bacteria was over control treatment (14.4 cm). In this study, it was determined that the first pod heights of *P. putida* strain BA-8 (14.0 cm), *B. subtilis* strain BA-142 (13.6 cm), *P. agglomerans* strain RK-92 (13.2 cm) and control treatment were higher than average first pod height (13.0 cm).

In this study, it was determined that *P. agglomerans* strain RK-123 (44.0 g) had the greatest impact on 100-seed weight; *P. agglomerans* strain RK-84 (42.7 g) received the same value.

It was determined that the effect of *P. agglomerans* strain RK-84 (696.9 g/25 plants), *B. pumilus* strain M-13 (655.4 g/25 plants), *B. megaterium* strain M-3 (625.8 g/25 plants) increased the yield according to control treatment (606.5 g/25 plants). In this study, *P. putida* strain BA-8 (576.4 g/25 plants) was over the average of the yield (546.2 g/25 plants).

Also, PGPBs were investigated for their ability to suppress diseases caused by natural plant pathogenic bacteria and/or fungal infections in field conditions. *B. megaterium* strain M-3, *E. rhapontici* strain RK-135 and *P. agglomerans* strain RK-123 suppressed the disease of bean caused by natural infections. It was found that there was only a low rate of Anthracnose disease in observations of the other applications. Natural infections caused bacterial and fungal pathogens in this study conducted year did not commonly observed disease on beans. For this reason, the effects of these bacterial strains on disease severity should be investigated later in detail.

DISCUSSION AND CONCLUSIONS

Our results indicated that some of the selected PGPRs are able to promote bean growth and yield and to suppress the disease of bean caused by natural infections. Similar results were reported in some of the previous studies showing that inoculation influenced early plant and root development, plant and root dry weight, grain yield, and the N-uptake efficiency of plants (Schwyn and Neilands, 1987; Dobbelaere et al., 2002; Çakmakçı et al., 1999; Çakmakçı et al., 2001; Çakmakçı et al., 2006). P-solubilizing and N_2-fixing bacteria improve the N and P nutrition of plants and thus stimulate plant growth and/or enzyme activities (Çakmakçı et al., 2006). The positive effect of the some tested strains on bean can be explained by their N_2 fixation ability, P-solubilizing ability, IAA and cytokinin production. It is well known that PGPR strains that produce plant hormones such as auxins and cytokinins can stimulate plant cell elongation or cell division, and/or change bacterial 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (Çakmakçı et al., 2007), which prevents the production of the plant growth-inhibiting hormone, ethylene (Patten and Glick, 2002; Penrose et al., 2001).

In previous studies, it was reported that application of *B. subtilis* strain BA 142 and *B. megaterium* strain M-3 strains used in the present study may stimulate yield and quality parameters in some plants such as sugar beet, barley (Çakmakçı et al., 1999), apricot (Banerjee et al., 2010), raspberry (Eşitken et al., 2002), and apple (Orhan et al., 2006). In addition, they were found to be capable of producing IAA and cytokinin, have N_2-fixing capacity, and *B. megaterium* strain M-3 has phosphate-solubilizing capacity (Orhan et al., 2007) and antimicrobial activity (Aslantaş et al., 2007). Our results are also in general agreement with previously reported data (Kotan et al., 1999; Lucas Garc'ıal et al., 2004; joseph et al., 2007; Khan and Patel, 2007; Penrase et al., 2001). Mineral fertilizers have long been used as the quickest way of improving crop productivity. However, due to their cost and associated environmental problems, continues use of fertilizers has resulted in a search for alternative approaches such as the use of plant growth promoting rhizobacteria. As a result of all experiments, some of the applications of PGPR increased growth and yield parameters. Consequently, our results indicated that some of tested bacteria including *Bacillus megaterium* strain M-3, *Erwinia rhapontici* strain RK-135 and *Pantoea agglomerans* strain RK-92 can be used as biofertilizer for bean production in sustainable and ecological agricultural systems.

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