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RESEARCH ARTICLE

Effect of Plant Growth-Promoting Bacteria (PGPB) on the Development of Pea crop (*Pisum sativum* L.)

Esin Dadasoglu

Atatürk University, Faculty of Agriculture, Department of Field Crops, Erzurum/Türkiye

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ABSTRACT

Microorganisms are of great importance in agriculture in terms of plant nutrients by reducing the need for chemical fertilization. In recent years, plant growth-promoting bacteria (PGPB) have been widely used as biological fertilizers (BF) in agriculture. This study was conducted to determine the effect of plant growth-promoting bacteria on the development of pea plants. Firstly the phosphate solubilization and nitrogen fixation potentials of the bacteria used in this study were determined. In the study, the effects of 4 different combinations, F1 [(*Rhizobium* sp. (FR-13) and *Pseudomonas alcaligenes* (FDG121)], F2 [(*Pseudomonas fluorescens* biotype F (FDG-7), *Rhizobium* sp. (FR-18) and *Bacillus-megaterium*-GC subgroup B(FDG-134)], F3 [*Arthrobacter oxydans* (FDG-72), *Bacillus-megaterium*-GC subgroup B (FDG-146), *Rhizobium* sp. (FR-11)] and F4 [*Acinetobacter genospecies* 9 (FDG-116), *Brevibacillus agri* (FDG-118), *Methylobacterium zatmanii* (FDG-123) and *Bacillus-megaterium*-GC subgroup A (FDG-153)] were investigated. Formulations made with bacteria that were found to be the best in terms of the properties specified among these strains were tested against pea plants under greenhouse conditions and their effects on the plant's total fresh and dry weight were investigated. The study was set up to have 3 replications. As a result of the statistical analysis made with the data obtained, the formulations used compared to the control; F2, F3 and F1 applications were important in total fresh weight, respectively, and F2 and F3 applications were important in total dry weight. As a result, these 3 formulations are especially effective on the yield of pea plants and can be used as potential biofertilizers.

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1. Introduction

Peas are farmed in about 84 countries all over the world. The pea crop (*Pisum sativum* L.) is a valuable leguminous crop and is a rich source of protein (Poblaciones & Rengel, 2016), phenolics, tannins and flavonoids, and antioxidants (Singh et al., 2017). Pea seeds have massive nutritional aspects, including high protein, carbohydrate, vitamin, phosphorus, and calcium contents (Maharjan et al., 2019; Janusauskaite, 2023). Pea crops are considered critical crops in sustainable agriculture (Powers & Thavarajah, 2019). This high predominance of peas is connected to their extraordinary growth, yield, and importance for human nutrition livestock.

Microorganisms are present near every living thing on the planet since they are ubiquitous in nature. When microorganisms connect with rhizosphere soil as biofertilizers, they colonize it and increase the take-up of plant nutrients (Demir et al., 2023). Nitrogen, phosphorus, and potassium are important macro-elements and are restricted nutrients for the expansion and evolution of plants. Utilizing microbial biofertilizers is a more eco-friendly strategy as they are ecologically gentler to plants and give plants the benefits of all the soil nutrients (Abeed et al., 2022).

Correspondence
E-mail address: edadasoglu@atauni.edu.tr

Bacteria that are free-living, promote plant growth, and are, used in biological control or as biological fertilizers (BF) are called plant growth promoting bacteria (PGPB). PGPB are able to exert a beneficial effect upon plant growth, N_2 fixing and P solubilizing and play a significant role as PGPB in the biofertilization of crops. These microorganisms are found in several genera including *Acinetobacter, Alcaligenes, Arthrobacter, Azospirillium, Azotobacter, Bacillus, Burkholderia, Enterobacter, Flavobacterium, Rhizobium and Serratia*. Although the mechanisms of PGPB are not fully understood, are thought to include: the ability to produce plant hormones; such as auxins, cytokinins and gibberellins, asymbiotic N_2 fixation, solubilization of inorganic phosphate and mineralization of organic phosphate and mineralization of organic phosphate and/or other nutrients and antagonism against phytopathogenic microorganisms by production of siderophores the synthesis of antibiotics enzymes and/or fungicidal compounds, and competition with detrimental microorganisms (Burdman et al., 2000; Bloemberg & Lugtenberg, 2001; Vessey, 2003; Antoun & Prevost, 2006; Çakmakçi et al., 2006; Fuentes-Ramirez & Caballero-Mellado, 2006; Niranjan Raj et al., 2006).

2. Materials and Methods

2.1. Isolation and Stocking of Bacterial Isolates

Soil samples were weighed 1 gr and bean nodules were sterilized, cut, transferred to tubes containing 2 ml of sterile water and left to mix in a hematological shaker for about 2 hours. Serial dilutions were then prepared from the solution in the tube with a sterile pipette. Nutrient Agar (NA) medium was used as isolation medium. Cultures were incubated at 25-30 °C for 24-72 hours and transferred to new media from each colony with different characters, especially those with dense growth, as much as possible from the formed colonies (Klement et al., 1990).

By giving a separate code number to each isolate, information about the isolation (location of isolation, date, etc.) was recorded stored at -80 °C in stock media containing 30% glycerol and Lauria Broth (LB) to be used in diagnosis and characterization processes and other studies.

2.2. Determination of Phosphate Solubizing Potential of Bacteria

24-hour bacterial cultures grown on nutrient agar were suspended in sdH_2O and their density was adjusted to 10^8 CFU/ml. Tubes containing 5 ml of NBRIP-BPB (The National Botanical Research Institute's phosphate-bromophenol blue) in each suspension were inoculated. After a 15-day incubation period, the phosphate solubilization ability of bacteria that showed discoloration in the medium was evaluated as positive (Metha & Nautiyal, 2001). In addition, the potential of the isolates to dissolve Mazıdağı Rock Phosphate was tested by adding Mazıdağı Rock Phosphate to the $Ca_3(PO_4)_2$ medium contained in NBRIP (Nautiyal, 1997).

2.3. Detection of Nitrogen Fixation of Bacteria

Bacteria from stock culture were drawn onto nitrogen-free medium Burk's and Ashby's solid medium (N-Free Solid Malate Sucrose Medium) using the scatter plate method and allowed to grow in an incubator set at 27 °C for 7-10 days. Bacterial growth in the medium was evaluated as nitrogen fixation positive (Wilson & Knight, 1952).

2.4. Testing Different Combinations of PGPB Strains in Greenhouse Conditions

Different combinations of 2, 3 and 4 strains have been created from strains that have good plant growth promoting properties. Pea applications were made for bacterial strains whose biofertilizer properties will be determined. For each strain, 100 ml of liquid medium was prepared and bacteria were grown in this medium on a shaker for 24 hours. Then, the bacterial solutions were adjusted to 10^8 CFU/ml and 9 seeds were added into it. These solutions containing seeds were mixed in a shaker for 2 hours, and finally, after the seeds were filtered and dried, 3 seeds were planted in each pot. Dry and fresh weights of the planted seeds were determined. Sterile NB medium, used to dilute the bacterial solution, was used as a negative control (Angın & Dadaşoğlu, 2022).

2.5. Statistical Analyses

The results obtained from the experiments were analyzed in the JMP (Version 4.0) statistical program and their arithmetic means and standard deviations were calculated. Duncan ($p \leq 0.01$) test was performed to determine the significance level of the differences between the applications.

3. Results and Discussion

In the study, 57 different bacterial strains were isolated from soil samples and nodules of bean plants in different areas in Erzurum province, and among these bacteria, 12 strains with the best nitrogen-fixing and phosphate-dissolving properties were used Table 1. Double, triple and quadruple formulations were created among these bacteria and the experiments were designed with 3 replications. In the study, the effects of the treatments on the total dry weight and total fresh weight of peas are given in Figure 1 and Table 2. According to the results obtained, in terms of total wet weight, an increase of 51%, 48% and 32% was observed in the F2, F3 and F1 formulations, respectively, compared to the control applications. However, the effect of the F4 formulation on total wet weight compared to the control was found to be statistically insignificant. Similarly, in terms of total dry weight, F2, F3 and F1 formulations increased by 64%, 57% and 19%, respectively, when compared to the control applications. However, according to statistical evaluation, the effect of F1 and F4

formulation applications on dry weight was found to be insignificant. In the study, observations were also made in terms of stem fresh weight, root fresh weight, stem dry weight and root dry weight, and the results are given in Table 2. F2, F3 and F1 formulations were found to be statistically significant in terms of stem fresh weight and root fresh weight, but F4 formulation was found to be statistically insignificant when compared to the control treatments. F2 and F3 formulations were found to be statistically significant in terms of stem dry weight and root dry weight, but F1 and F4 formulations were found to be statistically insignificant when compared to the control applications.

There are many studies in the world on the development of vegetables by bacteria that promote plant growth that PGPB, i.e., potato, tomato, onion, pepper, beans, and lettuce (Zahran et al., 2020; Sun et al., 2022; de Andrade et al., 2023). Although previous researchers reported that plant growth-promoting bacteria vary in diverse agriculture crops and even in different varieties of the same crops (Ummara et al., 2022), only a few research papers have studied their effects on pea. Shabaan et al. (2021) found that treating pea seeds with PGP bacteria improves plant height, shoot and root dry weights, and seed weight under heavy metal stress. In a study conducted with *Rhizobium* sp. inoculation of seeds and leaf application, successful results were obtained in pea production, and similar results were obtained in this study by *Rhizobium* sp. bacteria used in the formulations. In a study investigating the effects of 42 different PGPR strains on the development of peas and chickpeas, it was determined that Pseudomonas fluorescens and Bacillus cereus species increased the yield of both plants (Uslu, 2006). In this study, the most effective formulation, F2, includes *Pseudomonas fluorescens* strain and is parallel to the studies conducted on this species.

According to all these results; It was observed that especially F2 and F3 formulations provided a significant increase in the development of pea plants compared to the control. For this reason, it is thought that both formulations have the potential to be used as alternative biofertilizers to chemical fertilizers in pea cultivation.

Table 1. Bacterial strains and some plant growth parameters used in the study.

Strain No	Strain name	Host	Nitrojen fixing	Phosphat Solubilizing
FDG-7	*Pseudomonas fluorescens* biotype F	Soil	+	+
FDG-72	*Arthrobacter oxydans*	Soil	+	K^+
FDG-116	*Acinetobacter genospecies* 9	Soil	K+	+
FDG-118	*Brevibacillus agri*	Soil	K+	+
FDG-121	*Pseudomonas alcaligenes*	Soil	K+	+
FDG-123	*Methylobacterium zatmanii*	Soil	K+	+
FDG-134	*Bacillus-megaterium*-GC subgroup B	Soil	K+	+
FDG-146	*Bacillus-megaterium*-GC subgroup B	Soil	+	+
FDG-153	*Bacillus-megaterium*-GC subgroup A	Soil	K+	+
FR-11	*Rhizobium* sp.	Nodules of bean	K+	K+
FR-13	*Rhizobium* sp.	Nodules of bean	K+	K+
FR-18	*Rhizobium* sp.	Nodules of bean	K+	K+

+: Positive K^+: Strong positive.

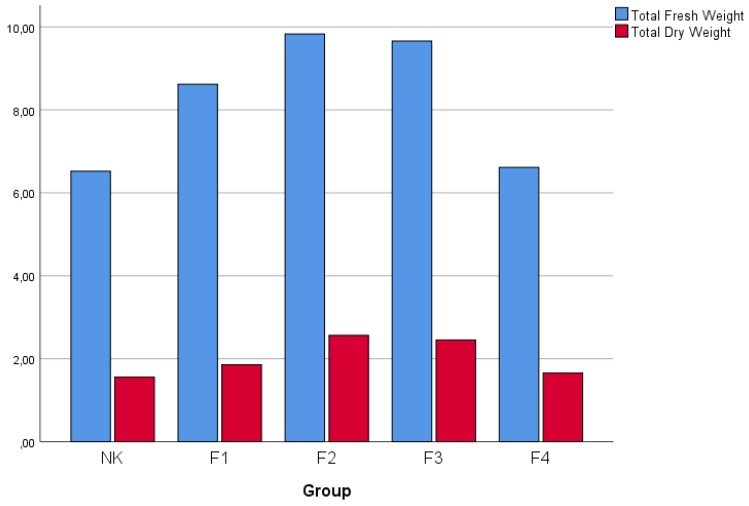

Figure 1. Total fresh weight and total dry weight indicators of the formulations compared to the negative control.

Table 2. Statistical results obtained from bacterial formulation applications.

					95% Confidence Interval for Mean			
		Mean	**Differences**	**Std. Error**	**Lower Bound**	**Upper Bound**	**Minimum**	**Maximum**
Total fresh weight	**NK**	6.521	B	0.436	4.643	8.399	5.833	7.330
	F1	8.618	A	0.439	6.731	10.506	7.833	9.350
	F2	9.876	A	0.212	8.962	10.790	9.488	10.220
	F3	9.662	A	0.585	7.143	12.181	8.735	10.745
	F4	6.614	B	0.311	5.277	7.952	6.107	7.179
Stem fresh weight	**NK**	5.324	B	0.294	4.058	6.589	5.000	5.911
	F1	6.790	A	0.239	5.763	7.818	6.313	7.045
	F2	7.033	A	0.135	6.453	7.613	6.788	7.253
	F3	7.322	A	0.429	5.478	9.166	6.736	8.157
	F4	5.331	B	0.116	4.834	5.828	5.119	5.517
Root fresh weight	**NK**	1.196	C	0.185	0.402	1.991	0.830	1.419
	F1	1.828	BC	0.256	0.725	2.931	1.520	2.337
	F2	2.843	A	0.078	2.509	3.177	2.700	2.967
	F3	2.135	AB	0.149	1.492	2.777	1.972	2.433
	F4	1.283	C	0.397	-0.426	2.993	0.750	2.060
Total dry weight	**NK**	1.555	C	0.291	0.301	2.809	1.238	2.137
	F1	1.854	ABC	0.200	0.992	2.716	1.477	2.160
	F2	2.563	A	0.189	1.752	3.374	2.296	2.927
	F3	2.453	AB	0.337	1.004	3.902	1.995	3.110
	F4	1.654	BC	0.175	0.902	2.405	1.441	2.000
Stem dry weight	**NK**	1.086	B	0.233	0.085	2.086	0.830	1.550
	F1	1.320	AB	0.167	0.603	2.037	1.000	1.560
	F2	1.761	A	0.157	1.084	2.439	1.537	2.065
	F3	1.650	AB	0.200	0.788	2.511	1.345	2.027
	F4	1.204	AB	0.098	0.782	1.626	1.100	1.400
Root dry weight	**NK**	0.469	B	0.059	0.216	0.723	0.408	0.587
	F1	0.534	B	0.036	0.380	0.688	0.477	0.600
	F2	0.802	A	0.031	0.668	0.935	0.759	0.862
	F3	0.804	A	0.140	0.202	1.406	0.650	1.083
	F4	0.450	B	0.078	0.116	0.784	0.341	0.600

Very significant at $p< 0.01$ level, insignificant at $p< 0.05$ level. Differences between numbers with the same letter in the same column are insignificant.

Conflict of Interest

The author has no conflict of interest to declare.

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