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SEARCHING POSSIBLE PGPR FROM NATURAL ECOSYSTEM

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Abstract

The utilization of Plant Growing Promoting Bacteria (PGPR) holds significant importance in agricultural systems, especially as a biofertilizer. This study aimed to select effective PGPR for maize to improve yield and nutrient content in a greenhouse pot experiment. Forty-five bacteria were isolated from three different ecosystems as forest, organic farm site, and pasture. The results indicated that PGPR application increased macro nutrients ranging from 12.5% to 50% compared to the control. With the PGPR isolated from forest application, the micronutrient content of Fe, Zn, Mn, and Cu in maize increased around 100%, 20%, 60%, and 100%, respectively. In terms of physiological parameters such as fresh and dry biomass weight, plant height and stem diameter in maize plants were statistically significant than the control treatment. The results proved that PGPR isolated from various ecosystem applications had a more stimulating impact on macro micronutrient content and physiological parameters in maize plants than non-PGPR applications. In general, organic farming sites would be the more promising starting point for PGPR isolation.

***Key words**: PGPR, ecosystem, nutrient, yield, maize.*

INTRODUCTION

Mazie is a widely cultivated crop plant around the world. One of the most widely utilized grains in the world, maize is used for a variety of purposes, including feed and biofuel. The top five countries producing maize worldwide are the United States of America, China, Brazil, Argentine, and Ukraine, in that order (FAO, 2023). While Turkey's maize cultivation areas were in the range of 6.6-5.9 million hectares between 2014-2018, it increased by 8% compared to the previous period and reached 6.4 million hectares in 2019. The production of maize increased from 6.5 million tons in 2020-2021 to 6.75 million tons in 2021-2022, an 3.84% rise (BÜGEM, 2022). However, one of the biggest costs in maize production is chemical fertilizer. Chemical fertilizers not only cost a large amount of money, but they also have a negative impact on the environment and human health (Sigua et al., 2005). Promoting the use of biofertilizers to reduce the usage of chemical fertilizer applications is the recent option for sustainable maize cultivation.

The rhizosphere is home to a group of bacteria known as plant growth-promoting rhizobacteria (PGPR) (Rodriguez & Fraga, 1999). The phrase ‘plant growth promoting bacteria’ refers to bacteria that colonize the roots of plants (rhizosphere) that enhance plant growth. Rhizosphere is the soil environment where the plant root is accessible and is a zone of maximum microbial activity resulting in a confined nutrient pool in which essential macro- and micronutrients are extracted. PGPR plays an important role in enhancing plant growth through a wide variety of mechanisms. Some examples of these mechanisms are nitrogen fixation (Montanez et al., 2009; Arruda et al., 2013), regulation of plant growth as well as phosphorus solubilisation (Perez et al., 2007), the ability to produce phytohormones Egamberdiyeva (2007) and the production of siderophores (Ahmad et al., 2006). Since PGPR has been used as one of the indicators of the quality of the soil, it could be attractive to evaluate some macro and micronutrient contents in maize to isolate bacteria from the soil in the different ecosystems. Interests in the beneficial rhizobacteria associated with the crops have increased recently and several studies clearly showed the positive and beneficial effects of PGPR on the growth and yield of different crops, especially maize in different environment under variable ecological conditions. Some indigenous bacteria such as *Bacillus* spp. (Zakry et al., 2012) and *Pseudomonas* spp. (Piromyou et al., 2011) from the rhizosphere has been qualified as PGPR to maize through phosphate solubilization and phytohormone production. Waday et al. (2022) reported that fresh weight (1.4 g), dry weight (0.45 g), and length (9.9 cm) of shoot maize plant inoculated with bacterial strain (JEC4) was significantly higher than control (0.6 g, 0.1 g, 6.8 cm). Similarly, Pande et al. (2017) studied the impact of some phosphate solubilizing bacteria on the growth of maize in a greenhouse pot experiment and found three isolated species showed a significant stimulating effect on maize growth in shoot height, fresh and dry weight compared to the control. The impact of the PGPR on maize production with six different bacteria in non-sterile and sterile pot experiments was reported by Gholami et al. (2009). As a result, non-sterile soil was found to have a greater stimulating effect on plant development than sterile soils. Previous research has assessed the impact of some PGPR bacteria on the growth and yield of several crops. The researchers have concentrated their efforts in recent decades on gathering PGPR from the intensive agricultural farm site. It was not fully determined how PGPR isolated from different ecosystems will affect growth and the number of macro-micro-nutrients. The main objective of this study was to isolate plant growth-promoting bacteria from different ecosystems such as forests, pastures, and organic farm sites using morphological characteristic features to increase yield and macro-micro nutrient content in maize plants.

MATERIALS AND METHODS

Soil sample

The soil for the pot experiment was transferred from International Agricultural Research and Traning Center's (IARTC) farm site. Soil samples were analyzed for pH, EC, lime and organic matter contents, and phosphorus and potassium concentrations. Results are shown in Table 1. Considering the soil analysis report, 150 ppm N fertilizer was applied using ammonium sulfate. Phosphorus and potassium were not applied due to their adequate abundance in the soil (Table 1). Through the experiment, herbicides or pesticides were not required. Maize (Tarex Albayrak) was sowed on 22 July 2022, 69 days after the seedling, and on 29 September 2022 plants were harvested. The study was arranged as a completely randomized design (CRD) with three replicate pots per treatment. Of the 45 bacteria isolated from different ecosystems, in particular, 17 isolates were from the forest, 16 isolate from organic farm sites, and 12 isolate from pasture. One positive and one negative control were also added to the experiment.

Bacteria application was done 10 days after corn planting. For each isolate, 1 ml of PGPR bacteria solutions was applied to the surface of the soil, just after irrigation was done. As a positive control, 1 ml sterile TSB medium solution was applied.

Sample collection and isolation of bacteria

The soil samples were collected from three different ecosystems as pastures, organic farm sites, and forest land around the West-Aegean region in Türkiye. Two composite samples were taken from pasture and five composite samples from the forest and organic farm site from 0 to 20 cm depth and each sample was mixed thoroughly. In total 13 samples were transferred to the laboratory to ensure uniformity and stored at 4°C before use. The serial dilution method was used to isolate PGPR. The first step in the dilution process was the addition of 10 g of soil to 90 ml of the extraction solution (0.85% saline solution) resulted in a 10^{-1} weight by volume dilution and repeated five more times (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}). Starting from 10^{-3} dilution, 1 ml of soil dilution was transferred to replicate agar plates (Tryptic Soy Broth Agar). Next, agar plates inoculated were placed in the incubator at 28 °C for 24 hours. After the incubation process, different bacterial isolates with distinct colony morphology were selected from each of three (10^{-3}, 10^{-4}, 10^{-5}) dilutions, and pure cultures were obtained by streaking on TSB agar plates. Forty-five bacterial isolates were chosen randomly considering the different sizes and shapes of the colonies for the greenhouse experiment. To measure plants' physiological parameters, before harvesting plants' height and diameters (2 dimensions) were measured. Then to determine biomass fresh weight (BFW), plants were harvested from each pot and weighed. Thereafter, samples were placed in an oven (65°C) until there was no change in the biomass weight. To determine macro and micronutrient analysis, oven-dried maize samples were milled with the grinder. Then sample masses of 400 mg substrates were microwave-assisted digested using 9 ml of 1 molar HNO_3 and 1 ml of perchloric acid. The program was performed in three steps: (1) 25 min to reach from 25°C to 180°C, (2) 15 min to hold 180°C, and (3) 15 min to cool down to room temperature. After cooling, the vessels were opened and transferred to a 50 mL volumetric flask. The final volume was made up to 50 mL with distilled water. P, K, Ca, Mg, B, Fe, Cu, Zn, and Mn, were determined in these solutions.

Table 1. Some of the soil properties

pH	EC ($dS \cdot m^{-1}$)	Lime (%)	O.M. Content (%)	Phosphorus ($kg\ ha^{-1}$)	Potassium ($kg\ ha^{-1}$)
7.88	630	4	1.5	138	1360
alkaline		Low	Low	Medium	High

Statistical analysis

A one-way analysis of variance (ANOVA) was performed to evaluate the effect of the PGPR application. JMP software version pro16 was used to analyze the experimental data. Tukey Honestly Significant Difference (HSD) test at the 5% level of significance ($p \leq 0.05$) was used for the comparisons of means.

RESULTS AND DISCUSSIONS

Macronutrients

In this study, average phosphorus (P) nutrients with PGPR treatments were as follows: isolated from pasture (0.189%), and organic farm site (0.186%) (Table 2). These Phosphorus nutrient rates were significantly higher than both positive (0.141%) and negative (0.144%) control treatments.

The phosphorus nutrient concentration was 0.161% higher in forest-isolated bacteria treatment which was significantly higher than the negative control (0.144%). This is an agreement with Kumar et al. (2014) who conducted an experiment on the influence of the PGPR on growth, yield and nutrient content in wheat. Phosphorus nutrient content in grain and straw with PGPR treatment was 1.81-fold and 1.72-fold higher than non-PGPR treatment. The second sampling point in the pasture and the fourth sampling point in the organic farm site had the highest average values of phosphorus nutrients in maize plants, respectively.

Additionally, PGPR treatments isolated from pasture in potassium nutrient content (3.82%) were the highest value compared to all other treatments. No statistical differences were observed for potassium nutrient content as inoculated by bacteria from the forest, organic farm site, and both negative and positive control. The first sampling points in the pasture had the highest average values of potassium nutrients in maize plants.

PGPR treatments isolated from all ecosystems in calcium (Ca) nutrient content in maize was significantly higher than the both positive and negative control treatment. Whereas the first sampling point in the organic farm site had the highest average values of calcium nutrients in maize plants, the first sampling point in the pasture was the lowest. While PGPR treatments isolated from organic farm sites and pasture in Mg nutrients content in maize were significantly higher than the positive control, no statistical differences were observed from the forest (0.205%) both negative (0.200%) and positive control (0.170%). The third sampling point in the organic farm site had the highest average values of magnesium nutrients in maize plants.

Our results showed that macronutrient content such as P, K, and Mg in maize under PGPR treatments isolated from different ecosystems was significantly higher than in negative control treatments. Similarly (Karthikeyan et al., 2010) used some PGPR such as *Azotobacter*, *Bacillus* and *Pseudomonas* separately or in combination to assess their impact on *Catharantus roseus*.

Table 2. Selected macronutrient contents

Ecosystem	Sampling point	Isolate	P (%)		K (%)		Ca (%)		Mg (%)	
Forest	1	1	0.164		3.44		0.437		0.230	
		2	0.166		3.54		0.412		0.212	
		3	0.158		3.51		0.441		0.212	
	2	1	0.161		3.58		0.483		0.213	
		2	0.150		3.32		0.444		0.196	
		3	0.145		3.48		0.441		0.200	
	3	1	0.171		3.15		0.524		0.218	
		2	0.158		3.43		0.488		0.201	
		3	0.148		2.98		0.461		0.186	
	4	1	0.185		3.79		0.392		0.229	
		2	0.170		3.25		0.461		0.180	
		3	0.160		3.35		0.464		0.205	
		4	0.154		3.08		0.492		0.192	
	5	1	0.184		3.76		0.446		0.237	
		2	0.148		3.17		0.470		0.192	
		3	0.155		3.84		0.431		0.194	
		4	0.161		3.07		0.435		0.187	
	Mean		*0.161*	*B*	*3.40*	*AB*	*0.454*	*A*	*0.205*	*BC*
Organic	1	1	0.180		3.48		0.421		0.229	
		2	0.191		3.56		0.425		0.241	
		3	0.195		3.69		0.441		0.247	
	2	1	0.183		3.77		0.533		0.265	
		2	0.193		3.30		0.427		0.232	
		3	0.191		3.30		0.439		0.229	
	3	1	0.194		3.53		0.482		0.249	
		2	0.187		3.55		0.494		0.260	
		3	0.186		3.81		0.454		0.251	
	4	1	0.182		3.83		0.575		0.267	
		2	0.201		3.85		0.438		0.248	
		3	0.195		3.63		0.430		0.240	
	5	1	0.166		3.33		0.495		0.239	
		2	0.172		3.81		0.525		0.277	
		3	0.190		3.60		0.406		0.252	
		4	0.172		3.41		0.400		0.230	
	Mean		0.186	*A*	3.59	*AB*	0.462	*A*	0.247	*A*
Pasture	1	1	0.184		4.39		0.447		0.263	
		2	0.194		4.27		0.387		0.233	
		3	0.176		4.30		0.439		0.243	
		4	0.195		3.43		0.484		0.243	
		5	0.197		3.67		0.431		0.249	
		6	0.171		3.42		0.414		0.231	
	2	1	0.188		4.02		0.485		0.262	
		2	0.194		3.87		0.389		0.233	
		3	0.188		3.88		0.406		0.249	
		4	0.180		3.42		0.491		0.262	
		5	0.196		3.67		0.417		0.253	
		6	0.200		3.51		0.422		0.237	
	Mean		0.189	*A*	3.82	*A*	0.434	*A*	0.247	*AB*
Control-		0	0.144	*C*	3.03	*B*	0.314	*B*	0.200	*ABC*
Control+		0	0.141	*BC*	3.05	*B*	0.232	*B*	0.177	*C*

Most of isolates showed all nutrient contents (N, P, K, Ca and Mg) increase compared to the control. However, only Ca content in maize was significantly higher than the both positive and negative control.

Micronutrients

PGPR treatments isolated from different ecosystems that were applied in the rhizosphere of maize plants resulted in a micronutrient content increased for all tested treatments (Table 3). The results for micronutrients such as Fe, Cu, Zn, Mn, and B were evaluated individually. iron (Fe) nutrients in maize with PGPR treatments isolated from the forest and organic farm site were significantly higher than the both positive and negative control. While iron (Fe) nutrients in maize with PGPR treatments isolated from the pasture were significantly higher than the positive control. The third sampling point in the forest and the fourth sampling point in the organic farm site had the highest average values of iron nutrients in maize plants, respectively. Rahimi et al. (2020) studied on the impact of PGPR on improving the acquisition of iron content in quince seedling. Their result showed that iron concentration increased 1.5-fold by PGPR application. Sharma et al. (2013) suggested that PGPR application can be affirmative strategy to solve the issue of iron deficiency in rice cultivation. The highest zinc (Zn) nutrients in maize were presented by PGPR treatments isolated from organic farm sites (53.2 ppm) followed by forest (52 ppm) and pasture (48 ppm). All PGPR treatments in terms of Zinc (Zn) nutrients in maize were significantly higher than the positive control. However, Zinc (Zn) nutrients in maize were significantly greater in organic farm sites than in both the positive and the negative control.

In comparison to both positive and negative controls, manganese (Mn) nutrients in maize were significantly higher in organic farm sites and forests. Manganese (Mn) elements in maize with PGPR treatments isolated from pasture were significantly greater than the positive control. The third sampling point in the forest and the fourth sampling point in the organic farm site had the highest average values of manganese nutrients in maize plants, respectively.

Copper (Cu) nutrients in maize with PGPR treatments isolated from all ecosystems were significantly higher than the both positive and negative control. While the highest value of copper nutrients in maize was PGPR treatments isolated from the forest (12.6 ppm), the lowest value was PGPR treatments isolated from organic (11.5 ppm). PGPR treatments isolated from all ecosystems in boron (B) nutrient content in maize was significantly higher than the both positive and negative control treatment. The second sampling point in the organic farm site had the highest average values of boron nutrients in maize plants.

In terms of micronutrient contents, while the highest values for Fe, and Mn in maize plants were noted in PGPR treatments isolated from forests followed by organic farm sites, the highest value of Cu content in maize plants was noted in the forest followed by pasture. In this study, micronutrient content (Fe, Zn, Mn) in maize under PGPR treatments isolated from different ecosystems was significantly higher than the negative control treatments. According to Rana et al. (2012) an enhancement of 28-60% in micronutrient content was recorded in treatments on wheat plant receiving the mix PGPR application with 2/3 recommended dose of NPK, as compared to full dose of fertilizer application. However, copper (Cu) and boron (B) were statistically significant than both negative control and positive control. The negative control had a larger micronutrient content than the positive control in the maize plant, even though there was no statistically significant difference between the control treatments.

Physiological parameters

The impact of different PGPR treatments on the physiological parameters in maize such as fresh and dry biomass weight, height, and shoot diameter is demonstrated in Table 4. Fresh biomass in maize ranged from 70.4 g per plant to 30.3 g per plant. Unexpectedly, positive control of fresh biomass with 70.4 g per plant performed the best result followed by PGPR treatment isolated from pasture with 66.7 g per plant and organic with 59.5 g per plant.

Table 3. Selected micronutrient contents

Ecosystem	Sampling point	Isolate	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	B (mg kg^{-1})
Forest	1	1	318	51.0	54.8	12.2	18.8
		2	259	43.1	51.4	12.1	14.5
		3	328	55.9	52.0	11.9	16.0
	2	1	235	41.1	56.9	12.3	14.0
		2	319	48.5	59.3	13.7	28.4
		3	325	49.7	50.6	12.2	24.6
	3	1	302	53.7	66.0	13.0	31.8
		2	526	57.5	66.0	13.2	28.4
		3	430	60.8	55.3	12.7	29.8
	4	1	261	46.3	45.0	11.2	23.6
		2	367	46.4	56.5	12.6	27.4
		3	308	51.7	49.4	13.1	24.0
		4	474	66.0	62.9	13.7	30.9
	5	1	235	51.2	43.9	11.2	26.0
		2	288	50.7	49.6	12.4	25.8
		3	292	50.6	46.7	12.5	24.7
		4	416	62.0	54.5	14.0	28.7
	Mean		*334 A*	*52.1 AB*	*54.2 A*	*12.6 A*	*24.6 A*
Organic	1	1	326	51.5	49.6	11.5	25.2
		2	292	50.1	46.3	11.6	24.8
		3	312	55.3	51.2	12.0	27.9
	2	1	301	51.7	50.4	12.6	26.4
		2	332	52.5	51.5	12.1	26.7
		3	348	49.6	54.1	11.1	30.2
	3	1	306	56.6	56.8	11.6	27.6
		2	346	53.8	55.7	10.9	25.7
		3	352	62.6	45.4	10.4	23.6
	4	1	423	58.0	59.5	12.0	26.5
		2	214	46.9	51.0	11.6	25.1
		3	406	58.4	52.0	11.7	30.6
	5	1	393	53.5	63.7	12.2	29.1
		2	402	54.9	57.5	11.5	21.1
		3	236	44.6	48.5	11.0	14.5
		4	288	47.8	44.1	10.5	15.8
	Mean		330 *A*	*53.2 A*	*52.2 A*	*11.5 B*	*25.4 A*
Pasture	1	1	238	42.3	47.8	13.2	26.8
		2	231	42.1	44.8	11.8	24.8
		3	284	49.7	44.6	11.4	26.7
		4	387	54.0	58.0	12.0	17.5
		5	289	47.7	49.6	12.0	17.3
		6	270	48.6	44.3	12.0	25.1
	2	1	301	51.6	51.8	13.1	24.8
		2	249	46.0	46.0	11.8	25.2
		3	303	46.7	42.8	11.0	25.8
		4	362	56.5	57.4	13.2	28.7
		5	233	49.8	47.3	12.8	22.4
		6	265	52.0	48.6	12.8	26.3
	Mean		284 *AB*	48.9 *AB*	*48.6 AB*	*12.3 AB*	*24.3 A*
Control-		0	164 *BC*	39.7 *BC*	34.7 *BC*	5.9 *C*	5.1 *B*
Control+		0	112 *C*	29.1 *C*	28.3 *C*	5.1 *C*	3.0 *B*

Table 4. Plant weight, height, and diameter at harvest

Ecosystem	Sampling point	Isolate	Fresh weight (g)	Dry weight (g)	Plant height (cm)	Stem diameter (mm)
Forest	1	1	52.8	6.73	134	7.59
		2	45.5	6.13	129	8.07
		3	50.2	6.83	135	8.07
	2	1	47.0	6.33	136	7.52
		2	34.1	5.10	132	7.51
		3	48.7	6.67	133	7.30
	3	1	36.7	5.27	131	6.64
		2	39.1	5.65	132	6.73
		3	45.0	6.07	130	7.42
	4	1	66.1	9.20	135	8.60
		2	44.2	6.20	127	7.24
		3	42.2	5.97	134	7.23
		4	37.7	5.33	122	6.76
	5	1	71.7	10.33	145	8.09
		2	54.3	7.73	133	7.52
		3	46.8	6.90	131	7.85
		4	42.2	6.17	128	7.23
	Mean		*46.9 B*	*6.50 B*	*132 A*	*7.49 C*
Organic	1	1	60.1	8.13	127	8.53
		2	70.9	9.63	138	8.88
		3	67.6	10.43	147	8.22
	2	1	50.9	6.73	131	7.63
		2	67.0	9.60	144	8.45
		3	70.6	10.93	140	8.99
	3	1	55.4	7.37	130	7.88
		2	57.0	8.27	141	8.20
		3	64.0	9.70	142	7.98
	4	1	57.2	7.57	135	8.66
		2	56.4	7.77	139	8.00
		3	61.0	9.33	141	7.82
	5	1	50.9	7.63	118	8.13
		2	52.5	7.03	140	7.03
		3	46.9	6.60	131	7.72
		4	55.1	7.95	141	8.30
	Mean		*59.5 A*	*8.50 A*	*137 A*	*8.20 B*
Pasture	1	1	62.9	9.60	110	10.26
		2	67.6	9.77	111	10.74
		3	76.0	12.63	134	9.57
		4	56.9	7.77	117	9.09
		5	66.4	9.17	128	7.95
		6	71.7	10.97	140	8.68
	2	1	59.0	8.43	113	9.55
		2	70.1	9.60	121	9.51
		3	75.6	11.77	137	9.94
		4	51.6	7.20	115	8.15
		5	72.3	9.60	134	8.74
		6	69.9	10.37	137	8.11
	Mean		*66.7 A*	*9.70 A*	*125 B*	*9.20 A*
Control-		0	30.3 *B*	4.70 *B*	110 *C*	5.42 *D*
Control+		0	70.4 *A*	11.27 *A*	117 *BC*	8.67 *ABC*

Whereas there were no statistical differences between the PGPR treatments isolated from the forest and the negative control for the fresh biomass weight of maize, they were statistically significant compared to the organic farm site and pasture, respectively. Sandini et al. (2019) reported that seed inoculation by *Pseudomonas fluorescens* to maize increased grain yield and biomass accumulation of maize plants.

Our result showed that the plant height in maize was also statistically significant by PGPR treatments isolated from different ecosystems. The height of the maize plants varied from 137 cm to 125 cm. In terms of stem diameter in maize, all PGPR treatments were statistically significant than the negative control. The highest stem diameter was presented by pasture at 9.2 mm followed by organic farm site at 8.2 mm, and forest at 7.5 mm.

CONCLUSIONS

This study aimed to investigate the impacts of PGPR practices isolated from various ecosystems on the nutrient content of macro-micro and yield quality in maize plants in the greenhouse condition. Of the factors analyzed, PGPR practices had a positive impact on macro-micro nutrient content and physiological parameters in maize. Even though the results from PGPR treatments isolated from forests and organic farm sites did not show statistically significant effects on the potassium content in maize compared to the control, they nevertheless contributed to higher potassium content in maize. Our results suggest that PGPR isolated from organic farm sites and PGPR isolated from pasture are the most effective PGPR treatments for positively influencing macronutrient content in maize plants in the greenhouse condition. PGPR applications isolated from organic farm sites are the most effective treatments for maize plants when evaluated for their macro-micronutrient composition.

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