Turk J
Field Crops
2022, 27(1),127-133
DOI: 10.17557/tjfc.1019160

INVESTIGATION OF THE EFFECT OF PGPR ON YIELD AND SOME YIELD COMPONENTS IN WINTER WHEAT (*Triticum aestivum* L.)

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Received: 04.11.2021

ABSTRACT

The plant growth-promoting rhizobacteria (PGPR) that live actively in plant roots and rhizosphere and support plant growth has gained widespread importance in agriculture. This study was carried out to obtain and identify the PGPR isolates from wheat soil and determine their ability and capacity on plant growth and yield. So, they were obtained from soil, and they were identified as *Bacillus* spp. (*B. simplex* and *B. pumilus*) by biochemical tests and MALDI-TOF MS (Matriks assisted lazer desorption ionization time of flight massspectrometry). After the wheat seeds (Flamura-85) were treated for PGPR, the field experiment was conducted with inoculated and non-inoculated seeds at the area of the Field Crops Department, Tekirdag Namık Kemal University in 2016-2018. The experiment was arranged in a split-plot design with three replicates for each treatment. In the experiment, some parameters such as plant height (PH), spike length (SL), number of grain per spike (NGPS), grain weight per spike (GWPS), and grain yield (GY) were evaluated and compared between treatments. The study has shown that PGPR treatments support plant growth and significantly increase yield between 9.6% and 29.29%. Especially, W3 and W4 strains (*B. simplex*) were showed a significant effect on the GY. According to the results, we can mention that using the PGPR promotes wheat growth and lead to increasing yield in the wheat. The use of PGPR can give promising results for sustainable and eco-friendly agricultural practices.

Keywords: PGPR, plant growth, seed treatment, winter wheat (*Triticum aestivum* l.), yield

INTRODUCTION

The plant growth-promoting rhizobacteria (PGPR) is the soil bacteria and one of the most important and agronomically useful soil microbiota (Kloepper and Schroth,1978; Bhattacharya and Jha, 2012). It can help plant growth indirectly by reducing plant pathogens, or directly by supporting the uptake of nutrients such as P, N, K also they can affect the biomass of the plants (Cakmakci et al., 2006; Ibiene et al., 2012; Khan et al., 2013). The PGPR can colonize and grow fastly onto the surface of the seed or the root, it can support plant growth, the germination rate of the seed, improve transplant emergence, and respond to stress conditions. For all those, it is an excellent alternative and tool to improve the agricultural system eco-friendly. Increasing crop production, the PGPR is given a good opportunity to prevent the use of a large number of chemical fertilizers and pesticides, which are generally overused in soil (Kumar et al., 2017). Many studies have demonstrated the abilities of plant growth-promoting microorganisms to increase plant nutritional status and reduce the use of pesticides (Meena et al., 2017; Aloo et al., 2019).

The PGPR can reduce the input of chemical fertilizers and pesticides without reducing yield. So, nowadays it is used as a popular biopesticide, photostimulation, and biofertilizer for sustainable agriculture (Rodrigurez et al., 2006; Santos et al., 2020). The PGPR can be applied to some different parts of the plant such as seed, root, and foliar. Even though the most common application of PGPR is known as a seed treatment, the root and foliar treatments also are used (Podile and Kishore, 2006). The application of PGPR supported most of the plants such as canola, grasses, maize, rice, and wheat. Recently studies indicated that PGPR is more effective on wheat yield. Some researchers obtained that PGPR providing P to wheat plants over the growing season, P-solubilizing rhizosphere bacteria at associated with wheat growth stages as vegetative biomass production and generative biomass production (Khan et al., 2013; Schadler et al., 2019; Asık and Arıoglu, 2020). All those results and documents try to understand the importance of PGPR for sustainable agriculture. The PGPR which is a productive and new alternative in the agricultural system also contains strains from genera such as *Pseudomonas*, *Serratia*, *Azospirillium*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Rhizobium*, *Erwinia*, *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, and *Flavobacterium* (Bashan and de-Bashan, 2005; Esitken et al., 2010) well recognized. However, *Bacillus* is an important genus within the PGPR group.

More studies referred to *Bacillus* spp. produce endospores that can tolerate extremes of the condition such as temperature, pH, and exposure to pesticides and fertilizers. Also, *Bacillus* spp. have been shown to improve root growth and morphology total root area in crops (Backman et al., 1997; Adesemoye and Kloepper, 2009; Duca et al., 2014; Shen et al., 2016; Nguyen et al., 2019).

This study aimed to collect and identify the PGPR strains in the wheat fields and also obtain their ability and capacity on plant growth and yield under the field conditions.

MATERIALS AND METHODS

Isolation of the PGPR from soil

Soil materials were collected from Tekirdag provinces during the 2015 and 2016 growing seasons. Nutrient agar (NA) media was used for isolation at 10^{-5} – 10^{-6} serial dilutions. The agar plates were incubated at 28 °C for 48 h. Each colony was used as an isolate in NA plates and followed by purification on the new NA plate with a repeated plating method (Schmidt and Belser, 1982). A total of 53 candidate PGPR isolates were isolated and used for further study.

Selection of the PGPR

Some tests such as hypersensitivity reaction test, soft rot test, and growth 37 °C were performed to select the candidate of PGPR isolates.

A hypersensitivity reaction test of the candidate PGPR isolates was carried out by inoculating tobacco leaves, using a highly concentrated bacterial suspension (~10^8 cfu/ml). The effectiveness of the bacterial isolates was evaluated by the absence of disease symptoms and hypersensitivity reaction. Candidate PGPR isolates were further checked for potato (*Solanum tuberosum* L.) soft rot test on potato slices. A loopful bacterial colony was taken from pure fresh culture and spread on surface-sterilized potato slices and incubated to obtain the occurrence of any soft rot on potato tissues. The existence of soft rot on potato slices showed the positive effect of pectolytic activities of the tested PGPR isolates. Lastly, the growth at 37 °C on the NA test was done. So human pathogenic isolates were eliminated with this test.

Identification of PGPR

Physiological and biochemical characteristics of the candidate PGPR isolates were determined with Gram reaction, oxidase, and production fluorescent pigmentation (Lelliot and Stead, 1987). After that, all isolates were analyzed with MALDI-TOF MS to be identified (Pavlovic et al., 2012). So, the raw spectra of the unknown PGPR isolates were used for pattern matching against the reference spectra of the database. The results of the pattern-matching process were expressed as proposed by the manufacturer, with log (scores) values ranging from 0 (no similarity) to 3 (absolute identity) (Carolis et al., 2012; Ziegler et al., 2012; Uysal et al., 2019)

Seed inoculation

The Flamura-85 wheat cultivar was used as experimental material in this study. Seeds of the cultivar were done surface-sterilization using 0.1% NaClO for 2 min and rinsed five times with sterilized water. Candidate PGPR isolates were grown on NA for experiments. A single colony from each isolate was transferred into a 50 ml flask, containing nutrient broth (NB) and kept in flasks overnight on a rotating shaker at 200 rpm. Bacteria grown on NB were diluted with sterile NB. to a final concentration was arranged as 10^8 CFU (colony forming unit) ml^{-1} in a spectrophotometer. For treatments, wheat seeds were inoculated with the candidate PGPR suspension of 10^8 CFU ml^{-1} along for 30 min before sowing. For control, non-inoculated seeds were used.

Field experiment

The experimental site and growing conditions: This study was carried out in the experimental area of Field Crops Department of Agricultural Faculty of Tekirdag Namik Kemal University, Tekirdag, Turkey during the 2016-2017 and 2017-2018 wheat growing seasons. Tekirdag district locates at latitude 40° 36'-40° 31' and longitude 26° 43'-28° 08'and altitude is 10 m. The climate data during the 2016-2017 and 2017-2018 wheat growing seasons and long-term average were given in Table 1. In the first year of the study, the total precipitation and the average temperatures were 360.3 mm and 9.8 °C, respectively. (Table 1). In the second year of the study, the total precipitation and the average temperatures were 612.7 mm and 12.1 °C respectively (Table 1).

Table 1. The monthly climatical data measured during the winter wheat-growing seasons in 2016-2017 and 2017-2018*

Months	Precipitation (mm)			Average temperature (°C)		
	2016-17	2017-18	Long term	2016-17	2017-18	Long term
November	43.1	85.2	55.2	3.8	11.7	11.4
December	43.1	94.8	86.2	3.8	9.5	7.2
January	107.0	76.5	69.9	1.9	6.6	4.4
February	38.8	95.3	54.7	6.5	7.2	5.3
March	32.2	63.7	55.6	9.1	10.2	6.8
April	51.6	10.6	42.9	11.2	14.0	11.5
May	16.7	114.2	37.6	16.8	15.2	16.6
June	36.8	75.4	37.8	22.0	22.4	22.5
Total	369.3	612.7	439.9	-	-	-
Average	-	-	-	9.38	12.1	28.9

*Source: Tekirdag meteorology station

Under field conditions, inoculated and non-inoculated wheat seeds were sown in Tekirdag on November 10, 2016, for first-year experiments and November 15, 2017, for second-year experiments with a density of 500 grains m^{-2}. The plot size was 5.10 m^2 (5 m x 1.02 m). The experiments were arranged in randomized complete block design with 3 replicates. For two years the experiment was fertilized at different times along season like sowing (20.20.0 composed fertilizer), tillering (urea, %46 N), and stem elongation (calcium ammonium nitrate %26N). The experiment was harvested in early July and yield and yield components were studied.

Statistical analysis

The JMP version 5.0 package statistical software was used for all data involving calculations and the comparison of each isolate for all measurements. The data were statistically analyzed using ANOVA. Duncan's multiple range test at a probability level of 0.05 was used to separate the means.

RESULTS

Isolation and selection of the candidate PGPR strains

A total of 53 candidate PGPR were isolated from wheat fields in Tekirdag. All isolates were subjected to growth at 37°C, tobacco (*Nicotiana tabacum*) HR, and potato rotting. Following the tests, none of the strains grown at 37 C, 46 isolates were found positive for tobacco HR. Any isolates weren't shown pectolytic activity on potato slices (Table 2). So, seven-candidate PGPR strains were retained for using further study.

Table 2. Identification of candidate PGPR strains

Strain code	Bacterial species	Gram reaction	Florescent	Oxidase	HR	Pectolytic activity	Growth at 37 °C
W1	*B. pumilus*	+	-	-	-	-	-
W2	*B. simplex*	+	-	+	-	-	-
W3	*B. simplex*	+	-	+	-	-	-
W4	*B. simplex*	+	-	+	-	-	-
W5	*B. simplex*	+	-	+	-	-	-
W6	*B. simplex*	+	-	+	-	-	-
W7	*B. pumilis*	+	-	-	-	-	-

W1: *B. pumilus*, W2: *B. simplex*, W3: *B. simplex*, W4: *B. simplex*, W5: *B. simplex*, W6: *B. simplex*, W7: *B. pumilus* They are code or name of bacterial strain that are identified as *B. pumilus* and *B. simplex*

Identification of candidate PGPR strains

According to the biochemical characterization of strains were identified *Bacillus* genera including *B. simplex* and *B. pumilus* (Table 2).

Two out of seven strains were selected according to their biochemical test results. Then these two strains were analyzed by MALDI-TOF MS. The strains were similarly identified like biochemical test results as *B. simplex* and *B. pumilus*.

Effect of plant growth and yield under field conditions

Results of variance analyses are presented in Table 3. The results indicated that grain yield (GY), spike length (SL), plant height (PH), number of grain per spike (NGPS), and grain weight per spike (GWPS) had some difference at 1% probability level (Table 3).

Table 3. ANOVA results of yield and yield components

S.O.V	GY	SL	PH	GWPS	NGPS
Replication	1732.646ns	0.175ns	15.484 ns	0.051 ns	0.776 ns
Year (Y)	403333.333**	0.630ns	48.682ns	0.745**	58.963**
Strain (S)	6725.131**	0.158ns	7.850ns	0.029ns	5.389ns
YxS	10476.762**	0.233ns	48.510**	0.189**	27.661**
Error	1636.268	0.181	13.930	0.034	3.925
General	12261.700	0.194	18.980	0.072	8.715

*: significant at α level %5, **: significant at α level %1, ns: non-significant,
S.O.V: source of variation, GY: grain yield, SL: spike length, PH: plant height, GWPS: spike length, NGPS: grain weight per spike

The morphological properties such as PH, SL, NGPS, GWPS, and GY were observed and evaluated for each of the PGPR strains treatment and compared with the non-inoculated control. The statical analysis indicated that interaction between grain yield and years was found significantly important (Table 3). Therefore, the years were evaluated separately (Table 4).

Table 4. Means of yield and yield components of wheat at different PGPR straints in the field trial run in the 2016 -17 and 2017-18 periods

2016-2017 field trails					
Treatment	PH (cm)	SL (cm)	NGPS	GWPS (g)	GY (kg ha^{-1})
W1	92.63	9.67	41.40b	2.08c	7236.6ab
W2	90.06	9.36	40.33b	2.03c	6963.3cd
W3	89.70	9.53	41.20ab	2.10abc	7443.3a
W4	88.53	9.57	44.26ab	2.44a	6790.0d
W5	86.76	9.74	46.20a	2.42ab	6566.6e
W6	94.20	9.77	41.93ab	2.19abc	6973.3bcd
W7	93.30	9.47	39.80ab	2.08bc	7016.6bc
Control	93.96	9.61	40.00ab	2.02c	6946.6cd
MSE	-	-	3.585	0.025	38.506
2017-2018 field trails					
W1	87.80	9.33a	42.16bc	2.00ab	4326.6ab
W2	85.36	9.50ab	44.40abc	1.93ab	4130.0b
W3	88.93	8.96b	43.43abc	1.61b	5163.3ab
W4	90.86	9.10b	41.76bc	1.73b	6090.0a
W5	94.46	8.90b	40.66c	1.63b	5403.3ab
W6	86.56	9.33ab	43.00bc	1.87b	5660.0ab
W7	90.73	9.43ab	46.56ab	2.43ab	5786.6ab
Control	85.93	10.00a	47.63a	2.33a	4710.0ab
MSE	-	-	4.362	0.040	3255.619

MSE: mean squared error , W1: *B. pumilus*, W2: *B. simplex*, W3: *B. simplex*, W4: *B. simplex*, W5: *B. simplex*, W6: *B. simplex*, W7: *B. pumilus* W1: *B. pumilus*, W2: *B. simplex*, W3: *B. simplex*, W4: *B. simplex*, W5: *B. simplex*, W6: *B. simplex*, W7: *B. pumilus* They are code or name of bacterial strain that are identified as *B. pumilus* and *B. simplex*

The number of grain per spike

In the first year, the application of PGPR was measured between 39.80 to 46.20 and 40.66 to 46.56 in the second year. Especially, W5 was increased number of grain pers pike compared to control. W5 strain showed highly effective at 15.5% (Table 4). Also, the mean values of NGPS in 2017-18 were higher than in 2016-17. This may have been caused by longer spikes in 2018 when the high rainfall was received. It can be said that high precipitation affects NGPS.

Spike length

Results of ANOVA indicated a statistically significant difference in spike length was not affected by any PGPR treatments (Table 3) when both years' experiments were evaluated. It varied between 9.47 and 9.77 cm in the first year and 8.90 to 9.43 cm in the second year (Table 4). Also, the first-year experiment showed that the W5 strain was enhanced SL at the rate of 1.35 (Table 4).

Plant height

All treatments were in the same group as statistically, W5 PGPR treatment (86.76 cm) in the first year and W2 PGPR treatment (85.36 cm) in the second year decreased the plant height (Table 4). The mean value of PH in 2016-17 was higher than in 2017-18 years. However, the mean values of SL in the 2016-17 year were lower than the 2017-18 year. These two results were parallel each together. The higher amount of precipitation in the first year was enhanced to PH. But it may get lower the mean value of SL (Table 4).

Grain weight per spike

It ranged from 2.03 g to 2.44 g in the first year and 1.63 g to 2.43 g in the second year (Table 4). Grain weight per spike was not affected by PGPR treatments (Table 3). However, in the first year, the W4 strain increased grain weight per spike at the rate of 20.79% (Table 4).

Grain yield

It was markedly influenced by PGPR treatments. The application of PGPR except in the first-year results indicated that W3 increased the value (7443.3 kg ha^{-1}) of GY at the rate of 7.15%. Also, W1 (7236.6 kg ha^{-1}) and W7 (7016.6 kg ha^{-1}) led to more grain yield when compared to non-inoculated control. However, second-year experiments, W3, W4, W5, W6, and W7 increased the GY between 9.62% and 29.29% (Table 4). According to results, strains were found the effects on plant growth during 2017-2018. The PGPR has supported wheat growth. So, our results indicated that as expected results, mean values of GY in 2016-2017 were higher than 2017-2018.

DISCUSSION

Wheat is one of the world's most important crops. Over 760 million tons of wheat were produced in 2020 worldwide (FAO,2021). The potential of wheat yield represents the yield of a cultivar grown in environments which is an adaptation of nutrients and water regime, pesticide application influences wheat yield and quality (Kovacevic, 2007). Recently, some studies that include a way to protect plants and soil against chemical damage, maintained to rise yield and quality of wheat. The use of PGPR-based products has given the opportunities that they

are considered safer than many of the chemicals now in use and not considered harmful to ecological processes or the environment (Lucas Garcia et al., 2004).

Here, the PGPR isolates were collected and identified as *Bacillus* spp. including *B. pumilus* and *B. simplex* strains by biochemical tests and MALDI-TOF MS assay. Following studies to obtain the PGPR strains ability on wheat growth and yield in field conditions. Previous studies indicated that *Bacillus* spp. associated with rhizosphere is one of the important PGPR. The first commercial bacterial fertilizer called Alinit was developed from *Bacillus* spp. They could support the plants in many ways such as biofilm could develop biofilms, solubilization phosphate, N fixing, or uptake nutrients. However, *Bacillus* spp. increased crop yield by 40% (Kilian et al., 2000; Haas and Defago, 2005; Beauregard et al., 2013; Lyngwi and Joshi, 2014; Berendsen et al., 2016).

In the present study, to determine whether PGPR interaction ability could make enhance plant performance, a field experiment was carried out with PGPR (*B. simplex* and *B. pumilus*) inoculated seeds and non-inoculated seeds. In the first-year experiment, PGPR showed increasing all plant parameters such as PH, SL, NGPS, GWPS, and GY. On the other hand, the second-year experiment indicated that PGPR just increased PH and GY. Also, both PH and GY values have been higher percentages than in the first-year experiment. Up to this point, when the results for both years were compared with control, the weather condition such as temperature and rainfall might have played a key role. PGPR strains were risen the GY by over 29% in the second year according to control. However, GY was increased over by 7% in the first year. Nguya et al. (2019) mentioned that the response of PGPR strains seems to correlate with air and soil temperate during winter. The low temperature might make a negative effect on the colonization and survival of PGPR when seeds are inoculated and sown in autumn. As a parallel with our results, the temperature for the first year in autumn was lower (3.8 °C) than the second year (11.7 °C). So, PGPR might not survive in the condition. Also, higher colonization at early stages helps PGPR to be more competitive. In addition, PGPR cannot better-adapted local microflora and soil microfauna (Bashan et al., 2014). Therefore, *Bacillus* spp. strains referred to in this study could not be successful to colonize in the first year because of weather and other microorganism population in the soil. So, in the second year, the temperature was suitable for *Bacillus* strains also they might have better-adapted ability and extent their population. For all those, *Bacillus* strains led to a rising rate of GY from 7% to 29%. In the present research, a significant increase in GY was recorded in treatments where all these PGPR species were inoculated. This increase in yield might be the result of N fixing and phosphate solubilization capacity of these inoculated strains (Krey et al., 2013; Puri et al., 2016). Grain yield is one of the significant parameters between yield and yield components. It is usually characterized by biotic and abiotic environmental factors. The present study mentions that PGPR led to having more grain yield. The results were in agreement with the finding of Dobbelaere et al. (2001) and Saber et al. (2012). Moreover, *Bacillus* strains were increased PH for two years. Similarly, Kumar et al. (2014) were reported that *B. megaterium* has a positive effect on the PH. Interestingly, Akbar et al. (2019) referred to species of *Bacillus* MCR-7, *B. subtilis* did not show promising results on GWPS. Conversely, our result indicated that *B. simplex* strain W4 increased GWPS in the first-year field experiment. It also pointed out variable significant differences in the bioactivity of different types of similar PGPR species (Shaharooa et al., 2008). However, Alsaady et al. (2020) point out the number of grains per spike. They indicated those treatments of PGPR such as *Pseudomonas fluorescens*, *Streptomyces*, *Azotobacter*, *Azospirillum*, *B. subtilis*, *B. pumilis*, and bacterial mixture found effective on NGPS. Similarly, the treatment of the W5 strain led to a significant increase in NGPS in this study.

The value of yield independence of yield components such as plant height, spike length, leaf area, or stem high were also found associated with the vegetative period. These components are in direct connection with productivity in wheat (Knezevic et al., 2007). So, the study results were given a similar outcome with previous studies. For two years field experiments were given evidence to see the effect of PGPR on plants growth and yield. Also, *Bacillus* strains referred to as *B. simplex* and *B. pumilus* increased the yield like as most studies (Turner and Backman 1991; Garcia et al., 2003; Kokolis-Burella et al., 2003; Adesomoye et al., 2008; Mısra et al., 2010; Yildirim et al., 2011; Ibiene et al., 2012). Besides, for each year, observed the yield parameters showed some changes. We have thought that the climate and environmental factors are caused the changes (Bouaziz and Hicks, 1990; Acevedo et al., 1991; de Freitas and Germida, 1992; Dobbelaere et al., 2001).

CONCLUSIONS

In conclusion, the PGPR has some ability such as the use of biofertilizer to grow plants and pesticides for plant diseases. Therefore, it increases agricultural productivity and has an important role in the sustainable agricultural industry. The present study indicates the beneficial effects of *B. simplex* and *B. pumilus* strains inoculation on wheat growth in field conditions. The results of grain yield indicated that *B. simplex* and *B. pumilus* have an advantage especially strain W3 and W4 led to a significant increase in grain yield. It is concluded that the application of PGPR performed better than non-inoculated application. Therefore, PGPR seems a good alternative instead of using synthetic chemical fertilizers. This approach could be reduced the application of synthetic chemical fertilizers. According to our results, PGPR has promising and eco- and environmentally-friendly, and economical outcomes for winter wheat growth and production. It should be developed for future studies as multidisciplinary for the sustainability of winter wheat production.

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