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Research Article

The Effect of Plant Growth Promoting Rhizobacteria on Root Growth in Bread Wheat (*Triticum aestivum* L.)

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Abstract. Bread wheat (*Triticum aestivum* L.) is the most produced cool-season cereal in the world and meets about 20% of our daily caloric intake. Climate change negatively affects grain yield, it is, therefore, necessary to improve climate-resilient wheat crops. It is a known fact that subsoil parameters are not mostly included in the breeding selection criteria due to some technical limitations. For this reason, it is essential to examine the root system, which has a fundamental role in drought tolerance, for morphological, anatomical, physiological, and architectural aspects, to understand the genetic mechanisms of these traits and to determine breeding strategies. In this study, the seeds of two different bread wheat varieties were inoculated by three different plant growth-promoting rhizobacteria (PGPR), which synthesize the ACC deaminase enzyme. When the results were evaluated, significant differences were observed between varieties and bacterial applications for the total root length and root growth angle. As a result of the study, it was observed that all three bacteria species had a positive effect on root development. *Brevibacillus choshinensis* was the most effective inoculation on total root length in Gerek 79 (95.4 cm), while it was *Arthrobacter agilis* in Bezostaja 1 (62.8 cm). We suggest that plant growth-promoting rhizobacteria have a positive effect on wheat root development and a detailed analysis of this effect should be carried out with future studies.

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Bitki Gelişimini Teşvik Edici Rizobakterilerin Ekmeklik Buğdayda (*Triticum aestivum* L.) Kök Gelişimine Etkisi

Özet. Ekmeklik buğday (*Triticum aestivum*), dünya üzerinde en fazla üretimi yapılan ve günlük kalori ihtiyacımızın yaklaşık %20'sini karşılayan bir serin iklim tahılıdır. İklim değişimi buğdayın verim değerlerini negatif yönde etkilemektedir. Islah çalışmalarında toprak altı parametrelerinin bazı teknik zorluklar nedeniyle çoğunlukla sürece dahil edilemediği bilinen bir olgudur. Bu nedenle kuraklık toleransında temel bir role sahip olan kök sistemini morfolojik, anatomik, fizyolojik ve mimari yönden incelemek, bu özelliklerin genetik mekanizmalarını anlamak ve yetiştirme stratejilerini belirlemek için esastır. Bu çalışma ile iki farklı ekmeklik buğday çeşidinin tohumlarına, ACC deaminaz enzimi sentezleme özelliğine sahip bitki gelişimini tetikleyici üç farklı rizobakteri (PGPR) inoküle edilmiştir. Sonuçlar incelendiğinde, toplam kök uzunluğu ve kök büyüme açısı parametrelerinde çeşitler ve bakteri uygulamaları arasında anlamlı farklılıklar gözlenmiştir. Çalışma sonucunda her üç bakteri türünün de kök gelişimi üzerine pozitif etkisinin olduğu saptanmıştır. *Brevibacillus choshinensis* Gerek 79'da 95.4 cm toplam kök uzunluğu ile en etkili inokülasyon iken, Bezostaja 1'de *Arthrobacter agilis* 62.8 cm ile en etkili inokülasyon olmuştur. Bitki gelişimini teşvik edici bakterilerin buğday kök gelişimine pozitif yönde etkisinin olduğu ve gelecekte yapılacak çalışmalar ile bu etkinin detaylı analizlerinin yapılmasını öneriyoruz.

INTRODUCTION

Three members of the Triticeae tribe, bread wheat (*Triticum aestivum* L.), corn (*Zea mays* L.), rice (*Oryza sativa* L.), and grain legumes represent the main food groups. Wheat can be cultivated on all continents except Antarctica. Since the world population is expected to exceed 9 billion by 2050, the yields of all crops, especially wheat, should increase by at least 50%. Wheat has the highest cultivation area among cool-season grains with more than 765 million metric tons of production as of 2019 (FAOSTAT, 2019). Wheat, alone, meets approximately 20% of the global calorie need. It is not feasible to create new agricultural land, and available lands are under the threat of salinity, flood, drought, and erosion (Döös, 2002). The global average wheat yield is about 2780 kg per hectare and it is 3547 kg in Turkey (FAOSTAT, 2019). These values are far below the genetic yield potential of wheat. The significant increase in the mean temperature due to climate change has shown that drought and high temperature will be the biggest obstacles to agricultural production (Trnka *et al.*, 2015). Simulation studies have shown that the yield increase will be about 10% lower than the expected values due to rising temperatures and drought stress (Reeves *et al.*, 2016). Turkey is in one of the most affected regions by climate change and drought, and urgently needs drought-tolerant wheat varieties (Trnka *et al.*, 2015). Developing high-yielding varieties that are tolerant to drought or high-temperature stresses will be possible by understanding the physiological and morphological responses of plants to stress conditions.

While the responses of aboveground plant organs to stress factors are investigated in vast detail in wheat pre-breeding studies, the root system, which is one of the most important organs for drought tolerance, has been mostly ignored due to technical difficulties. Since a well-developed root system reaches deeper and wider soil areas, it reduces the effects of low to moderate stresses by improved water and mineral uptake. The root system is the first organ to perceive the water deficit in the soil, and by sending hormonal signals to the stem, it can cause the plants to close the stomata, thus slowing down or completely halting photosynthesis. In the studies conducted with rice (*Oryza sativa*), the *Dro1* (*Deeper rooting 1*) gene, which allows the roots to grow at a steeper angle and deeper by making the roots more responsive to gravity, was cloned and it was reported that drought resistance was increased with the effect of this gene (Uga *et al.*, 2011). Manschadi *et al.* (2006), calculated that for every millimeter of extra water absorbed by the roots, yield increased by 55 kg h^{-1}. The effect of roots on drought tolerance has been proven many times since the pioneering works of Weaver (1926), Richards and Passioura (1981), Reynolds *et al.* (2007), and Passioura (1983; 2012). Considering all these factors, it is important to investigate plant root characters, increase the number and lengths of roots, total root length, and rooting depth, and find genes that determine root system architecture.

It has been known that Plant Growth Promoting Rhizobacteria (PGPR) contributes to plant growth (Biçer *et al.*, 2020). They can achieve this function by decreasing ethylene synthesis or increasing the synthesis of auxin and cytokinins (Glick *et al.*, 2007). 1-aminocyclopropane-1-carboxylic acid (ACC), a bacterial deaminase enzyme, can be synthesized by many microorganisms and inhibits ethylene biosynthesis. This enzyme prevents or reduces ethylene formation by breaking down ACC, which is a precursor in ethylene biosynthesis. Thus, as ethylene level decreases, the plant leaves do not receive stress signals and continue to grow and do normal activities. ACC deaminase enzyme was found to increase tap and lateral root elongation and provides tolerance against heavy metals and salinity in Arabidopsis (Glick *et al.*, 2007; Sharma *et al.*, 2016; Wintermans *et al.*, 2016).

Early root system analysis used in this study covers a phase where bacteria are active and contributes to plant growth. Besides, an in-depth observation can be obtained without environmental fluctuations (light, temperature, etc.) more commonly emerging in the later growth stages (anthesis, maturity, etc.). Therefore, the aims of this study were; 1) to evaluate the effects of three different rhizobacteria species that synthesize ACC deaminase (*Pseudomonas agarici*, *Arthrobacter agilis*, and *Brevibacillus choshinensis*), on the root development and architectures of two different bread wheat varieties (Gerek 79 and Bezostaja 1); 2) to determine the root development differences in the early stage when the bacteria are active, and 3) to determine appropriate bacteria race for enhanced root development at the seedling stage.

MATERIAL AND METHOD

This study was carried out in Siirt University, Faculty of Agriculture, Department of Agricultural Biotechnology Laboratory. Bread wheat varieties Gerek 79 and Bezostaja 1 were used to screen root development differences. Three bacteria species; *Pseudomonas agarici* (TV24C), *Arthrobacter agilis* (TV126C), and *Brevibacillus choshinensis* (TV53D) were used as the source of ACC deaminase synthesis (Table 1).

Table 1. Variety and bacteria abbreviations used in the study.
Çizelge 1. Çalışmada kullanılan çeşit ve bakteri kısaltmaları.

Bezostaja 1	Abbreviation	Gerek 79	Abbreviation
Bezostaja 1+ TV126C	Bez_126C	Gerek 79 + TV126C	Ger_126C
Bezostaja 1+ TV24C	Bez_24C	Gerek 79 + TV24C	Ger_24C
Bezostaja 1+ TV53D	Bez_53D	Gerek 79 + TV53D	Ger_53D

Bacteria Inoculation

Bacteria were taken from previously prepared stock cultures and transferred to nutrient broth medium and allowed to grow in this medium until reaching OD 600 = 0.02 concentration. Concentration was measured with Multiskan GO (Thermo Fisher Scientific, USA). Liquid bacteria culture is used for seed inoculation. The inoculation was carried out for three hours at room temperature. Thus, the seeds were swelled and inoculated with bacterial culture. Inoculated seeds were dried under sterile conditions and stored for use in germination studies. Seed inoculation was checked by testing the dried seeds on a growth medium.

Sowing Seeds

The sterilized seeds were inoculated with rhizobacteria, dried, and imbibed into Petri dishes for 24 hours for homogenous germination. Seeds with homogenous germination were transplanted into the germination papers (40x40 cm) covered with PVC plates and placed in containers filled with 20 cm water. Seedling growth was ensured by keeping a sufficient amount of water in the containers. Details of the PVC plate system are given in Hohn (2016).

Analysis of Root Parameters

In the study, early-stage (Z15) seminal root development (Zadoks *et al.* 1974) was analyzed in the PVC plates + germination paper system (modified cigar roll method) (Hohn, 2016). PVC plates were removed at the end of the 15th day and images were taken at 300 DPI resolution with a handheld document scanner (ISCAN Portable Handheld Scanner). Images were analyzed using ImageJ image analysis software (imagej.nih.gov, Rueden *et al.* 2017). As a result of the analysis, total root length, number of embryonic (seminal) roots, the longest root length, and root growth angles were measured. Root growth angle was calculated by measuring the growth angle between the 2nd level seminal roots that developed just after the first embryonic root.

Statistical Analysis

The experiment was conducted as three replications and 4 plants per replication. The data obtained were subjected to variance analysis (ANOVA) with Statistix 10 (Analytical Software; Tallahassee, FL, USA) software according to the "Factorial Design" and the differences between the application groups were determined with the Least Significant Difference (LSD) Multiple Comparison Test. Besides, the "compare with the best" function of the multiple comparison test was used to find the best bacteria x variety interaction.

RESULTS AND DISCUSSION

As a result of the study, germination problems or infections were observed in some bacteria applications, and some plants showing abnormal growth in Ger_53D and Ger_126C were eliminated. As a result of bacterial applications, a statistically significant difference was determined between wheat varieties and bacterial species according to the analysis of variance (ANOVA) in terms of total root length and root growth angle ($p < 0.05$), but no statistically significant difference was found in the longest root length.

Total Root Length

The effect of bacterial inoculation on the total root length of bread wheat varieties was significant. Variety, bacteria, and variety x bacteria interactions significantly affected the total root length ($p < 0.05$). The mean total root length of Gerek 79 (82.4 cm) was higher than that of Bezostaja 1(54.35 cm). When the effects of bacterial applications on the total root length on Bezostaja 1 were examined, Bez_126C had the longest root length (62.8 cm) and Bez_53D had the shortest with 47.1 cm. On the other hand, Ger_53D (95.38 cm) had the longest total root length while Ger_24C and Ger_126C had the shortest values in Gerek 79 bacteria applications (Table 2). This result showed that variation due to genetic differences between varieties was more than differences caused by bacterial species and each variety is affected by bacterial applications at different levels. Responses of each variety to three different bacteria were unique and seemed completely different from each other (Gerek 79 and

Bezostaja 1). This difference highlights the importance of genotypic characteristics and high heritability in seedling root development. According to the Least Significant Difference (LSD) analysis, three main groups were obtained in the total root length between control groups, bacterial applications, and varieties (Table 2).

Table 2. The effect of bacterial inoculation on the total root length of bread wheat varieties.
Çizelge 2. Bakteri inokülasyonunun ekmeklik buğday çeşitlerinin toplam kök uzunluğuna etkisi.

Applications	Varieties (cm)		Mean
	Bezostaja 1	Gerek 79	
Control	54.5±4.4 bc	81.6±5.6 a	68.1
1. Bacteria 24C	53.0±5.6 bc	76.3±4.4 a	64.7
2. Bacteria 53D	47.1±5.6 c	95.4±14.9 a	71.3
3. Bacteria 126C	62.8±7.5 abc	76.3±10.6 ab	69.6
Mean	54.4 B	82.4 A	68.4

*: Differences between means with the same letter are not statistically significant, $P<0.05$.

The Longest Root Length

Although no statistically significant difference was observed in the longest root length according to variance analysis, Ger_53D (28.99 cm) had the longest root, and Bez_53D (19.71 cm) had the shortest root length (Table 3). Shaharoona *et al.* (2006) reported that ACC deaminase activity promotes nodule formation as well as root and stem development in maize. In a similar study, Contesto *et al.* (2008) found that ACC deaminase activity positively affects root and root hair length in Arabidopsis. Similarly, in this study, the root length was longer in seeds inoculated with bacteria. The Bez_53D generally had the least developed root system. Opposite responses of two varieties to the same bacteria application (53D) are worth further evaluation. The contrasting responses make plant x rhizobacteria interactions far more complex than a simple symbiosis and need to be tested on large-scale experiments. The plasticity (Ehdaie *et al.* 2012) of the root system may have affected varieties' responses to bacterial applications. The LSD test did not reveal any grouping for the longest root length and significant differences were observed only between varieties and applications. Statistically significant differences were observed only among Bez_126C-Bezostaja, Bez_53D-Bez_126C, and between Ger_53D-Ger_24C (Table 4).

Table 3. The effect of bacterial inoculation on the longest root length in bread wheat varieties.
Çizelge 3. Bakteriyel inokülasyonunun ekmeklik buğday çeşitlerinde en uzun kök uzunluğuna etkisi.

Application	Varieties (cm)		Bacteria mean (cm)
	Bezostaja 1	Gerek 79	
Control	20.07±1.6	23.12±2.12	21.60
1. Bacteria 24C	22.60±2.12	23.15±1.66	22.87
2. Bacteria 53D	19.71±2.12	28.99±5.63	24.35
3. Bacteria 126C	26.85±2.84	21.76±4.01	24.31
Variety mean	22.30	24.26	23.29

Table 4. LSD all-pairwise comparison test of the longest root length.
Çizelge 4. En uzun kök uzunluğu LSD çoklu karşılaştırma testi sonuçları.

Genotype	Mean-cm	Bezostaja 1	Bez_126C	Bez_24C	Bez_53D	Gerek 79	Ger_126C	Ger_24C
Bezostaja 1	20.072							
Bez_126C	26.852	**6.779***						
Bez_24C	22.658	2.586	4.193					
Bez_53D	19.714	0.358	**7.137***	2.944				
Gerek 79	23.119	3.046	3.733	0.461	3.405			
Ger_126C	21.756	1.684	5.096	0.902	2.042	1.363		
Ger_24C	23.151	3.078	3.701	0.492	3.436	0.032	1.394	
Ger_53D	28.988	8.915	2.136	6.33	9.273	5.869	7.232	**5.837***
Alpha: 0.05	Standard Error for Comparison: 2.3437 to 6.5695							
Critical T Value: 2.04	Critical Value for Comparison: 4.7800 to 13.399							

*: Significant at $P<0.01$.

Root Growth Angle

The effects of variety, bacteria and variety x bacteria interactions for root growth angle were significant ($p<0.01$). When the mean root growth angles were examined, the widest (horizontal rooting) root growth angle was seen in Bez_126C, while other Bezostaja 1 applications had similar values (104.2-90.7°). Much narrower root growth angles (73.1-59.5°) were detected in Gerek 79 and bacterial applications in this variety. The widest root

growth angle was observed in Bez_126C and Ger_24C in each variety, and the effect of bacterial inoculation on root growth angle was significant (Table 5). Shahzad *et al.* (2008) found that ACC deaminase activity was effective in the root architecture and biomass of chickpea (*Cicer arietinum*), and Schwartz *et al.* (2013) reported an increase in root size, lateral root formation, and nodule development in pea (*Pisum sativum*). In this study, there was a wide variation between varieties, possibly due to genetic differences, and within the varieties, due to bacteria inoculation. These results are promising for re-defining root development terms in established varieties. Bacteria inoculation seems to be effective on root growth angle and it may improve drought tolerance by promoting deep root formation (Uga *et al.*, 2011; Lindh *et al.*, 2014).

Table 5. The effect of bacterial inoculation on root growth angle in bread wheat varieties.
Çizelge 5. Bakteri inokülasyonunun ekmeklik buğday çeşitlerinde kök büyüme açısına etkisi.

Application	Varieties		Bacteria mean (°)
	Bezostaja 1	Gerek 79	
Control	90.67±4.46	73.05±5.26	81.86
1. Bacteria 24C	100.68±5.26	83.12±4.04	91.90
2. Bacteria 53D	96.33±5.26	59.53±13.76	77.93
3. Bacteria 126C	104.21±6.97	70.49±9.81	87.35
Variety mean	97.97	71.54	84.76

In a comparative analysis, significant root growth angle differences were observed between all Bezostaja 1 and all Gerek 79 bacteria applications. There were contrasting differences between bacteria applications in each variety. The 126C, 24C, and 53D had significantly different effects on Bezostaja 1 and Gerek 79 (Table 6). Bezostaja 1 had a mean root growth angle of 97.97° while Gerek 79 had 71.55° among all applications and control. The differences in mean root growth angle may have been due to genetic and application factors.

Table 6. LSD all-pairwise comparison test of the root growth angle (°) between varieties.
Çizelge 6. Kök büyüme açısı LSD çoklu karşılaştırma testi sonuçları.

Variety/Bacteria	Mean°	Bez_126C	Bez_24C	Bez_53D	Bezostaja 1	Ger_126C	Ger_24C	Ger_53D	Gerek 79
Bez_126C	104.21								
Bez_24C	100.68	3.52							
Bez_53D	96.33	7.87	4.35						
Bezostaja 1	90.67	13.54	10.02	5.67					
Ger_126C	70.49	**33.72***	**30.19***	**25.84***	20.18				
Ger_24C	83.12	**21.08***	**17.56***	13.21	7.54	12.64			
Ger_53D	59.53	**44.68***	**41.15***	**36.80***	**31.14***	10.96	59.53	23.59	
Gerek 79	73.05	**31.15***	**27.63***	**23.28***	**17.61***	2.57	73.05	10.07	13.52
Alpha:	0.05	Standard Error for Comparison: 6.0181 to 16.003							
Critical T Value:	2.042	Critical Value for Comparison: 12.291 to 32.683							

*: Significant at P<0.01.

To observe the variation caused by bacteria applications, a "compare with the best value" test was conducted between varieties and applications. The angle difference of -33.72°, -21.08°, -44.68°, and -31.15° with Bez_126C was calculated with Ger_126C, Ger_24C, Ger_53D, and Ger_53D were obtained, respectively (Table 7).

Table 7. HSU'S "Compare with the Best Value" Multiple Comparison Analysis.
Çizelge 7. "En İyi Değerle Karşılaştır" HSU Çoklu Karşılaştırma Analizi sonuçları.

Variety	Mean°	Lower limit	Difference°	Upper limit
Bez_126C	104.21	-16.96	3.52	23.47
Bez_24C	100.68	-23.47	-3.52	16.96
Bez_53D	96.33	-27.82	-7.87	12.61
Bezostaja 1	90.67	-33.1	-13.54	6.73
Ger_126C	70.49	-61.3	**-33.72***	0
Ger_24C	83.12	-40.65	**-21.08***	0
Ger_53D	59.53	-80.86	**-44.68***	0
Gerek 79	73.05	-51.1	**-31.15***	0
Alpha:	0.05	Standard Error for Comparison: 8.059 to 14.91		
Critical D Value:	Varies	Critical Value for Comparison: 19.95 to 32.56		

*: Significant at P<0.01.

The Bez_126C showed the greatest trend for horizontal root growth while Ger_53D showed the trend for more vertical root growth. Gerek 79 is reported to be a drought-tolerant variety (Çekiç, 2007; Bayram *et al.*, 2015; Ayrancı *et al.*, 2017). The presence of narrow-root growth angle and vertical growth trend supports that the roots may reach deeper soil profiles. On the other hand, Bezostaja 1 is reported to be more sensitive to drought (Çekiç, 2007; Bayram *et al.*, 2015). According to root growth angle values, Bezostaja 1 had a wider root growth angle compared to Gerek 79. The complex interactions between each variety and bacterial inoculations are worth further evaluations to breed root ideotypes with tolerance to drought or nutrient-limited conditions (Lynch, 213; Gouache *et al.* 2015).

CONCLUSION

It has been known for a long time that bacteria break down the ACC precursor (deaminase) by synthesizing ACC deaminase causing ethylene biosynthesis to cease or decrease (Gontia-Mishra *et al.*, 2014; Govindasamy *et al.*, 2008 and 2009). It has also been reported that this mechanism prevents the formation of stress, allows the synthesis of hormones such as auxin, and increases root growth (Ali *et al.*, 2014; Carlos *et al.*, 2016). In this study, three different bacteria species known to synthesize ACC deaminase were tested in two different bread wheat varieties with different adaptation capacities. Significant but contrasting interactions were obtained. It is thought that it would be beneficial to inoculate plants at regular intervals from the leaf or root collar as well as from the seed. This study, which reveals the root structure of bread wheat and the effect of PGPR on the root system, is a prelude to understanding the relationships between the plant root system and beneficial bacteria.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to this article.

DECLARATION OF AUTHOR CONTRIBUTION

HB and YB designed and performed the experiments, analyzed data, and wrote the manuscript; MS, FÇ and Bİ provided feedback and run statistical analysis. All authors read and approved the final manuscript.

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