Selcuk Journal of Agriculture and Food Sciences

https://dergipark.org.tr/tr/pub/selcukjafsci

Research Article

(2023) 37 (2), 232-241 DOI:10.15316/SJAFS.2023.023 e-ISSN: 2458-8377 Selcuk J Agr Food Sci

Rhizobacteria Increases Growth and Development of Blackberry Saplings

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HIGHLIGHTS

- The results show that the applications have a positive effect on vegetative characteristics. SK-39, SK-50 and SY-43 bacterial strains increased the plant height and shoot length, SK-39 and SY-43 bacterial strains increased shoot diameter, SK-50 and SK-39 increased plant fresh weight, SK-50 increased plant dry weight. Treatments increased the root wet weight, SK-50 and SK-39 strains increased the root dry weight.
- Applications increased the leaf N, P, K, Ca, S, Zn, Cu and Na content while bacterial treatments reduced leaf Mn contents.
- Applications significantly increased the amount of leaf IAA, GA, cytokinin and zeatin content in the leaves, while decreased ABA content.

Abstract

In this study, the effects of rhizobacteria on vegetative growth of Chester Thornless blackberry cultivars saplings were investigated. In this context, plant height, shoot number, stem diameter, average shoot length, root length, plant fresh and dry weight, root fresh and dry weight, IAA, ABA, GA, cytokinin, zeatin hormones and N, P, K, Ca, Mg, S, Mn, Fe, Zn, Cu, Na nutrients were investigated. According to the evaluations, the highest plant fresh and dry weights and root fresh and dry weights were obtained from the SK50 bacteria strain. It was determined that plant height, stem diameter, average shoot length and root length maximum increased with SK-39 strain. Bacteria applications increased the IAA, GA, cytokinin and zeatin contents and decreased the ABA content in the leaves. With bacteria applications, leaf N, P, K, Ca, S, Zn, Cu and Na contents increased compared to control. According to the results of the research, it can be recommended to use *Herbaspirillum huttiense* SK-39, *Achromobacter xylosoxidans denitrificans* SK-50, *Pantoea agglomerans* GC subgroup SY-43 and *Microbacterium ester aromaticum* SY-48 bacteria strains in order to promote growth and development in the cultivation of blackberry cultivars.

Keywords: Blackberry; Rhizobacteria; Growth; Development

1. Introduction

Blackberry, which has a unique taste, color, and aroma like many berry fruits, is a member of the Rubus genus of the Rosaceae family. The fruits of the blackberry, which has a bushy feature, are juicy, soft, and edible.

Citation: Ameen ZAA, Pirlak L, Khalee AJK, Metin T, Dönmez MF (2023). Rhizobacteria Increases Growth and Development of Blackberry Saplings. *Selcuk Journal of Agriculture and Food Sciences*, 37(2), 232-241. https://doi.org/10. 15316/SJAFS.2023.023
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Received date: 15/10/2022
Accepted date: 10/03/2023

This type of fruit, which has a high economic value, is used in different ways such as jam, fruit juice, frozen fruit and cake production in the food industry (Ağaoğlu, 1986).

Pigments, phenols, flavones, flavonoids, vitamins, and fibers in blackberries, which contain high levels of antioxidants, are of great importance for human health. It has been determined in studies that these substances are higher than many fruit species such as banana, apple, and pear (Ravai, 1996; Kähkönen et al., 1999; Halvorsen et al., 2002).

In parallel with the increase in the world population, the need for food also increases and in order to meet this need, it is necessary to take measures to in-crease the yield in plants. It is known that chemical and synthetic substances applied to plants in agricultural areas adversely affect the natural balance, human and animal health and soil fertility by negatively affecting the environment. With the excessive and unconscious use of synthetic materials, the physical and chemical quality characteristics of agricultural soils deteriorate, soil and waters are polluted with toxic substances. Such problems can lead to the destruction of agricultural lands, which are important for the healthy and balanced nutrition of future generations, and to a decrease in the productivity of our lands. For this reason, organic fertilizers and various biological organisms are used for fertilization. Examples of these are farm manure, vermicompost, bat manure, bacteria, and mycorrhizae.

Bacteria can be applied to plants from roots or leaves, and it is seen among the promising applications especially in agricultural production. In agricultural areas, beneficial bacteria are used to increase the resistance of plants to biotic and abiotic stress factors, to accelerate plant growth and to increase yield.

Rhizobacteria, one of the most abundant microorganisms in the rhizosphere, have been reported as bacteria that are specific to the rhizosphere and colonize plant roots (Antoun and Kloepper, 2001). Approximately 2-5% of rhizobacteria have a beneficial effect on plant growth when reapplied to a soil containing competitive microflora and are therefore called plant growth-promoting rhizobacteria (Kloepper, 1978).

The use of rhizobacteria that promote plant growth is gaining worldwide importance and acceptance and looks promising for the future. Rhizobacteria, which promote plant growth, are also known to create resistance to various plant pathogens in crops such as cereals, legumes, ornamentals, vegetables, spices, and some perennial plants (Antoun and Prévost, 2005).

The use of biofertilizers consisting of beneficial microorganisms instead of synthetic chemicals in-creases plant growth, prevents damage to the environment and protects soil fertility (O'Connell, 1992). It is reported that there are many bacterial species living in common with plants in the plant root zone, and some of them increase yield and quality in plants. These bacteria, which belong to the genera Acinetobacter, Alcaligenes, Arthrobac-ter, Azospirillium, Azotobacter, Bacillus, Beijerinckia, Burkholdria, Enterobacter, Erwinia, Rhizobium and Serrotia, are named as 'Plant Growth Promoting Bacteria' (Rodriguez and Fraga, 1999, Nowak and Struz ve Struz, 2000; Sudhakar et al., 2000). Studies have shown that these bacteria increase plant growth and yield in many fruit species (Kloepper, 1989; De Silva et al., 2000; Sudhakar et al., 2000; Del Carmen Jaizme-Vega et al., 2004 Eşitken et al., 2006; Pırlak et al., 2007; Aslantaş et al., 2007; Pırlak and Köse, 2009; Eşitken et al. 2010; Arıkan and Pırlak, 2014; Ramos-Solano et al., 2014; García-Seco et al., 2015; Pırlak et al., 2020). Many bacterial species belonging to the genera Bacillus, Azotobacter, Azospirillum, Beijerinckia and Pseudomonas have nitrogen fixing properties (Reis et al., 1994). It was determined that the nitrogen fixing bacteria provided increases in plant growth and yield with foliar application in mulberry (Sudhakar et al., 2000), sweet cherry (Eşitken et al., 2006) and apple (Pırlak et al., 2007). It is also reported that bacteria promoting plant growth increase the synthesis of growth regulators in plants (Zahir et al., 2004).

This study was conducted to investigate the effects of some rhizobacterial strains that promote plant growth on growth and development of blackberry saplings.

2. Materials and Methods

2.1. Materials

The research was conducted in the Research and Application Greenhouses of the Department of Horticulture, Faculty of Agriculture, Selcuk University in 2021.

Chester Thornless blackberry saplings produced by tissue culture method were used as material.

Chester Thornless: A very vigorous, productive, large fruited, late maturing, moderately winter-hardy and thornless blackberry variety introduced in the USA in 1985. Chester Thornless is more productive than Hull Thornless and is similar in fruit size, col-our, firmness, quality, and seed size. On hot, sunny, and humid days, its fruit does not soften and lose its color as quickly as the fruits of other thornless semi-erect varieties (Galletta et al., 1998).

In the study, bacteria strains *Herbaspirillum huttiense* SK-4, *Herbaspirillum huttiense* SK-39, *Achromobacter xylosoxidans denitrificans* SK-50, *Pantoea agglomerans* GC subgroup SY-43 and *Microbacterium ester aromaticum* SY-48 were used. Bacteria were obtained from Iğdır University, Faculty of Agriculture, Department of Plant Protection. Determined characteristics of bacterial strains are given in Table 1.

Table 1. Determined characteristics of bacteria strains

Bacteria strains	N*	K*	Ca*	P*
SK-4	+	+	S^+	S^+
SK-39	S^+	+	+	S^+
SK-50	S^+	+	+	S^+
SY-43	S^+	+	W^+	S^+
SY-48	+	S^+	+	+

N: Nitrogen fixing, K: Potassium dissolving, Ca: Calcium using, P: Phosphorus dissolving, S+: Strong positive, W+: Weak positive, +: Positive

2.2. Methods

Bacteria used in the experiment were planted on Nutrient Agar in Iğdır University Faculty of Agriculture Department of Plant Protection laboratories and kept at 30°C for 24 hours. After the soaking process, a suspension was formed from the bacterial cultures in Nutrient Broth. The application of bacteria to plants was done at a density of 109 CFU ml-1 (Esitken et al., 2006).

Bacteria were first applied to the roots before planting, and then a total of 6 applications were made, 5 times with a one-month interval. After root pruning and cleaning of the seedlings in inoculation, the roots were kept in the bacterial suspension for 30 minutes. After bacterial inoculation, the saplings were planted in 12-liter pots containing soil: sand: fermented manure at a ratio of 2:1:1. The bacteria applications of the saplings planted in March were repeated in April, May, June, July, and August by pouring bacterial suspensions into the pots.

The following measurements and analyzes were made on the saplings removed at the end of the growing season.

The plant height of the saplings was measured with the help of a tape measure from the soil level. The number of bottom shoots of the saplings was deter-mined by counting. The shoot length of the seed-lings was measured with a tape measure. At the end of the experiment, wet weight measurements were taken by cutting the shoots determined before the experiment with the help of scissors and then kept in an oven at 72°C for 48 hours and their dry weights were weighed using precision balances. The diameters of the shoots were measured with the help of digital caliper. At the end of the experiment, the wet weight of the root parts of the plants removed from the pots was taken and then, after 48 hours in an oven at 72°C, their dry weights were weighed using precision balances. At the end of the experiment, the root length of the plants removed from the pots was measured with a tape measure (Ipek et al., 2014).

After the leaf samples were taken for nutrient analysis, they were brought to the laboratory, washed, and then dried at 65-70°C. The dried leaves were ground in a porcelain mortar and then the micro-Kjeldahl method was used for N determination, the vanado-molybdic yellow color method for P analysis, and the Mohr method for Cl analysis. The analyzes of K, Ca, Mg, Mn, Fe, Zn, Cu, B, Na nutrients were per-formed with the ICP device (Soltanpour et al., 1979).

For the analysis of plant growth regulators, 0.1 g leaves were homogenized and poured into 1.5 ml vials, by adding 500 µl 1-propanol/Water/HCL (2/1/0.002) solution and 10-50 ng internal standard, at 4°C for 30 minutes was kept. Then 1 ml of di-chloromethane was added and incubated at 4°C for 30 min. At the end of this period, it was centrifuged at 13000 xg for 5 minutes and 1 ml of the lower phase was evaporated and dissolved again with 0.3 ml of methanol. Growth regulator analyzes were performed with LC-MS/MS device (Pan et al., 2008).

2.3. Evaluation of data

The research was established according to the randomized plots experimental design, each application with 3 replications and 5 saplings for each replication. The data obtained from the research were subjected to the Duncan multiple comparison test and the analyzes were made at the 5% significance level. SPSS 23 package program was used for analysis.

3. Results

3.1. Effects of applications on vegetative characteristics

The effect of applications on plant height was found to be statistically significant. SK-39, SK-50 and SY-43 bacterial strains increased the plant height com-pared to the control, and the highest increase occurred with SK-39 bacteria application (Table 2).

Table 2. Effects of bacteria applications on vegetative properties

Treatments	Plant height (cm)	Shoot number per plant	Shoot length (cm)	Shoot diameter (cm)	Plant fresh weight (g)	Plant dry weight (g)
Control	49.40 c*	6.40 a	28.17 c	3.08 c	10.92 cd	8.76 bc
SK-4	48.00 c	4.40 c	29.23 c	3.35 bc	9.96 d	7.18 d
SK-39	55.20 a	5.20 bc	34.86 a	4.99 a	12.50 b	9.35 b
SK-50	52.60 ab	6.00 ab	33.46 b	3.64 bc	14.14 a	11.06 a
SY-43	52.60 ab	4.60 c	32.69 b	3.79 b	11.36 c	8.78 bc
SY-48	50.60 bc	6.20 a	27.09 d	3.31 bc	10.18 d	8.26 c
LSD	2.81	0.77	1.06	0.64	1.02	1.00

*: There is no difference between the averages shown with the same letter in the same column

The effects of bacteria applications on the number of shoots were found to be statistically significant. Bacterial treatments reduced the number of shoots compared to the control. While the number of shoots decreased in SK-4, SK-39, SY-43 applications compared to the control, there was no change in the number of shoots in SK-50 and SY-48 applications compared to the control.

The effect of the applications on the shoot length was found to be statistically significant. SK-39, SK-50 and SY-43 bacteria strains increased shoot length compared to the control, and the highest increase occurred with SK-39 bacteria application.

The effect of bacteria applications on shoot diameter was found to be statistically significant. SK-39 and SY-43 bacterial strains increased shoot diameter compared to control, but the effects of other treatments were not significant.

The effects of bacteria applications on “Chester Thornless” blackberry cultivar on plant fresh and dry weights were found to be statistically significant. SK-50 (14.14 g) and SK-39 (12.50 g) bacteria strains increased plant fresh weight compared to the control, while the effects of other treatments were found to be insignificant. While there was an in-crease in the dry weight of the plant only in the ap-plication of SK-50 (11.06 g) compared to the control, there was a decrease in the application of SK-4 (7.18 g) compared to the control. The effects of other applications were found to be insignificant.

The results show that the applications have a positive effect on vegetative characteristics. The effect of these bacteria promoting plant growth on increasing the sapling height can be explained by the growth promoting substance synthesis of rhizobacteria. In addition, nitrogen is required to support shoot growth. In this respect, the strong N-fixing properties of SK-39, SK-50, and SY-43 bacterial strains, which have the most increasing effects on plant and shoot length (Table 1), support the obtained result. In a study conducted on apple seed-lings, it was determined that rhizobacteria increased the sapling height (Parıltı, 2018). In addition, studies on different fruit species have reported that growth-enhancing bacterial strains significantly increase vegetative development (Eşitken et al., 2006; Aslantaş et al., 2007; Karlıdağ et al., 2007; Karakurt and Aslantaş, 2010; Coşkun and Pırlak, 2017; İpek et al., 2017b).

The effects of bacteria applications on root fresh and dry weights of “Chester” blackberry cultivar were found to be statistically significant. Except for the SY-48 bacterial strain, the treatments increased the root wet weight compared to the control. The highest increase was detected in the SK-50 application (94.42 g). The positive effect of bacteria strains on root dry weight was more limited. While SK-50 and SK-39 bacterial strains increased the root dry weight compared to the control, there was no change in other applications compared to the control (Table 3).

Table 3. Effects of bacteria applications on some root properties.

Treatments	Root fresh weight (g)	Root dry weight (g)	Root length (cm)
Control	65.76 e*	57.91 cd	65.20 b
SK-4	76.94 c	58.06 c	57.20 c
SK-39	79.70 b	66.38 b	69.60 a
SK-50	94.42 a	74.08 a	65.60 b
SY-43	72.24 d	56.91 cd	55.60 c
SY-48	61.62 f	55.88 d	73.00 a
LSD	2.36	2.00	6.59

*: There is no difference between the averages shown with the same letter in the same column

There are differences between the effects of bacteria applications on root length. SY-48 and SK-39 bacteria strains increased root length compared to con-trol, SK-4 and SY-43 decreased, and SK-50 did not cause any change compared to control. The application that increases the root length the most is SY-48. Similarly, in studies examining the effects of different bacteria strains on vegetative properties in rasp-berry and blackberry, it was reported that some bacteria increased root length and root fresh and dry weight compared to control, while others decreased (İpek et al., 2018; İpek and Eşitken, 2022).

3.2. Effects of Applications on Nutrient Content

The effects of bacteria applications on the macro and micronutrient contents of the examined leaves were found to be statistically significant, except for Mg (Table 4). Accordingly, applications increased the leaf N content significantly compared to the control, and the highest increase was determined in SY-43 and SK-39 bacterial strains. Similarly, leaf P contents increased with bacteria applications, and the applications that provided the highest increase were SK-50 and SK-39. Leaf K contents also in-creased with applications. The highest leaf K con-tents were determined in SK-4 and SK-39 bacteria applications. Ca contents also increased significantly with the applications, and the highest Ca contents were determined in SK-50 and SY-42 bacteria strains. The effects of bacteria applications on leaf Mg contents were found to be statistically insignificant. Applications also increased the leaf S contents in general compared to the control, and the highest increases were detected in SK-50 and SY-48 breeds. Bacterial treatments reduced leaf Mn contents. There are differences between the effects of applications on leaf Fe content. SK-50 bacteria strain increased the leaf Fe content compared to the control, decreased SY-43, SY48 and SK-39, and there was no change in SK-4 compared to the control. Applications significantly increased the leaf Zn content; the highest increase was determined in SK-39 bacterial strain. Likewise, the leaf Cu content increased as a result of the applications. The highest leaf Cu content was determined as 7.06 ppm in SK-4 application. The applications also increased the Na content of the leaves, and the highest increase was determined in the SK-50 bacterial strain. Leaf Na content, which was 109.54 ppm in the control, in-creased to 248.21 ppm in the SK-50 application.

It was determined that all bacteria strains used in this study increased all the nutrients examined in blackberry leaves, except for iron and manganese. Indeed, Somers et al. (2004) reported that bacteria that increase plant growth facilitate the uptake of nutrients by plants. Since the nitrogen fixing, potassium dissolving, calcium utilization and phosphorus dissolving properties of the bacteria used were determined as positive and strongly positive (Table 1), it is an expected result that they increase their in-take of this element. Mulberry (Sudhakar et al., 2000), strawberry (Köse, 2003), sour cherry (Arikan and Pirlak, 2016), pear (Ipek et al., 2017a), apple (İpek et al., 2017b) and It has also been reported in studies on raspberry (Ipek, 2019).

Table 4. Effects of bacteria applications on leaf nutrient content.

Treatments	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	Mn (ppm)	Fe (ppm)	Zn (ppm)	Cu (ppm)	Na (ppm)
Control	2.33 b*	0.19 b	1.56 c	0.76 b	0.19 a	0.13 b	30.93 ab	87.11 b	10.37 c	3.97 c	109.54 e
SK-4	2.90 a	0.29 a	2.25 a	0.98 ab	0.28 a	0.16 ab	34.06 a	86.15 b	16.68 b	7.06 a	148.91 d
SY-43	3.19 a	0.27 ab	2.03 ab	1.12 ab	0.23 a	0.16 ab	28.82 bc	73.80 c	15.52 b	6.75 a	164.42 cd
SY-48	2.68 ab	0.27 a	1.76 bc	1.03 ab	0.27 a	0.21 a	21.67 d	75.11 c	16.51 b	5.63 b	189.66 bc
SK-39	3.09 a	0.31 a	2.11 ab	1.07 ab	0.22 a	0.18 ab	23.74 cd	78.77 c	18.26 a	6.65 a	208.10 b
SK-50	2.82 ab	0.34 a	2.04 ab	1.22 a	0.22 a	0.23 a	27.15 bc	100.35 a	16.69 b	6.58 a	248.21 a
LSD	0.56	0.09	0.45	0.37	0.16	0.07	4.10	5.58	1.57	0.93	25.76

*: There is no difference between the averages shown with the same letter in the same column

3.3. The Effect of Applications on the Amount of Plant Growth Regulators

Indole acetic acid (IAA), gibberellic acid (GA), cytokinin, zeatin and abscisic acid (ABA) amounts were determined in leaf samples taken from plants belonging to Chester blackberry variety. The effects of bacteria applications on the amount of growth regulators were found to be statistically significant (Table 6). Applications significantly increased the amount of leaf IAA compared to the control, and the highest increases were detected in SK-39 and SY-43 breeds. Similarly, bacteria applications also in-creased the leaf GA contents, and the highest GA content was determined in SY-48 application. Ap-plications also significantly increased the cytokinin content in the leaves. Compared to the control, the highest increases occurred in SY-48 and SK-39 breeds. Applications also increased the leaf zeatin content in general. While an increase was observed in the SK-4, SY-43, SY-48 and SK-39 applications compared to the control, the SK-50 application was statistically in the same group as the control. Applications increased leaf IAA, GA, cytokinin and zeatin contents, while significantly decreased ABA content. All bacteria strains decreased the leaf ABA content compared to the control, and the ABA content, which was 10282 ng mg-1 in the control, de-creased to 1647 ng mg-1 in the SK-50 application.

Table 5. Effects of bacteria applications on the amount of plant growth regulators.

Treatments	IAA (ng mg^{-1})	GA (ng mg^{-1})	Sitokinin (ng mg^{-1})	Zeatin (ng mg^{-1})	ABA (ng mg^{-1})
Control	1.78 d*	3.06 d	2.45 d	1.18 d	10281 a
SK-4	3.20 c	5.34 b	6.73 c	2.77 a	5636 b
SK-39	7.41 a	6.82 a	11.66 a	2.07 bc	1819 d
SK-50	4.85 bc	4.08 c	6.31 c	1.57 cd	1647 d
SY-43	7.14 a	6.54 a	10.48 b	2.59 ab	4113 c
SY-48	5.26 ab	6.90 a	12.25 a	2.34 ab	2633 d
LSD	1.48	0.99	0.77	0.59	16.76

*: There is no difference between the averages shown with the same letter in the same column

According to the findings obtained in this study, it was determined that bacteria applications decreased ABA. It has also been reported in previous studies that rhizobacteria indirectly contribute to plant development by affecting the production of hormones such as indole acetic acid, cytokinin and zeatin (Lindow and Brandl, 2003; Vorholt, 2012; İmriz et al., 2014).

4. Conclusions

According to the findings, the bacterial strains used in the research generally increased plant and root growth, encouraged nutrient uptake, increased growth-promoting hormones, and decreased the amount of inhibitors.

When all data are evaluated, the use of *Herbaspirillum huttiense* SK-39, *Achromobacter xylosoxidans denitrificans* SK-50, *Pantoea agglomerans* GC subgroup SY-43 and *Microbacterium ester aromaticum* bacteria in order to promote growth and development in the cultivation of blackberry saplings.

Author Contributions: Conceptualization, L.P. and Z.A.A.A.; methodology, Z.A.A.A., M.T., M.F.D.; validation, L.P., A.J.K.K.; formal analysis, L.P., A.J.K.K.; resources, Z.A.A.A.; writing—original draft preparation, Z.A.A.A., L.P., A.J.K.K.; writing—review and editing, Z.A.A.A., L.P., A.J.K.K. All authors have read and agreed to the published version of the manuscript.

Funding: Please add: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

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