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The Effect of PGPR Applications on Bioactive Content and Fruit Characteristics of Different Apple Scion–Rootstock Combinations

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Abstract
In modern apple growing, plant and pomological characteristics as well as physiological behaviors of genotypes may vary according to the rootstock, changing growth ecology, and applications of biological control agents. The aim of this research is to determine the effects of rhizobacteria application on the biochemical substances (contents of total phenolics, total flavonoids and total anthocyanin and antioxidant activity) in fruits. This study was carried out on seven standard apple cultivars ('Scarlet Spur', 'Red Chief', 'Fuji', 'Jeromine', 'Galaxy Gala', 'Granny Smith' and 'Golden Reinders') grafted on M9 and MM106 rootstocks. Within the scope of the study, nitrogen + phosphorus solvent rhizobacteria were applied to each tree three times in 15 days in the spring period. On the other hand, in the study, the effects of rhizobacteria application on the biochemical contents of the fruits differed according to scion–rootstock combinations and these provided generally significantly positive contributions. Considering the fruit color data, the highest result was obtained from hue angle with 122.41 on 'Granny Smith' grafted to MM106 rootstock. According to the phenolic compound analysis, the highest phenolic compound content was epicatechin with 15.77 mg/kg, determined on 'Scarlet Spur' grafted to M9 rootstock. The highest positive contribution was 5.5% in total phenolic content, 4.5% in total flavonoid content, 3.3% in total anthocyanin content, and 5.7% in antioxidant activity. According to the results of this study, it has been determined that bacteria have positive effects on different fruit properties, but results may change with climate, growing conditions, environment and soil properties.

Keywords Individual phenolics · Rootstock · Rhizobacteria application · Biochemical content

Introduction

Rosaceae is a family of flowering plants under Rosales order. It contains the genera *Alchemilla, Sorbus, Crataegus, Cotoneaster, Rubus* and *Prunus*. Many different commercially grown fruits like apple, plum, apricot are in the Rosaceae family (Kant et al. 2018).

Horticultural plants have recently gained more popularity in. They include high content of non-nutritive, nutritive, and bioactive compounds such as flavonoids, phenolics, anthocyanins, phenolic acids, and as well as nutritive compounds such as sugars, essential oils, carotenoids, vitamins, and minerals. They also have a distinct flavor and taste, excellent medicinal value, and health care functions (Dogan et al. 2014a, b; Ersoy et al. 2018; Bolaric et al. 2021; Grygorieva et al. 2021).

Rootstocks have contributed significantly to the rapid developments in the cultivation in large areas of apple, which is the most produced species after banana in the world (Giorgi et al. 2005). All morphological, physiological, and biochemical events that occur in plants fall into the interaction area of rootstock and scion (de Oliveira Sousa 2022). In many countries of the world, studies on rootstock and

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scion interaction have been carried out for many years and efforts are made to illuminate the events that occur because of this interaction.

Türkiye is an important agricultural country with its agricultural production potential, the diversity of agricultural products produced and the structure of natural resources (Ercisli 2004). Many fruit species with different climate and soil requirements can be grown together. Apple has the highest production amount among fruit types in Türkiye with 4.3 million tons (TUIK 2021). Although the land areas where agricultural production is made remain constant, the world population is increasing gradually, and this requires obtaining more products per unit area. The way to get more products from a unit area is possible by using agricultural inputs such as fertilizer, seeds, water, pesticides at an adequate level and on time. The cultivation of our country's soils for years, the insufficient application of additives to improve the soil structure, the excessive and unconscious use of some chemical fertilizers and the effect of natural conditions have made our soils unproductive (Karaman 2006).

Plant growth promoting bacteria (Plant Growth Promoting Rhizobacteria [PGPR]) from free-living organisms in the soil are very useful in the production of agricultural products. These rhizobacteria species are usually bacteria included in the species *Pseudomonas* spp., *Azospirillum* spp., *Burkholderia* spp., *Bacillus* spp., *Enterobacter* spp., *Rhizobium* spp., *Erwinia* spp., *Serratia* spp., *Alcaligenes* spp., *Arthrobacter* spp., *Acinetobacter* spp. and *Flavobacterium* spp. (Koskey et al. 2017; Bargaz et al. 2018; Adeyemi et al. 2019). These rhizobacteria have many benefits on plant growth and productivity. They increase plant growth by increasing the nutrients in plants and include N fixation (Fukami et al. 2018) and P and K solubility (Soumare et al. 2020), which is the most studied pathway. In recent years, the use of rhizobacteria in sustainable agriculture has increased to increase soil fertility, improve crop products, and reduce the negative effects of chemical fertilizers on the environment. Rhizobacteria increase plant resistance against conditions that negatively affect plant growth, such as weed (Babalola et al. 2007), drought stress (Zahir et al. 2008), heavy metals (Kumar et al. 2009) and salt stress (Egamberdieva 2008; Kaymak et al. 2009), which are biotic and abiotic stress conditions. They provide yield increase and contribute to many morphological and physiological characteristics such as seed germination (Almaghrabi et al. 2014), root and shoot growth (Walker et al. 2012), leaf area, chlorophyll, protein, N and Mg contents in plants (Lucy et al. 2004; Selvaraj et al. 2008).

By using biofertilizers consisting of beneficial microorganisms instead of synthetic chemicals, plant growth is increased, environmental damage is largely prevented, and soil fertility is preserved (O'Connell 1992). Bacteria are generally grouped as biofertilizers that increase the nutrient ratio in the plant, phytostimulators that promote plant growth with plant hormone production, rhizoremediators that break down organic pollutants, and biopesticides that control diseases by producing antibiotics and antifungal metabolites. The use of these bacteria as biofertilizer and biocontrol agents in agriculture has increased especially in recent years (Basu et al. 2021; Wang et al. 2021).

The aim of this study is to determine the effects of nitrogen + phosphorus solvent bacteria (*Azospirillum* sp-245 + *Bacillus megaterium* M3) application on the biochemical properties of fruits in seven standard cultivars ('Scarlet Spur', 'Red Chief', 'Fuji', 'Jeromine', 'Galaxy Gala', 'Granny Smith' and 'Golden Reinders') grafted on two different rootstocks (M9 and M106).

Materials and Methods

Fruit Material and Experiment

The study was carried out in the Develi Plain, which has an area of approximately 1000 km^2, formed because of the volcanic movements of Mount Erciyes, between 2020 and 2021. The climate structure of the region is generally cold and snowy in winters and hot and dry in summers.

Experiments in the study were carried out on seven standard apple cultivars grafted on two rootstocks (M9 and MM106). 'Scarlet Spur', 'Red Chief', 'Fuji', 'Jeromine', 'Galaxy Gala', 'Granny Smith' and 'Golden Reinders' varieties were used as apple cultivars in the study. Soil samples of the orchard were taken as three samples from 0–30 cm and 30–60 cm depths before the applications and analyzed.

Table 1 Some nutrient content and physical and chemical properties of apple orchard soil

Soil depth	*P* *mg/kg*	*K* *mg/kg*	*Ca* *mg/kg*	*Mg* *mg/kg*	*Mn* *mg/kg*	*Zn* *mg/kg*	*Fe* *mg/kg*	*Cu* *mg/kg*
0–30 cm	11.5–15.1	151.3–241.5	1479.3–1750.0	228.1–258.9	18.2–29.7	2.66–3.58	1.10–1.53	1.38–2.12
30–60 cm	11.5–13.2	132.8–194.2	1512.0–1815.9	211.0–231.8	17.5–22.2	2.52–3.15	0.97–1.50	1.31–1.98
	Texture class	*EC* *(dS/m)*	*pH*	*Lime (%)*	*Organic matter (%)*		*Bacteria density (cfu/ml)*	
0–30 cm	Loamy	0.39–0.43	8.2–8.3	6.88–7.13	2.15–2.38		0.309×10^6–0.330×10^6	
30–60 cm		0.29–0.31	8.1–8.2	7.09–7.27	2.11–2.15		–	

The minimum and maximum values of the nutrient content and physical and chemical properties of apple orchard soil are given in Table 1.

The orchard was established in 2014 year with 75 cm within row and 4.0 m between row spacing for M9 rootstock, and at 1.5 m within row and 4.0 m between row spacing for MM106 rootstock. The plot of varieties grafted on M9 was established as a wire tree support system. Fertilizer application in the orchard was applied with drip irrigation (fertigation system) as 2 t/da of fertilizer every year.

Treatments

Azospirillum sp-245 and *Bacillus megaterium* M3 bacteria were used as rhizobacteria in the study. Bacteria were streak inoculated on Nutrient Agar and kept in a rotating shaker at 27 °C for 48 h. At the end of this period, the bacteria culture, which completed its growth aerobically, was transferred to bottles containing 15% glycerol and Nutrient Broth. The bacteria suspension was adjusted to 108 CFU/ml in sterile distilled water. Bacteria application was made to the crown projection areas of the trees, with 40 ml of solution per tree. The bacteria culture was sprayed with a low-pressure hand pump three times with an interval of 15 days after full flowering. No application was made to the control plants. The research was established according to the randomized blocks experimental design with three replications in each scion–rootstock combination and five trees in each replication. The effects of bacteria treatments were evaluated by determining biochemical contents in the fruits.

Color Characteristics

Color characteristics (L*, chroma and hue angle) was measured at opposite sides of each fruit with a colorimeter (Minolta, CR-400 model, Japan). The measurements were made in bright conditions from the points determined at two opposite poles of the equatorial part of each fruit. Values of L*, a* and b* were used to define a three-dimensional color space. The chroma value was calculated with the formula $C^* = (a^*2 + b^*2)1/2$, and the hue angle with $h^\circ = \tan^{-1} b^*/a^*$.

Total Phenolics, Total Flavonoids, Total Anthocyanins, and Antioxidant Activity

At the commercial harvest date, initially 15 fruits were selected from each replicate for biochemical contents. The seeds were removed, and flesh+skin was homogenized in blender. For the analysis of total anthocyanins, skins were also sampled. Resultant homogenates and skin samples were then stored at –20 °C until the analyses. Then, frozen flesh+skin samples were resolved at 21 °C. From the sample, 1 g was taken and 5 ml of 80% methanol solution was added. The mixture was shaken at 200 rpm for 60 min and centrifuged at 6000 rpm at 4 °C for 15 min. The obtained extract was used for the analysis of total phenolics, total flavonoids, and antioxidant activity.

The total phenolic contents were determined with Folin–Ciocalteu assay 100 µl extract was mixed with 400 mL distilled water and diluted (1/10) 1 mL Folin–Ciocalteu reagents. After an interval of 8 min, 5% Na_2CO_3 was added to 2 mL portions and the mixture was vortexed and incubated at room temperature for 90 min. Absorbance was then read at 765 nm wavelengths in spectrophotometer. Gallic acid was used as the standard. The results were expressed as mg of gallic acid equivalents (GAE) per 100 g of fresh weight.

Total flavonoids contents were determined in accordance with the principles specified by Karadeniz et al. (2005). A total of 1 mL extract was mixed with distilled water (4 mL) and 3% sodium nitrite ($NaNO_2$) solution (0.3 mL) followed by the addition of 0.6 mL of 10% aluminium chloride ($AlCl_3$) solution 5 min later. After 5 min, 2 mL of 1 mol L^{-1} sodium hydroxide (NaOH) and final solution made up to 10 mL with distilled water after 2 min. The solutions were then mixed, and absorbance was measured at 510 nm. The results were expressed as mg in 100 g catechin equivalents (CAE) on fresh weight basis.

Individual Phenolics

The individual phenolics were determined according to the procedure described by Singh et al. (2013). In the separation of phenolic compounds with ultra-high performance liquid chromatography (UHPLC; Thermo Scientific, Ultimate 3000, USA). The flesh+skin samples were extracted three times with 80% methanol solution. They were then centrifuged at 15,000 × g for 15 min. The supernatant was filtered with 0.45 µm millipore filters and then injected to UHPLC. The chromatographic separation was performed by using a DAD detector (DAD-3000, USA) in an UHPLC system. The analytes were separated by 250 × 3.0 mm, 5 µm Hypersil GD phenyl column (Thermo Scientific, USA) with temperature set at 30 °C. The elution solvents were aqueous 2.5% formic acid (solvent A) and 100% methanol (solvent B). The separation was conducted at 274 nm. Total run time took 40 min. Injection volume was 20 µL and the mobile phase flow rate was 1 ml min^{-1}. The results were expressed in mg kg^{-1}.

Data Analysis

The data obtained in the study were subjected to analysis of variance (ANOVA). The differences between the means were compared with the Tukey multiple comparison test. In addition, correlation analyzes were performed according

to the method specified by Sharma (Sharma 1996) to determine the relationships between enzymatic activity and other variables according to the applications.

Results and Discussion

The fruit skin color values in control and rhizobacteria application were statistically significant according to scion–rootstock interactions (Table 2). The data obtained from seven standard apple varieties on different rootstocks in the study are given in Table 2. The L* value, which indicates the brightness of the fruit skin, was determined the highest in 'Golden Reinders' variety and the lowest in 'Jeromine' variety grafted on both rootstocks in both control and bacteria application. While the effect of bacteria application on fruit skin L* value was generally positive, it was not in five combinations. The highest positive effect was observed in M9/Fuji combination with 8.8%.

Low values in the fruit skin color chroma value indicate dark color, while high values indicate light color. In control and bacteria application, the chroma value of the fruit skin color was determined to be the highest in 'Granny Smith' variety and the lowest in 'Fuji' variety, among the varieties grafted on both rootstocks. The effect of bacteria application on fruit skin color chroma value differed considerably according to scion–rootstock combinations. While bacteria application showed positive effect in five combinations, the effect was negative in other combinations.

In our study, hue angle values of cultivars with red fruit skin upper ground color were determined lower than other cultivars. The varieties with yellow and green fruit skin upper ground colors had higher hue angle than red ones. While the effect of bacteria application on fruit skin color hue value differed according to scion–rootstock combinations, a negative effect was observed in general. The highest increase in fruit skin color hue value because of bacteria application was detected in 'Fuji' (4.7%) and 'Jeromine' (3.8%) varieties grafted on M9 rootstock.

Although the color of the fruit skin is a variety feature, other factors also affect the coloration. For example, short days and cold night conditions have a strong effect on the coloration of fruit skins. As you go up to higher altitudes, the increased light intensity causes the fruits to be darker in color (Tonietto and Carbonneau 2004). On the other hand, it was determined that the bacteria application affected the fruit skin color values very little in the strawberry species (Ünal 2019). It is reported that the approach of the hue angle to zero indicates an increase in the red color tone in the apple, also the L* and chroma values generally decrease due to the increase in the red coloration (Rudell et al. 2002; Öztürk and Öztürk 2016).

The effects of scion–rootstock combinations on the phenolic compound contents and total of them in the apple fruits were statistically significant, except for hydroxy benzoic acid and caffeic acid in control group (Table 3). As a result of control group and bacteria application, the highest values in total phenolic substance, total flavonoid and total anthocyanin contents of the fruits were determined in 'Jeromine' variety grafted on both M9 and MM106 rootstocks. The lowest values were obtained in 'Granny Smith'/MM106 combination in total phenolic content, in 'Galaxy Gala' cultivar on both rootstocks in total flavonoid content, and in 'Granny Smith' and 'Golden Reinders' varieties in total anthocyanin content on both rootstocks. While the effects of bacterial application on

Table 2 The effect of rhizobacteria application on fruit skin color

Rootstock	Cultivars	L*		Chroma		Hue	
		Control	Bacteria	Control	Bacteria	Control	Bacteria
M9	'Scarlet Spur'	37.31 fg[(1)]	35.47 e	74.46 bc[(1)]	73.94 b	39.50 e[(1)]	40.09 d
	'Fuji'	52.35 d	56.96 c	24.03 i	24.36 f	39.74 e	41.61 cd
	'G. Smith'	67.71 bc	65.81 b	79.07 ab	79.72 a	121.91 a	115.89 a
	'G. Gala'	62.50 c	63.07 b	37.30 h	37.50 e	52.73 d	48.98 c
	'Golden Reinders'	75.05 a	76.03 a	41.88 gh	41.95 e	112.87 b	113.53 a
	'Red Chief'	46.35 de	45.47 d	42.41 gh	41.04 e	28.29 f	28.61 e
	'Jeromine'	35.85 g	36.75 e	68.47 cd	66.66 c	17.01 g	17.64 f
MM106	'Scarlet Spur'	42.65 ef	45.11 d	51.29 e	50.43 d	18.73 g	18.37 f
	'Fuji'	50.96 d	51.94 c	22.42 i	22.03 f	37.96 e	37.23 d
	'G. Smith'	70.56 ab	67.41 b	83.15 a	80.59 a	122.41 a	120.94 a
	'G. Gala'	63.31 c	63.50 b	41.27 gh	42.60 e	66.61 c	65.10 b
	'Golden Reinders'	74.84 a	73.40 a	44.33 fg	42.56 e	113.62 b	114.24 a
	'Red Chief'	42.15 ef	45.18 d	49.30 ef	49.09 d	22.22 fg	22.00 ef
	'Jeromine'	34.57 g	35.00 e	62.90 d	61.38 c	16.17 g	15.20 f

Differences within each treatment (control, bacteria effect) are shown with separate letters

Table 3 The effect of bacterial application on phenolic compounds (mg/kg) in scion–rootstock interactions

Rootstock	Cultivar	Amino-benzoic acid	Proto-catechuic acid	Hydroxy benzoic acid	Catechin	Chlorogenic acid	Caffeic acid	*p*-Coumaric acid	Epicatechin	Trans-ferulic acid	Rutin	Total phenolic compound
Control												
M9	'Scarlet S.'	10.15 b–e[1]	0.52 cd	0.01	3.83 e–g	14.19 a	0.59	1.37 ab	11.03 b–d	0.05 de	1.29 de	43.08 bc
	'Fuji'	12.66 bc	1.03 ab	0.03	7.81 ab	4.58 b	0.84	2.00 ab	7.03 ef	0.03 e	2.32 a–d	38.38 d–g
	'G.Smith'	9.28 de	0.84 a–d	0.03	4.49 d–f	4.68 b	0.50	2.24 ab	11.99 a–d	0.12 b–e	1.77 a–e	35.98 f–h
	'G.Gala'	10.39 b–e	0.44 d	0.03	2.70 g	6.66 b	0.46	1.54 ab	10.95 cd	0.07 c–e	1.49 c–e	34.77 f–h
	'Golden R.'	9.64 c–e	0.60 b–d	0.02	5.02 c–f	13.42 a	0.48	1.04 b	9.14 de	0.17 a–c	2.55 a–c	42.10 b–d
	'Red Chief'	11.50 b–e	0.79 a–d	0.03	4.58 d–f	13.59 a	0.59	2.16 ab	9.06 de	0.10 c–e	2.83 a	45.26 ab
	'Jeromine'	13.41 ab	1.05 ab	0.05	8.44 a	5.28 b	0.83	2.21 ab	14.24 ab	0.05 de	1.40 de	46.99 a
MM106	'Scarlet S.'	16.08 a	0.40 d	0.05	5.75 cd	8.23 b	0.61	1.75 ab	4.76 f	0.21 ab	2.67 ab	40.54 c–e
	'Fuji'	10.21 b–e	0.96 a–c	0.06	5.41 c–e	8.17 b	0.67	2.01 ab	9.17 de	0.26 a	1.46 c–e	38.41 d–f
	'G.Smith'	8.75 e	0.94 a–c	0.05	4.81 d–f	5.91 b	0.63	2.33 ab	9.29 de	0.11 b–e	0.71 e	33.58 h
	'G.Gala'	9.70 c–e	1.18 a	0.04	3.62 fg	5.45 b	0.55	1.90 ab	10.91 cd	0.14 b–e	1.07 e	34.58 gh
	'Golden R.'	12.13 b–d	0.84 a–d	0.08	3.95 e–g	5.47 b	0.57	2.63 a	9.59 de	0.12 b–e	1.73 a–e	37.14 e–h
	'Red Chief'	8.30 e	1.01 ab	0.06	6.56 bc	7.50 b	0.76	1.40 ab	13.75 a–c	0.15 b–d	1.71 a–e	41.22 cd
	'Jeromine'	9.52 c–e	0.94 a–c	0.05	5.82 cd	6.74 b	0.72	1.78 ab	14.98 a	0.10 c–e	1.67 b–e	42.36 bc
Rhizobacteria application												
M9	'Scarlet S.'	13.69 a	0.57 b–d	0.05 ab	4.43 cd	5.55 c–e	0.55 b–d	1.73 b–d	15.77 ab	0.20 a–c	2.37 cd	44.94 a–c
	'Fuji'	10.84 a–c	0.69 a–d	0.05 ab	9.02 a	7.78 bc	0.91 a	1.69 b–d	5.35 f	0.02 c	2.93 bc	39.32 e–g
	'G.Smith'	10.59 a–c	0.51 cd	0.07 ab	4.78 cd	4.79 de	0.60 b–d	1.65 b–d	10.61 de	0.10 a–c	3.72 b	37.44 f–h
	'G.Gala'	9.12 cd	0.39 d	0.02 b	2.68 d	8.80 ab	0.33 d	2.22 bc	10.08 de	0.06 bc	0.64 e	34.36 hi
	'Golden R.'	10.90 a–c	1.03 ab	0.05 ab	5.43 b–d	7.33 b–d	0.75 a–c	2.24 bc	14.27 a–c	0.19 a–c	1.84 c–e	44.06 b–d
	'Red Chief'	7.23 d	0.99 a–c	0.04 b	6.16 bc	8.95 ab	0.77 a–c	4.59 a	16.49 a	0.27 ab	1.64 c–e	47.16 ab
	'Jeromine'	11.08 a–c	0.96 a–c	0.06 ab	8.14 ab	5.72 c–e	0.78 a–c	2.58 bc	16.26 a	0.06 bc	1.67 c–e	47.35 a
MM106	'Scarlet S.'	9.73 cd	0.98 a–c	0.06 ab	7.59 ab	7.32 b–d	0.82 ab	1.54 cd	12.51 b–d	0.32 a	1.49 de	42.40 c–e
	'Fuji'	9.06 cd	0.45 d	0.03 b	4.82 cd	11.48 a	0.64 a–c	0.54 d	11.05 c–e	0.12 a–c	1.76 c–e	39.98 ef
	'G.Smith'	10.36 b–d	1.14 a	0.05 ab	3.06 d	4.16 e	0.63 a–c	2.16 bc	9.59 de	0.15 a–c	1.54 de	32.88 i
	'G.Gala'	10.98 a–c	0.75 a–d	0.05 ab	3.81 cd	9.51 ab	0.49 cd	2.35 bc	5.21 f	0.04 bc	1.03 e	34.26 hi
	'Golden R.'	13.08 ab	0.52 cd	0.02 b	4.10 cd	8.12 bc	0.63 a–c	1.47 cd	7.58 ef	0.07 bc	0.65 e	36.38 gh
	'Red Chief'	10.83 a–c	0.98 a–c	0.06 ab	4.78 cd	5.81 c–e	0.63 a–d	3.16 ab	12.23 cd	0.13 a–c	2.49 b–d	41.22 de
	'Jeromine'	11.66 a–c	0.69 a–d	0.11 a	7.73 ab	7.14 b–d	0.63 a–d	1.47 cd	5.40 f	0.21 a–c	7.31 a	42.37 c–e

[1]Differences within each treatment (control, bacteria effect) are shown with separate letter

Table 4 The effect of rhizobacteria application on biochemical properties of fruits

Rootstock	Cultivar	TPC (mg GAE 100 g^{-1})		TFC (mg CAE 100 g^{-1})		TAC (mg cyanindin 3-glycoside 100 g^{-1})		AA (% Inhibition)	
		Control	Bacteria	Control	Bacteria	Control	Bacteria	Control	Bacteria
M9	'Scarlet S.'	94.66c	99.60c	50.59 cd	52.37c	5.45b	5.54bc	69.13a	68.71a
	'Fuji'	75.15f	75.85g	45.68e	46.02de	3.80e	3.63d	53.67b–d	56.54e
	'G.Smith'	60.93h	59.58i	42.26e–g	42.29d–f	0.08f	0.07e	44.14e	46.05g
	'G.Gala'	71.37 fg	68.47gh	38.51gh	38.25f	4.14 cd	4.27d	50.46d	50.49f
	'Golden R.'	88.63de	89.75e	44.77e	45.16de	0.19f	0.18e	58.59b	59.09d
	'Red Chief'	107.40b	109.55b	60.01b	61.00b	4.22c	4.32d	66.69a	67.25a–c
	'Jeromine'	117.82a	118.43a	67.84a	67.48a	6.44a	6.53a	66.64a	65.94bc
MM106	'Scarlet S.'	89.26 cd	94.20de	46.34de	47.15d	5.34b	5.42c	66.87a	68.41ab
	'Fuji'	69.22 fg	71.15gh	43.75ef	44.60de	3.83de	3.60d	54.82b–d	54.52e
	'G.Smith'	54.82i	55.91i	40.00f–h	41.81ef	0.08f	0.10e	39.84e	38.37h
	'G.Gala'	65.83gh	67.77h	37.27h	38.44f	4.07c–e	4.16d	51.28 cd	51.07f
	'Golden R.'	83.19e	81.67f	45.56e	45.66de	0.22f	0.17e	55.69bc	58.85de
	'Red Chief'	94.98c	96.21 cd	55.21c	55.96c	4.11c–e	4.16d	64.56a	65.33c
	'Jeromine'	112.97ab	114.21ab	66.89a	67.22a	6.37 a	6.19b	64.64a	67.83a–c

TPC Total phenolics content, *TFC* Total flavonoids content, *TAC* Total anthociyanin content, *AA* Antioxidant capacity

total phenolic substance, total flavonoid and total anthocyanin contents differed in scion–rootstock combinations, the effect was generally positive (Tab. 4). Bacteria application provided the highest positive contribution in 'Scarlet Spur'/MM106 with 5.5% in total phenolic content, 'Granny Smith'/MM106 with 4.5% in total flavonoid content, and 'Galaxy Gala'/M9 combination with 3.3% in total anthocyanin content.

In the control application, the highest antioxidant activity was determined between 64.56% and 69.53% in six variety–rootstock combinations. The lowest antioxidant activity was obtained from the combination of 'Granny Smith' variety (39.84% and 44.14%, respectively) on MM106 and M9 rootstocks. The effect of bacterial application on antioxidant activity was generally positive. The highest increase in antioxidant activity because of bacteria application was observed in 'Golden Reinders'/MM106 combination with 5.7%, followed by 'Fuji'/M9 combination with an increase of 5.3% and 'Jeromine'/MM106 combinations with an increase of 4.9%.

Although the application of bacteria on the biochemical contents of fruits (total phenolics substance content, total flavonoids amount, total anthocyanin, and antioxidant activity) differed according to scion–rootstock combinations, it had a positive effect in general. Although there are findings on the positive effects of plant growth promoting bacteria on quality parameters such as fruit weight, especially in apple species, there is a gap in the literature regarding the effect on biochemical properties. On the other hand, it has been reported that bacteria strains generally improve the biochemical properties of fruits in raspberry (Ünal 2019).

Conclusion

According to the results, bacteria have positive effects on different fruit properties; however, with the effect of the environment on rootstocks, these results may change. Therefore, many different varieties grafted to different rootstocks are commercially grown around the world. Although our study has shown the effects of bacteria on different fruit properties, it also shows that there is a need for more detailed research.

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Conflict of interest M. Yaman, E. Yildiz, A. Sumbul, S. Ercisli, O. Sonmez, A. Gunes, A. Say, Y. M. Kece and H. T. Unsal declare that they have no competing interests.

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