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Effect of Rhizobacteria Application on Nutrient Content, Bioactive Compounds, Antioxidant Activity, Color Properties and Fruit Characteristics of Strawberry Cultivars

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Abstract: The aim of this study was to determine the effects of single and combined applications of plant-growth-promoting rhizobacteria (PGPR) bacteria on plant nutrition, biochemical content and fruit characteristics in Albion and Monterey strawberry cultivars. *Bacillus subtilis* OSU-142, *Bacillus megaterium* M3 and *Paenibacillus polymyx* were the PGPR used in the experiment. For each bacterial treatment, 10 mL of a 10^8 CFU mL^{-1} suspension was applied to the soil where Albion and Monterey cultivars were grown. PGPR bacteria were applied as single treatments and a mixture of equal amounts of these three bacterial species was applied as a mixed treatment. This study was carried out with a total of four different bacterial treatments and one control group. The highest fruit weight was obtained in the Monterey cultivar with 12.67 g in the Mix treatment and in the Albion cultivar with 11.79 g in the *Bacillus megaterium* M3 treatment. Regarding biochemical properties, *Paenibacillus polymyxa* was effective in influencing nutrient element content in fruits, while *Bacillus subtilis* OSU-142, *Paenibacillus polymyxa* and *Bacillus megaterium* M3 applications were more effective in leaf nutrient element content. It has been observed that the Mix treatment resulting from the combined use of bacteria, rather than their separate use, has a greater impact on fruit weight. Consequently, it has been understood that PGPR bacteria are potentially effective in improving the agronomic, pomological, and biochemical characteristics of strawberry cultivars and can be used in studies and breeding programs aimed at increasing strawberry yield and quality.

Keywords: strawberry; *Fragaria* x *ananassa* Duch; PGPR; bacterial inoculation

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1. Introduction

Strawberry is an important fruit species worldwide and is characterized by high adaptability. Strawberry belongs to the genus *Fragaria* in the Rosaceae family, Rosales order, and there are about 20 wild species in this genus [1]. The chromosome number of these species is x = 7 and there are four levels of polyploidy within the genus *Fragaria*: diploid, tetraploid, hexaploid, and octoploid [2]. The cultivated strawberry originated in the mid-18th century because of natural hybridization of *Fragaria chiloensis* L. and *Fragaria virginiana*

Duch. The strawberry cultivars used by breeders today usually belong to the species *Fragaria* x *ananassa* and have a polyploidy level of octoploid [3]. Strawberry plants, one of the most important berry fruits, can grow under various conditions, ranging from regions with an annual rainfall of less than 250 mm to areas at 3500 m altitude, and from 18 h day length to 12 h day length [4]. Türkiye's different climatic conditions allow strawberries to be grown in almost every region [5]. In 2022, approximately 9.6 million tons of strawberries were produced worldwide. China ranks first with a production of 3.36 million tons, while the USA, Türkiye, Mexico and Egypt are among the top five major producing countries. When Türkiye's strawberry production status is examined, an approximate doubling can be observed in the last 10 years. In 2022, strawberry production in Türkiye reached 728,112 tons [6].

Strawberry is a beneficial fruit for human health and nutrition [7]. According to the results of the previous studies, 100 g strawberry fruit contains 92 g water, 0.6 g protein, 0.4 g fat, 7.0 g carbohydrate, 0.5 g fiber, 166 g K and small amounts of P, Ca, Mg, Fe, Na, Mn and Cu, as well as 57 mg vitamin C and 522 mg amino acids [8]. Strawberries are a rich source of vitamin C, folate and minerals, as well as phenolic compounds with antioxidant and anti-inflammatory properties [9]. These properties suggest that strawberries have several health-promoting and disease-preventive effects, such as fighting inflammation, reducing oxidative stress, lowering the risk of obesity-related disorders, heart disease, and protecting against various types of cancer. Furthermore, strawberry polyphenols are also known to have effects on other pathways related to cellular metabolism and cellular survival [10].

The aim of modern fruit cultivation is to obtain high yields from a unit area with modern methods. For this purpose, greenhouse cultivation, and the use of chemical fertilizers and practices that increase the uptake of plant nutrients are among the effective methods [11]. Soil microorganisms improve nutrient uptake by plants and have great potential when used as biofertilizers. Free-living microorganisms used as biological control agents or biofertilizers are called plant-growth-promoting rhizobacteria (PGPR) [12]. PGPR are generally included in the genera *Acinetobacter*, *Achromobacter*, *Aereobacter*, *Agrobacterium*, *Alcaligenes*, *Artrobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Micrococcus*, *Pseudomonas*, *Rhizobium*, *Serratia* and *Xanthomonas* [13]. PGPR usually attach to the plant rhizosphere and inhibit or reduce the growth of soil-borne pathogens. In addition, the use of beneficial microorganisms instead of synthetic chemicals not only enhances plant growth but also prevents environmental damage and increases soil fertility [14].

Many positive effects of mycorrhizae and bacteria have been observed in previous studies in different plants. PGPR have been successfully applied in many different plant species such as quince [15], raspberry [16], cherry [17], sour cherry [18] and apple [19–21], and significant increases in plant yield and quality have been obtained. Recent studies have revealed the positive effects of the use of PGPR in strawberry cultivation for fruit quality and yield. Particularly, strawberry seedlings treated with *Azospirillum brasilense* and effective microorganisms produced larger and tastier fruits and increased the concentration of flavonoids and flavonols compared to the control group [22]. The root and foliar applications of PGPR improved strawberry plant growth parameters such as plant height, leaf area, and chlorophyll content, and physiological parameters such as photosynthesis, stomatal conductance and transpiration rate [23]. In strawberry plants, PGPR belonging to the genera *Bacillus* and *Pseudomonas* have been observed to promote the growth and significantly increase the population of other beneficial bacteria [24]. Furthermore, PGPR were found to protect strawberry plants from red spiders, strengthening the plants' natural defenses and increasing secondary metabolite production [25].

In strawberry cultivation, PGPR also become prominent in plant disease control. The use of the microalga *Chlorella fusca* as a potential biological control agent against Fusarium wilt disease in non-pesticide hydroponic strawberry cultivation was investigated. Microalgae treatment was found to be effective in improving strawberry plant health and yield by increasing the thickness of strawberry leaves and flowering rate and reducing

the incidence of Fusarium wilt [26]. PGPR applications are also effective as fertilizers in strawberry cultivation. The use of *Bacillus velezensis* strain in combination with various nitrogen fertilizers increased chlorophyll content and fruit yield [27]. It was concluded that PGPR-based biofertilizers improve the growth and physiological performance of strawberries by increasing nitrogen fertilization efficiency and are beneficial in terms of environmental and economic efficiency [28]. These findings indicate that the use of PGPR as a biofertilizer can improve strawberry quality and the growth of beneficial microorganisms and support agricultural sustainability.

The aim of this study was to determine the effects of single and combined applications of PGPR bacteria (*Bacillus subtilis* OSU-142, *Bacillus megaterium* M3, *Paenibacillus polymyx*) on strawberry growth and development in Albion and Monterey strawberry cultivars. For this purpose, plant nutrition, biochemical content and fruit characteristics were analyzed in response to bacterial treatments in strawberry. The results of this study reveal the potential of bacterial applications to increase yield and quality in strawberry cultivation.

2. Materials and Methods

2.1. Plant Material

Albion and Monterey strawberry cultivars (*Fragaria* x *ananassa* Duch), which constitute the study material, were selected from an open-field strawberry cultivation area in the Develi district of Kayseri in 2023. Three replications were used to carry out the experimental strategy, and ten plants were used for each treatment and cultivar being tested. After the treatments, fruit and leaf samples of the cultivars were taken. Samples taken from each strawberry plant for biochemical and morphological analyses were transferred to the laboratory in cold chain and kept at −80 °C until the analysis.

2.2. Methods

Bacillus subtilis, *Bacillus megaterium* and *Paenibacillus polymyxa* were used as bacteria in the areas selected for the experiment. All bacterial strains were obtained from Dr. Metin Turan (the culture collection of the Department of Genetics and Bioengineering, Faculty of Engineering and Architecture at Yeditepe University, Istanbul, Türkiye). These bacteria were reported to have properties that promote plant development and to have the potential to function as biocontrol agents against a broad variety of plant diseases [29,30]. The identification of bacteria in this study was performed by traditional methods such as biochemical tests for observation of bacterial colony characteristics and morphology. Homology analysis was recognized as a preferred method for rapid identification of bacteria due to its accuracy in identifying bacteria by genus [31]. Firstly, 16S rRNA sequence tests and homology analysis were performed; then, the culture was transferred to activated LB (Luria-Bertani) liquid medium and the fermentation broth was obtained. Centrifugation, acid precipitation and other methods were used to obtain the fermented supernatant and crude extract of bacteriocins. An antimicrobial round paper containing the fermentation broth and crude extract of bacteriocins was prepared and an inhibition test was performed, followed by identification. These bacteria, isolated and identified by Prof. Dr. Fikrettin ŞAHİN of Yeditepe University, were used in this study. Strains were characterized by Microbial Identification System (MIDI, Inc., Newark, DE, USA) software (MIS version no: 6:0) according to the commercial TSBA6 database. Bacteria were grown on nutrient agar by the streak plate inoculation method and kept at 27 °C for 48 h, and a single colony taken from the cultures that completed their growth at the end of this period was transferred to 250 mL of nutrient broth with 15% glycerol-containing flasks. They had grown aerobically in flasks on a rotating shaker (150 rpm) for 48 h at 27 °C (Merck KGaA, Darmstadt, Germany). The bacterial suspensions were then diluted in sterile distilled water to a final concentration of 10^8 CFU mL^{-1}. The resultant bacterial suspensions were used for treatment. In the treatment, 10 mL of each bacterial species was applied to the soil where Albion and Monterey cultivars were grown. *Bacillus megaterium* M3, *Bacillus subtilis* OSU-142, *Paenibacillus polymyxa* PGPR bacteria were applied as single treatments. In addi-

tion, a mixture of equal amounts of these three bacterial species was applied as a mixed treatment. This study was carried out with a total of four treatments and one control group. The experimental group without bacterial inoculation was considered the control group.

2.3. Pomological Analysis

Fruit sampling for the determination of fruit characteristics was carried out from the control and treatment plants, with 20 fruits representing the cultivar in each replicate. Fruit weight (g), fruit width and length (mm), pH, water-soluble dry matter (TSS) and titratable acid (TA) contents (%) and fruit peel color (according to C.I.E. L*a*b method with Minolta CR-300 chromometer in 2 opposite directions along the central axis of the fruit) were determined.

2.4. Biochemical Analysis

For the determination of the biochemical characteristics in fruit, fruit sampling was carried out in the control and treated plants, with 20 fruits representing the cultivar in each replicate.

Total phenolics: Total phenolic content was determined using the Folin–Ciocalteu reagent according to the method described by Slinkard and Singleton [32]. Accordingly, 80% methanol was added to 1 g of the sample taken from blended fruits and shaken at 200 rpm for 2 h at room temperature and then filtered with filter paper. Then, 400 µL of distilled water followed by 1 mL (1/10 folin/pure water) of Folin–Ciocalteu reagent were added to 100 µL of the obtained sample. After 8 min, 2 mL of 5% sodium carbonate (Na_2CO_3) was added to the solution and left to incubate in the dark for 90 min at room temperature. Finally, the absorbance of the blue-colored solution was recorded at a wavelength of 765 nm in a spectrophotometer. The results obtained were calculated in terms of Gallic acid as mg gallic acid equivalent (GAE)/100 g fresh weight.

Total flavonoid: Total flavonoid content was determined according to the method described by Karadeniz et al. [33]. Briefly, 1 g of blended fruit sample was weighed and mixed with 80% methanol, and then shaken at 200 rpm for 60 min at room temperature. After filtration, 1 mL of sample was taken and 4.0 mL of distilled water and 0.3 mL of 5% sodium nitrite ($NaNO_2$) solution were added and vortexed. After 5 min incubation, 0.6 mL of 10% aluminum chloride ($AlCl_3$) was added to the solution. The solution was incubated 5 min more and then 2 mL of 1 M sodium hydroxide (NaOH) was added and mixed. The final solution was kept for 2 min and then added to 10 mL with distilled water and after mixing, the absorbance values were read at 510 nm wavelength in a spectrophotometer. Total flavonoid content was calculated as mg CAE/100 g fresh weight using the standard curve obtained from catechin.

Total anthocyanin: Total anthocyanin content analysis was carried out based on the pH differential method according to Giusti et al. [34]. In this method, total monomeric anthocyanin content was determined at two different wavelengths (520 and 700 nm) and two different pH values (1.0 and 4.5). Then, 5 g of the blended fruit sample was weighed and 10 mL of methanol solution containing 1% hydrochloric acid (HCL) was added and the final mixture was kept overnight. Then, the vortexed solution was filtered with filter paper. To 1 mL of the sample, 4 mL of 0.025 M potassium chloride (KCl) buffer solution with a pH of 1.0 was added; to another 1 mL of the sample, 4 mL of 0.4 M sodium acetate buffer solution with a pH of 4.5 was added. The absorbances of the samples were recorded at 520 and 700 nm wavelengths in a spectrophotometer. Total anthocyanin content was calculated as mg cyanindin 3-glycoside/100 g fresh fruit.

Antioxidant activity: DPPH antioxidant activity of the fruits was determined by modifying the method described by Brand-Williams et al. [35]. Thus, 80% methanol was added to the sample and centrifuged at 200 rpm for 60 min at room temperature. The sample was then filtered through filter paper. The application of the method is as follows: 3900 µL of 0.2 mM DPPH (1.1-diphenyl-2-picryl-hydrazyl) solution was added to 100 µL of fruit extract and mixed with vortex and the mixture was incubated for 30 min at room

temperature in the dark. The absorbance values of the samples were determined in a spectrophotometer at 517 nm wavelength and the values were calculated according to the control and expressed as % inhibition.

2.5. Nutrient Analysis

The method applied by Sarıdaş [8] was used to determine the nutrient content of fruit samples. Fruits were harvested at full maturity. Leaf sampling was carried out in July by selecting healthy leaves that completed their development. Leaf and fruit samples were washed with pure water. The fruits and leaves were cut into small pieces, and dried in an oven at 65–70 °C to constant weight and ground. Nitrogen contents in 1 g of these samples were determined according to the "Kjheltec" method proposed by Lees [36]. Then, 1 g of the ground samples was weighed and dry-burned according to the method described by Kacar [37], and the phosphorus, potassium, calcium, magnesium, iron, zinc, manganese and copper contents of the samples were determined by ICP-OES [38].

2.6. Data Analysis

SPSS (Statistical Package for the Social Sciences) 15.0 package program was used for data analysis. "Duncan" multiple comparison test was used to evaluate the difference between the mean values of the characteristics. In addition, principal component analysis, heat map and correlation analysis were performed with JMP Pro 17 package program for the data obtained as a result of the treatments.

3. Results

In general, the effects of the bacterial treatments on the fruit characteristics (fruit weight, fruit width, fruit length, TSS, pH, TA and color space values) of the cultivars examined were found to be statistically significant (Table 1). Bacteria treatments showed mostly positive effects on fruit and leaf characteristics compared to the control group. The effects of the bacterial treatments on fruit weight, fruit width and fruit length varied among the cultivars. In the Albion variety, fruit weight increased from 8.87 g (control) to 11.79 g with the *B. megaterium* M3 treatment. The *P. polymyxa* (10.88 g) and Mix (10.78 g) treatments had the highest effect on fruit weight, while the *B. subtilis* OSU-142 treatment (7.85) showed a negative effect. In the Albion cultivar, the *B. megaterium* M3 treatment had the highest effect on fruit width (26.65 mm), and the Mix treatment (33.49) on fruit length. In the Monterey cultivar, only the Mix treatment (12.67 g) had a positive effect on fruit weight, while other treatments had a negative effect compared to the control. On fruit width and fruit length, the Mix treatment had the highest effect (25.31 mm and 34.73 mm, respectively).

Table 1. Effect of different bacterial treatments on fruit characteristics of strawberry.

Cultivar	Treatment	Fruit Weight (g)	Fruit Width (mm)	Fruit Length (mm)	TSS (%)	pH	TA	L*	a*	b*
Albion	Control	8.87 ab*	22.44 b	29.45 ab	9.10 d	3.72	0.93 a	33.04 a	80.13 abc	14.44 b
	B. subtilis OSU-142	7.85 b	23.18 b	28.84 c	10.16 ab	3.66	0.88 ab	29.81 b	86.78 a	14.65 b
	P. polymyxa	10.88 ab	22.63 b	28.81 c	9.76 bc	3.75	0.86 abc	31.97 a	83.53 ab	15.19 ab
	B. megaterium M3	11.79 a	26.65 a	31.43 ab	10.76 a	3.65	0.79 c	33.26 a	79.07 bc	16.58 a
	Mix	10.78 ab	25.02 ab	33.49 a	9.53 cd	3.76	0.84 bc	32.89 a	75.98 c	15.32 ab
Monterey	Control	9.56 ab	20.97 c	31.33 ab	9.73 b	3.91 ab	0.58	31.94 ab	89.17 ab	14.07 bc
	B. subtilis OSU-142	8.80 b	21.67 bc	27.82 b	9.26 b	4.02 a	0.57	30.52 b	83.01 ab	13.74 bc
	P. polymyxa	6.99 b	22.00 bc	25.72 b	11.13 a	3.73 b	0.55	35.62 a	70.06 c	16.48 b
	B. megaterium M3	6.57 b	24.96 ab	26.28 b	10.90 a	3.72 b	0.51	29.45 b	92.65 a	11.42 c
	Mix	12.67 a	25.31 a	34.73 a	11.03 a	3.88 ab	0.61	35.86 a	79.61 bc	20.89 a

* Differences between means indicated by different letters in each column are significant. Water-soluble dry matter (TSS), power of hydrogen (pH), titratable acid (TA); L* refers to the brightness of the fruit skin color, a* refers to the change in color of the fruit skin from green to red, b* refers to the color change in the fruit skin color from yellow to blue.

The effect of bacterial treatments on TSS was positive in both cultivars. In the Albion cultivar, the lowest TSS was found in the control treatment (9.10%) and the highest in the *B. megaterium* M3 treatment (10.76%). *B. substilis* OSU-142 (10.16%) had the second

highest effect on TSS. In the Monterey cultivar, the effect of bacterial treatments on TSS varied. The TSS, which was 9.73% in the control group, showed a negative effect with the *B. substilis* OSU-142 treatment (9.26%). In this variety, the highest TSS was determined in the *P. polymyxa* (11.13%), Mix (11.03%) and *B. megaterium* M3 (10.90%) treatments. The effect of bacterial treatments on pH and TA (titratable acidity) differed among varieties. The effect of bacterial treatments on pH in the Albion cultivar and TA in the Monterey cultivar was found not to be significant. Except *B. substilis* OSU-142, all other treatments had a negative effect on pH in the Monterey cultivar. In the Albion cultivar, all bacterial treatments had a negative effect on TA.

The effect of bacteria treatment on fruit color values differed according to treatments and cultivars. The *B. megaterium* M3 (33.26) and Mix (32.89) treatments had the highest effect on L* value, which indicates brightness, in the Albion variety, and the Mix (35.86) and *P. polymyxa* (35.62) treatments in the Monterey variety. In both varieties, the *B. substilis* OSU-142 treatment showed a negative effect compared to the control group. The highest a* value was determined in the *B. substilis* OSU-142 treatment (86.78) in the Albion cultivar and in the *B. megaterium* M3 treatment (92.65) in the Monterey cultivar. The highest b* value was determined in the *B. megaterium* M3 treatment (16.58) in the Albion cultivar and in Mix (20.89) in the Monterey cultivar.

The effects of bacterial treatments on the biochemical contents of fruits varied according to the treatment and cultivar (Table 2). In general, bacterial treatments positively affected the biochemical contents of the fruits. Total phenolic content of the Albion cultivar, which was determined as 377.01 mg GAE/100 g in the control group, was minimum in *B. substilis* OSU-142 (333.79 mg GAE/100 g), and maximum in the Mix (500.30 mg GAE/100 g) treatment. Bacterial treatments had a positive effect on total flavonoid content, while the highest value was determined in the *B. megaterium* M3 (75.54 mg CAE/100 g) treatment. *B. substilis* OSU-142 (173.87 mg cyn-3-gluc/100 g) showed a negative effect on total anthocyanin content, while *P. polymyxa* (230.190 mg cyn-3-gluc/100 g) showed the highest value. The minimum effect on antioxidant activity was observed in the Mix (30.30%) and control group (30.35%), while the maximum effect was observed in the *P. polymyxa* treatment (32.68%). Contrary to Albion cultivar, the effect of bacterial treatments on total phenolic, total flavonoid and total anthocyanin contents of the Monterey cultivar was not significant, while the Mix treatment (31.72%) had a negative effect on antioxidant activity and the *B. substilis* OSU-142 treatment (37.04%) had the highest value.

Table 2. Effect of different bacterial treatments on biochemical components of strawberry cultivars.

Cultivar	Treatment	Total Phenolic (mg GAE/100 g)	Total Flavonoid (mg CAE/100 g)	Total Anthocyanin (mg cyn-3-gluc/100 g)	Antioxidant Activity (% Inhibition)
Albion	Control	377.01 c*	61.91 b	220.47 bc	30.35 b
	B. subtilis OSU-142	333.79 c	64.31 ab	173.87 d	31.53 ab
	P. polymyxa	473.64 ab	63.18 b	283.84 a	32.368 a
	B. megaterium M3	430.29 b	72.54 a	230.190 b	30.52 ab
	Mix	500.30 a	67.21 ab	190.51 cd	30.30 b
Monterey	Control	350.37	71.47	193.87	33.06 ab
	B. subtilis OSU-142	320.45	64.61	223.60	37.04 a
	P. polymyxa	327.23	62.36	223.78	35.04 ab
	B. megaterium M3	360.53	64.02	200.58	34.88 ab
	Mix	347.45	64.91	197.21	31.72 b

* Differences between means indicated by different letters in each column are significant.

The effects of bacterial treatments on the macro- and micronutrient and heavy metal elements of strawberry fruits differed (Table 3). In the Albion variety, the effects of bacterial treatments on macronutrients such as K, Ca and S, micronutrients such as Fe, Mn and Zn, and heavy metals such as Al and Cr were found to be significant. The

highest results on the nutrients were obtained with *P. polymyxa* (8896.53 mg/kg) and *B. megaterium* M3 (8206.24 mg/kg) for K, control (1857.66 mg/kg) for Ca, *P. polymyxa* (461.83 mg/kg) for S, control (36.25 mg/kg) and *B. megaterium* M3 (34.42 mg/kg) for Fe, Mix (35.59 mg/kg) for Mn, *P. polymyxa* (10.80 mg/kg) and control (10.47 mg/kg) for Zn, *P. polymyxa* (22.31 mg/kg) for Al, and *B. megaterium* M3 (1.48 mg/kg) for Cr. The effects of bacterial treatments on macronutrients such as K, Ca and Mg, micronutrients such as B, Fe, Mn and Cu, and heavy metals such as Al and Cr were found to be significant in the Monterey variety. The highest results on nutrients were obtained with *P. polymyxa* (7081.86 mg/kg), Mix (6709.26 mg/kg) and *B. substilis* OSU-142 (6703.46 mg/kg) for K, *B. substilis* OSU-142 (1295.27 mg/kg), *B. megaterium* M3 (1183.22 mg/kg) and Mix (1150.93 mg/kg), *B. substilis* OSU-142 (1014.39 mg/kg) for Mg, *P. polymyxa* (10.56 mg/kg), *B. megaterium* M3 (61.30 mg/kg) for Fe, control (19.12 mg/kg) for Mn, *B. substilis* OSU-142 (1.07 mg/kg) and *P. polymyxa* (1.06 mg/kg) for Cu, *P. polymyxa* (24.41 mg/kg) for Al and control (0.72 mg/kg) and *P. polymyxa* (0.68 mg/kg) for Cr.

Table 3. Effect of different bacterial treatments on macro- and micronutrient and heavy metal contents of strawberry fruits.

		Macronutrients						Micronutrients					Heavy Metals	
Cultivar	**Treatment**	**N**	**P**	**K**	**Ca**	**Mg**	**S**	**B**	**Fe**	**Mn**	**Zn**	**Cu**	**Al**	**Cr**
Albion	Control	230.86	2199.96	7749.59 ab*	1857.66 a	1193.38	408.75 b	11.01	36.25 a	15.35 c	10.47 a	1.17	12.64 c	0.41 b
	B. subtilis OSU-142	224.26	2106.91	7659.51 b	1414.62 ab	1109.41	392.75 b	10.80	29.78 b	18.84 c	8.11 ab	1.44	16.85 abc	0.63 ab
	P. polymyxa	224.33	2476.26	8896.53 a	1540.04 ab	1232.76	461.83 a	13.10	31.77 ab	25.18 b	10.80 a	1.54	22.31 a	0.98 b
	B. megaterium M3	213.49	2203.56	8206.24 a	1294.06 b	1145.44	405.08 b	12.10	34.42 a	17.41 c	9.34 ab	1.45	20.17 ab	1.48 a
	Mix	203.25	2172.85	7851.68 ab	1457.68 ab	1120.76	380.55 b	11.26	27.21 b	35.59 a	9.25 ab	1.23	14.34 bc	0.57 ab
Monterey	Control	276.45	1547.35	5577.27 b	940.12 b	737.37 b	313.24	7.60 b	30.21 b	19.12 a	8.56	0.66 b	21.28 a	0.72 a
	B. subtilis OSU-142	224.74	1899.01	6703.46 a	1295.27 a	1014.39 a	387.01	9.94 a	39.41 b	5.95 c	9.50	1.07 a	10.49 b	0.33 b
	P. polymyxa	267.00	1715.17	7081.86 a	1011.70 ab	836.74 ab	398.95	10.56 a	56.61 a	7.15 b	8.15	1.06 a	24.41 a	0.68 a
	B. megaterium M3	206.55	1781.01	6197.52 ab	1183.22 a	880.68 ab	337.29	9.51 a	61.30 a	10.93 ab	7.79	0.95 a	13.27 b	0.44 b
	Mix	211.70	1891.03	6709.26 a	1150.93 a	939.91 ab	398.03	9.61 a	40.44 b	6.10 bc	9.12	0.80 ab	13.62 b	0.63 a

* Differences between means indicated by different letters in each column are significant.

The effects of bacterial treatments on the macro- and micronutrient and heavy metal elements of strawberry leaves varied (Table 4). The effect of bacterial treatments was positive on all elements except Cr in the Albion variety and on most elements except K, S, B and Al in the Monterey variety. The effects of bacterial treatments on N, P, K, Ca, Mg, Mn, Zn, Cu and Cr elements in the Albion cultivar and on N, P, K, Ca, Mg, S, B, Mn, Cu and Al elements in the Monterey cultivar were found to be significant. In the Albion cultivar, the *B. substilis* OSU-142 treatment had maximum effect on P (1954.54 mg/kg), Ca (4806.97 mg/kg), Mg (1505.61 mg/kg) and Cu (3.33 mg/kg). The *P. polymyxa* treatment had maximum effect on N (269. 67 mg/kg) and Zn (10.38 mg/kg). The *B. megaterium* M3 treatment had maximum effect on Mn (839.21 mg/kg). The Mix treatment had maximum effect on Cu (3.33 mg/kg). In the Monterey cultivar, the *P. polymyxa* treatment had the highest effect on Mn (30.60 mg/kg) and the *B. megaterium* M3 treatment had the highest effect on N (204.94 mg/kg), P (1134.29 mg/kg), Ca (2861.38 mg/kg) Mg (922.49 mg/kg) and Cu (5.02 mg/kg).

Table 4. Effect of different bacterial treatments on macro- and micronutrient and heavy metal contents in strawberry leaves.

		Macronutrients						Micronutrients					Heavy Metals	
Cultivar	**Treatment**	**N**	**P**	**K**	**Ca**	**Mg**	**S**	**B**	**Fe**	**Mn**	**Zn**	**Cu**	**Al**	**Cr**
Albion	Control	146.22 b*	1257.88 b	6209.45	2496.27 b	869.33 b	642.29	35.35	96.84	18.93 b	5.75 b	1.74 b	95.96	3.27 a
	B. subtilis OSU-142	227.54 a	1954.54 a	5013.82	4806.97 a	1505.61 a	513.47	43.20	87.44	38.63 a	8.45 a	3.33 a	101.56	2.82 ab
	P. polymyxa	269.67 a	1792.87 ab	5175.90	4119.10 a	1444.64 a	498.21	37.42	83.39	29.72 ab	10.38 a	3.21 a	91.16	2.81 ab
	B. megaterium M3	264.67 a	1883.53 a	5208.31	4319.38 a	1411.43 a	496.11	39.46	91.20	39.21 a	10.10 a	3.32 a	95.96	2.53 b
	Mix	203.23 ab	1694.55 ab	5191.68	4300.66 a	1345.79 a	527.82	44.87	80.92	35.36 a	8.77 a	3.33 a	97.72	2.31 b
Monterey	Control	150.25 c	810.32 c	7162.59 a	2051.69 b	690.87 c	688.42 a	62.98 a	79.94	21.08 b	4.94	1.40 b	155.75 a	3.81
	B. subtilis OSU-142	165.72 b	938.67 b	5128.02 bc	2629.25 a	733.01 c	488.05 b	40.15 b	74.22	30.03 a	5.96	3.06 ab	70.83 b	1.66
	P. polymyxa	173.88 b	860.89 bc	5226.35 bc	2532.83 a	778.39 b	473.53 b	49.93 ab	98.06	30.60 a	6.17	3.16 ab	97.23 ab	3.33
	B. megaterium M3	204.94 a	1134.29 a	5800.18 b	2861.38 a	922.49 a	609.68 a	44.32 ab	97.87	30.33 a	8.03	5.02 a	109.59 ab	5.25
	Mix	169.23 b	868.85 bc	4503.55 c	2734.17 a	815.84 b	452.60 b	38.56 b	72.07	27.41 a	6.11	3.05 ab	73.22 b	1.62

* Differences between means indicated by different letters in each column are significant.

As a result of PGPR applications, correlations between biochemical and morphological characteristics of the studied strawberry genotypes are presented in Figure 1. In general, there is a strong positive correlation ($r > 0.85$ ***) between fruit morphological characteristics. The highest positive correlation was found between fruit weight and fruit length ($r = 0.97$ ***). Similarly, there is a strong positive correlation between fruit length and fruit width ($r = 0.95$ ***). In particular, there is a positive correlation ($r > 0.36$ *) between the L* value and fruit characteristics. There is a significant positive correlation between L* and b* ($r = 0.84$ **). However, the a* value is negatively correlated with both the L* value ($r = -0.86$ ***) and the b value ($r = -0.73$ **). Fruit characteristics and the b value are positively correlated ($r > 0.68$ **). There is a strong positive correlation between fruit TA and pH ($r = 0.92$ ***). TSS is highly negatively correlated with both pH ($r = -0.87$ ***) and TA ($r = -0.90$ ***). Fruit width, length and weight are positively correlated with both pH ($r > 0.56$ *) and TA ($r > 0.48$ *). There is a strong positive correlation between the L* value and total phenolics ($r = 0.83$ ***). There are negative correlations ($r < -0.45$ *) between total anthocyanin and fruit characteristics (fruit width, length and weight), total flavonoids and pH. There are negative correlations ($r < -0.62$ **) between total antioxidants and fruit characteristics (fruit width, length and weight), the L* and the b* value.

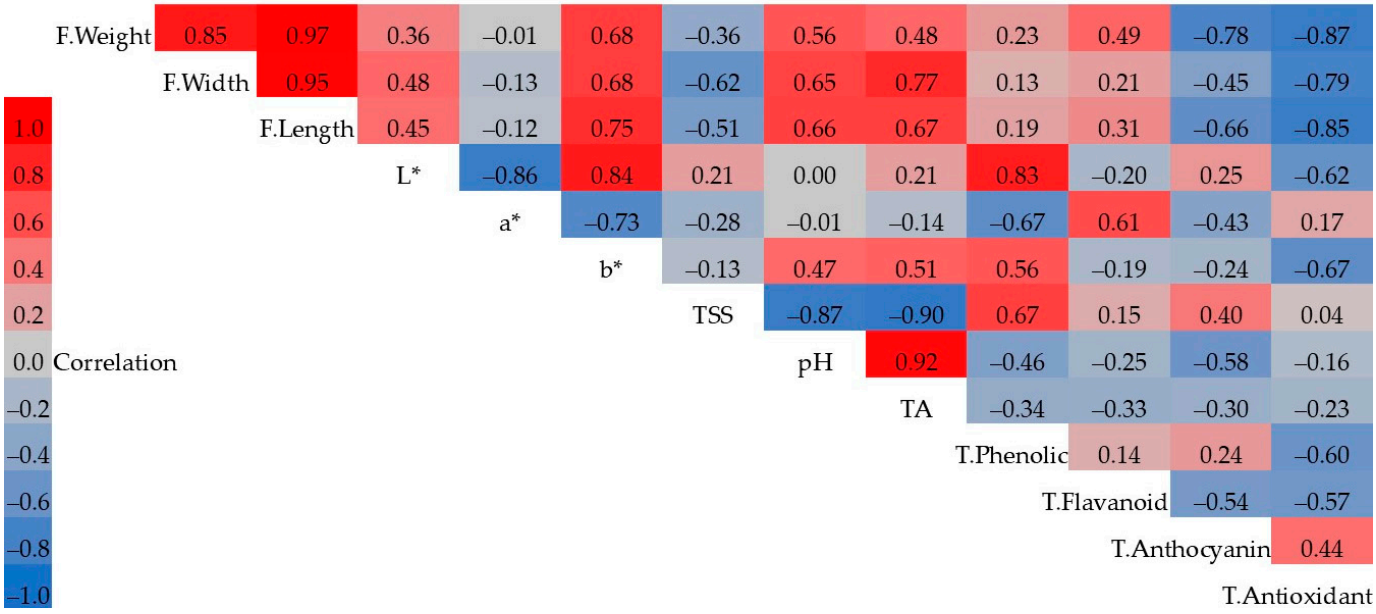

Figure 1. Correlations between biochemical, antioxidant and morphological characteristics of the strawberry cultivars. Fruit weight (F. Weight), fruit width (F. Width), fruit length (F. Length); L* refers to the brightness of the fruit skin color, a* refers to the change in color of the fruit skin from green to red, b* refers to the color change in the fruit skin color from yellow to blue, water-soluble dry matter (TSS), power of hydrogen (pH), titratable acid (TA), total phenolic (T.Phenolic), total flavonoid (T.Flavonoid), total anthocyanin (T.Anthocyanin), total antioxidant (T.Antioxidant).

The correlations between fruit and leaf nutrient contents of the strawberry genotypes are shown in Figure 2. A strong positive correlation ($r = 0.94$ ***) is found between fruit heavy metal contents (Al and Cr). Fruit B content shows a strong positive correlation with fruit contents such as Cr ($r = 0.83$ ***), Cu ($r = 0.82$ ***), Fe ($r = 0.71$ **), K ($r = 0.97$ ***), P ($r = 0.93$ ***) and S ($r = 0.86$ ***). Furthermore, fruit B content is positively correlated with leaf nutrient contents such as Mg ($r = 0.77$ **), N ($r = 0.85$ ***), P ($r = 0.70$ **) and Zn ($r = 0.83$ ***). Fruit Ca content has negative correlations with fruit Fe ($r = -0.89$ ***), leaf Mg ($r = -0.78$ **), Mn ($r = 0.71$ **), N ($r = 0.87$ ***), P ($r = 0.75$ **) and Zn ($r = 0.91$ ***). Fruit K content has strong positive correlations with fruit contents such as Cr ($r = 0.87$ ***), P and S ($r = 0.95$ ***). Fruit P content has strong positive correlations with fruit S ($r = 0.89$ ***) and

leaf Mg (r = 0.84 ***), N (r = 0.83 ***), Zn (r = 0.82 ***) contents. Leaf Al content has strong positive correlation (r > 0.97 ***) with leaf B, Fe, K, and S contents, and a strong negative correlation (r < −0.80 ***) with leaf Ca, Mg, Mn, and P contents. Leaf B content shows a strong positive correlation (r > 0.87 ***) with leaf contents of Fe, K, and S, and a strong negative correlation (r < −0.88 ***) with leaf contents of Ca, Cu, Mg, Mn, N, P, and Zn. Leaf Ca content has a strong positive correlation (r > 0.92 ***) with Cu, Mg, Mn, N, P, and Zn, while it has a strong negative correlation (r < −0.90 ***) with leaf Fe, K, and S contents. Leaf Cu content has a strong positive correlation (r > 0.93 ***) with leaf contents such as Mg, Mn, N, P and Zn. There is a strong positive correlation (r > 0.99 ***) between K and S contents, while they also show a positive correlation (r > 0.94 ***) with leaf Fe content. Leaf Mg, Mn, N, P and Zn show a strong positive correlation (r > 0.94 ***) with each other and a strong negative correlation (r < −0.80 ***) with leaf S content.

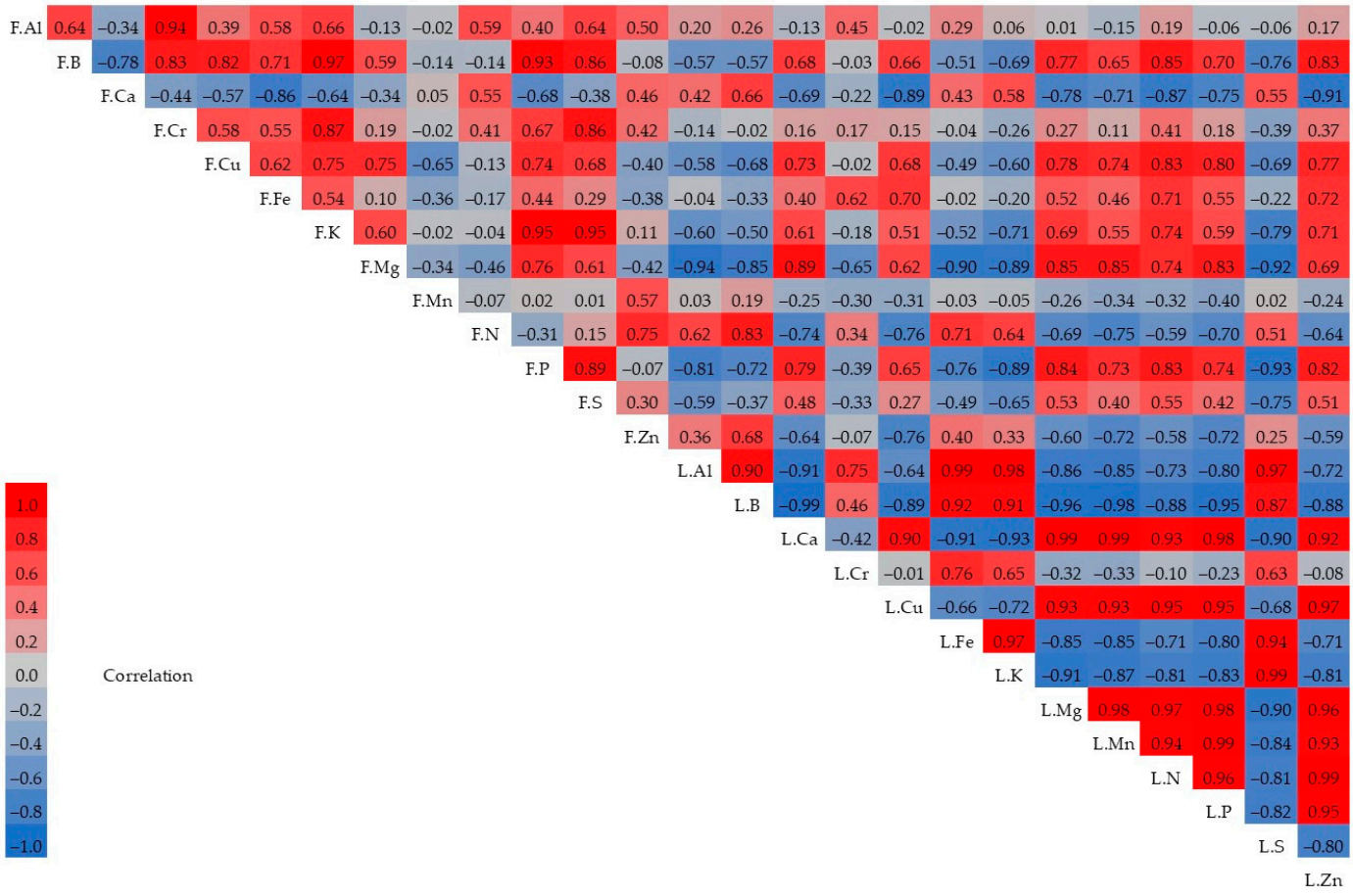

Figure 2. Correlations between fruit and leaf nutrient contents of the strawberry cultivars. F refers to strawberry fruits for macro- and micronutrient and heavy metal contents; L refers to strawberry leaves for macro- and micronutrient and heavy metal contents.

The evaluation of plant characteristic data with PCA is used to understand the relationships between these characteristics and to determine which ones explain more of the variance. PCA reduces the data to a small number of principal components, keeping most of the original variance in the dataset [39]. Principle component analysis of a total of 39 characteristics related to biochemical and morphological features of strawberry genotypes was performed within the scope of this study (Table 5; Figure 3). According to the results of principal component analysis, four main components (eigenvalues ≥ 1.00) explain 100% of the total variation. The third principal component (PCA 3) is reliable as it accounts for 87.61% of the total variation. The first (PCA 1) and second (PCA 2) principal components represent 50.36% and 69.50% of the total variation, respectively. When the

characteristics that contribute the most to the four main components are examined, each characteristic has different effects on the components. Fruit width and nutrient contents such as L. Zn, L. Mg, L. P, L. Mn, L. Cu, L. Ca, F. P, F. B and F. Cu contributed the most to PCA1. Fruit weight, fruit length, L*, a*, b*, L. Cr, L. Fe, L. Al, F. Mn and F. Fe had the most significant effect on PCA2. F. Al, F. Cr, F. Zn, F. S, F. Na, F. K, total anthocyanin, total flavonoid, L* and a* values had the highest contribution in PCA3. In PCA4, total phenolics, total flavonoids, total antioxidants, TSS, pH, TA and F. Ca, F. Mn and F had the highest contribution. Mg from nutrient contents had the highest contribution. In general, according to the results of PCA analysis, leaf nutrients and fruit characteristics (fruit width, length and weight) were prominent on PCA1 and PCA2, while biochemical contents and fruit nutrients were prominent on PCA3 and PCA4 (Table 5). This research provided the opportunity to distinguish the strawberry genotypes examined as a result of PGPR applications in terms of nutrient contents, phytochemical components and fruit quality characteristics depending on the treatments (Figure 3).

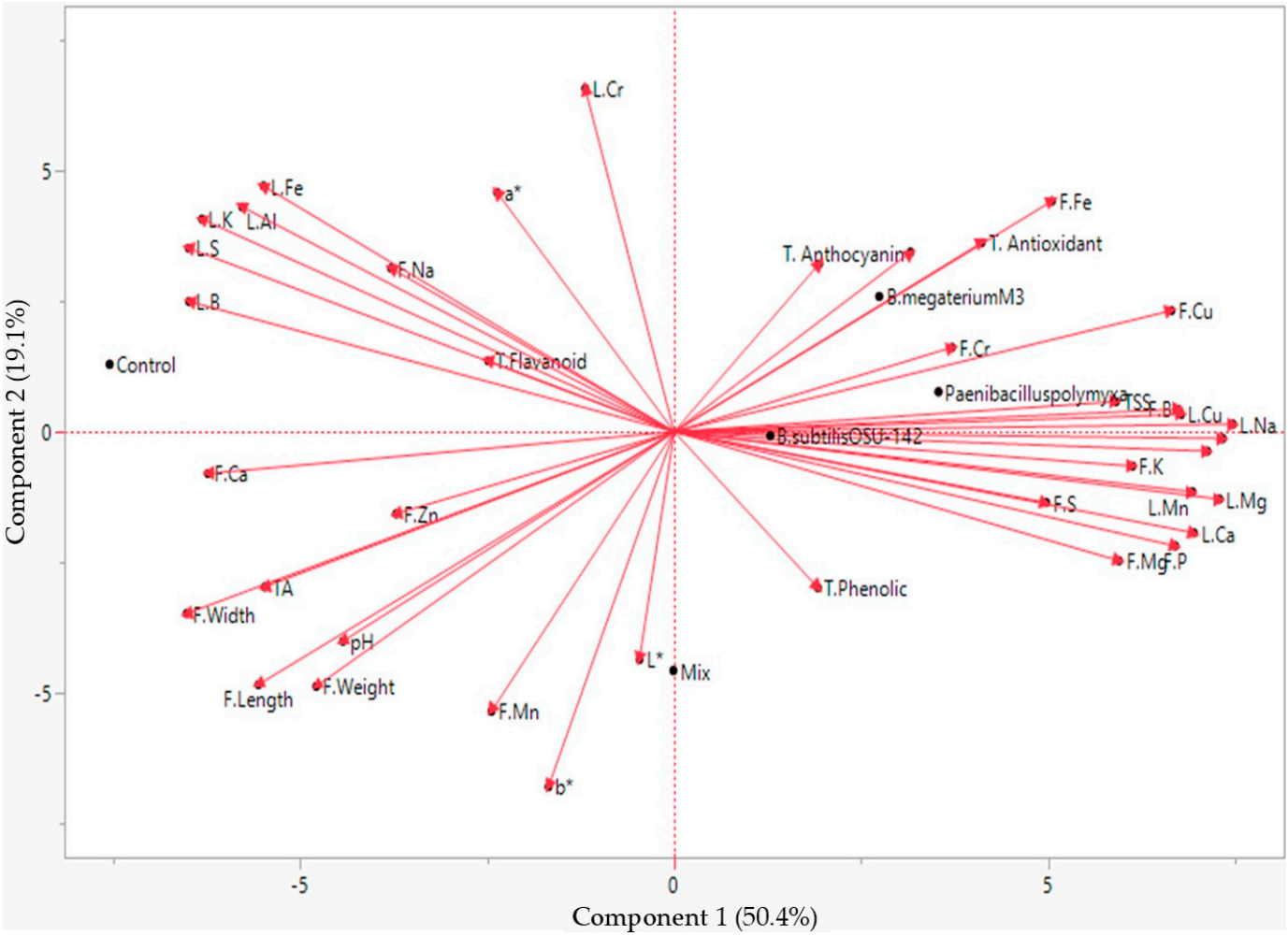

Figure 3. Biplot graph of the first two principal components in the strawberry cultivars examined based on the characteristics and treatments.

According to the PCA results, the bacterial treatments were divided into two main groups (Figure 4). The first group (A) included the control group of strawberry cultivars. The cultivars in this group showed higher averages than the other groups in terms of fruit width, TA and nutrient contents F. Ca, F. Na, F. Zn, L. Al, L. Fe, L. K, L. S and L. B. The second group (B) was divided into two subgroups. The first subgroup (B-1) included the cultivars treated with *Bacillus subtilis* OSU-142, *Bacillus megaterium* M3, *Paenibacillus polymyxa*. The

cultivars in this group had higher averages than the other groups in terms of pH, TSS, a*, total anthocyanins, total antioxidants, total flavonoids and nutrients F. Zn, F. Mg, F. Fe, F. Cu, F. B, F. K, F. P, F. S, L. Ca, L. Mg, L. Mn, L. Cr, L. P, L. Cu, and L. Zn. The second subgroup (B-2) consisted of the Mix group. The cultivars in this group showed higher fruit weight, fruit length, total phenolic, L* and b* values than the average of the other groups.

Table 5. Principal component analysis of strawberry genotypes regarding biochemical, antioxidant and morphological traits.

	PCA1	% Cont.	PCA2	% Cont.	PCA3	% Cont.	PCA4	% Cont.
Fruit Weight	−0.14	2.05	−0.24	5.59	−0.11	1.20	0.14	1.99
Fruit Width	−0.19	3.80	−0.17	2.85	0.05	0.30	0.07	0.42
Fruit Length	−0.17	2.76	−0.23	5.52	−0.03	0.09	0.09	0.81
L*	−0.01	0.02	−0.21	4.47	0.25	6.42	0.21	4.32
a*	−0.07	0.50	0.22	4.95	−0.27	7.42	−0.04	0.17
b*	−0.05	0.25	−0.33	10.86	0.13	1.57	0.08	0.59
Total Soluble Solids	0.18	3.12	0.03	0.08	0.01	0.01	0.28	7.89
pH	−0.13	1.75	−0.19	3.78	−0.04	0.14	−0.27	7.53
Titratable acidity	−0.16	2.68	−0.14	2.07	0.09	0.86	−0.23	5.35
Total Phenolic	0.06	0.33	−0.14	2.09	0.14	2.01	0.36	13.18
Total Flavonoid	−0.07	0.55	0.07	0.43	−0.24	5.73	0.31	9.41
Total Anthocyanin	0.09	0.89	0.17	2.78	0.30	8.73	0.01	0.02
Total Antioxidant	0.12	1.51	0.18	3.08	0.00	0.00	−0.31	9.80
Fruit Al	0.06	0.34	0.16	2.43	0.32	10.55	0.04	0.16
Fruit B	0.20	4.06	0.02	0.04	0.17	2.80	0.02	0.04
Fruit Ca	−0.19	3.47	−0.04	0.15	−0.01	0.02	−0.25	6.33
Fruit Cr	0.11	1.23	0.08	0.61	0.32	10.07	−0.02	0.03
Fruit Cu	0.20	3.95	0.11	1.27	0.03	0.08	−0.16	2.57
Fruit Fe	0.15	2.28	0.21	4.59	0.05	0.26	0.20	3.97
Fruit K	0.18	3.35	−0.03	0.10	0.22	4.66	−0.03	0.09
Fruit Mg	0.18	3.16	−0.12	1.43	−0.07	0.43	−0.22	5.01
Fruit Mn	−0.07	0.53	−0.26	6.73	0.14	1.95	0.23	5.28
Fruit N	−0.11	1.28	0.15	2.30	0.25	6.22	−0.17	2.85
Fruit P	0.20	4.01	−0.11	1.12	0.13	1.76	−0.03	0.08
Fruit S	0.15	2.21	−0.07	0.43	0.25	6.29	−0.14	1.84
Fruit Zn	−0.11	1.23	−0.08	0.58	0.32	9.97	−0.05	0.21
Leaf Al	−0.17	2.98	0.21	4.36	0.06	0.31	0.12	1.39
Leaf B	−0.19	3.76	0.12	1.46	0.15	2.16	0.00	0.00
Leaf Ca	0.21	4.31	−0.09	0.88	−0.11	1.17	−0.03	0.08
Leaf Cr	−0.04	0.13	0.32	10.16	0.04	0.13	0.21	4.28
Leaf Cu	0.20	4.10	0.02	0.03	−0.13	1.64	0.13	1.58
Leaf Fe	−0.16	2.69	0.23	5.21	0.09	0.74	0.08	0.62
Leaf K	−0.19	3.56	0.20	3.90	0.02	0.02	0.04	0.16
Leaf Mg	0.22	4.75	−0.06	0.39	−0.07	0.53	0.00	0.00
Leaf Mn	0.21	4.29	−0.06	0.31	−0.14	1.89	−0.01	0.02
Leaf N	0.22	4.99	0.01	0.01	−0.04	0.13	0.05	0.23
Leaf P	0.21	4.54	−0.02	0.03	−0.12	1.49	−0.01	0.01
Leaf S	−0.19	3.77	0.17	2.91	−0.02	0.06	0.09	0.81
Leaf Zn	0.22	4.81	−0.01	0.00	−0.04	0.17	0.09	0.90
Eigenvalues	19.64		7.46		7.05		4.83	
Variance (%)	50.36		19.14		18.10		12.39	
Cumulative Variance (%)	50.36		69.50		87.61		100.00	

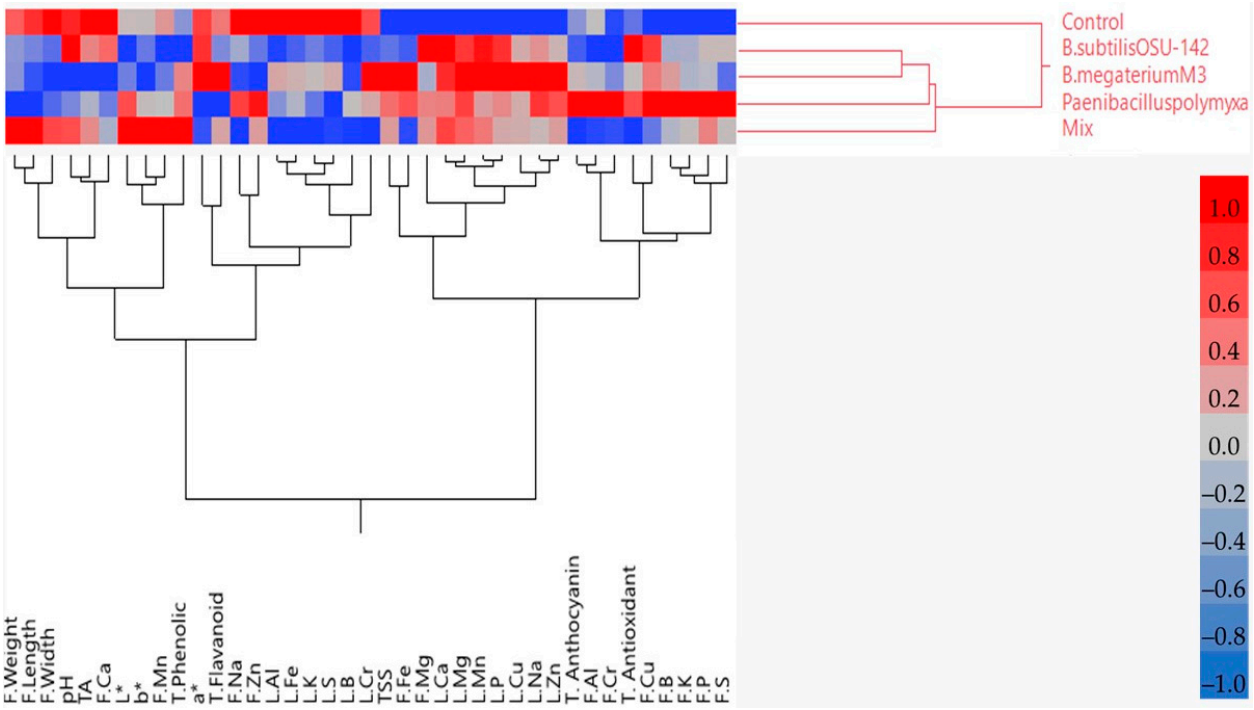

Figure 4. Heatmap analysis of the nutrient contents, phytochemical components, antioxidant activity, and fruit quality characteristics of the strawberry genotypes examined as a result of PGPR applications.

4. Discussion

In recent years, the use of PGPR has become more significant for achieving high quality and productivity in fruit cultivation, as well as for preserving soil fertility. Numerous studies have been conducted to investigate the impact of bacterial treatments on fruit cultivation. Research on the impact of bacterial treatments on fruit size has shown varying outcomes. It was reported that bacterial treatments had a positive effect on apple [20], quince [15] and raspberry [16], no effect on cherry [17], and a negative effect on sour cherry [18]. In studies conducted on different strawberry cultivars, various results on fruit characteristics were reported. It was reported that bacterial applications on fruit size were positive by some researchers [16,40–44] and ineffective by some others [23,45–49]. While it is generally considered that the positive effects of bacteria on fruit quality criteria are due to their ability to fix nitrogen, solubilize phosphorus and produce plant growth regulators [21], it has been reported that their negative effects may be due to cultivar, ecological factors and the characteristics of the bacteria used and the interactions between them [41,50–52].

The effects of bacterial applications on the quality criteria of fruits such as TSS, pH and TA have been investigated in many studies. As a result of factors such as ecology, bacterial species and varieties, it has been reported that bacterial treatments have positive and negative effects on TSS, pH and titratable acidity at different levels [23,24,41,42,45,48,52,53]. It was also reported that increased fruit weight and fruit size as a result of bacterial treatment may cause a decrease in the amount of TSS produced by the plants and its ratio per fruit [20].

Fruit color parameters are known as variety traits, but ecological factors are also effective on coloration. It was reported that bacterial treatments had no effect [47] or very little effect [54] on the color values of strawberry fruits. It was reported that there is a negative correlation between the L* value indicating the brightness of the fruits and the a* value indicating the redness [55]. Our study results supported this correlation. In addition, the enhancement in fruit color brightness with bacterial treatment was similar to the findings of Balcı et al. [45]. It was reported that the positive effect on fruit coloration was due to balanced nutrition and optimal growth and development [41,42,53,56,57].

In studies on the biochemical contents of strawberry fruits, bacterial treatments generally showed positive effects on phenolic and flavonoid contents and antioxidant activity. However, bacterial treatments had negative effects on anthocyanin contents [43,47,57–59]. In our investigation, the *B. subtilis* OSU-142 and Mix treatments resulted in a reduction in anthocyanin concentration in the Albion cultivar. The combination of treatments resulted in a reduction in antioxidant activity in the Monterey cultivar. The treatments led to an augmentation of phenolic and flavonoid concentrations in the Albion cultivar.

Many studies were conducted to investigate the effects of bacterial treatments on the leaf nutrients of different plant species. In most of the studies, it was reported that bacterial treatments increased leaf nutrients, and especially positive effects of bacterial treatments on leaf nutrient contents have been reported in cherry (increased P, K and Mg contents) [60], apricot (increased N, P, K, Ca and Mg contents) [61], sour cherry (increased N, Fe, Cu, Zn and Mn contents) [62], quince (increased P and K contents) [63], and pear (increased N, P, Mg, Mn and Zn contents) [64]. Bacterial treatments were shown to have beneficial impacts in our research, as they led to an increase in the absorption and accumulation of nutritional components in both strawberry kinds. On the other hand, the outcomes varied not only according to the different elements and varieties, but also according to the different kinds of bacteria that were used in the therapy. Çiyez [40] reported that there was an increase in the leaf N, Na, K, P, Fe, Cu, Ca, Mg and Mn contents of strawberry plants with *B. subtilis* bacteria and *G. etunicatum*, *G. fasciculatum* and *G. mosseae* mycorrhizal fungus treatments. Esitken et al. [52] reported that P and Zn contents in the leaves of organically grown strawberries increased with PGPR (*Pseudomonas* BA-8, *Bacillus* OSU-142 and *Bacillus* M-3) treatments. In addition, our findings are similar to the results of other related studies on strawberries [30,50,54,65]. The beneficial impacts of bacterial treatments on leaf and fruit nutrient contents can be attributed to the enhancement in nitrogen and phosphorus utilization in the soil by bacteria, the production of growth-stimulating compounds, the reduction in soil pH, and the augmentation of the absorption of elements such as Fe, Mn, Cu and Zn by plants [21].

According to our findings, strong positive correlations were found between strawberry fruit morphological traits (fruit weight, length and width). The research on strawberry plants demonstrates a significant and positive correlation between morphological traits, such as fruit weight, fruit length, and fruit breadth [66–69]. Milosavljević et al. [70] reported a strong positive correlation ($r = 0.81$) between total anthocyanins and total phenolics in the study of biochemical characterization of strawberry cultivars. Guiné et al. [71] observed that antioxidant activity, total phenolic and total anthocyanin contents were positively correlated ($r > 0.77$) in strawberry genotypes under various extraction conditions [70,71]. In the literature [70,71], positive correlations have been reported between anthocyanin content and phenolic compounds and antioxidant activity. However, in our results, negative correlations were found between total anthocyanin and fruit characteristics (width, length, weight). This difference may be due to the differences in strawberry genotypes used in our study, bacterial species used or environmental factors. In addition, the diversity of biochemical and morphological responses based on genotypes may also explain this difference. In a study conducted by Singh et al. [72], it was shown that there were substantial positive relationships between the N and Mg levels, as well as between the Ca and Cu levels, and the fruit yield. This situation is similar to the strong positive correlations observed between leaf N, Mg, P, Ca and Cu contents in our study. Khalil and Hammoodi [73] reported that high nutrient content in strawberry fruits was positively correlated with fruit firmness, TSS, and TA. In our study, TA and pH were found to have positive correlations with fruit width, length and weight. According to the report of Negi et al. [74], P and N contents directly affect fruit quality, while in our study, negative correlations were found between fruit Ca content and important leaf nutrients such as Fe, Mg, Mn, N, P and Zn. These results indicate that plant nutrition and element uptake may have been altered due to bacterial applications. PGPR may regulate nutrient uptake in plant roots, which may cause antagonistic effects of some elements. In addition, the relationship between fruit

quality and specific nutrient elements may have been affected by variables such as the type of PGPR applied, soil structure and environmental factors. Moreover, Yang et al. [75] reported that total Zn and Cu contents in strawberries were positively correlated.

Principal component analysis (PCA) is an important statistical method used to reveal the main structures in multidimensional datasets [76]. Lambrecht et al. [77] evaluated the relationships between developmental variables and fruit quality (fruit weight, TSS, TA, etc.) of strawberry cultivars using PCA and reported that the first two principal components explained more than 80% of the total variation for all characteristics examined. In our study, PCA1 and PCA2 together explained 69.50% of the total variance. However, in our study, the added PCA3 and PCA4 components explained 18.10% and 12.39% of the variance. This provided a more detailed analysis by considering more components in our results. Barth et al. [78] reported that the first two components explained 48.58% of the total variation according to the results of principal component analysis (PCA) in a study evaluating hybrid strawberry genotypes in terms of fruit weight, pH, TSS, TA, phenolic compounds, and anthocyanins. This result shows that they have a much lower variance explanatory power than our study. Amiri et al. [79] reported that PCA1 and PCA2 explained 77.03% and 17.11% of the total variation in the physical and chemical properties of strawberry fruits. Yildiz et al. [80] revealed that phytochemical properties (total phenolic and total anthocyanin contents), pH and TA had the highest contribution on PCA1, while PCA2 was positively correlated with phytochemical properties and pH according to PCA results. Similarly, in our study, it was observed that phytochemical properties had a significant effect on PCA3 and PCA4. Chiomento et al. [81] reported that total anthocyanin, flavonoids, phenolic contents, and antioxidant capacity had the highest contribution to PCA1 and TSS, and TA and plant yield had the highest contribution to PCA2 in different strawberry cultivars such as Albion, Monterey, Aromas and Camarosa. Their results largely overlap with those of PCA3 and PCA4 in our study. In our study, PCA3 had high contributions from phytochemical contents such as anthocyanin, flavonoid and S, while PCA4 had high contributions from chemical contents such as total phenolic, antioxidants and TSS. Amiri et al. [79] reported that TSS, TA, total phenolic, antioxidant and anthocyanin contents had the most significant effect on PCA1 and PCA2. In conclusion, our study, in addition to being in line with other studies in the literature, provided a deeper analysis by examining more components, which provided a more detailed characterization of strawberry genotypes in terms of nutritional contents, biochemical properties and fruit quality.

5. Conclusions

According to the results of this study, the treatments with *B. megaterium* M3, *B. substilis* OSU-142 and *P. polymyxa* bacteria had significant effects on macro- and micronutrient content, fruit weight, fruit size and biochemical characteristics of strawberry cultivars. The effects of the bacterial treatments varied according to the cultivars and the traits examined. It has been observed that the Mix treatment resulting from the combined use of bacteria, rather than their separate use, has a greater impact on fruit weight. Consequently, it has been understood that PGPR bacteria are potentially effective in improving the agronomic, pomological, and biochemical characteristics of strawberry cultivars and can be used in studies and breeding programs aimed at increasing strawberry yield and quality.

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