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Effect of rhizobacteria treatments on nutrient content and organic and amino acid composition in raspberry plants

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Abstract: Plant growth-promoting rhizobacteria (PGPR) have been found to be beneficial to plant growth, yield, crop quality, the environment, and sustainable agricultural production. Therefore, six bacterial strains were tested to determine their effects on raspberry's nutrient content and organic and amino acid composition. The experiment was performed from 2015 to 2017. Two-year-old raspberry plants were inoculated with bacterial suspensions by a dipping method and were planted in 30-L pots. The mineral content and organic acid and amino acid composition of the leaf and root were compared in the *Alcaligenes* 637Ca, *Staphylococcus* MFDCa1 and MFDCa2, *Agrobacterium* A18, *Pantoea* FF1, and *Bacillus* M3 bacterial strains. Nitrogen (N) content of the leaf was 2.55% in the A18 treatment, while N content of the root was 1.61% in MFDCa2. The leaf's iron (Fe) content was highest in the M3 treatment with 91.76 mg kg^{-1}, while 637Ca gave the highest root's Fe content with 107.80 mg kg^{-1}. The content of malonic acid (16.78 ng μL^{-1}), malic acid (4.59 ng μL^{-1}), citric acid (16.88 ng μL^{-1}), and fumaric acid (4.94 ng μL^{-1}) in leaves was higher in MFDCa2 than in the other treatments. In addition, 637Ca treatment had the highest root organic acid content in tartaric acid (5.94 ng μL^{-1}), butyric acid (15.19 ng μL^{-1}), and maleic acid (5.13 ng μL^{-1}). FF1 treatment was more effective than the other treatments for increasing the leaf's amino acid content, while the 637Ca, MFDCa1 FF1, and M3 treatments were more effective in increasing the root's amino acid content. As a result, it was determined that PGPR treatments play a significant role in mineral nutrient uptake and the organic acid and amino acid composition of the raspberry plant.

Key words: Amino acids, organic acids, plant growth-promoting rhizobacteria, plant nutrition, raspberry

1. Introduction

Raspberry (*Rubus idaeus* L.), a type of berry, is a member of the genus *Rubus* of the family Rosaceae. Raspberries are grown widely around the world except in desert areas. Raspberries originated in the Black Sea region of Turkey. They grow naturally in the region with high relative humidity and can usually be found at 1000 m or more above sea level (Jennings, 1988).

The world raspberry production is approximately 613,000 t. The Russian Federation is the largest raspberry producer, followed by Poland, the USA, Serbia, and Mexico. Raspberry production in Turkey was 4320 t in 2016. The world's highest yield per hectare of raspberry was obtained from Mexico with 15,200 kg, while raspberry yield per hectare in Turkey was 8850 kg (FAO, 2017). High yield and quality can be achieved by intensive agricultural techniques and practices. The intensive farming practices require the use of chemical fertilizers and agricultural mechanization. However, fertilization is costly and causes environmental problems such as water pollution, soil pollution, and air pollution (Savci, 2012). In addition, commercially synthetic fertilizers can have a negative effect on human health.

Currently, a number of bacterial species living in the rhizosphere have been found to be beneficial to plant growth and development, yield, crop quality, the environment, and sustainable agricultural production (O'Connell, 1992); they can also help reduce the use of synthetic fertilizers, pesticides, and herbicides in agriculture. These bacteria are named plant growth-promoting rhizobacteria (PGPR). Generally, these species are from the genera *Azospirillum*, *Bacillus*, *Azotobacter*, *Burkholderia*, *Enterobacter*, *Klebsiella*, and *Pseudomonas* (Vessey, 2003; Çakmakçı et al., 2010; Bhattacharyya and Jha, 2012). PGPR applications have been used for more than 100 years. However, PGPR came to prominence in the last 20–25 years. PGPR play a significant role in sustainable agriculture (Reddy, 2014). PGPR can contribute indirectly or directly to plant growth. They produce cytokines, oxines, GA_3, ACC-deaminase, and siderophores and release organic acids (Reddy, 2014).

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By their release of organic acids, they play important roles in plant nutrient uptake, especially of Fe, P, Zn, and B. The release of organic acids by the rhizosphere decreases the soil pH, and low soil pH converts insoluble forms of plant nutrients (Fe, Zn, P, and B) to soluble forms that are usable by plants. Organic acids such as malonic, acetic, oxalic, glycolic, and formic acids contribute to the acquisition of phosphorus, calcium, iron, zinc, and manganese by plants growing in low available nutrient soils (Ohwaki and Hirata, 1992; Marschner, 2011). Studies have shown that nutrient uptake ability in apricot, apple, cherry, citrus, mulberry, pear, raspberry, and strawberry is affected by IAA, GA_3, ACC-deaminase, and siderophore-producing rhizobacteria (Esitken et al., 2003, 2006; Köse, 2003; Ozturk et al., 2003; Orhan et al., 2006; Aslantaş et al., 2007; Ipek et al., 2014; 2017a, 2017b; Arikan and Pirlak, 2016). On the other hand, the main N-containing compounds, which are nitrate and amino acids, are transported by the xylem from the shoot to the leaf. The N content in the leaf and root is an indicator of amino acid content (Zimmermann, 1960).

There are limited studies about the effects of PGPR on organic acids and amino acids in plants, although there are numerous studies about PGPR abilities, such as promoting plant growth and development, synthesis of plant hormones, N-fixation, P-solubilization, and mineral uptake. The aim of the present study was to determine the effects of PGPR on raspberry plant nutrient content and organic and amino acid composition.

2. Materials and methods

2.1. Pot experiments

Pot experiments were carried out using the raspberry cultivar 'Heritage', which grows fruits on primocanes, and were planned according to a completely randomized design. In the experiment, there were three replicates per treatment and ten raspberry plants per replicate; in total, 210 plants were used in the experiment. Thirty-liter pots were filled with a mixture of 3 peat:1 perlite:1 sand ratio. Bacterial treatments were applied to the plant roots by the dipping method (Ipek et al., 2014). The plant roots were inoculated with the bacterial suspension 30 min prior to planting in the pots. The bacterial suspension was prepared at 10^9 CFU mL L^{-1} for the treatments. The control plants' roots were dipped into sterile water for 30 min. After planting, the bacterial suspensions were reapplied in June, July, and August of 2015. In addition, bacterial suspensions were also applied in May, June, July, and August of 2016. In both years of the experiment, plant nutrient content, organic acid content, and amino acid content were measured. After the first year, 15 plants in each treatment were removed from the pots for root analysis. The remaining plants were used in the second year of the experiment for leaf and root analysis.

2.2. Bacterial strains, culture conditions, and treatments

The bacterial strains *Alcaligenes* 637Ca, *Staphylococcus* MFDCa1 and MFDCa2, *Agrobacterium* A18, *Pantoea* FF1, and *Bacillus* M3 were obtained from Yeditepe University (Dr Fikrettin Şahin; personal communication) and Iğdır University (Dr M Figen Dönmez; personal communication). The 637Ca, A18, MFDCa1, and MFDCa2 strains were reported to be soluble in a carbonate buffer, and M3 and FF1 were reported to be soluble in a phosphate buffer in in vitro culture conditions (Orhan et al., 2006; Karakurt and Aslantaş, 2010). These bacterial strains are used as biofertilizers and plant growth promoters for horticultural plant species such as apple, apricot, cherry, grape, pear, raspberry, sour cherry, and strawberry (Sudhakar et al., 2000; Esitken et al., 2003, 2006; Köse, 2003; Orhan et al., 2006; Aslantaş et al., 2007; Pırlak and Köse, 2009; Ekinci et al., 2014; Ipek et al., 2014, 2017a, 2017b; Arikan and Pirlak, 2016).

The bacterial strains were stored in 15% glycerol at –86 °C until use. A single bacterial colony was taken from a bacterial culture that was grown on nutrient agar. Then it was transferred to flasks containing liquid nutrient broth (NB) and grown aerobically on a shaker rotating at 95 rpm for 1 day at 27 °C. The suspension of bacteria was diluted with sterile distilled water to a final concentration of 10^9 CFU mL^{-1}.

2.3. Plant nutrient element analysis

For leaf nutrient element analysis, leaves on the middle of the shoot were sampled in September in both experimental years. To dry the samples, the leaves were placed in an oven at 68 °C for 48 h and then ground with a mortar and pestle. The micro-Kjeldahl procedure was applied for the determination of N (Bremner, 1996); macroelement and microelement contents were determined after wet digestion of dried and ground subsamples using a HNO_3–H_2O_2 acid mixture (2:3 v/v) in three steps (step 1: 145 °C, 75% RF, 5 min; step 2: 180 °C, 90% RF, 10 min, and step 3: 100 °C, 40% RF, 10 min) in a microwave oven (Berghof Speedwave Microwave Digestion Equipment MWS-2; Berghof, Eningen, Germany). Inductively coupled plasma mass spectrometry (Optima 2100 DV, PerkinElmer) was then used to determine the P, K, Ca, Mg, Na, Fe, Mn, Zn, Cu, and B content (Mertens, 2005).

2.4. Leaf and root organic acid composition

Fresh leaf and root samples were picked from 1.0–1.5-cm shoot tips and the roots of the saplings. They were transferred to the laboratory on ice. To analyze the organic acid composition of the leaf and root, 1-g fresh leaf and root samples were homogenized with 10 mL of distilled water. After the homogenization phase, samples were centrifuged at 1200 rpm for 50 min. The supernatants were filtered and subjected to high-performance liquid chromatography (HPLC) using a Zorbax Eclipse-AAA 4.6

× 250 mm, 5 µm column (Agilent 1200 HPLC) at 220 nm. Flow speed was 1 mL min^{-1} and the column temperature was 25 °C. Organic acids were determined by using 25 mM potassium phosphate (pH 2.5) as the mobile phase (İpek et al., 2017b).

2.5. Leaf and root amino acid composition

Fresh leaf and root samples were homogenized with 0.1 N HCl and incubated for 12 h at 4 °C. After incubation, the samples were vortexed. The samples were then centrifuged at 1200 rpm for 50 min. The supernatants were filtered via a syringe filter with a 0.22-µm pore size. The supernatant that was transferred to fresh vials was analyzed via HPLC according to the methods of Aristoy and Toldra (1991), Antoine et al. (1999), and Henderson et al. (1999). Zorbax Eclipse-AAA 4.6 × 150 mm, 3.5 µm columns (Agilent 1200 HPLC) were used, readings at 254 nm were recorded, and the amino acids were identified by comparison with standards. Amino acids were quantified as nmol μL^{-1} after a 26-min derivation process by HPLC.

2.6. Statistical analysis

All data were analyzed using one-way analysis of variance (ANOVA) and significant differences among the means were compared by Duncan's multiple range test at $P = 0.05$ level using SPSS 23.0 (SAS Inc., Cary, NC, USA). The data were pooled to evaluate the entire 2-year study because no significant differences were found between the years.

3. Results

The results of the present study are given in Tables 1–6. The tables illustrate the effects of six bacteria strains and the control treatment on nutrient content and organic and amino acid composition of the raspberry cultivar "Heritage".

3.1. The nutrient content of leaf and root

The separate bacterial treatments compared with the control treatment had the highest nutrient content in both leaves and roots. The 637Ca treatments showed the best results among bacterial strains based on the leaf and root nutrient content. The N content in leaves and roots was 2.55% (A18) and 1.61% (MFDCa2), respectively. The A18 treatment increased the N content by approximately 14% compared to the control, while MFDCa2 increased it by 15%. The phosphorus content was 0.36% (in leaf) and 0.31% (in root) in raspberry plants treated with the M3 bacterial strain. The leaf and root K content in raspberry plants treated with 637Ca was 1.95% and 1.22%, respectively. The M3 bacteria strain increased the Ca content in leaves (1.16%), while 637Ca increased the root Ca content to 0.74%. The Mg content of leaves had the highest value after 637Ca treatment at 0.17%, while MFDCa1 treatment resulted in the highest value in roots at 0.1%. The Na content of leaves and roots were similar. In leaves, the highest Na content was measured after 637Ca (0.06%) treatment, while A18 gave the highest Na content in roots at 0.06%. The three bacterial strains A18 (30.1 mg kg^{-1}), M3 (28.53 mg kg^{-1}), and MFDCa2 (28.53 mg kg^{-1}) were more effective than the other treatments on leaf Zn content. MFDCa1 (35.26 mg kg^{-1}) and 637Ca (34.31 mg kg^{-1}) gave higher Zn content in roots than the other treatments. The M3 treatment increased the Fe content in leaves by approximately 9% compared with the control, while 637Ca increased Fe by 36% in the roots. The highest Mn content of leaves was found in the MFDCa2 (31.82 mg kg^{-1}), M3 (31 mg kg^{-1}), and FF1 (30.1 mg kg^{-1}) bacterial treatments, while the highest Mn root content was found with MFDCa1 (34.1 mg kg^{-1}) and 637Ca (32.9 mg kg^{-1}). The leaf copper content had the highest value for all treatments except the control and MFDCa2. In addition, MFDCa2 and 637Ca treatment gave the highest Cu content values in the roots with 30.2 and 29.7 mg kg^{-1}, respectively. The boron content in leaves was higher after A18 at 9.04 mg kg^{-1} than the other treatments. In the roots, the B content was higher after MFDCa1 treatment at 9.37 mg kg^{-1} than the other bacterial treatments (Tables 1 and 2).

3.2. The organic acid composition of leaf and root

The rhizobacterial treatments generally increased the amount of organic acids significantly compared to the control in leaves and roots (Table 3). The bacterial treatments increased organic acids from 1% to 21% compared with the control without oxalic acid and succinic acid. The control treatment gave higher concentrations of oxalic acid (1.13 ng μL^{-1}) and succinic acid (30.7 ng μL^{-1}) in the roots than bacterial treatments. The 637Ca treatment increased tartaric (5.95 ng μL^{-1}) and butyric acid (15.2 ng μL^{-1}) in roots and increased maleic acid in both leaves (5.37 ng μL^{-1}) and roots (5.13 ng μL^{-1}). The butyric acid (16.2 ng μL^{-1}) in leaves was increased by A18 treatment, which also increased citric acid (16.8 ng μL^{-1}) in the roots. The FF1 treatment increased the lactic acid (20.0 ng μL^{-1}) concentration in the roots. In addition to lactic acid, FF1 also increased oxalic (1.30 ng μL^{-1}), tartaric (6.66 ng μL^{-1}), and succinic (30.4 ng μL^{-1}) acid in the leaves. The M3 treatment was found to result in a lower concentration of all organic acids in both leaves and roots than the other bacterial strain treatments. The propionic acid (2.52 ng μL^{-1}) and lactic (22.0 ng μL^{-1}) acid contents of leaves were increased by MFDCa1. The MFDCa1 treatment also increased malonic (17.5) and malic (4.31) acid concentrations in the roots. Propionic acid (2.55 ng μL^{-1}) and malonic acid (16.8 ng μL^{-1}) in the roots, citric acid (16.9 ng μl^{-1}) in the leaves, and fumaric acid in both leaves (4.94 ng μl^{-1}) and roots (5.34 ng μl^{-1}) were increased by MFDCa2 treatment (Tables 3 and 4).

3.2. The amino acid composition of leaf and root

The PGPR treatments affected the amino acid contents of the raspberry plants. Generally, a bacterial strain was

Table 1. The effects of rhizobacteria treatment on the nutrient content of the leaves.

	Control	637Ca	A18	FF1	M3	MFDCa1	MFDCa2
N (%)	2.240^{g}	2.407^{e}	2.553^{a}	2.504^{b}	2.458^{c}	2.425^{d}	2.340^{f}
P (%)	0.340^{f}	0.324^{g}	0.352^{c}	0.353^{b}	0.356^{a}	0.348^{d}	0.341^{e}
K (%)	1.760^{g}	1.950^{a}	1.883^{b}	1.824^{e}	1.846^{d}	1.867^{c}	1.789^{f}
Ca (%)	1.110^{g}	1.139^{e}	1.145^{d}	1.114^{f}	1.162^{a}	1.152^{b}	1.146^{c}
Mg (%)	0.139^{g}	0.172^{a}	0.147^{e}	0.141^{f}	0.163^{b}	0.148^{d}	0.162^{c}
Na (%)	0.047^{g}	0.063^{a}	0.049^{f}	0.050^{e}	0.051^{d}	0.053^{c}	0.057^{b}
Zn (mg kg^{-1})	26.80^{b}	27.66^{b}	30.14^{a}	27.93^{b}	28.53^{ab}	27.47^{b}	28.53^{ab}
Fe (mg kg^{-1})	84.20^{d}	85.56^{cd}	87.71^{b}	86.49^{bc}	91.76^{a}	84.96^{cd}	88.16^{b}
Mn (mg kg^{-1})	27.70^{d}	28.96^{cd}	29.68b^{c}	30.10^{abc}	30.99^{ab}	29.45^{bcd}	31.82^{a}
Cu (mg kg^{-1})	23.90^{b}	27.94^{a}	28.59^{a}	28.66^{a}	27.45^{a}	27.70^{a}	25.11^{b}
B (mg kg^{-1})	6.13^{f}	7.85^{c}	9.04^{a}	8.08^{b}	8.02^{bc}	6.66^{d}	6.37^{e}

Table 2. The effects of rhizobacteria treatment on the nutrient content of the roots.

	Control	637Ca	A18	FF1	M3	MFDCa1	MFDCa2
N (%)	1.399^{f}	1.580^{b}	1.422^{e}	1.454^{cd}	1.443^{d}	1.465^{c}	1.611^{a}
P (%)	0.229^{g}	0.305^{b}	0.231^{f}	0.265^{c}	0.309^{a}	0.237^{e}	0.258^{d}
K (%)	0.935^{g}	1.221^{a}	0.956^{f}	1.032^{d}	0.963^{e}	1.110^{c}	1.113^{b}
Ca (%)	0.632^{g}	0.742^{a}	0.657^{f}	0.690^{d}	0.661^{e}	0.730^{b}	0.718^{c}
Mg (%)	0.080^{f}	0.096^{b}	0.095^{c}	0.095^{c}	0.087^{d}	0.099^{a}	0.086^{e}
Na (%)	0.046^{g}	0.052^{c}	0.060^{a}	0.051^{d}	0.049^{f}	0.055^{b}	0.050^{e}
Zn (mg kg^{-1})	25.85^{c}	34.31^{a}	26.16^{c}	31.75^{b}	23.67^{d}	35.26^{a}	27.68^{c}
Fe (mg kg^{-1})	79.15^{e}	107.80^{a}	83.49^{d}	95.45^{c}	84.25^{d}	100.60^{b}	93.84^{c}
Mn (mg kg^{-1})	26.53^{c}	32.87^{a}	26.60^{c}	30.89^{b}	27.63^{c}	34.11^{a}	29.57^{b}
Cu (mg kg^{-1})	23.96^{c}	29.71^{a}	26.61^{b}	26.14^{b}	27.13^{b}	26.45^{b}	30.22^{a}
B (mg kg^{-1})	5.41^{f}	8.39^{b}	5.54^{f}	7.09^{d}	6.90^{e}	9.37^{a}	8.01^{c}

Table 3. The effects of rhizobacteria treatment on the organic acid content (ng μL^{-1}) of the leaves.

	Control	637Ca	A18	FF1	M3	MFDCa1	MFDCa2
Oxalic acid	1.24^{b}	1.18^{d}	1.18^{d}	1.30^{a}	1.23^{bc}	1.19^{c}	1.16^{e}
Propionic acid	2.48^{b}	2.36^{e}	2.48^{b}	2.42^{c}	2.40^{d}	2.52^{a}	2.36^{e}
Tartaric acid	5.99^{d}	6.06^{c}	5.70^{f}	6.65^{a}	5.78^{e}	6.31^{b}	5.44^{g}
Butyric acid	15.38^{d}	13.04^{f}	16.18^{a}	15.57^{b}	15.31^{e}	12.95^{g}	15.44^{c}
Malonic acid	15.84^{d}	15.74^{e}	16.11^{c}	15.43^{g}	16.60^{b}	15.68^{f}	16.78^{a}
Malic acid	3.78^{f}	4.28^{c}	3.71^{g}	4.37^{b}	3.86^{e}	4.00^{d}	4.59^{a}
Lactic acid	20.25^{e}	20.89^{d}	21.79^{b}	21.01^{c}	20.20^{f}	21.98^{a}	19.09^{g}
Citric acid	15.97^{b}	15.19^{f}	15.47^{c}	17.08^{g}	15.42^{d}	15.231^{e}	16.88^{a}
Maleic acid	4.82^{d}	5.37^{a}	4.37^{f}	4.83^{d}	4.74^{e}	4.84^{c}	5.09^{b}
Fumaric acid	4.60^{f}	4.90^{b}	4.83^{c}	4.76^{e}	4.81^{d}	4.76^{e}	4.94^{a}
Succinic acid	25.38^{d}	29.04^{c}	24.36^{e}	30.38^{a}	23.52^{g}	29.14^{b}	23.58^{f}

prominent for each amino acid type in leaves and roots except for phenylalanine and proline contents of leaves and lysine content of roots (Tables 5 and 6). The FF1 treatment was more effective than the other bacterial strains in terms of aspartate, asparagine, glutamine, valine, and phenylalanine content in the roots, and glutamate, alanine, tyrosine, cysteine, valine, phenylalanine, isoleucine, and hydroxyproline content in the leaves. On the other hand,

Table 4. The effects of rhizobacteria treatment on the organic acid content (ng μL^{-1}) of the roots.

	Control	637Ca	A18	FF1	M3	MFDCa1	MFDCa2
Oxalic acid	1.13^{a}	1.05^{b}	1.02^{c}	1.00^{d}	1.05^{b}	1.04^{b}	1.04^{b}
Propionic acid	2.23^{g}	2.35^{d}	2.31^{e}	2.30^{f}	2.39^{c}	2.44^{b}	2.55^{a}
Tartaric acid	5.90^{b}	5.94^{a}	5.81^{d}	5.55^{f}	5.76^{e}	5.33^{g}	5.82^{c}
Butyric acid	13.57^{f}	15.19^{a}	12.81^{g}	13.89^{c}	13.79^{e}	14.72^{b}	13.84^{d}
Malonic acid	14.66^{f}	16.58^{c}	13.60^{g}	17.14^{b}	15.19^{e}	17.49^{a}	15.62^{d}
Malic acid	3.75^{f}	4.13^{c}	3.97^{d}	3.92^{d}	3.97^{d}	4.31^{a}	4.18^{b}
Lactic acid	18.89^{c}	18.74^{e}	17.47^{g}	19.97^{a}	17.82^{f}	18.87^{d}	19.95^{b}
Citric acid	16.11^{c}	14.85^{f}	16.79^{a}	15.81^{d}	14.90^{e}	16.43^{b}	14.47^{g}
Maleic acid	4.54^{f}	5.13^{a}	4.28^{g}	4.81^{d}	4.72^{e}	5.04^{b}	4.88^{c}
Fumaric acid	4.73^{e}	5.31^{b}	4.73^{e}	5.21^{c}	5.19^{c}	5.02^{d}	5.33^{a}
Succinic acid	30.71^{a}	26.78^{f}	28.67^{b}	25.91^{g}	27.24^{e}	27.87^{d}	28.01^{c}

Table 5. The effects of rhizobacteria treatment on the amino acid content (nmol μL^{-1}) of the leaves.

	Control	637Ca	A18	FF1	M3	MFDCa1	MFDCa2
Aspartate	1667^{d}	1804^{c}	1623^{f}	1828^{b}	1619^{g}	1839^{a}	1638^{e}
Glutamate	1477^{b}	1421^{d}	1402^{f}	1488^{a}	1408^{e}	1368^{g}	1454^{c}
Asparagine	2965^{d}	2879^{f}	2919^{e}	3004^{c}	3067^{b}	2819^{g}	3383^{a}
Serine	3635^{f}	3716^{d}	3807^{b}	3801^{c}	3833^{b}	3689^{e}	3856^{a}
Glutamine	2263^{g}	2591^{a}	2356^{e}	2586^{b}	2331^{f}	2494^{c}	2433^{d}
Histidine	786^{g}	872^{c}	794^{f}	877^{b}	801^{e}	898^{a}	811^{d}
Glycine	1147^{e}	1152^{d}	1171^{b}	1158^{c}	1173^{a}	1132^{f}	1105^{g}
Threonine	2162^{f}	2394^{d}	2131^{g}	2402^{c}	2423^{b}	2435^{a}	2172^{e}
Arginine	3201^{d}	3207^{c}	2997^{g}	3294^{b}	3042^{f}	3160^{e}	3348^{a}
Alanine	2269^{e}	2340^{b}	2291^{c}	2360^{a}	2282^{d}	2246^{f}	2311^{c}
Tyrosine	350^{g}	393^{c}	379^{f}	417^{a}	383^{e}	388^{d}	401^{b}
Cysteine	493^{e}	593^{b}	492^{e}	626^{a}	471^{f}	577^{c}	516^{d}
Valine	288^{e}	321^{b}	302^{d}	338^{a}	300^{d}	315^{c}	289^{e}
Methionine	591^{b}	501^{f}	566^{c}	509^{e}	543^{d}	478^{g}	599^{a}
Tryptophan	505^{g}	613^{a}	521^{f}	596^{c}	526^{e}	597^{b}	586^{d}
Phenylalanine	811^{f}	964^{a}	835^{d}	963^{a}	854^{c}	949^{b}	827^{e}
Isoleucine	644^{g}	680^{b}	650^{f}	728^{a}	656^{e}	677^{c}	658^{d}
Leucine	995^{b}	973^{c}	961^{d}	971^{c}	959^{e}	1019^{a}	952^{f}
Lysine	1244^{c}	1161^{g}	1254^{b}	1220^{d}	1210^{e}	1182^{f}	1281^{a}
Hydroxyproline	413^{f}	455^{b}	434^{c}	456^{a}	431^{d}	449^{b}	428^{e}
Sarcosine	2066^{d}	2212^{b}	2049^{e}	2187^{c}	2033^{f}	2243^{a}	1977^{g}
Proline	60^{bc}	55^{e}	62^{ab}	58^{d}	63^{a}	53^{f}	59^{cd}

Table 6. The effects of rhizobacteria treatment on the amino acid content (nmol μL^{-1}) of the roots.

	Control	637Ca	A18	FF1	M3	MFDCa1	MFDCa2
Aspartate	1866^{c}	1779^{f}	1814^{e}	2006^{a}	1823^{d}	1912^{b}	1775^{g}
Glutamate	1300^{g}	1463^{b}	1355^{f}	1413^{e}	1469^{a}	1423^{d}	1436^{c}
Asparagine	2982^{d}	2849^{g}	3055^{c}	3183^{a}	2902^{e}	3165^{b}	2871^{f}
Serine	3503^{g}	3855^{b}	3895^{a}	3671^{f}	3824^{d}	3829^{c}	3781^{e}
Glutamine	2413^{d}	2304^{f}	2253^{g}	2488^{a}	2402^{e}	2456^{c}	2474^{b}
Histidine	847^{d}	920^{a}	880^{c}	799^{f}	882^{b}	829^{e}	880^{bc}
Glycine	1198^{e}	1247^{b}	1238^{d}	1182^{g}	1242^{c}	1188^{f}	1266^{a}
Threonine	2329^{g}	2417^{c}	2372^{e}	2381^{d}	2473^{a}	2425^{b}	2352^{f}
Arginine	3207^{g}	3323^{e}	3346^{d}	3519^{b}	3359^{c}	3579^{a}	3312^{f}
Alanine	2212^{f}	2336^{a}	2225^{e}	2289^{c}	2281^{d}	2279^{d}	2312^{b}
Tyrosine	374^{e}	369^{f}	348^{g}	407^{b}	394^{c}	409^{a}	388^{d}
Cysteine	550^{d}	591^{a}	566^{c}	574^{b}	550^{d}	576^{b}	564^{c}
Valine	306^{f}	324^{c}	301^{g}	339^{a}	308^{e}	326^{b}	310^{d}
Methionine	553^{e}	507^{g}	587^{c}	623^{b}	540^{f}	626^{a}	557^{d}
Tryptophan	533^{g}	582^{e}	574^{f}	608^{d}	652^{a}	641^{b}	610^{c}
Phenylalanine	930^{e}	953^{b}	944^{c}	969^{a}	933^{d}	933^{d}	932^{d}
Isoleucine	658^{f}	696^{c}	659^{f}	702^{b}	660^{e}	710^{a}	691^{d}
Leucine	973^{g}	1069^{a}	1018^{d}	981^{f}	1037^{b}	1032^{c}	984^{e}
Lysine	1111^{e}	1238^{a}	1202^{c}	1176^{d}	1238^{a}	1203^{c}	1216^{b}
Hydroxyproline	416^{f}	493^{a}	461^{b}	432^{d}	455^{c}	420^{e}	431^{d}
Sarcosine	2181^{g}	2336^{b}	2237^{f}	2285^{e}	2287^{d}	2379^{a}	2311^{c}
Proline	57de	57^{d}	55^{e}	60^{c}	62^{b}	60^{c}	65^{a}

MFDCa1 application increased aspartate, histidine, threonine, leucine, and sarcosine in the leaves, and arginine, tyrosine, methionine, isoleucine, and sarcosine in the roots. According to the concentrations of amino acids in both leaves and roots, the 637Ca, MFDCa2, M3, and A18 treatments were followed by FF1 and MFDCa1. The organic acid concentration in the leaves and roots of raspberry plants treated by PGPR increased from 0.04% to 27.1% compared with the control plants.

4. Discussion

The increasing demand for food has directed farmers to employ new growing techniques. They use synthetic chemicals, fertilizers, and pesticides to increase food production, which is a challenge in today's agriculture. PGPR have been proven to be an environmentally friendly way of increasing crop yields by facilitating plant growth.

The features of PGPR in the present study positively affected plant nutrient elements of the raspberry cultivar "Heritage". Similar findings confirming the results of this study have been reported for other plant species such as pear, strawberry, raspberry, tomato, and pepper (Orhan et al., 2006; Karakurt and Aslantas, 2010; Ipek et al., 2014, 2017b; Seymen et al., 2015a, 2015b; Arikan and Pirlak, 2016). All PGPR strains increased leaf and root mineral content. Previous studies have demonstrated that M3 treatment increased P content (Çakmakçı et al., 2001; Elkoca et al., 2007; Esitken et al., 2010). This increase may have resulted from the P solubilization ability of the M3 bacterial strain. The nutrient content of leaf increased by 0.9%–47.5% while the nutrient content of root increased by 0.26%–73.19% in plants inoculated with bacteria compared with the control. The bacterial strains A18 and FF1, whose N fixing ability has not been reported, increased N content in leaves by about 14% and 12%, respectively. In addition, A18 and FF1 increased the B content of leaves by 47% and 32% compared to the control. Our results showed that bacterial strains may have different effects on plant nutrient element content in different plant species or cultivars.

The bacterial strains used in the present study were tested for the organic acid composition of pear (Ipek et al., 2017b), peach (Arıkan et al., 2018), and apple (Aras et al., 2017) leaves in high lime-containing soil. These bacterial

strains increased leaf organic acid content and resulted in increased plant nutrient elements. The bacterial strain MFDCa2 was more effective among bacteria strains in both the present study and previous studies in pear and peach. These bacterial strains increased leaf organic acid content 0%–21.42% and root organic acid content 0%–19.30%. The increase in inorganic acid content resulted in increased plant nutrient elements, especially Fe content, FC-R activity, and active Fe content in pear, peach, and apple leaves (Aras et al., 2017; Arıkan et al., 2018; Ipek et al., 2017b).

Plants synthesize all 20 common amino acids for protein synthesis. The key elements of an amino acid are carbon, hydrogen, oxygen, and nitrogen. The principal N compounds transported from roots to shoots and leaves via the xylem are nitrate and amino acids. The percentage of nitrate-N in xylem sap depends on the plant species, growth stage, and environmental conditions (Bollard, 1960). While nitrate is a major transported form of N, the roots are the main site of nitrate reduction for higher plants (Lewis, 1991). Asparagine and glutamine are the major amino acids transported through xylem vessels. The major N compounds in the phloem are also amino acids. A wide range of amino acids is detected in phloem sap, including glutamine and asparagine (Bollard, 1960). The amino acids produced by PGPR might promote plant growth, yield, and nutrient uptake under different growth conditions. There are limited studies about the amino acid content of leaves and roots affected by PGPR treatments. The present study showed that the bacterial strains increased N content in the leaves and roots. The increased N content in the leaves and roots of raspberry plants indicates that the amino acid content is increased (Zimmermann, 1960). The N content of the leaf or root was increased compared with the control of all PGPR strains in the present study and resulted in increases in the amino acid content of leaves and roots. The amino acid content in the leaf increased 0.3%–27% while the root amino acid content increased 0%–22.32%. Similar amino acid results have been reported in potato (Belimov et al., 2015), cauliflower (Ekinci et al., 2014), and wheat (Kudoyarova et al., 2014).

In conclusion, our results demonstrated that the PGPR bacterial strains *Alcaligenes* 637Ca, *Agrobacterium* A18, *Staphylococcus* MFDCa1, *Staphylococcus* MFDCa2, *Bacillus* M3, and *Pantoea* FF1 could be used in sustainable agricultural production. These bacterial strains can increase plant growth and might reduce the cost of synthetic mineral fertilizers. Reduced use of commercial synthetic fertilizers may also decrease the harmful effect of synthetic fertilizers on agricultural areas and the environment. The use of PGPR as fertilizer may become widespread in the next 10 years.

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