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Effects of plant growth promoting bacteria (PGPB) on yield, growth and nutrient contents of organically grown strawberry

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ABSTRACT

The effects of plant growth promoting bacteria (PGPB) on the fruit yield, growth and nutrient element content of strawberry cv. Fern were investigated under organic growing conditions between 2006 and 2008. The experimental plot was a completely randomized design with 3 replicates. Three PGPB strains (*Pseudomonas* BA-8, *Bacillus* OSU-142 and *Bacillus* M-3) were used alone or in combination as bio-fertilizer agent in the experiment. Data through 3 years showed that the use of PGPB significantly increased fruit yield, plant growth and leaf P and Zn contents. Root inoculation of M3 and floral and foliar spraying of OSU-142 and BA-8 bacteria stimulated plant growth resulting in significant yield increases. M3 + BA-8, BA-8 + OSU-142, M3, M3 + OSU-142 and BA-8 applications increased cumulative yield by 33.2%, 18.4%, 18.2%, 15.3% and 10.5%, respectively. Number of fruits per plant significantly increased by the applications of M3 + BA-8 (91.73) and M3 (81.58) compared with the control (68.66). In addition, P and Zn contents of strawberry leaves with bacterial inoculation significantly increased under organic growing conditions. Available P contents in soil were increased from 0.35 kg P_2O_5/da at the beginning of the study to 2.00, 1.97 and 1.82 kg P_2O_5/da by M3 + OSU-142, M3 + BA-8 and M3 + BA-8 + OSU-142 applications, respectively. Overall, the results of this study suggest that root inoculation of *Bacillus* M3 alone or in combination with spraying *Bacillus* OSU-142 or *Pseudomonas* BA-8 have the potential to increase the yield, growth and nutrition content of strawberry plant under organic growing conditions.

1. Introduction

Although commercial strawberry (*Fragaria* × *ananassa* Duch.) cultivation started towards the end of 1970 in Turkey, the country is currently one of the biggest strawberry producer in the world with 250,000 tons production annually (FAO, 2009). Recently some vegetable production areas of the Mediterranean region of Turkey have been converted to strawberry farms because of increased rentability of the Turkish strawberry production. The increased strawberry production in Turkey has initiated an increased interest to grow organic strawberries by farmers.

As well known, in organic agricultural system, the use of synthetic fertilizers, pesticides, growth regulators, and livestock feed additives are avoided. Instead, the use of bio-fertilization, crop rotations, crop residues, animal manures, legumes, green manures, off-farm organic wastes, mechanical cultivation, mineral-bearing rocks, and aspects of biological pest control to maintain soil productivity are promoted. However, yield reduction is an important problem in organic production system (Lind et al., 2003). The PGPB known as beneficial microorganisms used in place of synthetic chemicals, are capable to improve plant growth through supply of plant nutrients and may help to sustain environmental health and soil productivity (O'Connell, 1992; Esitken et al., 2005).

Recent studies confirmed that, a number of bacterial species mostly associated with the plant rhizosphere, are found to be beneficial for plant growth, yield and crop quality. They have been called 'Plant Growth Promoting Bacteria (PGPB)' including the strains in the genera *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillium*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholdria*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Rhizobium* and *Serrotia* (Bashan and de-Bashan, 2005).

The plant promoting effect of the PGPB is mostly explained by the release of metabolites directly stimulating growth. The mechanisms by which PGPB promote plant growth are not fully understood, but are thought to include: (a) the ability to produce plant hormones, such as auxins (Egamberdiyeva, 2005), cytokinins (Garcia de Salamone et al., 2001), gibberellins (Gutierrez-Manero et al., 2001), and inhibit ethylene production (Glick et al., 1995); (b) asymbiotic N_2 fixation (Sahin et al., 2004); (c) solubilization of

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inorganic phosphate and mineralization of organic phosphate and/or other nutrients (Jeon et al., 2003); (d) antagonism against phytopathogenic microorganisms by production of siderophores, the synthesis of antibiotics, enzymes and/or fungidical compounds and competition with detrimental microorganisms (Dobbeleare et al., 2002; Dey et al., 2004; Lucy et al., 2004).

Previous studies showed that PGPB stimulated growth and increased yield in several fruit species including apple, sweet cherry, citrus, raspberry, high bush blueberry, mulberry and apricot (Kloepper, 1994; De Silva et al., 2000; Sudhakar et al., 2000; Esitken et al., 2002, 2003, 2006; Orhan et al., 2006; Aslantas et al., 2007; Karlıdağ et al., 2007).

However, in our literature search, we found few reports covering the use of these microorganisms in organic strawberry production. Therefore, the objective of this study was to investigate the growth promoting effects of flower and foliar spraying and root inoculation of *Bacillus* (M3 and OSU-142) and *Pseudomonas* BA-8 bacteria strains on strawberry yield, growth and mineral content under organic growing conditions.

2. Materials and methods

2.1. The study site

This study was conducted at the Agricultural Research Station of Ataturk University located in Erzurum, Turkey (39°55′N and 41°16′E, 1835 m) during the summer periods (late May–late September) of 2006–2008. The soil was classified as an Aridisol with parent materials mostly consisting of volcanic, marn and lacustrin transported material (Soil Survey Staff, 1992). The experimental region has a semi-arid climate. The mean maximum temperature was 20–25 °C while the mean minimum temperature was 5–10 °C during the growing period.

2.2. The soil analyses

Before planting, soil samples were taken over 0–30 cm (20 subsamples) to determine baseline soil properties (Table 1). Soil samples were air-dried, crushed, and passed through a 2-mm sieve prior to chemical analysis. Cation exchange capacity (CEC) was determined using sodium acetate (buffered at pH 8.2) and ammonium acetate (buffered at pH 7.0) according to Sumner and Miller (1996). The Kjeldahl method (Bremner, 1996) was used to determine total N, plant-available P was determined by using the sodium bicarbonate method (Olsen et al., 1954). Soil pH was determined in 1:2 extracts, and calcium carbonate concentrations were determined according to McLean (1982). Soil organic matter was determined using the Smith-Weldon method according to Nelson and Sommers (1982). Ammonium acetate buffered at pH 7 (Thomas, 1982) was used to determine exchangeable cations. Microelements in the soils were determined by diethylene triamine pentaacetic acid (DTPA) extraction methods (Lindsay and Norwell, 1978).

2.3. Bacterial strain, culture conditions, media and treatment

Three PGPB strains (*Pseudomonas* BA-8, *Bacillus* OSU-142 and *Bacillus* M-3) were obtained from Yeditepe University, Dept. of Genetics and Bioengineering (Dr. Fikrettin Sahin, personal communication). These bacteria were reported as plant growth promoting bacteria and potential bio-control agents against a wide range of bacterial and fungal pathogens which cause important economical problems in agriculture (Cuppels et al., 1999; Kotan et al., 1999; Cakmakci et al., 2001; Esitken et al., 2002, 2003). Bacteria were grown on nutrient agar (NA) for routine use, and maintained in nutrient broth (NB) with 15% glycerol at −80 °C for long-term storage. A single colony was transferred to 500 ml flasks containing NB, and grown aerobically in flasks on a rotating shaker (150 rpm) for 48 h at 27 °C (Merck KGaA, Germany). The bacterial suspension was then diluted in sterile distilled water to a final concentration of 10^9 CFU ml^{-1}, and the resulting suspensions were used to treat strawberry plants.

2.4. Field experiments

Field experiments were carried out on "Fern" day-neutral strawberry cultivar. In 2006, an area was selected in Erzurum with no history of agricultural practice. Based on analysis results, 2 t/da manure and 10 kg/da rock phosphate (including 18% P_2O_5) was applied to this area, and because of optimal concentrations of available K in the soil, this element was not applied during the experiment. Nitrogen was supplied by fertigation using an organic certificated fertilizer (STYM 25: EU registration 2092/91). Eighth treatments in plots of 50 plants giving a total of 1200 strawberry plants were planted on raised bed at a spacing of 0.30 m × 0.35 m on a loamy soil with black plastic mulch in the beginning of May 2006. Application of *Bacillus* M3 was performed using a dipping method in which plant roots were inoculated with the bacterial suspensions of the concentration of 10^9 CFU ml^{-1} in sterile water about 30 min prior to plantation. Floral and foliar plant organs were sprayed with bacterial suspension (10^9 CFU ml^{-1}) of *Pseudomonas* BA-8 and *Bacillus* OSU-142 alone or combination

Table 1
Soil nutrient elements content of orchard when strawberry grown in 3 consecutive years under seven different PGPB application treatments on a calcareous Aridisol in Eastern Turkey in 2006–2008.

	Prior to planted	Control	BA-8	OSU-142	BA-8 + OSU-142	M3	M3 + BA-8	M3 + OSU-142	M3 + BA-8 + OSU-142
pH	7.55	7.56	7.39	7.43	7.53	7.46	7.36	7.37	7.45^{NS}
Available P (kg P_2O_5/da)	0.35 e	1.72 bc	1.53 cd	1.73 bc	1.78 b	1.61 cd	1.97 a	2.00 a	1.82 b***
Organic matter (%)	1.90	1.92	1.86	2.01	2.20	1.98	2.13	1.88	1.81^{NS}
Cu (ppm)	0.50	0.49	0.62	0.50	0.52	0.52	0.58	0.59	0.55^{NS}
Mn (ppm)	2.20 c	2.46 bc	3.17 a	2.88 ab	2.87 ab	3.08 ab	3.30 a	2.82 abc	3.34 a***
Fe (ppm)	3.40 c	5.15 bc	5.20 b	5.87 ab	5.58 b	5.52 b	5.74 ab	5.91 ab	7.51 a***
Zn (ppm)	2.25 cd	2.26 cd	2.84ab	2.84 b	2.65 b	2.82 b	2.44 bc	2.07 d	3.24 a***
Na (cmol/kg)	0.48	0.66	0.68	0.71	0.78	0.75	0.77	0.78	0.78^{NS}
Ca (cmol/kg)	12.18	11.82	12.60	12.21	12.01	12.18	11.72	11.02	11.21^{NS}
K (cmol/kg)	3.65 b	5.47 a	5.24 a	5.56 a	5.70 a	5.37 a	6.04 a	5.57 a	5.20 a**
Mg (cmol/kg)	1.75 b	2.36 a	2.35 a	2.28 a	2.31 a	2.37 a	2.30 a	2.35 a	2.28 a*
CEC (cmol/kg)	18.06 b	26.44 a	27.38 a	26.81 a	27.58 a	26.55 a	27.36 a	26.03 a	26.41 a**

NS, not significant; means separation within line by Duncan's multiple range test.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

until run off, 15 days intervals during growing period (June to October). Control plants were sprayed with sterile water and dipped into sterile water. Good quality underground water with an electrical conductivity of 0.28 dS m^{-1}, Na adsorption ratio of 0.40, and pH of 7.4 was used for drip irrigation during the experimental period.

During the experimental period (2006–2008) data were collected. Growth promoting effects of bacterial treatments were evaluated by determining cumulative yield (g/plant), average fruit weight (g), number of fruits per plant, leaf area (cm^2), fruit firmness (kg/cm^2), vitamin C (mg/100 ml), total soluble solids (TSS, %), reducing sugar (%) and titratable acidity (as citric acid, %). In addition, the effect of the bacterial treatments on the plant nutrient element (PNE) contents of leaves was evaluated. Soils of orchard were also analyzed at the end of experiment in 2006, 2007 and 2008 (at September in third years).

2.5. Leaf analyses

Fully developed mid-shoot leaves were sampled in August in the third years of the study. In order to determine the mineral contents of leaves, plants samples were oven-dried at 68 °C for 48 h and then grounded to pass 1 mm sieve. The total N was determined using Kjeldahl method and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany) (Bremner, 1996). Macro- (P, K, Ca, Mg and Na) and microelements (Fe, Mn, Zn, and Cu) were determined after wet digestion of dried and ground subsamples using a HNO_3–H_2O_2 acid mixture (2:3, v/v) with three steps (first step: 145 °C, 75%RF, 5 min; second step: 180 °C, 90%RF, 10 min; third step: 100 °C, 40%RF, 10 min) in a microwave oven (Bergof Speedwave Microwave Digestion Equipment MWS-2) (Mertens, 2005a). Tissue P, K, Ca, Mg, Na, Fe, Mn, Zn, and Cu were determined using an Inductively Couple Plasma spectrophotometer (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT, USA) (Mertens, 2005b).

2.6. Data analysis

All data in the present study were subjected to the analysis of variance (ANOVA) and means were separated by Duncan's multiple range tests. There was no statistical difference between years. Therefore, the data were pooled.

3. Results

The experiments in this study showed that bacterial treatments significantly affected fruit yield, vitamin C and number of fruit per plant (Table 2). Significant yield increase was obtained with *Bacillus* M3 (569.5 g/plant), *Pseudomonas* BA-8 (532.6 g/plant), BA-8 + OSU-142 (570.6 g/plant), M3 + BA-8 (642.1 g/plant) and M3 + OSU-142 (555.4 g/plant) treatments as compared with the control (481.9 g/plant) (Table 2). The percentage of yield increase was 33.2% when M3 + BA-8 were applied. However, there were no significant differences found between treatments concerning the average berry weight and other fruit properties tested such as fruit firmness, TSS, acidity and reducing sugar, except vitamin C content. The control treatment (71.58 mg/100 ml) provided the highest vitamin C content but there was no statistically significant difference between the control and bacterial applications except M3 + BA-8 + OSU-142. Similar to yield, M3 + BA-8, BA-8 + OSU-142 and M3 inoculations increased the number of fruit per plant (91.73, 82.77 and 81.58) compare to the control (68.66), respectively. In addition, there were no statistical differences between control and bacterial applications in terms of leaf area.

In this study we have found that bacterial treatments significantly increased PNE contents of strawberry leaves under organic growing conditions compared with the control (Table 3). In particular, root inoculation of M3 and foliar and floral BA-8 or OSU-142 spraying promoted P, Fe and Zn uptake of strawberry cv. Fern. The highest P (1.27%), Fe (23.99 ppm) and Zn (37.95 ppm) contents were obtained from M3 + BA-8 + OSU-142, BA-8 + OSU-142 applications, which increased P, Fe and Zn content of leaves by 53.0%, 20.1% and 77.9% compared to control, respectively (Table 3). Although bacterial treatments increased N, K and Na content of leaves, no significant differences were observed among treatments and the control (Table 3).

Bacterial applications significantly affected soil nutrient element contents (Table 1). Available P in soil was significantly affected by bacterial applications compared with the control. Available P content was 2.00 and 1.97 kg P_2O_5/da in M3 + OSU-142 and M3 + BA-8 applications while was 1.72 kg P_2O_5/da in the control. On the other hand, available P content in the soil was 0.35 kg P_2O_5/da at the beginning of treatment. At the beginning of the presented trials, soil K, Mg, Fe and Zn were determined as 3.65 cmol/kg, 1.75 cmol/kg, 3.40 ppm and 2.25 ppm, whereas soil K, Mg, Fe and Zn were 6.04 cmol/kg, 2.37 cmol/kg, 7.51 ppm and 3.24 ppm in M3 + BA-8, M3 and M3 + BA-8 + OSU-142 treatments, respectively. Bacterial applications significantly increased to soil Mn content compared with the situation prior to planting. Soil Mn increased from 2.20 ppm to 3.34 ppm by M3 + BA-8 + OSU-142, to 3.30 ppm by M3 + BA-8 and to 3.17 ppm by BA-8 applications.

4. Discussion

This is the first study demonstrating that PGPR can increase yield, growth and PNE contents of strawberry under organic growing conditions. Similar results are found in raspberry, sugar

Table 2
Yield, growth and fruit properties of strawberry cultivar when grown in 3 consecutive years under seven different PGPB application treatments on a calcareous Aridisol in Eastern Turkey in 2006–2008.

	Control	BA-8	OSU-142	BA-8 + OSU-142	M3	M3 + BA-8	M3 + OSU-142	M3 + BA-8 + OSU-142
Cum. yield (g/plant)	481.9 c	532.6 b	518.5 bc	570.6 b	569.5 b	642.1 a	555.4 b	490.1 c***
Fruit weight (g)	7.11	7.06	7.13	6.99	6.98	6.98	6.85	6.66^{NS}
Fruit firmness (kg/cm^2)	0.92	0.86	0.92	0.82	0.99	0.88	0.93	0.93^{NS}
TSS (%)	9.03	8.66	8.99	9.08	8.74	8.62	8.65	8.27^{NS}
Titr. acidity (%)	0.70	0.55	0.56	0.60	0.51	0.53	0.59	0.62^{NS}
Vitamin C (mg/100 ml)	71.58 a	66.03 ab	69.25 a	64.02 ab	64.65 ab	64.36 ab	70.00 a	59.25 b*
Reducing sugar (%)	5.51	5.15	6.02	6.49	5.18	5.39	5.84	5.44^{NS}
Cum. fruit number	68.66 c	76.24 bc	73.41 bc	82.77 ab	81.58 ab	91.73 a	81.21 abc	72.83 bc*
Leaf area (cm^2)	102.4	107.8	106.0	105.2	100.9	98.6	108.4	109.2^{NS}

NS, not significant; means separation within line by Duncan's multiple range test.
* $P < 0.05$.
*** $P < 0.001$.

Table 3
Leaf macro- and microelement concentration of strawberry cultivar when grown in 3 consecutive years under seven different PGPB application treatments on a calcareous Aridisol in Eastern Turkey in 2006–2008.

	Control	BA-8	OSU-142	BA-8 + OSU-142	M3	M3 + BA-8	M3 + OSU-142	M3 + BA-8 + OSU-142
N (%)	3.06	3.16	3.30	3.31	3.35	3.41	3.38	3.03^{NS}
P (%)	0.83 d	0.84 d	0.95 cd	0.92 cd	1.02 bc	1.11 b	1.13 ab	1.27 a***
K (%)	2.63	2.69	3.25	2.92	2.85	2.89	3.16	2.88^{NS}
Ca (%)	2.39	2.25	2.47	2.32	2.25	2.41	2.46	2.37^{NS}
Mg (%)	0.20	0.19	0.19	0.19	0.20	0.19	0.19	0.20^{NS}
Fe (ppm)	19.98 bcd	20.17 bc	17.54 cd	16.98 d	17.93 bcd	19.99 bcd	23.99 a	20.86 b***
Cu (ppm)	5.54 a	3.39 c	3.83 bc	4.48 abc	3.80 bc	5.12 ab	4.50 abc	5.57 a**
Mn (ppm)	46.70	45.30	43.06	45.52	39.87	43.03	45.45	42.93^{NS}
Zn (ppm)	21.33 c	33.18 ab	26.52 bc	37.95 a	32.11 ab	33.65 ab	27.49 bc	30.70 ab***
Na (ppm)	841.6	768.0	816.3	882.4	825.9	900.9	882.9	861.3^{NS}

NS, not significant; means separation within line by Duncan's multiple range test.
** $P < 0.01$.
*** $P < 0.001$.

beet, barley, corn and tomatoes. For example, Orhan et al. (2006) reported that under organic growing conditions, yield, cane length, number of cluster per cane and number of berries per cane of raspberry were increased by M3 and M3 + OSU-142 root inoculations. The use of OSU 142 and M3 stimulated yield and quality parameters of sugar beet and barley (Cakmakci et al., 2001) and tomatoes (Turan et al., 2004). Similarly, floral and foliar applications of *Bacillus* OSU-142 and BA-8 and OSU-142 increased yield, growth and PNE contents of leaves and decreased shot-hole disease in apricot, sweet cherry and apple (Esitken et al., 2002, 2003, 2006; Pirlak et al., 2007; Aslantas et al., 2007). The positive effects of OSU 142, BA-8 and M3 on the yield and growth of apricot, raspberry, tomatoes, sugar beet, apple, sweet cherry and barley were explained by promoting abilities of these bacteria for auxin and cytokinin production, N_2-fixation, phosphate solubilization and antimicrobial substance production (Sahin et al., 2000; Cakmakci et al., 2001; Esitken et al., 2002, 2003, 2006; Orhan et al., 2006; Pirlak et al., 2007; Aslantas et al., 2007; Karlıdağ et al., 2007). The yield and plant growth enhancement effects of bacteria used in this study on strawberry could be explained by the similar reasons. In addition, we found that the inoculation of bacteria increased P, Fe and Zn content of strawberry leaves, which provide the additional evidence supporting the finding of previous studies.

Higher P content of leaves in M3 inoculations may be explained by phosphate solubilizing capacity of this bacterium. This increase may also be explained by organic acids production by plants and bacteria in the rhizosphere, which in turn stimulates the availability of P, Fe and Zn. The findings obtained in this study were supported by a number of previous studies (Jakobsen, 1986; Smith and Read, 1997; Sundra et al., 2002; Shen et al., 2004). Phosphorus is an essential nutrient for plant growth and development and is one of the most important elements in crop production. Despite its wide distribution in nature, it is a deficient nutrient in most soils, especially in soils which have a high P-fixation capacity. Since a substantial amount of any applied P fertilizer is rendered unavailable and frequent applications of soluble forms of inorganic P are needed to maintain adequate P levels for the plant growth. It is a well known fact that a considerable number of bacterial species, mostly those associated with the plant rhizosphere, are able to exert a beneficial effect upon plant growth. Phosphate solubilizing microorganisms render these insoluble phosphates into soluble form through the process of acidification, chelatiation and exchange reactions (Banik and Dey, 1981; Bhattacharya et al., 1986). As we mentioned before, bacterial applications increased soil K, Mg, Fe, Zn and Mn content. In addition, CEC significantly increased by treatments compared with the situation prior to planting. Increased availability of mineral contents in soils during the cultivation and bacteria application in the soil can be attributed to increased mineralization of the organic complex and organic acid production by plants and bacteria in the rhizosphere (Jakobsen, 1986; Smith and Read, 1997; Sundra et al., 2002; Shen et al., 2004).

5. Conclusions

The results of the present study suggested that both root inoculation of *Bacillus* M3 and spraying of *Pseudomonas* BA-8 or *Bacillus* OSU-142 have potential to increase the yield, growth and P, Fe, Cu and Zn content of the strawberry plant and to increase the soil P, Fe, Zn, K, and Mg availability. Considering environmental pollution with excessive use of synthetic fertilizers and high costs in the production of N and P fertilizers, the bacteria tested in our study may is a promising alternative as a bio-fertilizer for fruit and vegetable production in sustainable and organic agricultural systems.

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