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Effects of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient contents in organically growing raspberry

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Abstract

During 2003 and 2005, plant growth promoting effects of two *Bacillus* strains OSU-142 (N_2-fixing) and M3 (N_2-fixing and phosphate solubilizing) were tested alone or in combinations on organically grown primocane fruiting raspberry (cv. Heritage) plants in terms of yield, growth, nutrient composition of leaves and variation of soil nutrient element composition in the province of Erzurum, Turkey. The results showed that *Bacillus* M3 treatment stimulated plant growth and resulted in significant yield increase. Inoculation of raspberry plant roots and rhizosphere with M3 and/or OSU-142 + M3, significantly increased yield (33.9% and 74.9%), cane length (13.6% and 15.0%), number of cluster per cane (25.4% and 28.7%) and number of berries per cane (25.1% and 36.0%) compared with the control, respectively. In addition, N, P and Ca contents of raspberry leaves with OSU-142 + M3 treatment, and Fe and Mn contents of the leaves of raspberry with M3 and OSU-142 + M3 applications significantly improved under organic growing conditions. Bacterial applications also significantly effected soil total N, available P, K, Ca, Mg, Fe, Mn, Zn contents and pH. Available P contents in soil was determined to be increased from 1.55 kg P_2O_5/da at the beginning of the study to 2.83 kg P_2O_5/da by OSU-142, to 5.36 kg P_2O_5/da by M3 and to 4.71 kg P_2O_5/da by OSU-142 + M3 treatments. The results of this study suggest that *Bacillus* M3 alone or in combination with *Bacillus* OSU-142 have the potential to increase the yield, growth and nutrition of raspberry plant under organic growing conditions.

Keywords: *Rubus idaeus*; *Bacillus* OSU-142; *Bacillus* M3; Root inoculation

1. Introduction

Intensive farming practices, that warrant high yield and quality, require extensive use of chemical fertilizers, which are costly and create environmental problems. Therefore, more recently there has been a resurgence of interest in environmental friendly, sustainable and organic agricultural practices (Esitken et al., 2005).

Organic agricultural is a production system, which avoids or largely excludes the use of synthetically compounded fertilizers, pesticides, growth regulators, and livestock feed additives. To the maximum extent feasible, organic agricultural systems rely upon 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. However, yield reduction is an important problem in organic production system (Lind et al., 2003). Use of biofertilizers containing beneficial microorganisms instead of synthetic chemical are known to improve plant growth through supply of plant nutrients and may help to sustain environmental health and soil productivity (O'Connell, 1992).

So far, considerable number of bacterial species mostly associated with the plant rhizosphere, have been tested and found to be beneficial for plant growth, yield and crop quality. They have been called 'plant growth promoting rhizobacteria (PGPR)' including the strains in the genera *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillium*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Rhizobium* and *Serratia* (Rodriguez and Fraga, 1999; Sturz and Nowak, 2000; Sudhakar et al., 2000). In previous studies, it was found that PGPR could stimulate

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growth and increase yield in apple, citrus, high bush blueberry, mulberry and apricot (Kloepper, 1994; De Silva et al., 2000; Sudhakar et al., 2000; Esitken et al., 2002, 2003).

The restricted availability of major nutrients like nitrogen and phosphorus limits plant growth and yield. Bio-fertilizers including microorganisms may add nitrogen to the soil by symbiotic or asymbiotic N_2 fixation. On a worldwide basis, it is estimated that about 175 million tons of nitrogen per year is added to soil through biological nitrogen fixation. Meanwhile super-phosphate fertilizer is expensive and in short supply, but bio-fertilizers can bridge the gap. There are several micro-organisms, which can also solubilize the cheaper sources of phosphorus, such as rock phosphate. Bacteria like *Pseudomonas* and *Bacillus* are widely used in organic production system and also important phosphorus solubilizing microorganisms, resulting in improved growth and yield of crops (Dobereiner, 1997).

The genus *Rubus* contains a large number of highly variably and heterogeneous species, which occur in all parts of the world except the dessert regions (Jennings et al., 1990). *Rubus idaeus*, red raspberry, is supposedly named after the region near Mount Ida in Asia Minor where they grew wild and were called 'Ida' fruit by the ancient Greeks (Jennings, 1988).

Red raspberries can be either of two types. Summer-bearing red raspberries have the typical biennial life cycle of a bramble: they bear their fruit from late June to August of their second year, and the canes die after fruiting. Primocane-bearing type such as Heritage is the exception to this life cycle: they bear fruit during the first year. Also called "everbearing" raspberries, they will fruit again in the spring, on the buds below those that fruited the previous fall. Because both of these red raspberries produce new canes (suckers) primarily from the root system, they are usually grown in a hedgerow. They are the most winter-hardy of the raspberries (Kuepper et al., 2003).

It is reported that bramble fruits are relatively easy to produce using organic methods if enough nutrients are available due to application of biofertilizer into the soil (Kuepper et al., 2003). However, so far there have been no attempts to study the effects of PGPR including N_2-fixing and phosphate-solubilizing bacteria on plant growth of raspberries. Therefore, in the present work, we report the effect of two N_2-fixing (*Bacillus* OSU-142 and M3) and a phosphate-solubilizing bacterial strain (*Bacillus* M3) treatments on raspberry plants growth in organic production system.

2. Material and methods

2.1. Bacterial strain, culture conditions, media and treatment

Strains of bacteria, *Bacillus* OSU-142 (N_2-fixing bacterium) and *Bacillus* M3 (N_2-fixing and phosphate solubilizing bacterium), were obtained from Dr. Fikrettin Sahin (Yeditepe University, Department of Genetics and Bioengineering). These bacteria were reported as plant growth promoting bacteria and potential bio-control agents against a wide range of bacterial and fungal pathogens causing economically important 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. For this experiment, the bacterial strains were grown on nutrient agar. 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 raspberry plants.

2.2. Field experiments

Experiments were carried out on "Heritage" primocane-fruiting raspberry cultivar. In 2003, an area was selected in Erzurum where no agricultural practices carried out on it previously. Soil samples were taken from 20 to 40 cm depth and analyzed before plantation (Table 3). Experiment area has sandy-loam soil including 31.0% clay, 35.5% silt, 33.5% sand, 3.8% organic matter and 0.96% lime. According to analysis results, 2 t/da manure and 10 kg/da rock phosphate (including 18% P_2O_5) has been applied to this area. One-hundred and twenty raspberry plants in total which divided four application groups including 10 plants with three replicates were planted at 1.0 m within row and 2.0 m between row spacing at the end of the April in a completely randomized design in 2003. Bacterial applications of *Bacillus* OSU-142, *Bacillus* M3 and their combination (OSU-142 + M3) were performed using dipping method in which plant roots were inoculated with the bacterial suspensions at the concentration of 10^9 CFU ml^{-1} in sterile water about 30 min prior to plantation. Control plants were dipped into sterile water. In the first year of the field experiment, canes did not bear fruit, therefore no data were collected. In the second and third years of experiment (2004 and 2005), canes bear fruit and the data were collected. Growth promoting effects of bacterial treatments were evaluated by determining total yield (g/plant), average berry weight (g), number of picks per plant, cane length (cm) and diameter (mm), number of cluster per cane, number of berries per cluster (on 60 cane), leaf area (cm^2), total soluble solids (TSS, %) and titratable acids (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 also analyzed at the end of experiment in 2004 and 2005 (at October in both years).

2.3. Leaf analysis

Fully developed mid-shoot leaves were sampled in September (harvest period) of 2 experiment years. In order to determine the mineral contents of leaves, plants samples were oven-dried at 68 °C for 48 h and then ground. The micro-Kjeldahl procedure was applied for determination of N. Potassium, Ca, and Mg contents were determined after wet digestion of dried and ground sub-samples in a H_2SO_4–Se–salisilic acid mixture. In the diluted digests, P was measured spectrophotometrically by the indophenol-blue method and after reaction with ascorbic acid. Potassium and Ca contents of plants were determined by flame photometry, Mg, Na, Fe, Mn, Zn, and Cu contents were determined by atomic absorption spectrometry using the method of AOAC (1990).

2.4. Soil analysis

Soil samples were taken from 20 to 40 cm depth at the beginning of experiment (April 2003) and at the end of experiment in second and third years (October 2004 and 2005). Soil pH was determined in 2:1 water–soil suspension by pH-meter (McLean, 1982). Exchangeable cations (K, Ca, and Mg) according to Thomas (1982) and plant available phosphorus according to Olsen and Sommers (1982) were determined. Organic matter and total nitrogen were also determined according to the instructions given by Nelson and Sommers (1982) and Bremner and Mulvaney (1982), respectively. Micro elements in the soils were determined by DTPA extraction methods (Lindsay and Norwell, 1969).

2.5. Data analysis

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

3. Results and discussion

3.1. Growth promoting effect of bacterial treatment in raspberry

Two years of trials (2004 and 2005) under organic growing conditions showed that bacterial treatments including *Bacillus* M3 and *Bacillus* OSU-142 + M3 applications significantly affected all parameters tested in this study (Fig. 1 and Table 1). The results showed that yield significantly increased ($p < 0.001$) by bacterial treatments except OSU-142 compared with control (Fig. 1). Significant yield increase was obtained with *Bacillus* M3 (554.5 g/plant) and OSU-142 + M3 (724.4 g/plant) treatments as compared with the OSU-142 (365.0 g/plant) and control (414.1 g/plant). The average percentage of yield increase was 33.9% and 74.9% when M3 and OSU-142 + M3 were applied, respectively. However, there were no significant differences between treatments concerning the average berry weight and other fruit properties (TSS and acidity) tested although there was a numerical increase in average berry weight of M3 and OSU-142 + M3 treatments. In addition, M3 (134.4 cm) and OSU-142 + M3 (136.1 cm) applications gave the highest cane length of raspberry representing increases over control (118.3 cm) of 13.6% and 15.0%, respectively, but there was no significant difference between these applications and the control in terms of cane diameter. Similarly, M3 and OSU-142 + M3 inoculations increased number of cluster per cane (15.3 and 15.7) and number of berries per cluster (9.67 and 10.51) as compared with OSU-142 (12.0 and 7.76) and the control (12.2 and 7.73), respectively. In addition, there were no differences between control and M3 and OSU-142 + M3 applications in terms of number of picks per plant. In general, OSU-142 application gave the lowest value at all of the parameters, except TSS and number of berries per cluster.

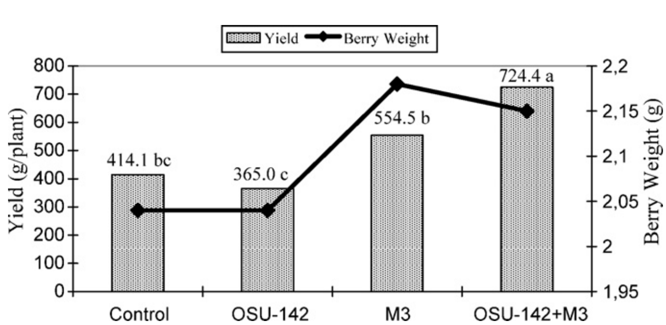

Fig. 1. The effects of treatments on yield (LSD 0.01: 151.8) and berry weight (n.s.) in raspberry.

This is the first study to demonstrate that PGPR can increase yield, growth and PNE contents of raspberry. De Silva et al. (2000) reported that the leaf area and stem diameter of high bush blueberry were increased by applying *Pseudomonas fluorescens* Pf 5. Some of the previous studies with the same PGPR strains tested on sugar beet, barley, corn and tomatoes have been reported similar findings confirming our data in the present work. The use of OSU 142 and M3 in sugar beet and barley (Cakmakci et al., 2001), corn (Ataoglu et al., 2004) and tomatoes (Turan et al., 2004) stimulated yield and quality parameters tested. In addition, floral and foliar application of *Bacillus* OSU-142 has increased yield, growth and PNE contents of leaves and decreased shot-hole disease in apricot (Esitken et al., 2002, 2003).

The yield and plant growth enhancement effects of bacteria used in this study on red raspberry could be explained with N_2-fixing and phosphate solubilizing capacity of bacteria. The positive effects of OSU 142 and M3 on the yield and growth of crops such as apricot, tomatoes, sugar beet, and barley were explained by N_2 fixation ability, phosphate solubilizing capacity and antimicrobial substance production (Sahin et al., 2000; Abbasi et al., 2001; Cakmakci et al., 2001; Esitken et al., 2002, 2003). In present study, we have also found that the inoculation of PGPR strains increased N and P content of raspberry leaves, which provide the additional evidence supporting the finding of previous studies. The data in the present study showed that bacterial application decreased pH level of experimental orchard's soil from 6.7 to 5.6–6.0. This result may be explained that bacterial colonization in soil decrease pH due to their organic acid production as a secondary metabolite which can a better soil condition for raspberry growing (Lind et al., 2003).

3.2. Effect of bacteria on plant nutrient element (PNE) contents of leaves

The PNE concentration of plant leaves treated by PGPR strains may provide important information about the effect of bacterial inoculation in PNE uptake. In this study, we have

Table 1
The effects of treatments on vegetative growth and berry properties in raspberry

	Mean of 2004 and 2005				LSD[a]
	Control	OSU-142	M3	OSU-142 + M3	
Cane length (cm)	118.3 b	99.6 c	134.4 a	136.1 a	0.01: 9.15
Cane diameter (mm)	11.25 a	8.66 b	11.94 a	11.95 a	0.05: 2.48
Number of picks	10.20 a	7.55 b	9.85 a	12.05 a	0.05: 2.30
Number of cluster	12.2 b	12.0 b	15.3 a	15.7 a	0.05: 3.11
Number of berries	7.73 b	7.76 b	9.67 a	10.51 a	0.01: 1.40
Leaf area (cm^2)	30.84	26.81	35.23	35.18	n.s.
TSS (%)	11.5	11.2	11.4	10.8	n.s.
Titratable acids (%)	1.37	1.32	1.40	1.35	n.s.

n.s., not significant. Means separation within line by Duncan's multiple range test.
[a] Least significant differences.

Table 2
The effects of treatments on PNE of leaves in raspberry

	Mean of 2004 and 2005				LSD[a]
	Control	OSU-142	M3	OSU-142 + M3	
N (%)	2.52 b	3.78 a	3.80 a	4.03 a	0.05: 0.86
P (%)	0.15 b	0.51 a	0.70 a	0.80 a	0.01: 0.34
K (%)	2.24	2.56	2.73	2.80	n.s.
Ca (%)	0.88 b	1.38 a	1.23 a	1.44 a	0.01: 0.33
Mg (%)	0.27	0.30	0.30	0.30	n.s.
Fe (ppm)	90 b	123 ab	158 a	148 a	0.01: 47.9
Cu (ppm)	16	18	31	25	n.s.
Mn (ppm)	47 c	50 bc	87 ab	102 a	0.01: 38.0
Na (ppm)	77	80	97	98	n.s.
Zn (ppm)	69	76	88	85	n.s.

n.s., not significant. Means separation within line by Duncan's multiple range test.
[a] Least significant differences.

found that bacterial treatments increased PNE contents of red raspberry leaves under organic growing conditions (Table 2). In particular, all bacterial inoculations promoted N, P and Ca uptake of red raspberry cv. Heritage. The highest N (4.03%), P (0.80%) and Ca (1.44%) contents were obtained from OSU-142 + M3 application, which increased N, P and Ca content of leaves by 60%, 433% and 64% compared with control treatment (Table 2). All bacterial treatments, except OSU-142, were also resulted in significant Fe and Mn increase in leaves. Inoculation with M3 and OSU-142 + M3 increased Fe and Mn content of leaves by 75.6% and 64.4% and 85.1% and 117.0%, respectively (Table 2). This increase may also explained by organic acids production by plants and bacteria in the rhizosphere, which decrease soil pH and stimulate the availability of P, Ca, Fe and Mn. These findings in the present study were supported by a number of previous studies

Table 3
The effects of treatments on soil nutrient elements content of orchard

	Prior to planted	Mean of 2004 and 2005				LSD[a]
		Control	OSU-142	M3	OSU-142 + M3	
Total N (%)	0.24 a	0.19 b	0.23 a	0.18 b	0.18 b	0.05: 0.04
Available P (kg P_2O_5/da)	1.55 d	1.48 d	2.83 c	5.36 a	4.71 b	0.01: 0.31
K (cmol/kg)	1.6 a	1.47 ab	1.37 b	1.39 b	1.37 b	0.01: 0.14
Ca (cmol/kg)	8.6 ab	8.1 bc	8.7 a	7.8 c	8.2 abc	0.05: 0.51
Mg (cmol/kg)	4.05 a	3.68 b	3.77 b	3.63 b	3.52 c	0.01: 0.13
Fe (ppm)	0.25 b	0.19 b	0.21 b	0.31 a	0.32 a	0.01: 0.06
Mn (ppm)	0.20 b	0.16 b	0.17 b	0.19 b	0.25 a	0.01: 0.04
Cu (ppm)	0.14	0.12	0.12	0.16	0.14	n.s.
Zn (ppm)	0.12 b	0.11 b	0.12 b	0.16 a	0.13 ab	0.01: 0.03
pH	6.7 a	6.4 b	6.0 c	5.6 d	5.7 d	0.01: 0.27

n.s., not significant. Means separation within line by Duncan's multiple range test.
[a] Least significant differences.

(Jakobsen, 1986; Smith and Read, 1997; Sundra et al., 2002; Shen et al., 2004). Although bacterial treatments increased K, Mg, Cu, Na and Zn content of leaves, no significant differences occurred among treatments and the control (Table 2).

3.3. Variation of soil nutrient elements

Bacterial applications significantly changed soil nutrient element contents (Table 3). At the beginning of trials, soil total N was determined as 0.24%. However, after PGPR applications total N contents were determined as 0.23%, 0.18%, 0.18% and 0.19% in soils inoculated with OSU-142, M3 and OSU-142 + M3 treatments and control, respectively. Available P in soil significantly affected by bacterial applications compared to the control. Available P contents were calculated as 2.83, 5.36 and 4.71 kg P_2O_5/da in OSU-142, M3 and OSU-142 + M3 applications while it was 1.48 kg P_2O_5/da in the control. On the other hand, available P content in the soil was 1.55 kg P_2O_5/da at the beginning of treatment. Phosphorus and nitrogen are known to be the most important elements and essential nutrients for plant growth and development in crop production. Despite its wide distribution in nature, it is a deficient nutrient in most soils. Many soils are defined as having high P-fixation capacity, since a substantial amount of any applied P fertilizer is render unavailable and frequent applications of soluble forms of inorganic P are need to maintain adequate P levels for plant growth. It is well known 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, chelation and exchange reactions (Banik and Dey, 1981; Bhattacharya et al., 1986). In the present study, soil pH significantly decreased by bacterial treatments. Soil pH reduced from 6.7 to 6.0 by OSU-142, to 5.6 by M3 and to 5.7 by OSU-142 + M3 applications. Decrease in pH may be explained by production of organic acids as reported by a number of researchers in the previous studies (Gaind and Gaur, 1989; Turan et al., 2006). In addition, applications of PGPR strains had significant effects on K, Ca, Mg, Fe, Mn, and Zn contents of the soil in 2004. Increasing availability of mineral contents in soils due to bacterial applications can be attributed decreased pH of soil rhizosphere solution and increased mineralization of organic complex. Negative effects of OSU-142 application on the growth of raspberry cv. Heritage may be related by high amount of auxin production or some other unknown secondary metabolites (OSU-142 produce IAA, unpublished data), which may cause inhibitory effect on the growth of some plant species (Davies, 2004; Zahir et al., 2004). However, application of OSU-142 + M3 did not show any inhibitory effect on raspberry cv. Heritage tested. This result may be explained by balanced auxin and cytokinin production since M3 is able produce Zeatin (unpublished data).

In conclusion, M3 and OSU-142 + M3 may have potential to be used in development of biofertilizer need organic agricultural production because of render these insoluble phosphates into soluble form and increasing organic matter mineralization through the process of acidification, chelation and exchange reactions in plant growth media.

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