EFFECTS OF SOME PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) STRAINS ON PLANT GROWTH AND LEAF NUTRIENT CONTENT OF APPLE

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(Received August 20, 2009/Accepted April 6, 2010)

ABSTRACT

This investigation was conducted to evaluate the effects of four strains of plant growth promoting rhizobacteria (*Agrobacterium rubi* A-18, *Bacillus subtilis* OSU-142, *Burkholderia gladioli* OSU-7 and *Pseudomonas putida* BA-8) on growth and leaf nutrient content of ‘Starking Delicious’, ‘Granny Smith’, ‘Starkrimson Delicious’, ‘Starkspur Golden Delicious’ and ‘Golden Delicious’ apple cultivars grafted on semi-dwarf rootstock MM-106. The applications of bacterial strains increased the leaf number and area as well as number of annual shoots and their diameter, although OSU-7 application suppressed annual shoot length. BA-8 application resulted in the highest annual shoot number (52.4) and OSU-142 in the largest leaf area (16.12 cm^2). The applications of A-18 bacteria decreased the concentration of N, K and Cu and increased the concentration of P and Zn in the leaves. OSU-142 application resulted in the highest Mg (0.13%) and Fe (32.7 ppm) contents and OSU-7 in the highest Mn content (40.3 ppm). None of the applications affected the concentration of Na and Ca in the leaves.

Key words: apple, PGPR, plant growth, leaf nutrient content, Erzurum

INTRODUCTION

Apple is one of the most important fruit crops grown and consumed in the world. Apple culture covers almost the whole region of the temperate climate in the Northern and Southern Hemispheres (Way et al., 1990).

Intensive agriculture relies on the use of chemical fertilizers to provide high fruit quality and yield. On the other hand, excessive use of chemical fertilizers causes problems not only in terms of financial cost but also in terms of the cost to the environment. The interest in sustainable agriculture recently has increased. The development and application of sustainable agricultural techniques and biofertilization are vital to alleviating environmental pollution (Rodriguez and Fraga, 1999; Esitken et al., 2003; Vessey, 2003).

Many bacterial species, mostly associated with 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)”. These bacterial species include the strains in the genera *Serratia, Pseudomonas, Burkholderia, Agrobacterium, Erwinia, Xanthomonas, Azospirillum, Bacillus, Enterobacter, Rhizobium, Alcanigenes, Arthrobacter, Acetobacter*, *Acinetobacter, Achromobacter, Aerobacter, Artrobacter, Azotobacter, Clostridium, Klebsiellla, Micrococcus, Rhodobacter, Rhodospirrilum* and *Flavobacterium* (Rodriguez and Fraga, 1999; Bloemberg and Lugtenberg, 2001; Esitken et al., 2003).

The mechanisms of PGPR action are not fully understood but are thought to include: a) the ability to produce plant hormones, such as auxins (Jeon et al., 2003; Egamberdiyeva, 2005), cytokinins (García de Salamone et al., 2001), and gibberellins (Gutiérrez-Mañero et al., 2001); b) asymbiotic N_2 fixation (Sahin et al., 2004; Canbolat et al., 2006); c) solubilization of inorganic phosphate and mineralization of organic phosphate or other nutrients (Jeon et al., 2003) and d) antagonism against phytopathogenic microorganisms by production of siderophores, the synthesis of antibiotics, enzymes or fungicidal compounds and competition with detrimental microorganisms (Döbbelaere et al., 2002; Dey et al., 2004; Lucy et al., 2004). It was reported by different researchers that due to N fixing or P solubilization bacterial applications increased growth rate and yield of apricot (Esitken et al., 2003), peanut (Dey et al., 2004), and apple (Aslantas et al., 2007) in long-term conditions.

Plant-growth promoting bacteria are important in managing plant growth because of their effects on soil conditions, nutrient availability and tree growth. However, there has been no available comprehensive investigation done about the effects of PGPR on nutrient uptake and vegetative growth of apple. Preliminary experiments performed by us showed that some of these bacteria increased vegetative growth and also had effects on leaf nutrient contents. Hence, we carried out research to determine the effects of inoculations with selected PGPR on plant growth and content of nutrient elements in the leaves of five economically and commercially important apple cultivars grown in orchards in Erzurum region in Turkey.

MATERIAL AND METHODS

Bacterial strains

Agrobacterium rubi (A-18), *Bacillus subtilis* (OSU-142), *Burkholderia gladioli* (OSU-7) and *Pseudomonas putida* (BA-8) were used to investigate effects on plant growth and nutrient element content of five apple cultivars. Strains of bacteria were obtained from Dr. Fikrettin Sahin from Yeditepe University, Department of Genetics and Bioengineering, Istanbul, Turkey. These bacteria were reported as plant growth promoting and potential biocontrol agents against a wide range of bacterial and fungal pathogens causing economically important plant disorders (Esitken et al., 2006). 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, 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, Germany) (Aslantas et al., 2007). The bacterial suspension was diluted in sterile distilled water to a final concentration of 10^9 cfu ml^{-1}, and the resulting suspensions were applied to the apple trees.

Orchard experiment

This study was carried out at the Research and Application Orchard of the Department of Horticulture of the Agriculture Faculty at Atatürk University in 2004 and 2005 in Erzurum, Turkey. 11-year-old apple trees cvs 'Starking Delicious' (SD), 'Granny Smith' (GS), 'Starkrimson Delicious '(SCD), 'Starkspur Golden Delicious' (SSGD) and 'Golden Delicious' (GD) grafted on MM-106 were used in the experiment. A total of 25 apple trees in a completely randomized design were used. The bacterial suspensions were sprayed on the main branches randomly selected from 4 different directions of each tree and these were evaluated as replication. The first application was done at first bloom and the second application was sprayed at full bloom. A water-spray treatment was designated as the control (Esitken et al., 2003). All the sprays were applied to runoff.

Plant growth parameters

Annual shoot length (cm) and shoot thickness (mm) were measured and shoot number (unit) was counted. Leaf area was measured with a CI 202 portable digital area-meter (Pirlak et al., 2003). All measurements were carried out at the end of the vegetative growth period (Aslantas et al., 2007).

Determination of leaf nutrient content

Fully developed mid-shoot leaves were sampled at the end of June in both years to determine the content of minerals. The samples were oven-dried at 68 °C for 48 h and then ground. Amicro-Kjeldahl procedure was applied for determination of nitrogen (N). For other analyses, dried and ground sub-samples were digested in H_2SO_4-Se-salicylic acid mixture. Phosphorus (P) was measured spectrophotometrically by the indophenol-blue method and after reaction with ascorbic acid. Potassium K, natrium (Na) and calcium (Ca) contents were determined by flame photometry; magnesium (Mg), iron (Fe), cupper (Cu), manganese (Mn) and zinc (Zn) contents were determined by atomic absorption spectrometry using the method of AOAC (1990).

Data analysis

All data were processed by SPSS software and the means were separated by Duncan's multiple range tests (Duzgunes et al., 1993). Since there were no significant differences between results obtained in both years, the data obtained were analyzed and evaluated together.

RESULTS AND DISCUSSION

Growth promoting effect of bacterial application

'Starkspur Golden Delicious' cultivars had the highest mean annual shoot length (56.07 cm). The bacteria applications increased shoot length relative to the control. The BA-8 application had the highest effect (39.33 cm).

The application of OSU-7 bacteria promoted increase of annual shoot diameter compared to the control. The difference among apple cultivars was significant ($p < 0.001$) and 'Granny Smith' cultivar had the highest shoot diameter (5.81 mm).

Bacteria applications significantly affected number of annual shoots. The differences among cultivars were significant. 'Starkspur Golden Delicious' cultivar had the highest shoot number (84.4) when compared to the control (44.6) and the BA-8 application gave the highest shoot number (52.4 units).

The differences among bacterial applications and cultivars were statistically significant in terms of leaf area. The 'Golden Delicious' cultivar had the highest leaf area (17.89 cm^2) and the OSU-142 application gave the highest leaf area (16.85 cm^2) compared to the control application (14.58 cm^2) (Tab. 1).

Two years of trial under orchard conditions showed that bacterial inoculation and cultivar, significantly affected the shoot number and leaf area (Tab. 1). Similar results were reported with bacterial applications in rabbiteye blueberry (De Silva et al., 2000) in mulberry (Sudhakar et al., 2000), and in apple (Aslantas et al., 2007).

We can point out that all the applications increased vegetative growth by promoting the production of, for example, auxins (Jeon et al., 2003; Egamberdiyeva, 2005), cytokinins (García de Salamone et al., 2001) and gibberellins (Gutiérrez-Mañero et al., 2001). OSU-7 and BA-8 can show positive effects in plant growth as the increase in cell growth, by affecting significantly cytokinin synthesis (Aslantas et al., 2007). Many studies have been performed to determine the effects of bacterial applications on vegetative growth in apricot (Esitken et al., 2003); in wheat (Khalid et al., 2004); in sweet cherry (Esitken et al., 2006); in sugar beet (Cakmakci et al., 2006); and in apple (Aslantas et al., 2007). In these studies it was documented that bacteria applications increased annual shoot length. The plant growth enhancement effects of bacteria used in this study on apple, could be explained with N_2-fixing, indol-3-acetic acid (IAA) producing capacity

Table 1. The effect of PGPR on vegetative growth of apple cultivars

Cultivar (C)	Shoot length [cm]	Shoot diameter [mm]	Shoot number (unit)	Leaf area [cm²]
SD	41.53 bc	5.09 b	48.0 b	15.64 b
GS	31.67 c	5.81a	39.4 c	15.73 b
SCD	37.8 b	5.35 ab	32.2 e	14.11 bc
SSGD	56.07 a	5.51 ab	84.4 a	13.69 c
GD	18.67 d	3.95 c	32.6 d	17.89 a
Treatment (T)				
Control	34.27	5.09	44.6 d	14.58 bc
A-18	37.93	4.93	48.8 b	16.12 ab
OSU-142	37.03	5.19	48.0 c	16.58 a
OSU-7	37.17	5.25	42.8 e	13.71 c
BA-8	39.33	5.24	52.4 a	16.08 ab
Source df				
C 4	***	***	***	***
T 4	NS	NS	***	**
CXT 16	NS	*	***	***
Error 50				

Means followed with the same letter within each column are not significantly different, by Duncan's New Multiple Range Test
NS – not significant, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$

and also antimicrobial effects (Sahin et al., 2000; Abbasi et al., 2001; Cakmakci et al., 2001; Esitken et al., 2003).

Effects of bacteria on leaf nutrient content

The bacterial applications in this in study only slightly affected leaf nutrient contents. The effects of bacterial applications significantly affected concentrations of N, P, Mg, Fe, Mn and Zn but not K, Ca, Na and Cu contents compared to the control. All bacterial applications decreased leaf N contents except the BA-8 application, and 'Starkrimson Delicious' had the highest N content (2.12%). The A-18 among all applications had the highest effect and 'Golden Delicious' among all cultivars had the highest P content. All applications had significant and decreasing effect on K content compared to the control and 'Starkspur Golden Delicious' had the highest K content (3.18%). The OSU-142 caused the highest Mg (0.12%) and Fe (32.7 ppm) accumulation in leaves. The A-18 application had the biggest effect on Zn accumulation and 'Starkspur Golden Delicious' had the highest Zn content (17.2 ppm) (Tab. 2).

Many studies have been carried out to determine the effects of PGPR on plant nutrient contents. The bacteria applications affected P, Zn and Cu contents in rabbiteye blueberry

Table 2. The effect of PGPR and cultivars on some nutrient compositions of apple leaves

Cultivar (C)		N [%]	P [%]	K [%]	Mg [%]	Ca [%]	Na [%]	Fe [ppm]	Mn [ppm]	Zn [ppm]	Cu [ppm]
SD		1.91b	0.17d	1.94d	0.13c	0.27b	0.059	17.70c	25.53d	12.2c	5.6ab
GS		1.57d	0.16d	1.85d	0.12d	0.26c	0.059	25.20b	26.27d	14.9b	3.2c
SCD		2.12a	0.23b	2.11c	0.13b	0.28a	0.060	28.20b	45.51b	14.0b	6.0ab
SSGD		1.89b	0.21c	3.18a	0.14a	0.27b	0.061	25.20b	54.76a	17.2a	5.0b
GD		1.75c	0.39a	2.88b	0.11e	0.26c	0.058	45.60a	38.85c	5.9d	6.8a
Treatment (T)											
Control		1.94a	0.21c	2.45	0.12b	0.27	0.059	27.9b	38.5	12.7b	5.8
A-18		1.79b	0.30a	2.41	0.12b	0.27	0.059	31.2ab	34.8	17.2a	5.2
OSU-142		1.83ab	0.21c	2.29	0.13a	0.27	0.058	32.7a	39.2	11.4bc	5.2
OSU-7		1.74b	0.21c	2.39	0.12b	0.27	0.060	22.5c	40.3	10.7c	5.4
BA-8		1.94a	0.23b	2.40	0.12b	0.27	0.059	27.6b	38.1	12.2bc	5.0
Source	df										
C	4	***	***	***	***	***	NS	***	***	***	***
T	4	*	***	NS	*	NS	NS	***	NS	***	NS
CXT	16	NS	***	***	***	***	NS	***	***	***	**
Error	25										

Means followed with the same letter within each column are not significantly different, by Duncan's New Multiple Range Test
NS – not significant, *P < 0.05, **P < 0.01, ***P < 0.001

(De Silva et al., 2000); N, P, K, Ca and Mg contents in apricot (Esitken et al., 2003); N, P and K contents in winter wheat (Egamberdiyeva and Höflich, 2003); N, P and K contents in corn (Wu et al., 2005); and N, P, and K contents in apple (Shirkot and Sharma, 2006). PGPR strains significantly affected plants growth and development by biological nitrogen fixation, by increasing inorganic phosphate solubilization of organic phosphorus compounds, by affecting Fe adsorption with siderophore production and by affecting the uptake absorption and translocation of miconutrients (Esitken et al., 2003; Cakmakci et al., 2006; Aslantas et al., 2007). It should be stated that plant species may show different reactions against these bacteria. In addition, we can add that regional differences play a part in the effectiveness of these bacteria.

In conclusion, this study showed that all the tested bacterial strains (OSU-7, BA-8, OSU-142, and A-18) have an important potential. In this experiment, this was the potential to increase especially plant growth because PGPR strains are able to synthesize plant growth regulators such as auxins (Jeon et al., 2003; Egamberdiyeva, 2005), cytokinins (García de Salamone et al., 2001), and gibberellins(Gutiérrez-Mañero et al., 2001). We may conclude that apple cultivars can show different reactions to treatments with bacteria and the regional differences may also affect effectiveness of these bacteria. The inoculation with PGPR contributed to the increase in vegetative growth and nutrient content of apple when compared to the control, but it strongly depended on the cultivars and bacterial strain tested. Plant-growth responses were variable and depended on the inoculant strain, apple cultivar and growth parameters evaluated. The effect of PGPR, as a complex process, depends on bacterial strains and population, plant-bacterial strain combination, plant genotype, growth parameters evaluated, and environmental conditions (Cakmakci et al., 2006, 2007; Sahin et al., 2004). The bacteria applications showed the desirable effects on plant growth and plant nutrient element contents in the present study performed to investigate the effects of 4 different bacterial strains on 5 apple cultivars in the local district. These applications are safe, effective and easily adopted by growers. Therefore, they may be considered as biofertilizer for fruit, vegetable and ornamental plant production in sustainable and ecological agricultural systems.

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WPŁYW WYBRANYCH SZCZEPÓW RYZOBAKTERII STYMULUJĄCYCH WZROST ROŚLIN NA WZROST JABŁONI I ZAWARTOŚĆ SUBSTANCJI ODŻYWCZYCH W ICH LIŚCIACH

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STRESZCZENIE

Badania miały na celu ocenę wpływu czterech szczepów ryzobakterii stymulujących wzrost roślin (*Agrobacterium rubi* A-18, *Bacillus subtilis* OSU-142, *Burkholderia gladioli* OSU-7 i *Pseudomonas putida* BA-8) na wzrost jabłoni odmian 'Starking Delicious', 'Granny Smith', 'Starkrimson Delicious', 'Starkspur Golden Delicious' i 'Golden Delicious' szczepionych na podkładce półkarłowej MM-106 i na zawartość substancji ożywczych w ich liściach. Traktowanie bakteriami spowodowało zwiększenie liczby liści na drzewach oraz grubości i powierzchni ich blaszek, a także liczby jednorocznych pędów, aczkolwiek szczep OSU-7 spowodował zmniejszenie ich długości. Drzewa traktowane szczepem BA-8 miały największą liczbę jednorocznych pędów (52,4), a traktowane szczepem OSU-142 największą powierzchnię liści (16,12 cm^2). Traktowanie szczepem A-18 spowodowało spadek zawartości N, K i Cu i wzrost zawartości P i Zn w liściach. Liście drzew traktowanych szczepem OSU-142 zawierały największą ilość Mg (0,13%) i Fe (32,7 ppm), a te z drzew traktowanych szczepem OSU-7 największą zawartość Mn (40,3 ppm). Żaden szczep bakterii nie miał wpływu na zawartość Na i Ca w liściach.

Słowa kluczowe: jabłoń, PGPR, wzrost roślin, zawartość substancji odżywczych w liściach, Erzurum