TÜBİTAK

Turk J Biol
35 (2011) 283-291

doi:10.3906/biy-0908-35

Effects of plant growth promoting rhizobacteria on fruit set, pomological and chemical characteristics, color values, and vegetative growth of sour cherry (*Prunus cerasus* cv. Kütahya)

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Received: 21.08.2009

Abstract: This study was performed to determine effects of 4 plant growth promoting rhizobacteria (*Bacillus subtilis* OSU - 142, *Bacillus megaterium* M - 3, *Burkholderia cepacia* OSU – 7, and *Pseudomonas putida* BA - 8) alone and in combinations on fruit set of sour cherry trees (*Prunus cerasus* L., cv. Kütahya), and to investigate their resulting pomological and chemical characteristics as well as vegetative growth. All the tested bacterial strains alone or some of their combinations have a great potential to increase especially fruit set and plant vegetative growth, and indirectly affect fruit pomological and chemical characteristics. Therefore, they may be considered as biofertilizer for fruit, vegetable, and ornamental plant production in sustainable and ecological agricultural systems.

Key words: *Bacillus*, fruit set, PGPR, plant growth, *Pseudomonas*, sour cherry

Bitki büyümesini teşvik eden bakterilerin vişnenin (*Prunus cerasus* cv. Kütahya) vejetatif gelişimi, renk değerleri, meyve tutumu, pomolojik ve kimyasal özellikleri üzerine etkileri

Özet: Bu çalışma bitki büyümesini teşvik eden 4 bakterinin (*Bacillus subtilis* OSU - 142, *Bacillus megaterium* M - 3, *Burkholderia cepacia* OSU - 7 ve *Pseudomonas putida* BA - 8) tek başlarına veya kombinasyonlar halinde vişnenin (*Prunus cerasus* cv. Kütahya) vejetatif gelişimine olduğu kadar pomolojik ve kimyasal özellikleri üzerine de etkilerini değerlendirmek için yürütülmüştür. Kullanılan bütün bakteri suşlarının tek başlarına ya da kombinasyonlarının özellikle meyve tutumu ve vejetatif gelişme üzerine doğrudan; meyvelerin pomolojik ve kimyasal özellikleri üzerine dolaylı olarak olumlu yönde bir etkisinin olduğu görülmüştür. Bu yüzden bu bakteri suşlarının sürdürülebilir ya da ekolojik tarım sistemlerinde meyve, sebze ve süs bitkileri yetiştiriciliğinde biyogübre olarak kullanılabileceği düşünülmektedir.

Anahtar sözcükler: *Bacillus*, meyve tutumu, PGPR, bitki gelişimi, *Pseudomonas*, vişne

Introduction

Sour cherry (*Prunus cerasus* L.) is one of the most important fruit crops grown in Turkey, with approximately 170 tons of fruit produced annually in Anatolia alone (1).

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 environmentally friendly, sustainable, and organic agricultural practices (2). Uses of bio-fertilizers containing beneficial microorganisms instead of synthetic chemicals are known to improve plant growth through the supply of plant nutrients. This may help to sustain environmental health and soil productivity (3-5).

Bacterial species called plant growth promoting rhizobacteria (PGPR) are found in several genera including *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillium*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Rhizobium,* and *Serratia* (6-13). The mechanisms of PGPR are not fully understood, but are thought to include: (a) the ability to produce plant hormones, such as auxins (14), cytokinins (15), and gibberellins (16); (b) asymbiotic N_2 fixation (17); (c) solubilization of inorganic phosphate and mineralization of organic phosphate and/or other nutrients (14); and (d) antagonism against phytopathogenic microorganisms by production of siderophores, the synthesis of antibiotics, enzymes and/or fungicidal compounds, and competition with detrimental microorganisms (18-20).

Numerous studies have shown an improvement in plant growth and development in response to seed or root inoculation with microbes capable of producing plant growth regulators (21). However, less has been shown on the promoting effects on yield and plant growth when PGPR capable of producing plant growth regulators in fruit trees are introduced by floral or foliar inoculation. *Bacillus subtilis* OSU-142, *Pseudomonas putida* BA-8, and *Burkholderia cepacia* were previously selected as biological control agents for the management of various plant diseases (22-25). More recent studies showed that *Bacillus megaterium* M-3 and *B. subtilis* OSU-142 were able to fix N_2 asymbiotically and promote plant growth and yield in barley, sugar beet, tomato, pepper, and apricot (9,22,26-29). In addition, *P. putida* BA-8 produces transzeatin (9) and *B. subtilis* OSU-142 produces IAA (30).

The objectives of this research were to determine effects of 4 bacterial treatments (*Bacillus subtilis* OSU-14, *Bacillus megaterium* M-3, *Burkholderia cepacia* OSU-7, and *Pseudomonas putida* BA-8) alone and in combinations on fruit set of sour cherry trees (*Prunus cerasus* L., cv. Kütahya) in Turkey, and to investigate their resulting pomological and chemical characteristics as well as vegetative growth.

Materials and methods

Bacterial strains

Bacterial strains (*Bacillus subtilis* OSU-142, *Bacillus megaterium* M-3, *Burkholderia cepacia* OSU-7, and *Pseudomonas putida* BA-8) used in this study were obtained from Dr. Fikrettin Şahin (Yeditepe University, Department of Genetics and Bioengineering, İstanbul, Turkey). Bacterial cultures 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.

The application procedure to plants

For this experiment, the bacterial strains were grown on NA. A single colony was transferred to a 500 mL flask containing NB, and grown aerobically on a rotating shaker (150 rpm) for 48 h at 27 °C (Merck KGaA, Germany) (30). The bacterial suspension was diluted in sterile distilled water to a final concentration of 10^9 cfu/mL and applied to the sour cherry trees.

Plant materials used

An important 12-year-old sour cherry cultivar (*Prunus cerasus* L., cv. Kütahya) was grafted on regional Mahaleb rootstock (*Prunus mahaleb* L.). This particular cultivar has been widely used in the past due to its suitability to the ecological conditions of the district and its high commercial potential.

Orchard experiments

This study was conducted at the Research and Application Orchard of the Department of Horticulture of Agriculture Faculty at Atatürk University in Erzurum, Turkey, in 2007. The 4

bacterial strains (OSU-142, M-3, OSU-7, and BA-8) alone and in combination with 2 (OSU-142 + M-3 and OSU-142 + OSU-7), 3 (OSU-142 + OSU-7 + BA-8, and OSU-142 + BA-8 + M-3), and 4 strains (OSU-7 + BA-8 + M-3 + OSU-142) and were applied to the sour cherry trees. This experiment was organized in a completely randomized design. The control treatment was a water spray (9). Main branches that were randomly selected from 4 different directions of each tree were spray inoculated and these data were evaluated as replicates.

Observations of fruit set

The flowers on main branches in swelling/blooming periods from 4 different directions of trees were determined by counting and labeled. Fruit set rates were determined by after final drop. The values obtained by counting were calculated by flower number and obtained percent values.

Fruit pomological traits and color values

Ten fruits were randomly harvested from each branch to identify pomological traits. The fruit and fruit stone weight were obtained by weighing on a scale with 0.01 g sensitivity. Fruit volume was determined by displacement while diameter, length, width, stalk length, and thickness were found by a digital compass in each fruit (31-33). Fruit external and internal brightness, redness, and yellowness color values were determined by a Minolta colorimeter (33).

Fruit chemical analyses

Some chemical analyses were performed on the same fruit on which pomological analyses were carried out. A digital refractometer was utilized to find the amount of total soluble solids (TSS). Ascorbic acid, malic acid, glucose, and fructose were quantified using a RQflex plus 10 refractometer. Acidity was determined by 0.1 N NaOH titration and pH in fruit juice by a pH meter (31).

Plant growth parameters

All measurements of plant growth were carried out at the end of the vegetative growth period (30). The measurements taken included average shoot length (cm), shoot thickness (mm), and leaf area (cm^2). Leaf area was measured with a CI 202 portable digital area-meter (31).

Data analysis

All data in the present study were processed by SPSS and the means were separated by Duncan's multiple range tests. The statistical analyses of percentage values in relation to fruit set were performed using transformed values.

Results

The results of fruit set rate and pomological properties on sour cherry are provided in Table 1. There were some significant differences ($P < 0.01$) among the treatments in terms of fruit set rate. All bacterial applications increased the fruit set rate, but only 2 different combinations (OSU-142 + M-3 and OSU-7 + BA-8 + M-3 + OSU-142) significantly increased the rate compared to the control. The combined treatment of all 4 bacteria (OSU-7 + BA-8 + M-3 + OSU-142) had the highest fruit set rate (27.04%). For all pomological properties, except fruit stalk thickness, some effects of the bacteria treatments were statistically significant ($P < 0.01$). The single M-3 bacterial treatment had the highest fruit diameter (21.82 mm), fruit length (19.44 mm), fruit width (18.76 mm), fruit weight (5.60 g), and fruit volume (5.08 mL), but only the diameter, width, and weight were significantly greater than the control. OSU-142 + M-3 had the highest fruit stalk length (58.21 mm) and OSU-7 + BA-8 + M-3 + OSU-142 had the highest fruit stone weight (0.37 g). Furthermore, OSU-7 + BA-8 + M-3 bacteria application had the lowest fruit diameter (19.39 mm), fruit length (17.26 mm), fruit width (17.08 mm), fruit stalk length (50.50 mm), fruit weight (3.99 g), fruit volume (3.67 mL), and fruit stone weight (0.26 g).

The results of the external and internal brightness, redness, and yellowness color values on sour cherry are provided in Table 2. According to these results, OSU-7 had a statistically increasing effect on the external redness (5.97) color values ($P < 0.05$). OSU-7 and OSU-7 + BA-8 + M-3 combination increased the internal brightness color values, but this increase was not statistically significant.

Effects of the bacterial treatments on fruit chemical composition are displayed in Table 3. There were significant differences ($P < 0.01$) between treatments for all but malic acid ($P \leq 0.05$). OSU-7

Table 1. Effects of PGPR strains on sour cherry (cv. Kütahya) in terms of fruit set rate and pomological properties.

Treatments	Fruit set rate (%)	Fruit diameter (mm)	Fruit length (mm)	Fruit width (mm)	Fruit stalk length (mm)	Fruit stalk thickness (mm)	Fruit weight (g)	Fruit volume (mL)	Fruit stone weight (g)
Control	12.93 c	20.55 bc	18.60 ab	17.76 bc	56.05 ab	0.93	4.81 cd	4.67 ab	0.29 bc
BA-8	18.02 bc	20.96 abc	18.72 ab	18.53 ab	55.41 ab	0.97	5.05 abc	4.83 ab	0.30 bc
OSU-7	15.22 bc	20.42 bc	18.62 ab	17.87 bc	55.10 ab	0.98	4.79 cd	4.08 bcd	0.33 ab
M-3	15.60 bc	21.82 a	19.44 a	18.76 a	57.16 ab	0.97	5.60 a	5.08 a	0.31 b
OSU-142	16.23 bc	20.59 bc	18.60 ab	18.61 ab	56.52 ab	0.96	5.38 ab	4.83 ab	0.33 a
OSU-142+OSU-7+BA-8	16.70 bc	20.03 cd	17.68 bc	17.10 c	45.08 d	0.96	4.36 de	3.83 cd	0.33 ab
OSU-142+M-3	21.12 ab	20.81 bc	19.09 a	18.32 ab	58.21 a	1.00	5.16 abc	4.92 ab	0.36 a
OSU-7+BA-8+M-3	15.62 bc	19.39 d	17.26 c	17.08 c	50.50 c	0.91	3.99 e	3.67 d	0.26 c
OSU-7+BA-8+M-3+OSU-142	27.04 a	20.45 bc	18.89 ab	18.15 ab	57.11 ab	0.98	4.98 bc	4.58 abc	0.37 a
OSU-142+OSU-7	14.52 bc	21.09 ab	18.54 ab	18.05 ab	54.39 b	0.93	5.23 abc	4.95 a	0.36 a
Significant level	**	**	*	**	**	NS	**	**	**

NS: non-significant ($P < 0.05$ or $P < 0.01$); *: significant ($P < 0.05$); **: significant ($P < 0.01$); The statistical analysis of percentage values was performed using transformed values.

Table 2. Effects of PGPR strains on sour cherry (cv. Kütahya) in terms of fruit L (brightness level), a (redness level), and b (yellowness level) values.

Treatments	External			Internal		
	Brightness level	Redness level	Yellowness level	Brightness level	Redness level	Yellowness level
Control	24.71	16.03	3.77 b-d	20.78 a-c	21.78	7.68
BA-8	24.82	14.32	3.69 b-d	19.84 bc	27.33	11.03
OSU-7	25.72	16.55	5.97 a	26.09 a	30.12	14.32
M-3	26.07	12.44	2.85 d	17.87 c	26.10	9.69
OSU-142	25.43	16.45	4.31 a-d	23.32 a-c	30.02	13.60
OSU-142+OSU-7+BA-8	26.90	18.77	5.09 a-c	24.60 ab	29.46	14.12
OSU-142+M-3	24.18	13.95	3.01 cd	23.19 a-c	32.32	16.40
OSU-7+BA-8+M-3	27.42	15.16	4.37 a-d	26.44 a	27.39	12.98
OSU-7+BA-8+M-3+OSU-142	24.71	17.96	5.19 ab	25.43 ab	30.36	14.91
OSU-142+OSU-7	24.02	15.13	3.58 b-d	21.01 a-c	27.30	11.09
Significant level	NS	NS	*	*	NS	NS

NS: non-significant ($P < 0.05$ or $P < 0.01$); *: significant ($P < 0.05$)

Table 3. Effects of PGPR strains on sour cherry (cv. Kütahya) in terms of fruit chemical composition.

Treatments	Ascorbic acid (%)	Titrable acidity (%)	Malic acid (%)	pH	Glucose (%)	Fructose (%)	Total soluble solids (%)
Control	0.22 a	0.65 e	0.40	3.75 ab	4.75 f	7.25 f	16.00 d
BA-8	0.19 bc	0.65 e	0.35	3.75 ab	6.55 b	7.05 g	13.05 h
OSU-7	0.20 b	0.96 a	0.54	3.55 cd	4.75 f	7.95 cd	15.35 f
M-3	0.22 a	0.71 d	0.33	3.85 a	5.35 d	7.85 d	16.85 c
OSU-142	0.18 c	0.71 d	0.41	3.55 cd	5.75 c	8.05 c	15.75 de
OSU-142+OSU-7+BA-8	0.20 b	0.89 b	0.37	3.65 bc	3.95 g	8.75 b	15.50 ef
OSU-142+M-3	0.18 c	0.65 e	0.31	3.85 a	5.15 e	7.95 cd	17.90 b
OSU-7+BA-8+M-3	0.19 bc	0.65 e	0.42	3.55 cd	6.95 a	9.05 a	18.85 a
OSU-7+BA-8+M-3+OSU-142	0.20 b	0.89 b	0.36	3.65 bc	5.25 de	6.15 h	14.55 g
OSU-142+OSU-7	0.19 bc	0.77 c	0.32	3.45 d	3.95 g	7.65 e	15.50 ef
Significant level	**	**	NS	**	**	**	**

NS: non-significant ($P < 0.05$ or $P < 0.01$); **: significant ($P < 0.01$)

had the highest titratable acidity (0.96%) content and malic acid (0.54%) content. M-3 had the highest ascorbic acid (0.22%) and pH (3.85) contents, although neither of these is significantly different from the control. OSU-7 + BA-8 + M-3 combination had the highest glucose (6.95%), fructose (9.05%), and total soluble solids (18.85%) contents.

Vegetative growth parameter results are provided in Table 4. There are some statistically significant differences ($P < 0.01$) amongst the bacterial treatments for shoot thickness and leaf area, but not for shoot length ($P > 0.05$). OSU-142 + OSU-7 combination had the highest shoot thickness (3.65 mm) and OSU-7 + BA-8 + M-3 + OSU-142 combination had the highest leaf area (11.90 cm^2).

Discussion

In previous studies, it was reported that application of OSU-142 and M-3 strains used in the present study may stimulate yield and quality parameters in some plants, such as sugar beet, barley (28), apricot (9,23), raspberry (33), and apple (30). In addition, they were found to be capable of producing IAA and cytokinin, have N_2-fixing capacity, and M3 has phosphate-solubilizing capacity (9,23,28,30,34). In this study, we found foliar applications of *B. subtilis* OSU-142, *B. megaterium* M-3, *B. cepacia* OSU-7, *P. putida* BA-8, and some of their combinations had significant effects on fruit set, pomological properties, color values, chemical compositions, and vegetative growth parameters of sour cherry. Some combined treatments had more effect on the evaluated parameters as compared to the control, but some were less effective or ineffective.

The positive effect of the 4 individual tested strains and some of their combinations on sour cherry can be explained by their N_2 fixation ability, P-solubilizing ability, IAA and cytokinin production, and antimicrobial substance production in plant shoots.

Application of the bacterial treatments during full bloom is thought to have a positive effect on fruit set. A direct effect of auxin on fruitlet abscission

Table 4. Effects of PGPR strains on sour cherry (cv. Kütahya) in terms of vegetative growth parameters.

Treatments	Shoot length (cm)	Shoot thickness (mm)	Leaf area (cm^2)
Control	15.40	2.81 d	8.24 d
BA-8	17.30	3.01 cd	10.39 a-c
OSU-7	18.97	3.05 cd	8.64 cd
M-3	22.60	3.47 ab	9.94 b-d
OSU-142	21.37	3.15 bc	11.80 a
OSU-142+OSU-7+BA-8	19.97	3.32 bc	8.63 cd
OSU-142+M-3	20.53	3.29 bc	10.66 ab
OSU-7+BA-8+M-3	19.00	3.15 bc	10.83 ab
OSU-7+BA-8+M-3+OSU-142	25.20	3.22 bc	11.90 a
OSU-142+OSU-7	19.33	3.65 a	10.47 ab
Sig. Level	NS	**	**

NS: non-significant ($P < 0.05$ or $P < 0.01$); **: significant ($P < 0.01$)

may potentially result in a delay of fruitlet abscission and/or an increase in set. This effect is only evident when ethylene synthesis is low or prevented (35). It is well known that PGPR strains that produce plant hormones, such as auxins and cytokinins, can stimulate plant cell elongation or cell division, and/or change bacterial 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (36), which prevents the production of the plant growth-inhibiting hormone, ethylene (37).

In addition, we report that foliar applications of the bacterial strains tested in this study also increased vegetative growth parameters, such as leaf area and shoot development of sour cherry. Thus, higher quality fruit may be obtained from sour cherry trees that were treated with a foliar application of these bacterial strains. The bacterial treatments also caused significant changes in fruit chemical composition. Fruit quality may be affected by the bacterial treatments indirectly because the phytohormones produced by bacteria have effects on fruit set, fruit pomological traits, and vegetative growth.

Significant correlations were found amongst several of the measured parameters. There were significant negative correlations ($r = -0.398^*$) between fruit weight and fruit fructose content, and negative correlations between fruit pomological properties and fruit brightness level, redness level, and yellowness color values. OSU-7 + BA-8 + M-3 bacteria treatment decreased all the pomological properties to the control in sour cherry. However, some treatments had the highest increasing effect on glucose, fructose, and total soluble solids contents. This can be explained by the fact that when fruit sugar content and color hues decrease, fruit enlargement increases (11). As brightness level, redness level, and yellowness level values increase, color hues become darker (33). There were significant positive correlations between plant vegetative growth and glucose, fructose, and total soluble solids contents. More vegetative growth can provide more photosynthesis activity and more synthesis of soluble matter, such as sugars, which affect fruit quality characteristics, such as size, composition, and color.

Table 5. The correlations among all the measured parameters on sour cherry.

	FS	FD	FL	FW	FSL	FST	FW	FV	FSW	SL	ST	LA	L_{ex}	a_{ex}	b_{ex}	L_{in}	a_{in}	b_{in}	Asc	pH	MA	TA	Glc	Frc	TSS
FS	1				0.4*	0.3*			0,3*	0.4*								0.3*	0.4*			0.5**			
FD		1	0.7**	0.8**	0.5**	0.3*	0.8**	0.6**					−0.3*	−0.4	−0.4**	−0.5*				0.3*	−0.5**				
FL			1	0.7**	0.6**	0.4**	0.8**	0.6**					−0.4*			−0.4**				0.4**	−0,3*			−0.4*	
FW				1	0.7**	0.4**	0.8*	0.7**						−0.4*		−0.4**								−0.424*	
FSL					1		0.6**	0.6**					−0.3*	−0.4*										−0.5**	
FST						1	0.3*																		
FW							1	0.7**	0.4*				−0.4**		−0.3*	−0.4					−0.4*			−0.3*	
FV								1					−0.4*	−0.4*	−0.4*	−04*					−0.4			−0.4*	
FSW									1	0.3*	0.4*										−0.4*	0.362*	−0.4**		
SL										1	0.3*						0.3*								
ST											1										−0.5**				
LA												1											0.4*		
L_{ex}													1		0.4*								0.4*		
a_{ex}														1	0.6**							0.3*			
b_{ex}															1						0.4*	0.5**			
L_{in}																1		0.5**				0.3*			
a_{in}																	1	0.7**							
b_{in}																		1							
Asc																			1	0.5**					
pH																				1					
MA																					1	0.3*		−0.5**	
TA																						1	0.6**		
Glc																							1		
Frc																								1	
TSS																									1

FS: Fruit set; FD: Fruit diameter; FL: Fruit length; FW: Fruit weight; FSL: Fruit stalk length; FST: Fruit stalk thickness; FW: Fruit weight; FV: Fruit volume; FSW: Fruit stone weight; SL: Shoot length; ST: Shoot thickness; LA: Leaf area; L_{ex}: L external: a_{ex}: a external; b_{ex}: b external; L_{in}: L internal; a_{in}: a internal; b_{in}: b internal; Asc: Ascorbic acid; MA: Malic acid; TA: Titrable acidity

In conclusion, this study suggests that *B. subtilis* OSU-142, *B. megaterium* M-3, *B. cepaciai* OSU-7, and *P. putida* BA-8 have great potential to increase fruit set, plant growth, and fruit quality. Because these strains are able to synthesize plant growth regulators, such as IAA and cytokinin, and can fix nitrogen and solubilized phosphorus, they may be effective means for biological control of bacterial and fungal plant diseases. These treatments are safe, effective, and easily adopted by growers. Therefore, they should be considered as bio-fertilizer for fruit, vegetable, and ornamental plant production in sustainable and ecological agricultural systems.

Acknowledgements

We wish to thank Alissa B. Kriss (The Ohio State University, Department of Plant Pathology, USA) for helping to review the manuscript.

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