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Full Length Research Paper

Effects of some plant growth promoting rhizobacteria treated twice on flower thining, fruit set and fruit properties on apple

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The objective of this study was to determine the effects of inoculation bacteria (*Agrobacterium rubi* A-18, *Bacillus subtilis* OSU-142, *Burkholderia gladioli* OSU-7 and *Pseudomonas putida* BA-8) treated twice on flower thinning, set and fruit properties of apple cultivars, Starking Delicious, Granny Smith, Starkrimson Delicious and Starkspur. Golden Delicious cultivars grafted on MM 106 were used as plant materials. The suspensions prepared with bacteria strains were applied to crown of trees by spraying at the periods of first blooming and full blooming. Fruit set rate showed significant differences among the cultivars. Starkspur Golden Delicious cv. had the highest fruit set with a rate of 12% while Granny Smith cv. had the lowest fruit set with a rate of 4%. Bacterial treatments decreased fruit set by 12% (OSU-142) and 33% (A-18 and BA- 8). The bacterial treatments had no significant effect on fruit size, width and height of fruit; but it decreased specific gravity of fruit, stalk thickness, stalk length and stalk hole deepness. The bacterial treatments generally reduced the rate of total soluble solid, total sugar, reduced sugar, ascorbic acid contents, titrable acidity and pH rate.

Key words: Apple, plant growth promoting rhizobacteria (PGPR), fruit set, fruit properties.

INTRODUCTION

Apple (*Malus domestica* Borkh.) is one of the most important fruit crops grown and consumed all around the world, and the apple culture has distributed almost across the whole region of the temperate climate in the Northern and Southern hemispheres (Way et al., 1990).

In recent time, some bacteria strains have been used to increase yield and quality in a lot of crops, in addition to this, fight bacterial, fungal and viral diseases and pests. Moreover, it was determined that some of these bacteria strains have flower thinning effect when used in excessive dose (Esitken et al., 2003; Vessey, 2003). There have been bacterial species called “plant growth promoting rhizobacteria”’ (PGPR) including the strains in the genera *Pseudomonas*, *Burkholderia*, *Agrobacterium*, *Erwinia*, *Azospirillum*, *Bacillus*, *Rhizobium* (Rodriguez and Fraga, 1999; Esitken et al., 2003; Sıddıqui, 2006; Niranjiyan et al., 2006; Dursun et al., 2008). The mechanisms of PGPR are not fully understood, but are thought to include: (a) The ability to produce plant hormones, such as auxins (Jeon et al., 2003), cytokinins (Garcia de Salamone et al., 2001), and gibberellins (Gutierrez-Manero et al., 2001) (b) Asymbiotic N_2 fixation (Sahin et al., 2004); (c) solubilization of inorganic phosphate and mineralization of organic phosphate and/or other nutrients (Jeon et al., 2003; Aslantas et al., 2007); and (d) antagonism against phytopathogenic microorganisms by production of siderophores, the synthesis of antibiotics, enzymes and/or fungicidal compounds and competition with detrimental microorganisms (Dey et al., 2004; Lucy et al., 2004). A pretesting experiment of this study indicated that while these bacteria had increasing effect on fruit set at single dose treatment, the bacteria treatments more than single dose showed thinning effect changes to different cultivars. Therefore, this study was

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Abbreviations: A-18, *Agrobacterium rubi*; **OSU-142,** *Bacillus subtilis*; **OSU-7,** *Burkholderia gladioli*; **BA-8,** *Pseudomonas putida*; **PGPR,** plant growth promoting rhizobacteria; **SD,** starking delicious; **GS,** granny smith; **SCD,** starkrimson delicious; **SSGD,** starkspur golden delicious; **TSS,** total soluble solids.

performed to determine the effects of bacteria inoculations on flower thinning, fruit set and fruit properties in economically and commercially important four apple cultivars grown in a local orchard. Also, this study is significant in terms of determining fruit set and fruit pomological traits on apple.

MATERIALS 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 fruit set and fruit properties of four apple cultivars in this study. For this experiment, the bacterial strains were grown on nutrient agar. 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 researh and treatment orchard of the Department of Horticulture, Faculty of Agriculture Ataturk University in 2004 and 2005. Starking Delicious (SD), Granny Smith (GS), Starkrimson Delicious (SCD) and Starkspur Golden Delicious (SSGD) of 11 years old apple trees grafted on MM-106 were used as plant materials. These cultivars are suitable to ecological conditions of the district and their commercial potentials are high. In this experiment, twenty apple trees were used in completely randomized design. The main branches selected/assesed from 4 different directions of each tree were treated by PGPR and each branch was considered as replication. The first treatment was sprayed at first blooming and the second at full blooming period to the apple trees until running off and drinking water was sprayed to control trees.

Observations concerning fruit set

The flowers on main branches in swelling/blooming periods from 4 different directions of applied trees were determined by counting and labeling. Fruit set rates were determined by counting after small fruit drop (I. drop) and after final drop; the values obtained by counting in 2 periods were calculated by flower number and obtained percent values (II. drop).

Determination of fruit pomological traits

Ten fruit were randomly harvested from each branch to determine pomological traits of fruit. The mean weight of these fruit were obtained by balance with 0.01 g sensitivity, fruit volume by calculating overflowing water, specific gravity of fruit as d = m/V, fruit width, height, stalk hole deepness, hole deepness of sepal circle, length and thickness of fruit stalk by digital compass, fruit flesh firmness by hand penetrometer and filled pip number by counting in each fruit (Pırlak et al., 2003).

Fruit chemical analyses

Some chemical analyses were performed on same fruit on which pomological analyses were done. Total soluble solids (TSS) by digital refractometer, ascorbic acid by titration method 2,6-dichlorofenolindolfenol dye solution[17], acidity by 0.1N NaOH titra-tion, pH in fruit juice by pH meter, total and reduced sugar contents by dinitrophenol method were determined (Pırlak et al., 2003).

Data analysis

The data of this study were evaluated using statistical package for the social sciences (SPSS) software program and means were separated by Duncan's multiple range tests. Variance analyses were performed after the data with percent (%) values were applied to angle transformation (Duzgunes et al., 1993). Since, there were no statistical differences between the years, the data of both years were evaluated together.

RESULTS

Fruit set rate and pomological properties

There were significant differences ($P < 0.05$) among the treatments in terms of fruit set. Also, the effects of the treatments and interaction in the periods of fruit set were statistically significant ($P < 0.05$) among the cultivars. SSGD cultivars had the highest-set rate both in I. (17%) and II. periods (12%) (Table 1).

It was determined that bacterial treatments had no significant effects on fruit weight, fruit diameter and fruit height at $P > 0.05$ significant level. The effect of bacteria treatment on fruit volume was also statistically non-significant. But the difference among cultivars ($P < 0.05$) and the interaction of C x T ($P < 0.001$) was statistically significant (Table 1).

The specific gravity of fruit decreased with all bacteria treatments as compared to the control and was found statistically significant ($P < 0.05$). The changing range of fruit specific gravity was obtained from 0.85 g (GS) to 0.94 g ml^{-1} (SSGD) (Table 1).

The bacterial treatment had very significant effect ($P < 0.001$) on stalk hole deepness of fruit in all cultivars. All treatments decreased stalk hole deepness compared to the control (7.93 mm) and OSU-142 treatment had the lowest value (6.23 mm). The difference among the cultivars was significant and SSGD had the highest (7.69 mm) and GS had the lowest (6.23 mm) values in terms of the parameter (Table 1).

The bacterial treatment significantly ($P < 0.05$) decreased filled pip number of fruit. OSU-7 treatment had the highest decreasing effect (24.6%) as compared to the control. There was relationship between filled pip number and fruit weight. Bacterial treatments generally decreased fruit weight, but differences were not statistically significant (Table 1).

The difference among cultivars and bacteria treatments in fruit flesh firmness were statistically significant at $P < 0.001$ significant level. As compared to the control (4.46 kg cm^{-2}) while BA-8 had the highest value (4.60 kg cm^{-2}), A-18 had the lowest value (4.35 kg cm^{-2}) among the cultivars, SCD cultivar had the highest value (4.58 kg cm^{-2})

Table 1. PGPR effects on fruit set, yield and pomological traits in apple cultivars.

Cultivar		Fruit set (%)		Fruit weight (g)	Fruit diameter (mm)	Fruit height (mm)	Fruit volume (ml)	Specific gravity of fruit (g ml^{-1})	Stalk hole deepness (mm)	Hole deepness of sepal circle (mm)	Stalk length (mm)	Stalk thickness (mm)	Filled pip number	Flesh firmness (kg cm^{-2})
		1	2											
SD		9b	6b	133.7	68.0	62.4	143.9b	0.91a	7.64a	6.04a	27.2b	2.35ab	750a	4.26b
GS		7b	4b	132.9	67.5	60.3	156.9a	0.85b	6.23b	4.71b	2.7c	2.19b	6.20b	4.54a
SCD		8b	6b	123.5	65.5	62.3	137.4b	0.91a	6.41b	6.23a	2.2c	2.41a	7.60a	4.58a
SSGD		17a	12a	125.1	66.1	62.3	134.7b	0.94a	7.69a	6.02a	29.6a	2.16b	8.00a	4.49a
Treatment														
Control		12	9	131.1	67.2	61.9	144.3	0.93a	7.93a	6.13	27.21	2.43	8.13a	4.46bc
A-18		8	6	130.9	67.1	62.5	144.6	0.90ab	6.84bc	5.76	26.58	2.13	8.00a	4.35d
OSU-142		11	8	128.8	66.8	62.3	144.4	0.89ab	6.23c	5.93	26.34	2.24	6.75ab	4.53ab
OSU-7		10	7	125.8	66.2	60.9	144.1	0.87b	6.70bc	5.25	26.48	2.28	6.13b	4.42dc
BA-8		9	6	127.4	66.5	61.5	138.8	0.92a	7.26ab	5.69	25.53	2.31	8.00a	4.60a
Source	**df**													
C	3	***	***	NS	NS	NS	*	***	***	***	***	*	***	***
T	4	*	*	NS	NS	NS	NS	*	***	NS	NS	NS	*	***
C x T	12	* *		***	*	***	***	***	*	*	NS	NS	***	***
Error	20													

of fruit flesh firmness (Table 1).

Fruit chemical analyses

Bacterial treatment had statistically significant effect ($P < 0.001$) on decreasing TSS in all apple cultivars. OSU-142 (11.60%) produced the highest decreasing rate in TSS as compared to the control (12.24%). In terms of cultivars, SCD cultivar had the lowest TSS value (11.02%) (Table 2).

Bacteria treatments had the significant differences in terms of total sugar contents except BA-8. Also, it was determined that all bacterial treatments decreased reduced sugar contents as compared to the control (7.46%). OSU-7 treatment (6.97%) gave the lowest reduced sugar contents. GS had the highest total sugar contents (11.51%), and SSGD cultivar had the highest reduced sugar contents (7.59%) among the cultivars (Table 2).

Bacteria treatment had no significant effect on ascorbic acid contents ($P > 0.05$). However, C x T interaction was statistically significant ($P < 0.001$) (Table 2). The effect of bacterial treatments on titrable acid contents was significant ($P < 0.05$) and the differences among the cultivars were statistically significant ($P < 0.001$). All treatments decreased pH levels of fruit juice as compared to the control (3.78). The lowest pH was measured with OSU-7 treatment (Table 2).

DISCUSSION

The cultural treatments such as hormones and bacteria treatments affected fruit set (Ngugi et al., 2005). Bacterial treatments decreased fruit set by 12% (OSU-142) and 33% (A-18 and BA-8). It was determined that some PGPR strains had flower thinning effect as used by excessive dose (Esitken et al., 2003; Vessey, 2003). It was reported by Aslantas et al. (2007) that OSU-7; OSU-142 and BA-8 bacteria strains had high indol-3-acetic acid (IAA) production capacity. This

Table 2. PGPR effects on some fruit contents in apple cultivars.

Cultivar		Total soluble solids (%)	Total sugar (%)	Reduced sugar (%)	Askorbic acid (mg 100 ml^{-1})	Titrable acid (%)	pH of fruit juice
SD		11.48c	10.51b	7.17b	7.94	0.39c	4.02b
GS		12.38b	11.51a	7.09b	8.68	1.33a	3.25d
SCD		11.02d	10.44b	6.85c	7.58	0.39c	4.05a
SSGD		12.55a	11.49a	7.59a	7.58	0.80b	3.52c
Treatment							
Control		12.24a	11.19a	7.46a	8.25	0.79a	3.78a
A-18		11.69c	10.91b	7.06c	7.94	0.69b	3.73b
OSU-142		11.60c	10.75b	7.08c	7.95	0.74ab	3.69c
OSU-7		11.67c	10.75b	6.97c	7.64	0.71b	3.67e
BA-8		12.08b	11.34a	7.30b	7.95	0.73b	3.68d
Source	**df**						
C	3	***	***	***	NS	***	***
T	4	***	***	***	NS	*	***
C x T	12	***	***	***	***	**	***
Error	20						

Means followed with the same letter within each column were not significant different; NS = Non significant; *P < 0.05; **P < 0.01; ***P < 0.001.

situation may indicate that these bacteria strains cause flower thinning because of their effects of increasing auxine synthesis as they were treated twice.

Bacterial treatments had no significant effects on fruit weight, fruit diameter and fruit height and volume. These results had a good agreement with the results given by Ngugi et al. (2005) who reported that some bacterial treatments did not change fruit weight in rabbit eye blueberry.

The specific gravity of fruit decreased with all bacteria treatments as compared to the treatments as compared to the control and was found statistically significant. Increasing fruit specific gravity caused higher fruit flesh firmness. This may also be caused by fruit volume and seed core greatness. As seed core greatness of fruit increases, fruit volume increases and therefore specific gravity of fruit decreases. All treatments decreased stalk hole deepness compared to the control (7.93 mm) and OSU-142 treatment had the lowest value (6.23 mm). There are some other factors affecting these parameters. As a matter of fact, there are relations among blooming time, stalk hole deepness of fruit and fruit stalk length (Aslantas and Karakurt, 2007a). Also, it was reported that early blooming apple cultivars had higher fruit stalk hole deepness, and stalk length values compared to late blooming cultivars as well as blooming time delayed by increasing altitude and this situation affected fruit stalk hole deepness, fruit stalk length and thickness (Aslantas and Karakurt, 2007b).

The bacterial treatment significantly decreased filled pip number of fruit. Decrease of filled pip number means small apple weight. Of course, the reaction of cultivars against treatments can show great differences. Biofungucide treatment with *B. subtilis* decreased filled pip number as compared to untreated fruit of rabbit eye blueberry (Ngugi et al., 2005). The present results supported the researcher's findings.

The bacterial treatments generally reduced the rate of total soluble solid, total sugar, reduced sugar, ascorbic acid contents, titrable acidity and pH rate. However, Ebrahim and Ally (2004) in wheat; Cakmakcı et al. (2001) in barley and sugar beet reported increases in TSS by bacteria treatments. This situation may result from different reaction of each cultivar against different treatments. All bacterial treatments increased shoot number and length, therefore fruit had lower TSS,

total sugar, reduced sugar, ascorbic acid, titrable acid and pH contents because of exposure to lower light density (data were not shown in this MS but evaluated).

Conclusion

Bacterial treatments produce different results depending on the crop species and cultivars. Bacterial treatment is safe, effective and easily adopted by farmers. PGPR's had increasing effect on fruit set and quality in previous studies when used in one time and suitable concentration. However, the results of this study showed that *A. rubi* (A-18), *B. subtilis* (OSU-142), *B. gladioli* (OSU-7) and *P. putida* (BA-8) had flower thinning effect when treated twice. This situation can benefit farmers and producers in horticultural treatments by PGPR. Hence, its importance is recognized by farmers as well as researchers. Therefore, these may be considered as biofertilizer for fruit and vegetable production in sustainable and ecological agricultural systems. In addition, they may be used to thin fruit trees with heavy flower load. Therefore, this can help determine fruit load and quality.

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