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ORIGINAL ARTICLE / ORIGINALBEITRAG

The Influence of the Rootstocks on Biochemical and Bioactive Compound Content of '0900 Ziraat' Sweet Cherry Fruit

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Abstract
The study was conducted to determine the effects of different rootstocks (Ma × Ma 14, GiselA 5, GiselA 6 and SL 64) on quality properties and bioactive compounds of '0900 Ziraat' sweet cherry fruit. Fruit weight, thickness and length of Ma × Ma 14 and SL 64 was higher than the GiselA 5 and GiselA 6. Firmness of GiselA 6 was lower than the other rootstocks (Ma × Ma 14, GiselA 5 and SL 64). The chroma value of GiselA 5 was greater than the other rootstocks, whereas the hue angle of SL 64 was lower than the other rootstocks. While the lowest SSC and acidity was obtained in GiselA 5, the highest vitamin C was determined in Ma × Ma 14. Total phenolics and total flavonoids, thus antioxidant activity of GiselA 6 and SL 64 rootstocks was higher than Ma × Ma 14 and GiselA 5 rootstocks. Catechin was the major phenolic acid in '0900 Ziraat' sweet cherry fruit. GiselA 6 had higher catechin and 4-hydroxybenzoic acid content than the other rootstocks. As a result, it was revealed that sweet cherry rootstocks were effective on bioactive compounds and fruit quality characteristics.

Keywords Antioxidant · Catechin · Firmness · Flavonoids · Phenolics · Vitamin C

Einfluss verschiedener Veredlungsunterlagen auf den Gehalt biochemischer und bioaktiver Inhaltsstoffe in Früchten der Süßkirschensorte '0900 Ziraat'

Schlüsselwörter Antioxidans · Catechin · Festigkeit · Flavonoide · Phenole · Vitamin C

Introduction

Sweet cherry is a fruit species that is conspicuous due to its taste, color, appearance and high antioxidant properties, which has positive effects on human health, and its production is increasing production day by day. Sweet cherry is very rich in terms of bioactive compounds such as sugars, organic acids, vitamins, antioxidants, phenolic compounds and flavonoids, which is effective in the formation of quality characteristics and in determining on health value of the fruit (Usenik et al. 2008; Fazzari et al. 2008; Yildiz et al. 2018). Bioactive compounds such as antioxidants, phenolic compounds and flavonoids have various positive effects on the human health like anti-inflammatory and anticarcinogenic effects (Kroon and Williamson 1999) and they are significant in the human nutrition (Usenik et al. 2008). Furthermore, these compounds have shown protective effects on neuronal cells (Kim et al. 2005). In this context, due to the positive effects on human health, the studies on fruit cultivation focus on the factors affecting phenolic compounds. The concentration of these bioactive compounds vary on depending upon the factors such as climate, soil and the rootstock (Usenik and Setampar 2002; Spinardi et al. 2005), the cultivar (Mozetic et al. 2002; Kim et al. 2005; Usenik et al. 2008), and cultural treatments such as irrigation, fertilization, pruning (Serra et al. 2011). With the different studies with *Prunus* sp. (Tareen and Tareen 2004; Gonçalves et al. 2006; Whiting et al. 2005; Čme-

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lik and Družić-Orlić 2008; Long and Kaiser 2010; Cantin et al. 2010; Tavarini et al. 2011; Sitarek and Bartosiewicz 2012; Aglar and Yıldız 2014; Popescu and Popescu 2015; Lopez-Ortega et al. 2016; Pal et al. 2017), it has been determined that the rootstock have an effect on the vegetative development, fruit quality and yield efficiency of the cultivar. However, the rootstock may affect the mineral content (Jimenez et al. 2007) and the phenolic compounds concentration in the tissues of the cultivar (Usenik and Štampar 2002). The research conducted by Spinardi et al. (2005), it was determined that the rootstock was effective on biologically active compound contents such as the polyphenol and anthocyanin content of sweet cherry fruit. On sweet cherry cultivation, the cultivars grafted on generative and clone rootstocks of species such as *P. avium* and *P. mahaleb* are used (Treutter et al. 1986). With the increase of interest in sweet cherry cultivation, new rootstocks, which are appropriate for the cultivation, have been developed (Jimenez et al. 2007). Therefore, it is necessary to determine the effect of the rootstocks on the bioactive content of the cultivar. In the literature, there is no study about the effect of the rootstock on biochemical and bioactive content of '0900 Ziraat' sweet cherry known as Turkish cherry. Thus, the aim of the study was to determine the effect of the rootstocks (Gisela-5, Gisela-6, Ma × Ma 14 and SL 64) on the bioactive compound content in '0900 Ziraat' sweet cherry fruit.

Materials and Methods

Experimental Design

5-year old uniform sweet cherry trees (*Prunus avium* cv. '0900 Ziraat') grafted on different rootstocks [GiselA 5 (*Prunus cerasus* × *Prunus canascens*), GiselA 6 (*Prunus cerasus* × *Prunus canascens*), Ma × Ma 14 (*Prunus mahaleb* × *Prunus avium*), Saint Lucie GF 64 (SL 64, selection of *Prunus mahaleb*)] in Suşehri, Sivas Province, Turkey (40° 10′ 09.67″N latitude, 38° 06′ 37.14″E longitude and 952 m altitude), with annual precipitation and temperature, respectively, 252 mm and 11 °C (pH: 7.9, clay-loam soil), were selected for the experiment. The trees were planted in an east-west direction with 4.0 m row spacing and 3.5 on-row tree spacing and trained according to the Spanish Bush system. Standard cultural practices (irrigation, fertilization, pruning, disease control) were regularly applied during the experimental period. Irrigations were applied by drip irrigation. Macro-micro nutrients were supplied in three aliquots on March 1, April 1 and May 1. A total of 12 g N (nitrogen), 20 g K_2O (60%, potassium oxide), 5 g $NH_4H_2PO_4$ (monoammonium phosphate) and 20 g K_2SO_4 (potassium sulphate) were supplied per tree. Additionally, 5 g calcium nitrate [Ca (NO_3)] per tree was supplied once in May 15. Any symptoms of nutritional deficiency were not observed in the leaf or fruit during the growing season.

Fruit Weight, Fruit Dimensions and Firmness

Fruit weight was measured using a digital scale (±0.01 g) (Radvag PS 4500/C/1, Poland). Fruit dimensions (length, thickness and width) were determined with a digital caliper (±0.01 mm) (Model CD-6CSX, Mitutoyo, Japan). Fruit firmness was measured with a digital portable durometer (nondestructive device, Agrosta® 100 Field, Agrotechnologie, France) and the results were expressed as DurofelUnits (%). In Durofel Units, 0 indicates that the fruit is too soft and 100 indicates that the fruit is too firm. Twenty fruit each replicate were used to determine the fruit weight, length, width and firmness.

Color Characteristics

A colorimeter (Konica-Minolta, model CR–400, Japan) was used to measure L*, chroma and hue angles from opposite sides of each fruit. CIE (Commission Internationale de l'Eclairage) system was employed in chromatic analyses. Color characteristics were measured in 10 fruits randomly selected from each replicate. 3-D color space was defined with the aid of L*, *a** and *b** values. Equations in parenthesis were used to calculate chroma [$C^* = (a^{*2} + b^{*2})^{1/2}$] and hue angle ($h^\circ = \tan^{-1} b^*/a^*$).

Soluble Solids Content (SSC), Titratable Acidity and Vitamin C

Initially 20 fruits were selected from each replicate for SSC, titratable acidity and vitamin C analyses. Fruit stones were removed, and juice was extracted with the aid of an extractor (HR1855/70, Philips, Turkey). SSC (%) was measured by a digital refractometer (PAL-1, McCormick, USA). About 10 ml extract was diluted with 10 ml distilled water for titratable analyses. The amount of 0.1 N sodium hydroxide used for titrating the resultant solution to a pH of 8.2 was expressed in mg malic acid 100 g^{-1}. About 0.5 ml extract was completed to 5 ml with 0.5% oxalic acid for vitamin C analyses. The ascorbic acid test strip (Catalog no: 116,981, Merck, Germany) was immersed in the resultant solution for 2 s and excess liquid over the test strip was removed. Readings were performed in a reflectometer (Merck RQflex plus 10), expressed as mg 100 g^{-1} fresh weight.

Total Phenolics, Total Flavonoids and Antioxidant Activity

Initially 20 fruits were selected from each replicate for total phenolics, total flavonoids and antioxidant activity. The stones were removed, and pulps were homogenized in blender. Resultant homogenates were then stored in 3 tubes at –20 °C until the analyses. Frozen homogenates were thawed at room temperature (≈21 °C) and re-homogenized with a blender. Fruit juice was separated from the pulp through centrifuging the slurry at 12.000 × g at 4 °C for 30 min. Resultant juice was then diluted with distilled water and refrozen at –20 °C in multiple aliquots to be used later on for phenolics, flavonoids and antioxidant analyses.

The total phenolics were determined with the aid of an automated UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan) in accordance with principles specified by Beyhan et al. (2010). Gallic acid was used as the standard. The results were expressed as microgram (µg) of gallic acid equivalents (GAE) per hundred grams of fresh weight (fw) (µg GAE g^{-1} fw).

Total flavonoid contents were determined in accordance with the principles specified by Chang et al. (2002). Since this time quercetin was used as test standard, results were expressed as µg in g quercetin equivalents (QE) on fresh weight basis (µg QE g^{-1} fw).

Two different procedures as of DPPH radical scavenging activity (Blois 1958) and Ferric ions (Fe^{+3}) reducing antioxidant power assay (FRAP) (Benzie and Strain 1996) were employed for antioxidant activity of the sweet cherry. Results were expressed as µmol in g^{-1} Trolox equivalent (TE) on fresh weight basis (µmol TE g^{-1}) in both assays.

Individual Phenolics

In the study, catechin, chlorogenic acid, rutin, caffeic acid, protocatechuic acid, 4-hydroxybenzoic acid, 4-aminobenzoic acid and p-coumaric acid were measured. In the separation of phenolic acids with ultra-high performance liquid chromatography (UHPLC, Thermo Scientific, Ultimate 3000, USA), the method described by Ozturk et al. (2015) was used. The samples were distilled with distilled water at the ratio of 1:1 and after they were centrifuged at 15,000 × g for 15 min. The supernatant was filtered with 0.45 µm millipore filters and then injected to UHPLC. The chromatographic separation was performed by using a DAD detector (DAD-3000, USA) in UHPLC system. The analytes were separated by 250 × 3.0 mm, 5 µm Hypersil GD phenyl column (Thermo Scientific, USA) with temperature set at 30 °C. The elution solvents were aqueous 2.5% formic acid (solvent A) and 100% methanol (solvent B). The separation was conducted at 274 nm. Total run time took 40 min. Injection volume was 20 µL and the mobile phase flow rate was 1 ml min^{-1}. The results were expressed in mg kg^{-1}.

Statistical Analysis

The normality of the data was confirmed by the Kolmogorov-Smirnov test and the homogeneity of variances by the Levene's test. The results obtained in each analysis were analyzed with SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA) software. Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test. All analyses were performed with a 95% confidence level ($p < 0.05$).

Result and Discussion

Fruit Size and Firmness

Large fruit, which is preferred by the consumers due to its positive effects of the fruit on the visual attractiveness, taste and the fresh fruit ratio (Blazkova et al. 2002) and significantly affects the economic value and the amount of the sweet cherry, (Whiting et al. 2006) is one of the main goals of sweet cherry cultivation (Zhang and Whiting 2011). In sweet cherry, although the fruit size varies depending on the cultivar, the factors such as yield (Gonçalves et al. 2006), the ripening stage (Diaz-Mula et al. 2009) and rootstock (Usenik et al. 2010) can affect the fruit size. In the study, which to determine the effects of rootstocks on bioactive

Table 1 Effects of rootstocks on fruit weight, fruit sizes and firmness of '0900 Ziraat' sweet cherry fruit

Rootstocks	Quality characteristics				
	Weight (g)	Width (mm)	Thickness	Length (mm)	Firmness (N)*
Ma × Ma 14	9.50 a	21.88 a	24.12 a	22.19 a	44.67 a
GiselA 5	8.96 c	22.41 a	22.90 b	21.69 b	46.20 a
GiselA 6	8.87 c	22.10 a	20.27 c	21.65 b	38.73 c
SL 64	9.25 a	22.44 a	24.81 a	22.89 a	42.40 b

The scale ranges from 0 to 100 for very soft to very firm surfaces
$n = 60$ for the firmness (three replicate × ten fruit × two different measurements for each fruit)
$n = 60$ for the weight and fruit sizes (three replicate × twenty fruit)
Means in columns with the same letter do not differ according to Tukey's test at $P < 0.05$

compounds on sweet cherry, it was determined that the fruit size varied depending on the rootstock. The lowest values were recorded in GiselA rootstocks (GiselA 5 and GiselA 6), while the largest fruits were obtained from the trees of Ma × Ma 14 rootstock.

However, there was no statistically significant difference between Ma × Ma 14 and SL 64 rootstocks (Table 1). The findings of the study are consistent with the results of the studies about the effect of cultivar-rootstock interaction on fruit quality of sweet cherry (Gonçalves et al. 2006; Cantin et al. 2010; Lopez-Ortega et al. 2016). Sitarek and Bartosiewicz (2012) reported that the trees on the Gisela 3 rootstock have the smallest fruits, the largest fruit has been obtained from the trees on the F 12/1 rootstock. Again, Whiting et al. (2005) stated that the difference between rootstocks in terms of fruit size is significant, and the fruit on the Mazzard rootstock was 16% heavier than the fruits of the GiselA rootstocks (GiselA 5, GiselA 6). In the dwarf rootstocks, the deterioration of physiological balance due to their generative development superiority may be the reason for obtaining smaller fruit in these rootstocks.

The fruit firmness in sweet cherry is one of the most significant factors affecting the market value of the fruit and the marketing period. The fruit firmness varies depending upon the fruit ripening stage (Blazkova et al. 2002). As fruit ripening or fruit size increase, the decreasing on fruit firmness may occur (Diaz-Mula et al. 2009). However, the ecological and genetic factors can affect the fruit firmness. Hajagos et al. (2012) reported that there were the differences between cultivars in terms of the fruit firmness, 'Regina' cherries have generally better firmness than 'Kordia's'. Usenik et al. (2010) and Gonçalves et al. (2006) reported that the effect of rootstocks on fruit firmness was significant, and the fruit firmness values were lower in vigorous rootstocks. In our study, it was determined that the fruit firmness values varied depending on the rootstock used, and the trees on GiselA 6 rootstock had softer fruits. GiselA 5 rootstocks were determined as rootstock having the highest values in terms of the fruit firmness. However, there was no statistically significant difference between Ma × Ma 14 and GiselA 5 rootstocks in terms of the fruit firmness (Table 1). In considering this situation, it can be concluded that the effect of rootstock on the fruit firmness cannot be related to rootstock strength. Lopez-Ortega et al. (2016) determined that there is no relationship between fruit firmness and productivity, but fruit firmness and SSC were positively correlated (r = 0.691ab, $P < 0.01$).

Fruit Color

The fruit brightness in the sweet cherry is a significant quality parameter in terms of market value (Crisosto et al. 2003). The L * value of the color parameters refers to the brightness. In the study, there was no statistically significant difference between rootstocks in terms of L * value. It has been determined that the effect of the rootstock on chroma value, which expresses the color viability, is significant. The highest chroma value was recorded with fruit on GiselA rootstock, whereas SL 64 rootstock had the lowest chroma value. The decreasing in hue angle values means an increase in the red color of the fruit. In the study, the effect of rootstock on hue angle value was significant. It was found that the fruit of SL 64 rootstock compared to the other three rootstocks was more red (Table 2). In the studies conducted by Gonçalves et al. (2006) and Lanauskas et al. (2014), it has been reported that the effect of rootstock on fruit coloration is significant. Tareen and Tareen (2004) reported that the differences between the rootstocks in terms of fruit color data were significant, and the fruits on Mazzad rootstock were better colored than Colt rootstock's. However, Lopez-Ortega et al. (2016) suggested that the rootstock on fruit color had no significant effect.

Table 2 Effects of rootstocks on color characteristics of '0900 Ziraat' sweet cherry fruit

Rootstocks	Color characteristics		
	L*	Chroma	Hue angle
Ma × Ma 14	33.63 a	36.91 b	21.85 a
GiselA 5	34.61 a	38.49 a	22.10 a
GiselA 6	34.42 a	35.55 b	21.61 a
SL 64	33.08 a	34.47 b	19.75 b

$n = 60$ for the color characteristics (three replicate × ten fruit × two different measurements for each fruit)
Means in columns with the same letter do not differ according to Tukey's test at $P < 0.05$

SSC, Titratable Acidity and Vitamin C

In the study, the content of SSC and titratable acidity in fruit has varied depending on the rootstock used. The fruit on GiselA 5 rootstock had lower SSC and titratable acidity content (Table 3). In contrast to the study results, Lopez-Ortega et al. (2016) reported that there was no significant difference between the rootstocks in terms of SSC and titratable acidity content. They showed that SSC and titratable acidity contents vary depending on the years and yield, and a negative correlation was found between these parameters (SSC ve titratable acidity). However, Gonçalves et al. (2006) and Usenik et al. (2010) found that in sweet cherry, the rootstock affects SSC content, and the dwarf rootstocks have higher SSC. Similarly, Daza et al. (2008) and Rato et al. (2008) have reported that in the plum, the rootstock has a significant effect on the quality parameters such as SSC and titratable acidity.

There is 7 mg of vitamin C 100 g^{-1} fresh weight in sweet cherry fruit (Ferretti et al. 2010). In the study, vitamin C

Table 3 Effects of rootstocks on SSC, titratable acidity and vitamin C of '0900 Ziraat' sweet cherry fruit

Rootstock	Biochemical characteristics		
	SSC (%)	Titratable acidity (g malic acid 100 g^{-1})	Vitamin C (mg 100 g^{-1} fresh weight)
Ma × Ma 14	12.03 a	0.45 a	7.57 a
GiselA 5	11.60 b	0.38 b	5.83 b
GiselA 6	12.00 a	0.45 a	6.03 b
SL 64	12.10 a	0.46 a	5.23 c

$n = 9$ for the SSC, titratable acidity and vitamin C (three replicate × three different measurements for each replicate)
Means in same columns with the same lowercase letter do not differ according to Tukey's test at $P < 0.05$

Table 4 Effects of rootstocks on bioactive compounds of '0900 Ziraat' sweet cherry fruit

Rootstock	Bioactive compounds			
	Total phenolics µg GAE g^{-1}	Total flavonoids µg QE g^{-1}	DPPH µmol TE g^{-1}	FRAP µmol TE g^{-1}
Ma × Ma 14	136.25 d	95.97 c	0.95 c	7.00 c
GiselA 5	183.40 c	103.21 b	1.65 b	9.20 b
GiselA 6	332.08 a	186.35 a	1.94 a	11.43 a
SL 64	279.17 b	159.82 a	1.88 a	11.10 a

$n = 9$ for the bioactive compounds (three replicate × three different measurements for each replicate)
Means in same columns with the same letter do not differ according to Tukey's test at $P < 0.05$

content ranged from 5.23 mg (SL 64) to 7.57 mg as mg 100 g^{-1} fresh weight (Ma × Ma 14). The effect of the rootstook on vitamin C content was found to be significant. However, there was no statistically significant difference between GiselA rootstocks (GiselA 5 and GiselA 6) in terms of vitamin C content (Table 3). Spinardi et al. (2005) reported that the vitamin C content in sweet cherry cultivars depending on the rootstock, and Mainla et al. (2008) determined that the effect of the rootstock on the vitamin C concentration in the apple is significant.

Bioactive Compounds

Sweet cherry is very rich in terms of bioactive compounds such as sugars, organic acids, vitamins, antioxidants, phenolic compounds and flavonoids, which is effective in the formation of quality characteristics and in determining oh health value of the fruit (Fazzari et al. 2008; Usenik et al. 2008). The concentration of these bioactive compounds varies depending upon the ecological factors such as climate and soil, the rootstock (Usenik and Štampar 2002; Spinardi et al. 2005), the cultivar (Mozetic et al. 2002; Kim et al. 2005; Usenik et al. 2008). The results of our study confirm this information. In the study, the effect of rootstock on bioactive compounds of the fruit was found to be significant. In terms of total phenolic, total flavonoid and antioxidant activity, the highest values were recorded with GiselA 6 rootstock and the lowest values were obtained with Ma × Ma rootstock. It was determined that the difference between the total phenolic values of GiselA 6 and SL 64 rootstocks occurred, while there was no statistically significant difference between these rootstocks in terms of total flavonoid and antioxidant activity (Table 4).

It was also determined in the study conducted by Usenik and Štampar (2002) that the effect of the rootstock on bioactive compounds was significant. Furthermore, in the research conducted by Spinardi et al. (2005) has been determined that the rootstock in sweet cherry influences biologically active compound contents such as the polyphenol and anthocyanin content in the fruit. Gonçalves et al. (2006) showed that the rootstocks affect the tree physiology (water relations, leaf gas exchange, chlorophyll fluorescence, light canopy permeability, leaf photosynthesis pigments and metabolites). The differences in the concentration of bioactive compounds in fruit can be explained by the effects of rootstocks on tree physiology.

Individual Phenolics

The major phenolic compounds in sweet cherry are hydroxycinnamic acids. Neochlorogenic acid and p-coumarylquinic acid are the highest concentration hydroxycinnamic acids (Kim et al. 2005). However, sweet cherry contains small amounts of chlorogenic acid (Kim et al. 2005), ferulic acid and hydroxybenzoic acids (phydroxybenzoic acid) (Matilla et al. 2006).

Usenik et al. (2010), have determined that sweet cherry fruit has individual phenolic compounds such as neochlorogenic acid, p-coumaroylquinic acid, chlorogenic acid, rutin, catechin, epicatechin, and procyanidin. In our study, it was observed that the highest individual phenol was catechin, followed by chlorogenic acid, rutin, caffeic acid, protocatechuic acid, 4-hydroxybenzoic acid, epicatechin and

Table 5 Effects of rootstocks on individual phenolics compounds of '0900 Ziraat' sweet cherry fruit

Individual phenolics	Rootstocks			
Compounds (mg kg^{-1})	Ma × Ma 14	GiselA 5	GiselA 6	SL 64
Catechin	481.0 c	494.3 c	927.9 a	792.4 b
Chlorogenic acid	23.31 b	25.33 b	31.56 a	30.89 a
Rutin	5.32 b	13.63 a	12.09 a	11.35 a
Caffeic acid	2.44 b	2.40 b	3.60 a	3.37 a
Protocatechuic acid	2.74 b	2.93 a	2.91 a	2.99 a
4-hydroxybenzoic acid	2.95 b	3.18 b	4.31 a	2.59 b
Epicatechin	4.52 b	8.28 a	Nd	Nd
4-aminobenzoic acid	0.98 a	0.96a	0.81 b	0.93 a

nd not determine

$n = 9$ for the individual phenolics compounds (three replicate × three different measurements for each replicate)

Means in same line with the same letter do not differ according to Tukey's test at $P < 0.05$

4-aminobenzoic acid respectively. Generally, the individual phenolic content varied depending on the rootstock (Table 5). Similarly, Jakobek et al. (2009) and Usenik et al. (2010) reported that there were significant differences between rootstocks in terms of individual phenolic concentration in sweet cherry. In general, while the GiselA 6 rootstock had the highest concentration, the lowest values were obtained from the Ma × Ma 14 rootstock. In terms of individual phenolic content, the difference between rootstocks may be a result of the effect of the rootstock on the vegetative and the generative development of the cultivar, or it can be said that the incompatibility problem has an effect on the difference between the rootstocks. An earlier research by Usenik and Štampar (2002) indicated that different rootstocks could induce different effects regarding the phenolic compound concentrations in the scion tissues. Usenik and Štampar (2000) found that low compatibility resulted in a pronounced accumulation of polyphenols, namely p-coumaric acid above the graft union of 'Lapins' grafted on different rootstocks (F 12/1, GiselA 5, Weiroot 158), as a stress response to grafting. The higher p-coumaric content above the graft union in the apricot cultivar grafted on the heterospecific rootstocks, which cause incompatibility, was detected (Usenik et al. 2006).

As a result, it can be said that in term of the fruit quality characteristics such as fruit size, fruit color, firmness, SSC and acidity, vigorous rootstocks (SL 64 and Ma × Ma 14) are more suitable rootstocks for sweet cherry cultivation. However, bioactive compounds determine the value on human health of the fruit. In considering this situation, it can be said that GiselA 6 and SL 64 rootstocks are more suitable rootstocks.

Conflict of interest O. Karakaya, B. Ozturk, E. Aglar and H.I. Balik declare that they have no competing interests.

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