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Research article

Effects of Rootstocks on Sugar and Organic Acid Contents of 'Deveci' Pear

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Abstract. The aim of present study was to determine the effects of three rootstocks [quince BA 29 and EMC (*Cydonia oblonga*), and seedling of *P. communis*] on sugars and organic acid contents in 'Deveci' pear (*Pyrus communis* L.) cultivar during 2011-2015 years. In the study, oxalic, tartaric, malic, ascorbic, acetic, citric, fumaric and propionic acid as organic acids and fructose, sucrose, glucose and total sugar as sugars in the fruit samples were investigated in terms of rootstocks. Major organic acids for 'Deveci' pear were malic, ascorbic and propionic acids. Malic and ascorbic acid in BA 29 rootstock were higher than EMC and seedling rootstocks. Contrarily, propionic acid was higher in seedling rootstock. In terms of malic acid, BA 29 had the highest malic acid content (3425.3 mg $100g^{-1}$) while seedling rootstock had the lowest amount (2976.5 mg $100g^{-1}$). Significant differences were observed between rootstocks for tartaric, ascorbic, acetic, citric and fumaric acids. Tartaric, ascorbic, citric and fumaric acids in BA 29 and EMC rootstock were higher than the content of seedling rootstock. The most abundant sugars in 'Deveci' pear were fructose (32.4, 39.27 and 42.4 g kg^{-1} fw, respectively) and glucose (18.7, 22.3 and 26.7 g kg^{-1} fw) for BA 29, EMC and seedling rootstocks. The total sugar content of the seedling rootstock (74.0 g kg^{-1} fw) was higher than BA 29 (53.0 g kg^{-1} fw) and EMC (65.8 g kg^{-1} fw).

'Deveci' Armudunun Şeker ve Organik Asit İçeriği Üzerine Anaçların Etkileri

Anahtar kelimeler:
Askorbik asit, fruktoz, malik asit, *Pyrus communis*, toplam şeker

Özet. Bu çalışmanın amacı 2011-2015 yılları arasında 'Deveci' armudunun şeker ve organik asit içeriği üzerine 3 farklı anacın [BA 29 ve EMC (*Cydonia oblonga*) ayva ve armut çöğürü (*P. communis)*] etkilerini belirlemektir. Araştırmada anaçlar bakımından meyve örneklerinde organik asit olarak; okzalik asit, tartaric asit, malik asit, askorbik asit, asetik asit, sitrik asit, fumarik asit ve propiyonik asit, şekerler olarak ta; fruktoz, sukroz, glikoz ve toplam şeker incelenmiştir. 'Deveci' armudunda malik, askorbik ve propiyonik asit baskın organik asitler olarak belirlenmiştir. Malik ve askorbik asit içeriği BA 29 anacından elde edilen meyvelerde, EMC ve çöğür anacından daha yüksek bulunurken propiyonik asit içeriği çöğürde daha yüksek bulunmuştur. Malik asit içeriği BA 29 anacında en yüksek (3425.3 mg $100g^{-1}$), çöğür de ise en düşük (2976.5 mg $100g^{-1}$) miktarda bulunmuştur. Tartarik, askorbik, asetik, sitrik ve fumarik asit bakımından anaçlar arasında önemli farklılıklar belirlenmiştir. Tartarik, askorbik, sitrik ve fumarik asit içeriği BA 29 ve EMC anacında çöğür anacındakinden daha yüksek belirlenmiştir. BA 29, EMC ve çöğür anacı üzerine aşılanan 'Deveci' armudunda fruktoz (sırasıyla, 32.4, 39.27 ve 42.4 g kg^{-1} taze ağırlık) ve glikoz (sırasıyla, 18.7, 22.3 ve 26.7 g kg^{-1} kuru ağırlık) en fazla miktarda bulunan şekerler olmuştur. Çöğür anacı üzerine aşılı bitkilerden elde edilen meyvelerin toplam şeker içeriği (74.0 g kg^{-1} taze ağırlık) BA 29 (53.0 g kg^{-1} taze ağırlık) ve EMC (65.8 g kg^{-1} taze ağırlık) anacındakinden daha yüksek belirlenmiştir.

INTRODUCTION

The pear is one of the most commonly consumed pome fruit species in Europe (Ozcagiran *et al*., 2004; Hancock and Lobos, 2008). In Turkey, pears are the second most important pome fruit after the apple and these species are grown in almost all parts of the country. The most common pear species grown in Turkey is the *P. communis* (Ercisli 2004).

World pear production reached 25.7 million ton in 2014 and Turkey is in 5th place in pear production in the world with 462.336 t of annual production and it is one of the major pear producing countries in Europe being ranking second after Italy (FAO 2017). In Turkey, pear production has been increasing year by year with such cultivars as 'Deveci', 'Santa Maria' and 'Williams' extensively used in pear production. The 'Deveci' pear cultivar, the main one in Turkey, is one of the best winter-type pear cultivars, originating from Anatolia, gaining in popularity during the last few years in Turkey because of high fruit quality, production and storage capacity (Sen *et al*., 2009).

Pear fruits are preferred by consumers due to their juiciness, crispness, sweetness and aroma characteristics. Although pears have contain low level of protein and fat, they have high level of vitamin C, E, B complex vitamin, sugar and organic acids contents (Jackson 2003; Ozcagiran *et al*., 2004). Most pear fruit is consumed directly as a source of monosaccharaides, minerals when fully mature, according to commercial practice. The edible part of the fruit contains considerable amounts of sugars, vitamins, organic acids, polyphenols and minerals and other nutrients. The nature and the concentration of sugars and organic acids are important factors influencing the organoleptic properties of fruit and fruit products (Silva *et al*., 2002: Chen *et al*., 2007). Among the most important constituents of pear and related products are sugars and acids (Nour *et al*., 2010). Pear fruits contain sugar such as sucrose, fructose, glucose, sorbitol and organic acids such as fumaric, malic, quinic, shikimic, and citric acid (Colaric *et al*., 2006). Differences in chemical components of fruit might occur depending on the maturity stage, environmental factors (Colaric *et al*., 1999), cultural practices applied in an orchard (Peck *et al*., 2006), storage conditions (Roth *et al*., 2007).

The purpose of this study was to determine the effects of different rootstocks on sugars and organic acid contents of `Deveci' pears during the first five year (2011-2015) period.

MATERIALS AND METHODS

Chemicals

Organic acids, malic, oxalic, tartaric, ascorbic, acetic, citric, fumaric and propionic acids and fructose, glucose, sucrose were supported from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). In all cases bidistilled water was used, and purified in a Barnstead Easy Pure water purification system by Thermo Scientific. Sugars and organic acids standards were prepared in water and diluted in methanol.

Plant Materials

This study was performed during the 2011-2015 years. Pear trees, grafted on three rootstocks, were planted in May 2010 with 1-year-old scions, at the farmer orchard in Samsun (41°22'N; 36°10'E; altitude 182 m), located in the Black Sea Region on the north coast of Turkey. Samsun has a warm and humid climate in summer, and winters are cool and damp. Precipitation is heaviest in late autumn and early winter. According to long term climatic data of Samsun, the mean maximum temperature is 26.2 °C; the mean minimum is 3.3 °C, and the mean annual temperature is 14.1 °C (TSMS, 2017). The soil traits of experimental area based on the result of soil samples taken from 20 cm are clay (83%), low lime (0.50%), salt-free (0.105%), pH (6.6), phosphorus (63.2 kg da^{-1}), potassium (236 kg da^{-1}) and high organic matter (5.76 %).

The following three rootstocks were tested: quince BA 29, quince EMC (*Cydonia oblonga*) and a seedling of *Pyrus communis*, obtained from local wild pear genotype. The plant material was in the same condition for all rootstocks. The plants grafted on the BA 29 and EMC rootstocks were spaced at 3.5 x 1.2 m intervals, the plants grafted on the seedling was spaced at 4 x 4 m, headed at 80 cm and trained according to the modified leader system. Plant grafted onto quince rootstocks were tied from three wires at 0.5, 1.0 and 1.5 m in the training system and seedlings were not wired. Pruning was done regularly every year. Irrigation was done at one week intervals in response to the plant's water needs with drip irrigation system. Fertilization, NPK solution, was applied by the irrigation system based on the fruit trees development (up to 40 N–10 P_2O_5–60 K_2O in the last two years).

From all rootstocks the undamaged pear fruits, each replication has 15 fruits, were harvested

observantly by hand at their commercial maturity stage and transferred to the laboratory in cooled polythene bags to reduce water loss during transport. The fruits were cleaned to remove all foreign matters such as dust, dirt and cut into thin slices and stored at −20 °C until preparation of the samples. The pear slices were homogenized to a puree with a homogenizer (T25 basic Ultra Turrax, IKA, Staufen, Germany).

Extraction of Sugars, Organic Acids

Extraction of sugars were similar to those previously described by Colaric *et al*. (2006) and Muir *et al*. (2009), with minor modifications. For sugars, homogenized fruit samples were weighed into a beaker and 80 mL of hot distilled water at 80 °C was added. The beaker was placed on a hot-magnetic stirrer and stirred with heat bath (around 80 °C) (JP Selecta SA, Barcelona, Spain) for 15 min. until the sample was completely dispersed. The solution was then cooled to room temperature and then quantitatively transferred to a 100 mL volumetric flask; the volume was adjusted to 100 mL. Samples were stirred, and 9 mL samples were taken in a test tube and then 0.5 mL Carrez I (dissolving 21.9 g of crystallized zinc acetate and 3 mL of glacial acetic acid in 100 mL of distilled water) and 0.5 mL Carrez II (dissolving 10.6 g of potassium hexacyanoferrate (Fe^{+2}) in 100 mL of distilled water) were added and then vortexed about 30 sec. and centrifuged (NF 200, Nuve, Ankara, Turkey) at 2500 rpm for 10 min. The clear supernatant was then taken and further filtered through 0.22 µm sterile Millex syringe filter (Millipore, Carrigtwohill, Co. Cork, Ireland) and transferred to a vial and used for HPLC analyses of sugars.

Extraction of organic acids were similar to previously described by Chen et al. (2007), with minor modifications. 1 g homogenized fruit samples were weighed into a test tube and 10 mL 0.01 N perchloric acid was added and vortexed 1 min. The test tube was centrifuged (NF 200, Nuve, Ankara, Turkey) at 7000 x g at 4 °C for 7 min. The clear supernatant was then taken and further filtered through 0.45 µm sterile Millex syringe filter (Millipore, Carrigtwohill, Co. Cork, Ireland) and transferred to a vial and used for HPLC analyses of organic acids.

Sugar (fructose, glucose, sucrose) content was analyzed using HPLC equipment (Shimadzu, Tokyo, Japan). Separation of sugars was carried out using an Inertsil NH_2 column (5 µm, 250 mm x 4.6 mm; GL Sciences, Torrence, Calif., USA) operated at 40 °C. The mobile phase was acetonitrile: bidistilled water (75:25, v/v) and injection volume was 20 µL and flow rate was 0.1 mL min^{-1}; total run time was 60 min and a refractive index (RI) detector (RID-10A model) was used for monitoring eluted carbohydrates according to the method of Muir et al. (2009).

Organic acids were analyzed using HPLC equipment (Agilent 1260 infinity, California, USA). Separation of organic acids was carried out using a Shim-Pack column (4 µm, 150 mm x 4.6 mm,; Tokyo, Japan) associated with a UV detector set at 210 nm as described by Colaric *et al*. (2006). The column temperature was set at 35 °C. The elution solvent was 10 mM perchloric acid in bidistilled water at a flow rate of 0.6 mL min^{-1}. The injection volume was 20 µL and the duration of the analysis was 30 min.

Analyzed compounds were identified by addition of standard solutions in combination with retention times as well as by comparing their spectra with those of corresponding standards. Quantification was achieved according to the concentrations of a corresponding external standard. Concentrations of analyzed compounds are expressed in mg per 100g or g per kg of fruit fresh weight (FW).

Data Analyses

The experimental design was a randomized complete block with 4 replications and 5 trees per replicate. Statistical significance was determined by the one-way analysis of variance (ANOVA) using the SPSS (Version 16.0) program (SPSS Inc. Chicago, USA).The Duncan multiple range test was used to compare treatments when an analysis of variance showed significant differences among means. Means were presented as an average of first five years in Tables.

RESULTS AND DISCUSSION

The effects of different rootstocks on the organic acids contents of the 'Deveci' pear are shown in Table 1. There were statistically significant differences among the rootstocks in terms of organic acid contents, except for oxalic, malic and propionic acid (Table 1). The malic, propionic and ascorbic acid were detected as major organic acid in the pear fruits. The acetic and fumaric acid were detected as minor organic acid in the 'Deveci' pear fruits. These results are accordance with the findings of Colaric *et al*. (2006) and Hudina and Stampar (2000) and Sha *et al*. (2011).

The highest acetic acid content was obtained from the BA 29 quince rootstock (50.7 mg $100g^{-1}$) and the lowest content in the EMC rootstock (27.9 mg $100g^{-1}$). The highest ascorbic and citric acid were obtained from BA 29 (1838.3 mg $100g^{-1}$ and 121.7 mg $100g^{-1}$,

respectively) and the EMC (1566.5 mg $100g^{-1}$ and 105.6 mg $100g^{-1}$, respectively) followed by the seedling (522.0 mg $100g^{-1}$ and 88.9 mg $100g^{-1}$) (Table 1). Colaric *et al*. (2006) reported that citric acid content is mostly dependent on a ripening stage and cultivar. Compared to the findings of Sha *et al*. (2011), similar acetic and oxalic acid contents were identified in this study while malic, citric acid contents were higher in the some *Pyrus communis* pear cultivars.

Table 1. Comparison between rootstocks for the organic acids content of 'Deveci' pear.
Çizelge 1. 'Deveci' armudunun organik asit içeriği bakımından anaçların karşılaştırılması.

Organic acids	Rootstocks		
(mg $100g^{-1}$)	BA 29	EMC	Seedling
Acetic acid	50.7 a*	27.9 b	39.0 ab
Ascorbic acid	1838.3 a	1566.5 a	522.0 b
Citric acid	121.7 a	105.6 a	88.9 b
Fumaric acid	32.8 a	51.2 a	10.9 b
Malic acid	3425.3 a	3065.9 a	2976.5 a
Oxalic acid	133.5 a	122.0 a	141.0 a
Propionic acid	1394.0 a	2019.0 a	2030.3 a
Tartaric acid	175.2 a	122.1 a	52.6 b

* The difference between means shown on the same line with the same letter is not significant according to Duncan's Multiple Range test at $P<0.01$.

The highest fumaric acid was observed from the EMC (51.2 mg $100g^{-1}$) and the BA 29 (32.8 mg $100g^{-1}$) and the lowest in the seedling rootstock (10.9 mg $100g^{-1}$). Statistically significant differences were observed for the tartaric acid content of the 'Deveci' pear fruit grafted onto different rootstocks. The highest tartaric acid content was observed from BA 29 (175.2 mg $100g^{-1}$) and EMC (122.1 mg $100g^{-1}$), the lowest in the seedling (52.6 mg $100g^{-1}$) (Table 1). Dziezak (2003) cited that fumaric acid concentration was the lowest among the quantified organic acids; nevertheless, the taste effect of fumaric acid on the flavour of fruits is stronger than the effect of citric acid.

The fruits of the 'Deveci' pear grafted on the BA 29 had the higher malic acid content than the EMC and seedling rootstocks. Seedling had the higher oxalic and propionic acid content (141.0 mg $100g^{-1}$ and 2030.3 mg $100g^{-1}$, respectively) than the BA 29 and EMC rootstock (Table 1). Silva et al. (2002) and Colaric *et al*. (2006) reported that malic acid is predominant organic acid content in the fruits. The content of malic acid of the 'Deveci' pear fruit in the present study are in accordance with Sha *et al*. (2011) who reported that malic acid is the major component of organic acids in the examined 40 pear cultivars.

Statistically significant differences were observed for the sugar content (fructose and sucrose) of the 'Deveci' pear fruit grafted onto different rootstocks, except for the glucose and total sugars. Fructose was the most abundant sugar component in the pear fruit of 'Deveci'. The fructose content of the fruits varied from 42.4 g kg^{-1} to 32.4 g kg^{-1}. The fruits of the 'Deveci' pear grafted on the seedling had the higher fructose content than the EMC and seedling rootstocks (Table 2). The content of fructose in the present study are in accordance with Colaric *et al*. (2006) who reported that fructose is the most abundant sugars component in the 'Williams' pear.

Table 2. Comparison between rootstocks for the sugars content of 'Deveci' pear.
Çizelge 2. 'Deveci' armudunun şeker içeriği bakımından anaçların karşılaştırılması.

Sugars	Rootstocks		
(g kg^{-1})	BA 29	EMC	Seedling
Fructose	32.4 b*	39.2 a	42.4 a
Glucose	18.7 a	22.3 a	26.7 a
Sucrose	2.0 b	4.3 a	4.9 a
Total sugars	53.0 a	65.8 a	74.0 a

* The difference between means shown on the same line with the same letter is not significant according to Duncan's Multiple Range test at $P<0.01$.

The highest sucrose content was observed from seedling and EMC (4.9 g kg^{-1} and 4.3 g kg^{-1}, respectively), the lowest in the BA 29 (2.0 g kg^{-1}). The fruits of the 'Deveci' pear grafted on the seedling had the higher glucose and total sugars than the EMC and BA 29 rootstocks (Table 2). These results were very similar to with the findings of Dolenc and Stampar (1997). And also, these results are similar consistent with Colaric *et al*. (2006), who reported that pears contained up to 73.54 g kg^{-1} FW of fructose, 9.42 g kg^{-1} FW of glucose, 7.94 g kg^{-1} FW of sucrose. Gundogdu et al. (2014), who determined values of glucose between 6.67-13.89 g $100g^{-1}$ FW, fructose between 8.52-18.37 g $100g^{-1}$ FW, saccharose between 0.954-1.564 g $100g^{-1}$ FW in hawthorn species (*Crataegus spp*.).

CONCLUSION

In the study was determined the sugars and organic acid contents of 'Deveci' pear grafted on different rootstocks. Statistical analysis showed significantly higher content of acetic, ascorbic, citric, fumaric and tartaric acid in fruits on the BA 29 quince rootstock. Significantly, the highest content of fructose, glucose, sucrose and total sugars were determined in the fruits of 'Deveci' pear grafted on the seedling rootstock. Malic, ascorbic and propionic acids

were detected as major organic acids. Malic and ascorbic acid in BA 29 rootstock was higher than EMC and seedling rootstocks. Contrarily, propionic acid was higher in seedling rootstock. Tartaric, ascorbic, citric and fumaric acids in BA 29 and EMC rootstock were higher than the content of seedling rootstock. Fructose and glucose were the two most abundant sugars in 'Deveci' pear for BA 29, EMC and seedling rootstocks. The total sugar content of the seedling rootstock was higher than BA 29 and EMC. The results of this study showed that there were significant effects of rootstocks on sugar and organic acid content of pear fruits. However, more detailed studies are needed to confirm and clarify how those differences occur.

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