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Research Article

Correlation between Pomegranate Genotypes and Phenolic Compounds

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Abstract. In this study, the individual phenolic compounds and relationship with each other of 21 genotypes of Siirt (Şirvan) region were determined. Protocatechuic acid, vanillic acid, gallic acid, rutin, quercetin, catechin, chlorogenic acid, caffeic acid, syringic, p-coumaric, ferulic acid, and phloridzin content were recorded. Catechin was identified to be dominant phenolic. Statistically, 56 ŞİR 10 genotype had come to the fore with five phenolic compounds. The lowest content of three phenolic compounds was measured in 56 ŞİR 20 genotype. Syringic was recorded to be the lowest level phenolic compound. It was observed that there were positive correlation rutin, caffeic, vanillic and ferulic acid. However, the negative correlation was determined between syringic and caffeic acid. Pomegranate genotypes of Şirvan have been found to be rich in phenolic compounds which have significant health effects.

Fenolik Bileşikler ve Nar Genotipleri Arasındaki Korelasyon

Anahtar kelimeler:
Korelasyon, fenolik bileşik, *Punica granatum*

Özet. Bu çalışmada, bireysel fenolik bileşikler ve Siirt (Şirvan) bölgesinde yetişen 21 genotipin birbirleriyle ilişkisi belirlenmiştir. Protocatechuic asit, vanillik asit, gallik asit, rutin, kersetin, kateşin, klorojenik asit, kafeik asit, şırınga edici, p-kumarik, ferulik asit ve ploridzin içerikleri kaydedilmiştir. Kateşin baskın fenolik bileşik olarak tanımlanmıştır. 56 ŞİR 10 genotipi, istatistiksel olarak, en yüksek beş fenolik bileşik içeriğine sahip olarak ön plana çıkmıştır. 56 ŞİR 20 genotipi ise üç fenolik bileşik açısından en düşük içeriğe sahip olmuştur. Syringic en düşük seviye fenolik bileşik olarak kaydedilmiştir. Rutin, kafeik, vanilic ve ferulik asit arasında pozitif korelasyonu olduğu görülmüştür. Ancak, siringik ve kafeik asit arasında negatif korelasyon belirlenmiştir. Şirvan yöresindeki nar genotiplerinin, sağlık üzerinde önemli etkileri olan fenolik bileşikler açısından zengin olduğu tespit edilmiştir.

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INTRODUCTION

The homeland of pomegranate fruit, one of the oldest cultivated agricultural products; especially Iran, including the southern and south-eastern Turkey, including the Middle East, the Caucasus and northern India is known (Stover and Mercure, 2007; Kurt and Şahin, 2013). Pomegranate (*Punica granatum L*.) is one of the important fruit grown in both tropical and subtropical conditions (Korkmaz *et al*., 2016). Pomegranate cultivation in our country, especially in the Mediterranean Region, Aegean and Southeast Anatolia is distributed. According to 2017 data; it is being carried out intensively in Antalya, Muğla and Mersin (TÜİK, 2019). Due to its ecological conditions or not too selective, pomegranate cultivation has increased in recent years (Kurt and Şahin, 2013). The use of pomegranate in different food industries such as fruit juice, pomegranate syrup, wine, liqueur, concentrate, syrup, syrup, vinegar, jam and jelly, in addition, the production of drugs due to its compounds has also increased the tendency for pomegranate.

Pomegranate fruit contains significant amounts of acid, sugar, vitamins, polysaccharides, polyphenols and minerals (Tamer, 2006). In addition to being rich in nutrients such as potassium, phosphorus and magnesium, it contains plenty of K and C vitamins (Anonymous, 2016). But the composition of the fruit varies depending on genetic factors, growing conditions, ecological factors (Poyrazoglu *et al*., 2002), the maturity and storage conditions (Tamer, 2006).

Phenolic compounds are substances that contribute to the color and sensory properties of many fruits, vegetables and beverages. Research which is conducted in recent years has shown that phenolic compounds commonly found in plant products are very useful compounds for health reasons due to their antioxidant activities (Okatan *et al*., 2015; Okatan *et al*., 2018). The phenolic compounds contained in the pomegranate fruit increase the value of antioxidant and their health benefits. Studies have shown that pomegranate has the effect of antioxidant, antiinflammatory, anticancer (Toklu *et al*., 2007), antimicrobial (Duman *et al*., 2009) and antimalarial (Reddy *et al.,* 2007). It is reported in studies that the pomegranate prevents colon cancer and stop disease progression (Larrossa *et al*., 2010). Also, it is known that it is used in the treatment of prostate cancer and prevents calcification (Malik *et al*., 2005). In addition to its antioxidant content, pomegranate fruit provides liquids the blood, lowers cholesterol and benefits for Alzheimer's and heart diseases (Singh *et al*., 2008). These phenolic compounds, which are abundant in pomegranate fruit, provide the flavor formation of the fruit and are effective in the formation of the harsh taste. So, they have important effects on the fruit juice processing industry. It is also known to cause the formation of residues.

To be found southeast of Tukey, Siirt is one of the distribution areas of pomegranate genotypes (Kurt and Şahin, 2013). In addition, this province is seen as an important region, taking second place in the region in terms of pomegranate production (TÜİK, 2019). There are microclimate areas in the district and pomegranate cultivation is common in these regions.

The composition of pomegranate fruit and its mechanisms of action must be known due to positive effects on health. For this purpose, it was aimed to identify individual phenolic compounds of different pomegranate genotypes grown in Şirvan district of Siirt province.

MATERIAL AND METHOD

This study was carried out in Şirvan district of Siirt province between 2008-2009. Located in the north of the province of Siirt, Şirvan district has a mountainous and rugged terrain. The continental climate prevails in the district so winters are cold and summers are hot. Autumn and winter are rainy. In Şirvan, the average annual temperature is 14.8 °C and the average annual rainfall is 821 mm. July is the hottest month and August is the driest month. Peanut, pomegranate, walnut, grape and rice are the leading products of agricultural production.

The samples collected from the fully matured fruits in each genotype were brought to the laboratory and stored at -20 °C until laboratory analysis. The gallic acid, catechin, chlorogenic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, phloridzin, protocatechuic, vanillic acid, rutin and quercetin were determined in the research. The method of Rodriguez-Delgado *et al*. (2001) was developed and used for the separation of phenolic compounds with HPLC. The samples collected were diluted with distilled water at the ratio of 1:1. They were centrifuged at 15000 rpm for 15 min., the supernatant was filtered with 0.45 µm millipore filters and then injected to HPLC. The chromatographic separation was conducted by using DAD detector (Agilent. USA) and 250*4.6 mm, 4 µm ODS colon HiChrom, USA) in Agilent 1100 (Agilent) HPLC system. Solvent A Methanol-acidic

acid-water (10:2:88), Solvent B Methanol-acidic acid-water (90:2:8) were used as the mobile phase. The separation was conducted at 254 and 280 nm and the flow rate was determined as 1 mL/min. and the injection volume was determined as 20 µL.

The data were subjected to statistical analysis in SPSS package program and Duncan multiple comparison test was used to determine the differences. The principal component analysis (PCA) was used to determine the correlation between pomegranate genotypes and phenolic compound contents. XLSTAT 2016 (Addinsoft, New York, USA) was used to perform statistical analyses.

RESULTS AND DISCUSSION

In this study, 12 phenolic compounds were determined in pomegranate by HPLC and shown in Table 1 and 2. The differences between genotypes were statistically significant ($P< 0.05$). Catechin was identified to be dominant phenolic whereas syringic acid was recorded to be the lowest level phenolic compound. Catechin was followed by gallic acid, rutin and protocatechuic acid . Also, the highest phenolic content was recorded as 5.761 g L^{-1} (gallic acid). It was found that 56 ŞİR 10 genotype had the highest content of five phenolic compounds statistically (quercetin, catechin, chlorogenic acid, ferulic acid, phloridzin). The highest protocatechuic acid, ferulic and caffeic acid were determined in 56 ŞİR 19 genotype. The lowest content of three phenolic compounds (quercetin, gallic acid, catechin) was measured in 56 ŞİR 20 genotype (Table 1, 2). A linear correlation was obtained between rutin, caffeic, vanillic and ferulic acid. Also, it was observed that there were positive correlation chlorogenic acid, catechin and phloridzin. However, the negative correlation was determined between syringic and caffeic acid (Figure 1a).

As shown Table 1, protocatechuic acid content ranged from 0.988 (56 ŞİR 19) to 0.079 g L^{-1} (56 ŞİR 03). The highest vanillic, quercetin and rutin were measured as 0.277, 0.780 and 2.918 g L^{-1} respectively. The highest gallic acid content was recorded in 56 ŞİR 03 (5.761 g L^{-1}) but the highest catechin content was recorded in 56 ŞİR 10 (5.228 g L^{-1}). As shown Table 2, it was found that 56 ŞİR 16 had the highest syringic and p-coumaric acid but it had the lowest phloridzin (0.174 g L^{-1}) content. Chlorogenic acid content ranged from 0.033(56 ŞİR 18) – 0.317 g L^{-1} (56 ŞİR 10). 56 ŞİR 15, 17, 18 and 19 genotypes had high rutin and caffeic acid content (Figure 1c). Both catechin and chlorogenic acid content were recorded higher in 56 ŞİR 03, 07, 10, 11 genotypes. Similarly, 56 ŞİR 04 and 56 ŞİR 19 genotypes had high ferulic, vanillic, gallic and protocatechuic acid (Figure 1c). It was determined that 56 ŞİR 12 and 13 genotypes had close phenolic contents (Figure 1b). A similar situation was found between 56 ŞİR 15 and 56 ŞİR 18. Also, It was observed that 56 ŞİR 01, 56 ŞİR 08 and 56 ŞİR 09 genotypes showed similar values in terms of phenolic contents (Figure 1b).

The different results were found in the studies on the phenolic compound content of pomegranate. Mphahlele *et al.* (2016) found that catechin was dominant phenolic compound and it was followed by rutin and gallic acid. But the other researchers determined that the highest phenolic compounds content was gallic acid (0.034-30.86 g L^{-1}) and the second phenolic compound was catechin in Turkey pomegranate (Poyrazoğlu *et al.*, 2002). Our results are consistent with Mphahlele *et al.* (2016) and are partially similar to Poyrazoğlu *et al.* (2002). In a study conducted in Germany, the researchers measured as 1.8 mg L^{-1} vanillic acid and 3.6 mg L^{-1} ferulic acid. Also, they found that caffeic acid ranged from 5.2-10.8 mg L^{-1} (Fisher *et al.,* 2011). Although catechin content was the dominant phenolic compound (5.225 g L^{-1}) in our findings, it was recorded 0.4 mg 100 ml^{-1} by some researchers in Spain (de Pascual-Teresa *et al.,* 2000). In addition, in the study conducted with different genotypes in Siirt region, the highest phenolic compound was found as catechin (Gundogdu and Yılmaz, 2012). Also, gallic, ferulic and vanillic acid contents in our study was higher to those reported by (Gundogdu and Yılmaz, 2012), but caffeic, syringic, phloridzin, chlorogenic and p-coumaric acid contents was similar. Contrary to our study, Turgut and Seydim, (2013) determined that catechin was the lowest phenolic compound (0.04-0.035 mg L^{-1}) in Mediterranean Region. The researchers measured the highest gallic, rutin and chlorogenic acid content as 4.26, 2.17, 1.63 mg L^{-1} respectively. In a different study, gallic acid was determined to be the dominant phenolic in Bhagwa cultivar and it was followed by ellagic acid and catechin (Fawole and Opara, 2013a). Similarly, in other study conducted with Ruby cultivar, the researchers also stated that the highest value of gallic acid and this content was lower than our findings (Fawole and Opara, 2013b). In accordance with our results, Mphahlele *et al.* (2014) stated that catechin was the most dominant flavonoids while gallic acid was the dominant phenolic acid in pomegranate juice. It is seen that there are differences between studies. It is thought that the reasons such as ecological factor and different genetic resources provide this difference.

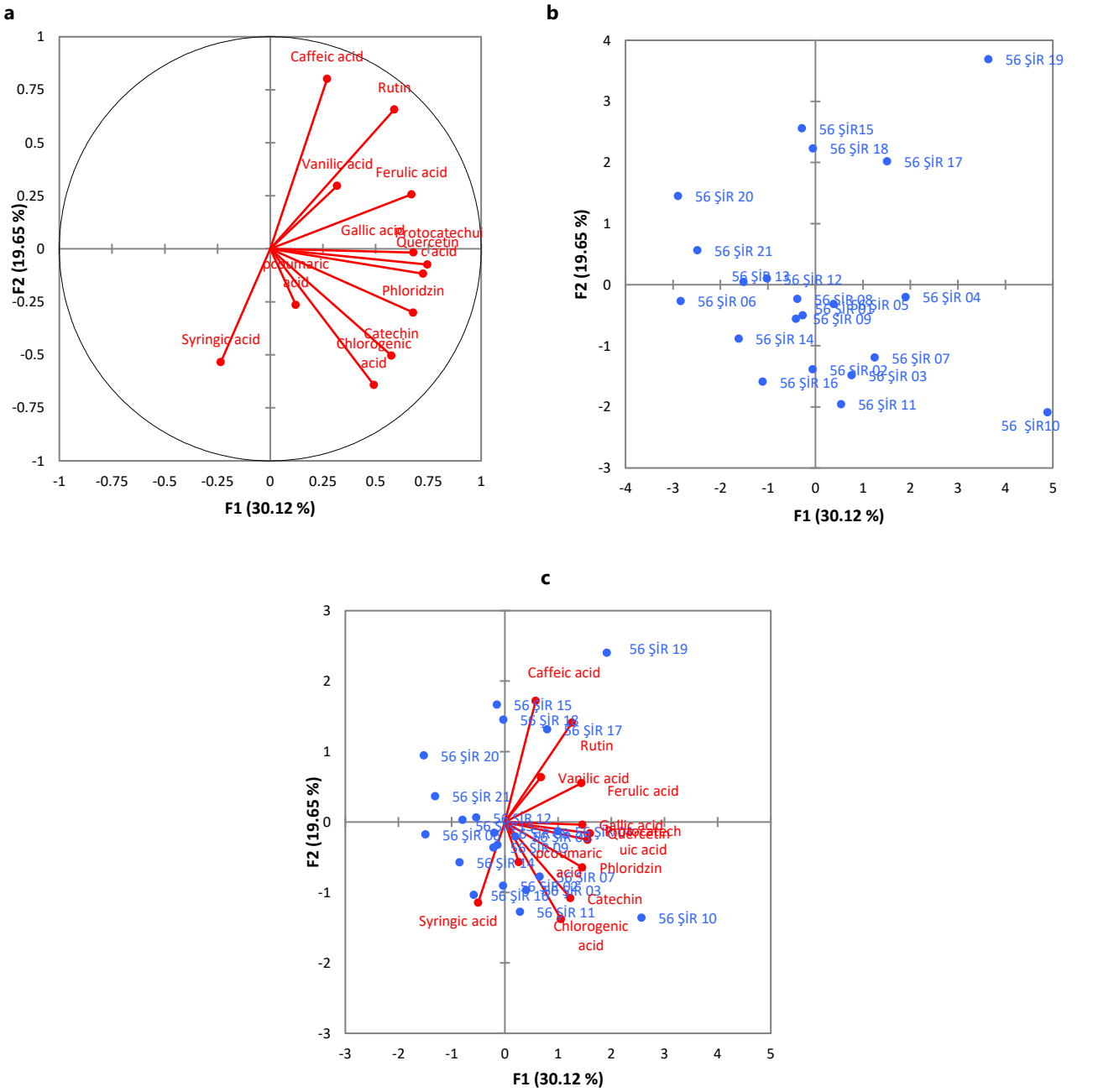

Figure 1. Correlation relationships a)Correlation between phenolic compounds b)Correlation between genotypes c)Correlation between genotypes and phenolic compounds.

Şekil 1. Korelasyon ilişkileri a)Fenolik bileşikler arasındaki korelasyon b)Genotipler arasındaki korelasyon c)Genotipler ile fenolik bileşikler arasındaki korelasyon.

Table 1. Protocatechuic acid, vanillic acid, gallic acid, rutin, quercetin, catechin content of pomegranate genotypes (g L^{-1}).
Tablo 1. Nar genotiplerinin protokateşuik asit, vanilik, rutin, kuersetin, gallic asit ve kateşin içerikleri (g L^{-1}).

Genotypes	Chlorogenic acid	Caffeic acid	Syringic acid	p-coumaric acid	Ferulic acid	Phloridzin
56 ŞİR 01	0.127 ± 0.006 gh*	0.017 ± 0.000 e	0.042 ± 0.001 d	0.051 ± 0.002 b	0.223 ± 0.017 hi	0.409 ± 0.016 e
56 ŞİR 02	0.111 ± 0.011 i	0.016 ± 0.001 e	0.017 ± 0.000 hi	0.094 ± 0.003 b	0.144 ± 0.010 l	0.693 ± 0.022 b
56 ŞİR 03	0.240 ± 0.013 b	0.021 ± 0.000 de	0.011 ± 0.000 k	0.124 ± 0.011 b	0.158 ± 0.008 jkl	0.295 ± 0.015 i
56 ŞİR 04	0.173 ± 0.005 e	0.018 ± 0.001 e	0.019 ± 0.002 h	0.141 ± 0.016 ab	0.344 ± 0.009 de	0.614 ± 0.011 c
56 ŞİR 05	0.159 ± 0.007 f	0.021 ± 0.000 de	0.011 ± 0.009 k	0.104 ± 0.012 b	0.441 ± 0.014 c	0.306 ± 0.014 hi
56 ŞİR 06	0.091 ± 0.009 k	0.021 ± 0.002 de	0.048 ± 0.007 c	0.054 ± 0.008 b	0.127 ± 0.009 m	0.176 ± 0.009 n
56 ŞİR 07	0.187 ± 0.006 d	0.022 ± 0.000 cde	0.029 ± 0.004 f	0.074 ± 0.005 b	0.357 ± 0.007 d	0.502 ± 0.017 d
56 ŞİR 08	0.115 ± 0.010 hi	0.019 ± 0.001 de	0.015 ± 0.001 ij	0.084 ± 0.011 b	0.238 ± 0.015 gh	0.351 ± 0.020 g
56 ŞİR 09	0.136 ± 0.015 g	0.017 ± 0.000 e	0.012 ± 0.009 jk	0.087 ± 0.006 b	0.335 ± 0.022 e	0.233 ± 0.009 k
56 ŞİR 10	0.318 ± 0.016 a	0.037 ± 0.003 b-e	0.043 ± 0.007 d	0.080 ±0.007 b	0.549 ± 0.020 a	1.255 ± 0.034 a
56 ŞİR 11	0.207 ± 0.011 c	0.020 ± 0.001 de	0.062 ± 0.005 b	0.091 ± 0.003 b	0.156 ± 0.008 kl	0.252 ± 0.010 j
56 ŞİR 12	0.109 ± 0.003 i	0.025 ± 0.000 cde	0.029 ± 0.001 f	0.057 ± 0.006 b	0.454 ± 0.011 c	0.303 ± 0.021 i
56 ŞİR 13	0.063 ± 0.001 l	0.033 ± 0.001 b-e	0.025 ± 0.000 g	0.087 ± 0.001 b	0.164 ± 0.017 jk	0.294 ± 0.013 i
56 ŞİR 14	0.095 ± 0.006 jk	0.011 ± 0.000 e	0.063 ± 0.003 b	0.063 ± 0.003 b	0.358 ± 0.010 d	0.190 ± 0.009 m
56 ŞİR 15	0.110 ± 0.002 i	0.055 ± 0.001 bc	0.011 ± 0.000 k	0.082 ± 0.010 b	0.244 ± 0.013 fg	0.200 ± 0.017 lm
56 ŞİR 16	0.038 ± 0.007 m	0.014 ± 0.003 e	0.076 ± 0.010a	0.278 ± 0.019 a	0.257 ± 0.011 f	0.174 ± 0.018 n
56 ŞİR 17	0.038 ± 0.003 m	0.025 ± 0.001 cde	0.012 ± 0.000 jk	0.094 ± 0.013 b	0.512 ± 0.016 b	0.318 ± 0.014 h
56 ŞİR 18	0.034 ± 0.002 m	0.061 ± 0.003 b	0.015 ± 0.000 ij	0.094 ± 0.006 b	0.244 ± 0.007 fg	0.403 ± 0.019 e
56 ŞİR 19	0.040 ± 0.004 m	0.096 ± 0.007 a	0.011 ± 0.000 k	0.077 ± 0.009 b	0.563 ± 0.006 a	0.358 ± 0.017 g
56 ŞİR 20	0.067 ± 0.006 l	0.053 ± 0.004 bcd	0.024 ± 0.001 g	0.053 ± 0.003 b	0.218 ± 0.013 i	0.206 ± 0.009 l
56 ŞİR 21	0.106 ± 0.007 ij	0.036 ± 0.009 b-e	0.033 ± 0.003 e	0.044 ± 0.008 b	0.176 ± 0.014 j	0.389 ± 0.017 f

*There are significant ($P < 0.05$) differences between values with different letters in the same lines.

Table 2. Chlorogenic acid, caffeic acid, syringic, p-coumaric, ferulic acid, phloridzin content of pomegranate genotypes (g L^{-1}).
Tablo 2. Nar genotiplerinin klorojenik, kafeik, siringik, p-kumarik, ferulik ve phloridzin içerikleri (g L^{-1}).

Genotypes	Chlorogenic acid	Caffeic acid	Syringic acid	p-coumaric acid	Ferulic acid	Phloridzin
56 ŞİR 01	0.127 ± 0.006 gh*	0.017 ± 0.000 e	0.042 ± 0.001 d	0.051 ± 0.002 b	0.223 ± 0.017 hi	0.409 ± 0.016 e
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56 ŞİR 04	0.173 ± 0.005 e	0.018 ± 0.001 e	0.019 ± 0.002 h	0.141 ± 0.016 ab	0.344 ± 0.009 de	0.614 ± 0.011 c
56 ŞİR 05	0.159 ± 0.007 f	0.021 ± 0.000 de	0.011 ± 0.009 k	0.104 ± 0.012 b	0.441 ± 0.014 c	0.306 ± 0.014 hi
56 ŞİR 06	0.091 ± 0.009 k	0.021 ± 0.002 de	0.048 ± 0.007 c	0.054 ± 0.008 b	0.127 ± 0.009 m	0.176 ± 0.009 n
56 ŞİR 07	0.187 ± 0.006 d	0.022 ± 0.000 cde	0.029 ± 0.004 f	0.074 ± 0.005 b	0.357 ± 0.007 d	0.502 ± 0.017 d
56 ŞİR 08	0.115 ± 0.010 hi	0.019 ± 0.001 de	0.015 ± 0.001 ij	0.084 ± 0.011 b	0.238 ± 0.015 gh	0.351 ± 0.020 g
56 ŞİR 09	0.136 ± 0.015 g	0.017 ± 0.000 e	0.012 ± 0.009 jk	0.087 ± 0.006 b	0.335 ± 0.022 e	0.233 ± 0.009 k
56 ŞİR 10	0.318 ± 0.016 a	0.037 ± 0.003 b-e	0.043 ± 0.007 d	0.080 ±0.007 b	0.549 ± 0.020 a	1.255 ± 0.034 a
56 ŞİR 11	0.207 ± 0.011 c	0.020 ± 0.001 de	0.062 ± 0.005 b	0.091 ± 0.003 b	0.156 ± 0.008 kl	0.252 ± 0.010 j
56 ŞİR 12	0.109 ± 0.003 i	0.025 ± 0.000 cde	0.029 ± 0.001 f	0.057 ± 0.006 b	0.454 ± 0.011 c	0.303 ± 0.021 i
56 ŞİR 13	0.063 ± 0.001 l	0.033 ± 0.001 b-e	0.025 ± 0.000 g	0.087 ± 0.001 b	0.164 ± 0.017 jk	0.294 ± 0.013 i
56 ŞİR 14	0.095 ± 0.006 jk	0.011 ± 0.000 e	0.063 ± 0.003 b	0.063 ± 0.003 b	0.358 ± 0.010 d	0.190 ± 0.009 m
56 ŞİR 15	0.110 ± 0.002 i	0.055 ± 0.001 bc	0.011 ± 0.000 k	0.082 ± 0.010 b	0.244 ± 0.013 fg	0.200 ± 0.017 lm
56 ŞİR 16	0.038 ± 0.007 m	0.014 ± 0.003 e	0.076 ± 0.010a	0.278 ± 0.019 a	0.257 ± 0.011 f	0.174 ± 0.018 n
56 ŞİR 17	0.038 ± 0.003 m	0.025 ± 0.001 cde	0.012 ± 0.000 jk	0.094 ± 0.013 b	0.512 ± 0.016 b	0.318 ± 0.014 h
56 ŞİR 18	0.034 ± 0.002 m	0.061 ± 0.003 b	0.015 ± 0.000 ij	0.094 ± 0.006 b	0.244 ± 0.007 fg	0.403 ± 0.019 e
56 ŞİR 19	0.040 ± 0.004 m	0.096 ± 0.007 a	0.011 ± 0.000 k	0.077 ± 0.009 b	0.563 ± 0.006 a	0.358 ± 0.017 g
56 ŞİR 20	0.067 ± 0.006 l	0.053 ± 0.004 bcd	0.024 ± 0.001 g	0.053 ± 0.003 b	0.218 ± 0.013 i	0.206 ± 0.009 l
56 ŞİR 21	0.106 ± 0.007 ij	0.036 ± 0.009 b-e	0.033 ± 0.003 e	0.044 ± 0.008 b	0.176 ± 0.014 j	0.389 ± 0.017 f

*There are significant ($P < 0.05$) differences between values with different letters in the same lines.

CONCLUSION

Şirvan has an important potential for pomegranate genotypes and cultivation. With this study, it has been thought that the diversity in the region will be revealed and this diversity will be gained to the local and national markets. As a result, phenolic compounds of Siirt (Şirvan) pomegranates were found to be high. It was observed that particularly, 56 ŞİR 10 and 56 ŞİR 19 genotypes contained to be high phenolic compounds. It has been concluded that these species should be brought into the pomegranate sector which is an important fruit type in terms of food and pharmaceutical industry. Primarily, genotypes should be considered on a preferential basis in breeding studies. Hybridization and registration should be done with these genotypes and results should be gained to the regional market. In addition, it should be laid out a garden with these genotypes and pomegranate potential in the region should be increased, table and industry processing sector should be revived more. In addition to this, taking into consideration that the studies on pomegranate in the eastern and southeastern part of our country are few, care should be taken to increase these and similar studies.

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