

## COMPOSITIONAL DIVERSITY IN FRUITS OF ROWANBERRY (*Sorbus aucuparia* L.) GENOTYPES ORIGINATING FROM SEEDS

Mehmet Ramazan BOZHUYUK<sup>1\*</sup>, Sezai ERCISLI<sup>2</sup>, Rayda Ben AYED<sup>3</sup>, Tunde JURÍKOVA<sup>4</sup>,  
Hafize FIDAN<sup>5</sup>, Gulce ILHAN<sup>2</sup>, Gursel OZKAN<sup>2</sup>, Halil Ibrahim SAGBAS<sup>2</sup>

<sup>1</sup>Igdir University, Faculty of Agriculture, Horticulture Department, Igdir, Turkey

<sup>2</sup>Ataturk University, Faculty of Agriculture, Horticulture Department, Erzurum, Turkey

<sup>3</sup>University of Sfax, Centre of Biotechnology of Sfax, Tunisia

<sup>4</sup>Institute for Education of Pedagogics, Faculty of Central European Studies, Constantine the  
Philosopher University in Nitra, Drazovska 4, SK-949 74 Nitra, Slovak Republic

<sup>5</sup>Department of Catering and Tourism, University of Food Technologies, Plovdiv, Bulgaria

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Turkey has great ecological, topological and geographical diversity within the country and this diversity has contributed not only to a high genetic diversity, but has also allowed the successful introduction and cultivation of a great number of fruit tree taxa. Turkey is also known to have a great richness of wild edible fruits with regard to variety and biological diversity. Rowanberry or mountain ash (*Sorbus aucuparia* L.) is one of the wild edible fruits naturally found most parts of Turkey. Present study describes morphological (tree growth habit, fruit color) and biochemical fruit traits (vitamin C, organic acids, specific sugars, total phenolic content, total anthocyanin content, total antioxidant capacity) of 17 seed propagated rowanberry genotypes. We found significant differences among almost all studied parameters. The genotypes had diverse *L*, chroma and hue values, which found between 28.76-42.38%; 24.11-29.45% and 33.13-42.66%, respectively. Among sugars and organic acids, Glucose and Malic acid were dominant in rowanberry fruits, respectively. Total phenolic content, total anthocyanin content, vitamin C and antioxidant activity varied from 123-189 mg GAE per 100 g, 18-57 mg per 100 g, 25.6-40.2 mg per 100 g and 3.36-6.92 mM trolox equivalent per 100 g of fresh weight (FW) basis. Results suggest using rowanberry fruits in production of functional foods with high biologically active properties.

*Keywords:* *Sorbus aucuparia* L., seed propagation, functional foods.

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*Corresponding authors:* Mehmet Ramazan Bozhuyuk, Igdir University, Faculty of Agriculture, Horticulture Department, Igdir, Turkey. E-mail: [mrbozhuyuk@gmail.com](mailto:mrbozhuyuk@gmail.com)

## INTRODUCTION

Underutilized, wild edible or neglected horticulture plants show great variability in nature and represent part of diet of Mediterranean, Middle East, Africa and Middle Asia countries, because there is close relationship between cultural heritage and biological diversity. Therefore a new social, cultural and economic value must be given to these local valuable resources in order to maintain traditions for future generations (VIJAYAN *et al.*, 2008; ZIA-UL-HAQ *et al.*, 2014; GUNEY *et al.*, 2019).

The northeastern part of Turkey is responsible for more than half of the country's wild edible fruit production with potential for expansion, as there is a growing market for its consumption, since the population has searched for foods that in addition to nourishing, can offer compounds that provide health benefits. The region has also rich for the local horticultural plants (SAHIN *et al.*, 2002; ERCISLI *et al.*, 2008; ALP *et al.*, 2016; EYDURAN *et al.*, 2015; 2016).

Rowanberry (*Sorbus aucuparia* L.), 'kuş üvezi' in Turkish is one of the most important wild edible fruits found in Turkey's flora. The specie is found in European countries, Caucasia and Siberia. In Turkey, seed propagated rowanberry trees mostly found between 800-2500 meter above sea levels (BALTACIOGLU, 2006).

Rowanberry fruits are much appreciated by consumers, and its popularity stems mainly from its pleasant taste, nutritional characteristics, appearance and suitability for fresh consumption, being also widely used for canning, jellies and sweets (JURIKOVA *et al.*, 2014; MIKULIC-PETKOVSEK *et al.*, 2017). It is accepted functional fruit that can provide not only basic nutritional and energy, but also an additional physiological benefit. Usually, the functionality of rowanberry fruits is related to some of its ingredients because of which consumers increasingly prefer to consume these fruits.

Therefore, in last two decades, there has been a greater interest in studying and quantifying biological active substances with health-promoting properties including vitamin C, phenolic metabolites, minerals, organic acids, specific sugars present in wild edible fruits viz. rowanberry, rose hip, cornelian cherry, barberry, myrtle, elderberry etc. (ERCISLI *et al.*, 2008, 2012; SERCE *et al.*, 2010). Their interest as a source of nutraceuticals has been highlighted in recent studies (LEONTI, 2002; DOGAN *et al.*, 2014; MIKULIC-PETKOVSEK *et al.*, 2012, 2015).

In addition to this, wild edible fruits may have great potential as a source of unusual colours and flavours (SALVATORE *et al.*, 2002). Due to continuous seed propagation of wild edible fruit species, there is a great variability for most of the morphological and biochemical characteristics of wild non-cultivated fruits that could be important for breeding activities aiming to obtain healthier fruits.

Until now, fruit quality effects have been much more studied in *Sorbus domestica* L. than in *Sorbus aucuparia* L. and little is known about the influence of genotypes with different genetic origin on morphological and biochemical composition of the fruit. Thus, in this research, we aimed to determine and compare some important morphological and biochemical features of a wide number of *Sorbus aucuparia* L. genotypes, naturally growing in Artvin province in Turkey due to their potential application as functional foods.

## MATERIALS AND METHODS

### *Plant material*

Ripe fruits of *Sorbus aucuparia* L. trees naturally grown in Artvin province located in the Northeastern part of Turkey were sampled. A total 17 pre-selected seed propagated rowanberry genotypes were used which fruits were harvested in August 2017. Average age of the investigated genotypes were 20.

### *Morphological characteristics*

Tree growth habit and external fruit colors were determined. In the entire fruits, values of  $L^*$  (brightness or lightness),  $a^*$  ( $-a^*$  = greenness,  $+a^*$  = redness),  $b^*$  ( $-b^*$  = blueness,  $+b^*$  = yellowness),  $C^*$  (chroma) and Hue (lightness's angle) were measured using a chromameter, (CR-400 Konica Minolta, Tokyo, Japan) (MCGUIR, 1992)

### *Sample preparation and extraction*

For organic acids, specific sugars, total phenolic contents, total anthocyanin content and total antioxidant capacity, the harvested fruit was immediately frozen and stored at  $-20\text{ }^{\circ}\text{C}$  until further analysis. During the analysis, the fruits were taken from refrigerator and thawed to  $24\text{-}25\text{ }^{\circ}\text{C}$ . A laboratory blender was used to homogenise the fruit samples (100 g lots of fruits per genotypes) and a single extraction procedure (taking 3 g aliquots transferred inside tubes and extracted for 1 hour with 20 mL buffer including acetone, water (deionized), and acetic acid (70:29.5:0.5 v/v) was carried out (SINGLETON and ROSSI, 1965).

### *Extraction of sugars and organic acids*

Five grams of samples slurries were mixed with deionized water or metaphosphoric acid (2.5%) for the analysis of individual sugars (Glucose, Fructose, Sucrose and Sorbitol) and organic acids (Malic, Citric and Tartaric acid), respectively. The obtained homogenates were centrifuged at 10000 rpm for 10 min. The samples were filtered into HPLC vials using  $0.45\text{ }\mu\text{m}$  PTFE membrane filter for analysis. All HPLC solvents were sonicated. All samples and corresponding standard injection were repeated three times and the mean values were calculated.

### *Chromatographic conditions*

The Perkin Elmer HPLC system controlled by Totalchrom navigator software (version 6.2.1), consists of a pump and UV detector was used for analysis of the samples. Organic acids determination was performed according to method reported by SHUI and LEONG (2002). The sugars were determined using the method of BARTOLOME *et al.* (1995) with help of HPLC with refractive index (RI) detector. The separation was carried out on SGE SS Exsil amino column ( $250 \times 4.6\text{ mm ID}$ ). The isocratic elution was performed using acetonitrile (80%) and deionized water (20%) with a flow rate of  $0.9\text{ mL/min}$ . The column was operated at  $30\text{ }^{\circ}\text{C}$  and the sample injection volume was  $20\text{ }\mu\text{L}$ . Quantification of organic acids and sugars were performed against the reference standards.

### *Vitamin C*

Flesh parts of fruit samples were used to assess pH and vitamin C. Vitamin C (mg/100

g) was determined with the reflectometer set of Merck Co (Merck RQflex) (ERCISLI *et al.*, 2012).

#### *Total phenolic content*

The total phenolic content (TPC) of the samples was evaluated using the method of SINGLETON and ROSSI (1965). In this procedure, each extract (1 mL) was mixed with Folin-Ciocalteu's reagent and water 1:1:20 (v/v). The samples were incubated for 8 min. Then sodium carbonate (10 mL) having a concentration of 7% (w/v) was added. After incubation for 2 h, the absorbance at 750 nm was measured. The total phenolic content was calculated against the reference standard calibration curve of Gallic acid. The TPC was expressed as mg of Gallic acid equivalents (GAE) per 100 g of sample (FW).

#### *Total anthocyanin content*

Total anthocyanins contents were measured using a pH differential method according to GIUSTI and WROLSTAD (2001) with help of UV-visible spectrophotometer. The absorbance was measured both at 533 and 700 nm in buffers solution at pH 1.0 and 4.5. The total anthocyanins were calculated from the absorbance values and molar extinction coefficient value of 29,600. Total anthocyanins contents were expressed as mg cyanidin 3-galactoside per 100 g of sample (FW).

#### *Ferric Reducing Antioxidant Power Assay (FRAP)*

FRAP (Ferric reducing antioxidant power) assay was used for antioxidant capacity analysis. For this purpose, acetonic fruit extract (50  $\mu$ L), FRAP reagent (2.95 mL), acetate buffer (0.1 mol/L), TPTZ (10 mmol/L), and ferric chloride of 20 mmol/L (10:1:1 v/v/v) were used. The values of samples absorbance were compared with those of the reference standard calibration curves in the range of 10-100  $\mu$ mol/L of Trolox to determine FRAP values. The FRAP was expressed as mM per 100 g of Trolox equivalent on the basis of the fresh weight of the fruit (BENZIE and STRAIN, 1996).

#### *Statistical Analysis*

The experiments were performed in quintuplicate. The SPSS software package was used for analysis. For analysis of variance, the obtained data were used for means calculation. Duncan multiple range tests were performed at the significant level of  $p < 0.05$ .

## RESULTS AND DISCUSSION

Tree growth habit of tested rowanberry genotypes was shown in Table 1. The majority of genotypes had vase like growth habit and rest of the genotypes had upright growth habit (Table 1).

As shown in Table 1, the fruit color lightness ( $L$ ) color intensity (chroma) and color appearance (hue) of the rowanberry genotypes was found to be statistically significant. The genotype T4 had the highest  $L$  (42.38%) and genotype T11 had the highest chroma (29.45%) values than rest of the genotypes (Table 1). Hue values were found between 33.13% (T7) and 42.66% (T4). Fruit skin color is considered to be the most important index of quality and maturity. As previously reported by MIKULIC-PETKOVSEK *et al.* (2017) there were strong

relationships between maturity and color development. They found *L*, chroma and hue values in 2 rowanberry cultivars in Czech Republic between 33-45%; 28-30% and 33-43%, respectively which is in agreement with our results.

Table 1. Growth habit and fruit color indices of rowanberry genotypes

Genotype	<i>L</i>	Chroma	Hue	Growth habit
T1	33.44de	26.41cd	35.50d	Semi upright
T2	29.70gh	24.11f	34.44de	Vase
T3	32.78f	28.39b	34.89de	Semi upright
T4	41.55ab	28.04bc	42.66a	Vase
T5	28.76h	26.55cd	41.78ab	Vase
T6	35.67de	25.20e	34.41de	Vase
T7	34.20e	28.00bc	33.13e	Vase
T8	40.36b	29.90ab	39.73bc	Semi upright
T9	36.67d	26.30d	38.86bc	Vase
T10	41.23ab	24.50ef	38.14c	Vase
T11	35.45de	29.45a	40.04bc	Vase
T12	38.41c	28.87ab	36.64cd	Vase
T13	29.03gh	26.67cd	37.11cd	Vase
T14	42.38a	27.43c	40.24b	Semi upright
T15	41.04ab	27.12cd	34.98de	Semi upright
T16	34.88de	29.50ab	35.10de	Vase
T17	30.34g	29.02ab	40.13bc	Vase

\*Different letters indicate the statistical difference within the same column among genotypes at 5% level.

The organic acid content in the fruit of the 17 genotypes of the *Sorbus aucuparia* L. is shown in Table 2. Malic acid is being the major constituent in all of the 17 genotypes (17.51-26.22%) and followed by Citric acid (2.25-7.15%) and Tartaric acid (1.15-2.04%), respectively. There were statistically significant differences in Malic, Citric and Tartaric acid contents among genotypes. MIKULIC-PETKOVSEK *et al.* (2017) found that Malic acid represents almost 60-88% of total organic acids in *Sorbus* fruits. They also point out that content of citric and tartaric acid together represented 7-39% of total analyzed acids. Organic acids content is an important indicator of the flavour and nutritional quality of fruits (SALLES, 2003). Some level of organic acids in fruits can enhance appetite and facilitate digestion (PERDOK *et al.*, 2003). The composition and content of organic acids in fruits are comprehensive traits that constitute multiple factors. Malic acid has a mild sour flavour with a refreshing taste, and compared to citric acid, it produces slow, but long-lasting taste stimulation. Thus, fruits with high malic acid content usually possess excellent flavour. These results indicate the presence of various organic acids in rowanberry fruits with differences in their compositions among different genotypes. Due to the differences in the organic acids composition and content in the fruits, tested genotypes of rowanberry show different quality and flavour.

Table 2. Organic acids content (g/100 g) in fruit of rowanberry genotypes

Genotype	Malic	Citric	Tartaric
T1	20.18d	3.89de	1.44bc
T2	26.22a	4.07d	1.92ab
T3	20.78cd	2.56ef	1.15c
T4	18.09e	5.67bc	1.70ab
T5	25.22ab	2.25f	1.28bc
T6	22.07c	6.02bc	1.36bc
T7	19.44de	4.34cd	1.74ab
T8	19.84de	5.14c	1.83ab
T9	21.20cd	3.56de	1.98ab
T10	18.70de	3.12e	2.04a
T11	17.51ef	4.60cd	1.30bc
T12	20.55cd	7.15a	1.79ab
T13	21.39cd	5.35bc	1.67ab
T14	18.40de	5.03cd	1.49bc
T15	24.14b	6.22b	1.60ab
T16	21.56cd	4.01de	1.55b
T17	22.56bc	4.89cd	1.50bc

\*Different letters indicate the statistical difference within the same column among genotypes at 5% level.

Table 3. Sugar contents (g/100 g) in fruits of rowanberry genotypes

Genotype	Glucose	Fructose	Sorbitol	Sucrose
T1	38.84ef	31.88bc	20.44bc	1.04 <sup>NS</sup>
T2	37.94fg	30.60c	19.91bc	1.56
T3	42.22d	35.14b	23.90ab	2.67
T4	41.31de	35.78ab	24.08ab	2.14
T5	36.98fg	29.22cd	26.09ab	1.44
T6	48.45a	39.56a	27.18a	2.87
T7	43.68cd	36.27ab	24.42ab	2.45
T8	37.65fg	27.65cd	20.66bc	2.25
T9	46.64b	38.24ab	25.15ab	1.96
T10	38.25f	28.56cd	18.79bc	2.14
T11	40.26e	34.02bc	23.04b	2.02
T12	37.07fg	26.44cd	18.25c	1.35
T13	48.09ab	39.27ab	26.87ab	1.76
T14	36.69fg	29.51cd	21.02bc	1.56
T15	38.06g	31.45bc	22.55bc	1.85
T16	36.11g	25.82d	19.07bc	1.47
T17	44.04c	39.04ab	26.46ab	2.66

NS: Non significant; \*Different letters indicate the statistical difference within the same column among genotypes at 5% level.

Glucose was the sugar present at the highest concentration (36.11-48.45%), as previously reported in rowanberries (MIKULIC-PETKOVSEK *et al.*, 2017) followed by Fructose

(25.82-39.56%), Sorbitol (18.25-27.18%) and Sucrose (1.04-2.87) (Table 3). Their levels differed statistically significant among genotypes, except for sucrose. Rowanberry fruits found rich for sorbitol. Sorbitol is more beneficial than other sugars with regard to diet control, dental health and to avoid gastrointestinal problems, and it can be used as a Glucose substitute (FORNI *et al.*, 1992). MIKULIC-PETKOVSEK *et al.* (2017) reported Sorbitol content between 26.83-27.80% in fruits for rowanberry cultivars grown in Czech Republic.

Vitamin C (ascorbic acid), total phenolic, total anthocyanin and total antioxidant capacity of the rowanberry genotypes are given in Table 4. There were statistically significant differences ( $p < 0.05$ ) for vitamin C (Ascorbic acid), total phenolic, total anthocyanin and total antioxidant capacity among seed propagated rowanberry genotypes. Vitamin C content was between 25.6-40.2 mg/100g FW (Table 4). Previous studies indicated that vitamin C content of rowanberry in Turkey was 35 mg/100 g (BALTACIOGLU, 2006). PIIR *et al.* (2003) reported the range of vitamin C content between 12-86 mg/100 g FW in rowanberry fruits in Estonia, whereas KAMPUSS *et al.* (2009) reported 10-51 mg/100 g in rowanberry fruits in Latvia.

Table 4. Vitamin C, total phenolic content, total anthocyanin content and antioxidant activity (FRAP) of rowanberry genotypes

Genotypes	Vitamin C (mg/100 g)	Total phenolic content (mg GAE/100 g)	Total anthocyanin content (mg/100 g)	FRAP (mM TE/100 g)
T1	27.8cd	144c	50b	4.24c
T2	30.5c	135cd	22de	3.80cd
T3	32.4bc	123d	29cd	3.36d
T4	25.6d	139cd	18e	3.67cd
T5	31.4bc	130cd	28cd	3.55cd
T6	33.6bc	152bc	42b	4.98bc
T7	38.0ab	176ab	38bc	6.35ab
T8	26.8cd	189a	26d	6.92a
T9	37.6ab	181ab	31cd	6.56ab
T10	28.8cd	165b	46ab	5.35bc
T11	35.1bc	135cd	22de	3.95
T12	40.2a	171ab	57a	5.58b
T13	27.0cd	140c	20de	4.11cd
T14	38.8ab	160bc	18e	5.90ab
T15	30.5c	148bc	26d	4.76bc
T16	39.4ab	176ab	34c	6.11ab
T17	35.9b	160bc	53ab	5.11bc

\*Different letters indicate the statistical difference within the same column among genotypes at 5% level.

The total phenolic and total anthocyanin content of rowanberry genotypes differed significantly whereby total phenolic content ranged from 123 mg to 189 mg GAE per 100 g FW (Table 4). Previously, total phenolic content was found between 134-220 mg GAE per 100 g FW and differed between rowanberry genotypes/cultivars (BALTACIOGLU, 2006; MIKULIC-PETKOVSEK *et al.*, 2017). The result indicates rowanberry fruits had high polyphenol content

which is comparable with polyphenols consisted in the other berry fruits, such as elderberry, blackberry, and raspberry.

Total anthocyanin content of rowanberry genotypes were found between 18-57 mg/100 g FW and the differences among genotypes were statistically significant (Table 4). HUKKANEN *et al.*, (2006) reported a wide variation (6-156 mg/100 g fresh weight) in total anthocyanin content among rowanberry cultivars and they also indicated that the anthocyanin fraction in rowanberries consists mainly cyanidin 3-galactoside (main fraction), cyanidin 3-glucoside (minor fraction), and cyanidin 3-arabinoside (minor fraction). MIKULIC-PETKOVSEK *et al.* (2017) also reported a wide variation of total anthocyanin content among rowanberry cultivars between 24-101 mg/100 g FW.

The rowanberry genotypes exhibited statistically significant differences ( $p < 0.05$ ) for total antioxidant capacity (FRAP values). Total antioxidant capacity varied between 3.36 and 6.92 mM TE per 100 g FW (Table 4). MIKULIC-PETKOVSEK *et al.* (2017) reported total antioxidant capacity between 3.4 and 4.9 mM TE/100 g FW of rowanberry cultivars grown in Czech Republic which is in accordance with our results. These differences may be caused by factors such as genotyping and growing condition differences. When compared with other fruit species, *Sorbus* fruits have higher or similar antioxidative activity like plums, pear etc. which have 2.91-5.87 mM trolox/kg FW (OZTURK *et al.*, 2009; KAULMANN *et al.*, 2014).

### CONCLUSION

The results of this study demonstrate the significant effect of genotypes on morphological and biochemical characteristics of rowanberry fruits. More specifically, this research shows the important role of the organic acid and sugar profiles in rowanberry fruit flavour quality, as well as the studied health promoted compounds which may contribute to enhance of this wild fruit consumption.

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**DIVERZITET PLODOVA ROGAČA (*Sorbus aucuparia* L.) DOBIJENIH IZ SEMENA**

Mehmet Ramazan BOZHUYUK<sup>1\*</sup>, Sezai ERCISLI<sup>2</sup>, Rayda Ben AYED<sup>3</sup>, Tunde JURÍKOVA<sup>4</sup>,  
Hafize FIDAN<sup>5</sup>, Gulce ILHAN<sup>2</sup>, Gursel OZKAN<sup>2</sup>, Halil Ibrahim SAGBAS<sup>2</sup>

<sup>1</sup>Igdir Univerzitet, Poljoprivredni Fakultet, Departman za hortikulturu, Igdir, Turska

<sup>2</sup>Ataturk Univerzitet, Poljoprivredni Fakultet, Departman za hortikulturu, Erzurum, Turska.

<sup>3</sup>Univerzitet Sfax, Centar za Biotehnologiju Sfax, Tunis

<sup>4</sup>Institut za obrazovanje i pedagogiju, Fakultet za centralnoevropske studije, Konstantin filozof  
Univerzitet u Njitra, Drazovska 4, SK-949 74 Njitra, Slovačka Republika

<sup>5</sup>Departman za catering i turizam, Univerzitet za tehnologiju hrane, Plovdiv, Bugarska

**Izvod**

Turska ima veliku ekološku, topološku i geografsku raznolikost i ta raznolikost je doprinela ne samo velikoj genetskoj raznolikosti, već je omogućila i uspešno uvođenje i gajenje velikog broja drvenastog voća. Turska je takođe poznata po bogatstvu divljih jestivih plodova s obzirom na diverzitet varijeteta i biološku raznovrsnost. *Sorbus aucuparia* L. je jedna od divljih jestivih plodova koje prirodno nalazimo u većini delova Turske. Ova studija opisuje morfološka (osobine rasta drveća, boja ploda) i biohemijska svojstva voća (vitamin C, organske kiseline, specifični šećeri, ukupni fenolni sadržaj, ukupan sadržaj antocijanina, ukupni antioksidativni kapacitet) 17 genotipova rogača razmnoženih iz semenki. Otkrili smo značajne razlike među gotovo svim ispitivanim parametrima. Među šećerima i organskim kiselinama glukoza i jabučna kiselina su bili dominantni u plodovima rogača. Ukupni sadržaj fenola, antocijanina, vitamina C i antioksidativna aktivnost varirali su od 123-189 mg GAE na 100 g, 18-57 mg na 100 g, 25.6-40.2 mg na 100 g i 3.36-6.92 mM ekvivalenta troloksa na 100 g sveže težine (FW). Rezultati ukazuju na mogućnost upotrebe plodova rogača u proizvodnji funkcionalne hrane sa visokim sadržajem biološki aktivnih supstanci.

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