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Original Article

Some Morphological and Biochemical Characteristics of Wild Grown Caucasian Whortleberry (*Vaccinium arctostaphylos* L.) Genotypes from Northeastern Turkey

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Abstract

Some important morphological such as fruit weight, fruit external color, fruit shape, the number of berry per cluster, cluster color, plant crown habit, harvest date and biochemical characteristics including sugars, organic acids, total phenolics, total anthocyanins, and antioxidant capacity of thirteen wild grown Caucasian whortleberry sampled from Coruh valley, in northeastern Turkey, were determined. Antioxidant capacity was measured as FRAP assay (ferric reducing antioxidant power). Significantly differences were found among genotypes for most of the selected morphological and biochemical features. The genotypes had in general black fruit color and round shape. Harvest date varied from 2 August to 12 August. Fruit weight ranged from 0.37 to 1.08 g. Genotypes had only fructose and glucose in their fruits as sugars. Citric acid was dominant organic acids and was found between 8.87 and 11.20 g per kg of fresh fruits and followed by tartaric acid (2.85-3.30 g/kg). Fruits of genotypes exhibited very high total phenolic content, which ranged from 3740 to 5541 µg per g on a fresh weight basis. Total anthocyanin contents were between 81 and 172 µg of delphinidin-3-glucoside equivalent in per g fresh fruit indicating great diversity.

Keywords: Caucasian whortleberry; composition; diversity; pomology

Introduction

Fruits in particular wild relatives show great morphological and biochemical diversity. They are a rich source of organic acids, sugars, fibers, minerals, etc. Wild edible fruits have also rich in ascorbic acid, tocopherol, anthocyanins, phenolics, and carotenoids such as β-carotene. These compounds contribute significantly to their antioxidant activity. Amongst fruits, berries are one of the richest groups which serve as powerful antioxidants due to its wide variety of anthocyanins and high phenolic contents. Red, blue, purple and black colored berries indicating high anthocyanins and they exhibit a wide variety of biological activity and promote health (Veberic *et al*., 2009; Paredes-Lopez *et al*., 2010; Ercisli *et al*., 2012a; Landete, 2012; Milivojevic *et al*. 2012).

The *Vaccinium* genus includes over 400 species however a few of them for using human consumption, which include Lowbush blueberry (*V. angustifolium*), Cranberry (*V. macrocarpon*), *V. myrtillus* (Bilberry), *V. erythrocarpum* (Bearberry), *V. ashei* (Rabbiteye Blueberry), *V. pallidum* (Blue Ridge blueberry), and *V. arctostaphylos* (Caucasian whortleberry) (Ozgen *et al*., 2014). In food industry particularly developed countries, highbush blueberry and bilberry fruits have been using mostly and commercial blueberry production in those countries mainly carried out with selected cultivars of highbush blueberry (*V. corymbosum* L.) (Ozturk *et al*., 2016).

V. arctostaphylos L. or locally known as "Ayı Üzümü" in Turkey is a perennial and deciduous plant with purple-black to blackberries. They grow as a shrub or woody bush and widespread throughout of Northeastern Black Sea region forests under acidic and rainy soils (Ozgen *et al*., 2014). *Vaccinium arctostaphylos* L. is a medicinal plant due to high

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phenolic compounds and anthocyanins. The plant is well known and used in Turkish folk medicine for the antidiabetic and antihypertensive agent (Baytop, 1999). Rural peoples harvest its fruits for direct consumption and also sell them on the roads.

Caucasian whortleberry still remains to be an underutilized fruit if compared to commercial fruits such as apricots, peaches, sweet cherries, banana, orange etc. Unfortunately, the majority of consumers has no information about the morphology of this plant and also health-promoting the content of its fruits. In fact, Turkey has huge biodiversity of *V. arctostaphylos* a few studies have been reported related to some morphological and biochemical content.

Fruit breeding programs based on variability to improve plant quality, productivity, and nutritional value. It is well known that genotypic effect is the main factor to increase nutritional and functional quality but environmental conditions, cultural practices, ripening and post-harvest conditions have an effect on those characters (Capocasa *et al.*, 2008).

Thus, the aim of this investigation was to report some important fruit characteristics of wild Caucasian whortleberry genotypes to add more information about this plant in literature.

Materials and Methods

Plant material

Commercially ripe berries of *V. arctostaphylos*, naturally wild grown in Coruh valley situated Northern east parts of Turkey were harvested. In commercial harvest period, dark-blue color Caucasian whortleberry fruits were collected in August during the year 2014 and 2015 from 13 pre-selected *V. arctostaphylos* genotypes that show higher yield, pest and diseases free and more attractive bigger fruits.

Morphological characteristics

Fruit weight was measured by using 0.01 sensitivity electronic balance and randomly harvested 50 fruits from different directions of shrubs with five replications were used for measurement.

Biochemical and bioactive composition

Sample preparation and extraction

For the organic acids, specific sugars, total phenolic, total anthocyanin contents and total antioxidant capacity analyses, harvested fruits immediately brought to the cooler state and stored at -20 °C until further analyses. During analysis, the cold fruits were taken and thawed to room temperature. A laboratory blander used to homogenise fruit samples (100 g lots of fruits per genotypes) and a single extraction procedure (taking 3 g aliquots and transferring it inside tubes and extracted for one hour with 20 mL buffer including acetone, water (deionized), and acetic acid (70:29.5:0.5 v/v) (Singleton and Rossi, 1965).

Extraction of sugars and organic acids

Five grams samples slurries were mixed with deionized water or metaphosphoric acid (2.5%) for the analysis of individual sugar and organic acid, respectively. The obtained homogenates were centrifuged at 10000 rpm for 10 min. The samples were filtered into HPLC vials using 0.45 μm 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 software Totalchrom navigator (version 6.2.1), consists of a pump and UV detector was used for analysis of the samples. Organic acids separation and determination were performed as per the 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 × 4.6 mm ID). The isocratic elution was performed using acetonitrile (80%) and deionised water (20%) with a flow rate of 0.9 mL/min. The column was operated at 30 °C and the sample injection volume was at 20 μL. Quantification of organic acids and sugars were performed against the reference standards.

Total phenolic contents

Total phenolic contents (TPC) of the samples were 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 the addition of sodium carbonate (10 mL) having a concentration of 7% (w/v) was performed. After incubation for 2 h, the absorbance at 750 nm was measured. Total phenolic contents were calculated against the reference standard calibration curve of gallic acid. The TPC was expressed as μg of gallic acid equivalents (GAE) per g of sample (fresh weight (FW) basis).

Total anthocyanin content

Total anthocyanins contents were measured using a pH differential method of Giusti and Wrolstad (2005) 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 μg of delphinidin-3-glucoside equivalent in per g of fresh sample.

Ferric Reducing Antioxidant Power Assay

FRAP (Ferric reducing antioxidant power) assay was used for antioxidant capacity analysis. For this purpose, acetonic fruit extract (50 μL), 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 μmol/L of Trolox was used to determine FRAP values of samples. The FRAP was expressed as μmol per g of Trolox equivalent on the basis of the fresh weight of fruits (Benzie and Strain, 1996).

Statistical analysis

All samples were five times replications for each experiment. 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

There were no significant differences in morphological and biochemical characteristics during the selected two years, thus the data were pooled from both years.

Morphological properties

Fruit weight compounds are important quality contributors and have an important role on consumers. Fruit weight found to vary significantly ($p < 0.05$) among Caucasian whortleberry genotypes ranged from 0.37 to 1.08 g depicting almost three-fold variations (Table 1). Caucasian whortleberry is underutilized fruit and there was no much report on fruit weight. Celik and Koca (2013) reported fruit weight of 6 Caucasian whortleberry genotypes naturally grown under forests as wild in Rize province of Turkey. between 0.52 and 1.19 g indicating a wide variability. Islam *et al.* (2009) also reported genotype-dependent fruit weight between 0.32 and 1.05 g among 46 Caucasian whortleberry genotypes from Northeastern Turkey. These results are in agreement with above-mentioned literature for fruit weight. Some of the most important changes that occurred during the domestication and improvement of horticultural crops were increased fruit weight.

Six Caucasian whortleberry genotypes had black, four genotypes had dark purple and three genotypes had dark blue external fruit color indicating diversity on fruit color among genotypes (Table 1). Celik and Koca (2013) found diverse fruit color (Dull black, Shiny black, purplish black, blue) among Caucasian whortleberry genotypes. Islam *et al.* (2009) reported black and dark blue fruit color among Caucasian whortleberry genotypes. The results clearly indicating that Caucasian whortleberries had darker fruits similar *Vaccinium myrtillus* compared to cultivated blueberry cultivars.

The majority of genotypes had round fruit shape (7 genotypes), followed by oblate and oval (3 genotypes equally). Islam *et al.* (2009) reported that Caucasian whortleberry genotypes had in general round fruit shape and a few genotypes had oblate and ellipse fruit shape (Table 1). Phenotypic diversity within wild and cultivated horticultural crops is particularly evident for fruit shape and size (Ercisli *et al.*, 2008a; Ercisli *et al.*, 2008b; Ercisli *et al.*, 2012b). Fruit shape is genetically controlled mostly and four genes that control fruit shape have been cloned (Rodriguez *et al.*, 2011)

The most of the genotypes exhibited spread crown habit (6 genotypes) and red cluster color (7 genotypes). The number of berries per cluster was between 4.83 and 8.24 among genotypes. Harvest date varied from 2 August and 12 August (Table 1). Celik and Koca (2013) reported harvest date between 25 July and 10 August and the number of berries per cluster between 5.30-7.90 among Caucasian whortleberry genotypes. Islam *et al.* (2009) reported mostly red green and red cluster color among Caucasian whortleberry genotypes. Akbulut *et al.* (2013) found the number of berries per cluster between 6.5-10.5 among Caucasian whortleberry genotypes.

Biochemical contents

As indicated in Table 2, statistically significant ($p<0.05$) variability for fructose, glucose and total sugars was observed among samples (Table 2). Fructose content was found a little bit higher than glucose for Caucasian whortleberry genotypes. Fructose content ranged from 29 to 56 g/kg and glucose content ranged from 27 to 48 g/kg. Ozgen *et al.* (2014) reported that among the fruits of 6 Caucasian whortleberry genotypes, the amount of fructose was 45.1 g/kg, whereas glucose contents were 41.2 g/kg. Total sugar contents were between 56 and 104 g/kg among genotypes (Table 2). Sugars contribute to particular food's appearance, food texture and also food shelf-life. Sweetness is one of the main drivers of consumer preference and thus is given high priority in fruit breeding programmes (Guan *et al.*, 2015).

Citric acid was dominant organic acids and was found between 8.87 and 11.20 g per kg of fresh fruits and followed by tartaric acid (2.85-3.30 g/kg). Malic and ascorbic acid

Table 1. Morphological characteristics of Caucasian whortleberry genotypes (mean of 2014-2015)

Genotypes	Fruit weight (g)	Fruit color	Fruit shape	Cluster color	Crown habit	Harvest date	Berry number per cluster
VA-1	0.69d	Black	Round	Red	Spread	7 August	6.86bc
VA-2	0.55e	Dark Purple	Oblate	Red	Erect	2 August	7.48ab
VA-3	0.96b	Dark Blue	Round	Red	Semi-Erect	10 August	5.88c
VA-4	0.63de	Black	Oval	Red-Green	Spread	8 August	6.95b
VA-5	0.81c	Dark Purple	Round	Red	Erect	10 August	6.31bc
VA-6	0.37f	Black	Oblate	Red-Green	Spread	5 August	7.90a
VA-7	0.59de	Dark Purple	Round	Red	Semi-Erect	4 August	7.12ab
VA-8	1.03ab	Black	Oval	Red	Erect	12 August	5.67cd
VA-9	0.61de	Black	Round	Red-Green	Semi-Erect	4 August	7.37ab
VA-10	0.86bc	Dark Blue	Round	Red-Green	Erect	9 August	6.07bc
VA-11	1.08a	Dark Purple	Oval	Red	Spread	3 August	5.30d
VA-12	0.77cd	Black	Oblate	Green	Spread	4 August	6.56bc
VA-13	0.51e	Dark Blue	Round	Red-Green	Spread	6 August	7.69ab

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

were between 0.07-0.41 and 0.07 and 0.21 g/kg fresh weight basis (Table 3). As indicated in Table 3, genotypes statistically differed each other ($p<0.05$) for all organic acids. Ozgen *et al.* (2014) reported that main organic acids in 6 genotypes of Caucasian whortleberry was citric acid (average 9.85 g/kg) and followed by tartaric acid (3.25 g/kg) indicating similarities with our results. Organic acids content in fruits influences flavors, nutritive value, quality etc. (Shui and Leong, 2002). It is important to determine the authenticity of fruits for preparing juices and beverages and also levels and ratios among them useful to determine fruit juice yield because each fruit species and cultivars have a unique organic acid pattern (Wrolstad, 1981; Coppola and Starr, 1986; Camara *et al.*, 1994).

Total phenolic content varies significantly ($p < 0.05$) among genotypes and ranged from 3740 to 5541 µg GAE per g fresh weight basis could be explained genotypic background differences of Caucasian whortleberry which totally arising from heterozygote seeds. Ozgen *et al.* (2014) reported total phenolic content on 6 Caucasian whortleberry genotypes in Turkey between 3900 to 5780 µg GAE per g fresh weight. Phenolic compounds have multifunctional properties and are important contributors to functional quality of fruits and vegetables. They act as a strong antioxidant by quenching singlet oxygen and scavenge free radicals. Therefore phenolic richness of Caucasian whortleberry fruits implies its significance for antioxidants as well due to the strong correlation of phenolic content and antioxidant potential.

Total anthocyanin content was between 81 and 172 µg of delphinidin-3-glucoside equivalent in per g fresh fruit indicating great diversity (Table 4). An earlier study on Caucasian whortleberry has shown to contain 75-194 µg of delphinidin-3-glucoside equivalent in per g fresh fruit (Ozgen *et al.*, 2014). Anthocyanins comprise the main share of the phenolic profiles in Caucasian whortleberry fruits. The anthocyanins possess significant health effects as well as antioxidant properties (Morazzoni and Bombardelli, 1996). Results from Latti et al. (2009) indicated that the most predominant anthocyanins in this species were delphinidin (41%), petunidin (19%), and malvidin (19%). Also, a similar conclusion was raised from Caucasian whortleberry samples originated from Iran (Nickavar and Amin, 2004).

The total antioxidant capacity (FRAP) in the present study of Caucasian whortleberry samples was in the range of 18.0-23.4 µmol Trolox equivalent per g in FRAP method as shown in Table 4. Earlier results of the Giovanelli and Buratti (2009) showed that values of antioxidant power were 24 µmol Trolox equivalents per g of the fruits of blueberry cv. Bluecrop. The values reported ere significantly higher than most of the berries reported in the literature (Moyer *et al.*, 2002; Sun *et al.*, 2002). The present results provide enough evidence to show that Caucasian whortleberry has the strong antioxidant potential. The presence of high levels of phenolics, flavonoids and other polyphenolic compounds in berries in general, exhibit elevated antioxidant capacities and are effective scavengers of several reactive oxygens species.

Table 2. Sugars in fruits of Caucasian whortleberry genotypes (mean of 2014-2015)

Genotypes	Glucose (g/kg)	Fructose (g/kg)	Total sugar (g/kg)
VA-1	37b	44bc	81b
VA-2	48a	56a	104a
VA-3	35b	40bc	75bc
VA-4	45ab	52ab	97ab
VA-5	43ab	46b	89ab
VA-6	27c	29d	56c
VA-7	33bc	36c	69bc
VA-8	37b	42bc	79b
VA-9	35b	41bc	76bc
VA-10	37b	43bc	80b
VA-11	39ab	47b	86ab
VA-12	41ab	50ab	91ab
VA-13	29bc	32d	61c

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

Table 3.Organic acid contents (g/kg) of Caucasian whortleberry genotypes (mean of 2014-2015)

Genotypes	Citric acid	Tartaric acid	Malic acid	Ascorbic acid
VA-1	9.06c	2.85b	0.14ab	0.10ab
VA-2	9.35bc	2.91ab	0.20ab	0.14ab
VA-3	9.74bc	3.07ab	0.33ab	0.10ab
VA-4	10.64ab	3.30a	0.25ab	0.11ab
VA-5	8.87cd	3.17ab	0.07b	0.21a
VA-6	10.12b	2.90ab	0.38ab	0.16ab
VA-7	9.45bc	2.95ab	0.18ab	0.18ab
VA-8	10.40ab	3.00ab	0.41a	0.16ab
VA-9	8.97cd	2.91ab	0.29ab	0.14ab
VA-10	11.20a	3.10ab	0.36ab	0.07b
VA-11	9.62bc	3.06ab	0.30ab	0.11ab
VA-12	10.35ab	3.00ab	0.36ab	0.17ab
VA-13	10.68ab	3.15ab	0.17ab	0.20a

Different letters indicate the statistical difference within the same column among accessions at 5% level.

Table 4. Bioactive characteristics of Caucasian whortleberry genotypes (mean of 2014-2015)

Genotypes	Total phenolics µg GAE/g FW	Total anthocyanins µg del-3-gly/g FW	FRAP µmolTE/g FW
VA-1	5337ab	160ab	21.1ab
VA-2	4641c	118d	18.5b
VA-3	3836de	110de	16.9bc
VA-4	5541a	172a	21.7a
VA-5	4405cd	130cd	18.1bc
VA-6	5268ab	165ab	20.8ab
VA-7	4153d	98e	17.7bc
VA-8	5110b	155b	19.9ab
VA-9	4981bc	147bc	19.5ab
VA-10	3942de	93ef	17.3bc
VA-11	3774e	81f	16.1c
VA-12	4862bc	140c	19.1ab
VA-13	3740e	87ef	16.5bc

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

Conclusions

The present results clearly indicated that the Caucasian whortleberry genotypes had different levels of morphological and biochemical characteristics. Thus to determine the superior genotypes of Caucasian whortleberry based on high bioactive content could increase the intake of health promoting compounds and may have aided in the prevention of chronic human diseases. These genotypes may be highly desirable in germplasm breeding programs to breed quality varieties of Caucasian whortleberry with high fruit size and antioxidant potential.

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