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Determination of genetic diversity among some naturally growing *Arbutus andrachne* L. genotypes in Türkiye using morphological, biochemical, and molecular markers

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Abstract: The strawberry tree (*Arbutus andrachne* L.) is a wild edible fruit that grows naturally in the Mediterranean climate zone, including Türkiye. In this study, the genetic morphological, biochemical, and genetic diversity of 10 superior strawberry tree genotypes growing naturally in Antalya Province in Türkiye were investigated. Morphological and biochemical characterization were performed based on some important fruit characteristics. In addition, genetic variation was detected at the molecular level using simple sequence repeat (SSR) markers. The genotypes exhibited fruit weight, width, length, and shape index values of 1.22–2.12 g, 12.07–15.28 mm, 10.15–14.24 mm, and 0.99–2.22, respectively. The biochemical parameters soluble solid content (SSC), vitamin C, total antioxidant capacity, malic acid, citric acid, fructose, and glucose were in the range of 22.17%–27.2%, 49.37–151.33 mg 100 g^{-1}, 17.08–34.18 µmol TE g^{-1} fw, 3.01–6.02 g 100 g^{-1}, 0.43–2.30 g 100 g^{-1}, 4.55–9.05 g 100 g^{-1}, and 3.30–6.70 g 100 g^{-1}, respectively. Gallic acid was the major phenolic acid (3.10–12.13 mg 100 g^{-1}). In molecular analysis using 5 SSR loci, a total of 39 polymorphic alleles were determined. The highest number of alleles was obtained from CA421f (13 alleles) and VCC_12 (10 alleles). Expected heterozygosity and observed heterozygosity ranged from 0.61 to 0.70 and from 0.65 to 0.78, respectively. The results of this study indicate the importance of germplasm resources of *Arbutus andrachne* L.

Key words: *Arbutus andrachne* L., diversity, biochemical composition, SSR

1. Introduction

Arbutus andrachne is common in the coastal regions of Türkiye, generally found together with maquis and red pine forests in limestone/serpentine/volcanic areas and arid and rocky places. In Europe and the Middle East, Albania, Bulgaria, Cyprus, the East Aegean, Greece, Iraq, Crete, Crimea, Lebanon and Syria, Palestine, the Transcaucasus, and Türkiye were reported among the natural distribution areas of *Arbutus andrachne* L. (synonymous *Arbutus idaea* Gand., *Arbutus integrifolia* Lam., *Arbutus lucida* Steud., and *Arbutus sieberi* Klotzsch).[1]

A. andrachne is a species with edible fruits, known as sandal ağacı, hartlap, and kızılbacak in Turkish, that spreads naturally in the Mediterranean climate zone. The flowers bloom in March and April. Fruits ripening in autumn are 1.0–1.5 cm in diameter and orange yellow or light red. They have an excellent root system adapted to adverse conditions (Kayacik, 1982; Onursal and Gozlekci, 2007). The species is regarded as an underutilized wild edible fruit (Yildiz and Aktoklu, 2010; Fortalezas et al., 2010). It has become a symbolic plant of the Mediterranean vegetation with its different stem structure, color, large leaves, and spectacular fruits. Since the wood texture of *A. andrachne* is hard, it is used as a material in handicrafts. Its fruits have a bitter flavor as they contain large amounts of tannins. Moreover, its young shoots and leaves are used in traditional medicine due to their arbutin content to treat some human diseases (Onursal and Gozlekci, 2007).

In recent years, consumers have given importance to consuming completely natural and healthy foods that do

1 https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:326280-2

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not contain chemical preservatives, due to their better medicinal properties and high nutritional values. In this context, wild edible fruits have gained more importance (Bolaric et al., 2021; Grygorieva et al., 2021; Bozhuyuk, 2022). As a wild edible fruit, it has been reported that the fruits of this species have a positive effect on preventing cardiovascular diseases (Hu, 2003; Ikram et al., 2009), some types of cancer (Ikram et al., 2009), immune system-related problems, and brain dysfunction (Leong and Shui, 2002). Its fruit is reported to contain antioxidant substances such as flavonoids, phenolics, carotenoids, organic acids, and vitamins (Prior et al., 2003; Heber, 2004; Rangkadilok et al., 2007; Kubola et al., 2011). Organic acids, which affect the taste of fruits, act depending on the acid–sugar balance (Savran, 1999). Organic acids in fruits affect flavor (reducing sweetness and increasing tartness). Moreover, the total acid to sugar ratio determined in fruits is a maturity criterion (Savran, 1999). Another important content of the fruits is sugars, which affect their taste (Colak, 2019; Sagbas et al., 2020). Phenolic compounds, which have effects on color, flavor, and durability in fruits and vegetables, also provide benefits in terms of human health due to their antioxidant, anticarcinogenic, antimutagenic, and antimicrobial activity effects (Argiles et al., 1998; De Groot and Rauen, 1998; Parthasarathy, 1998; Smith et al., 2000; Gyawali and Ibrahim, 2014). Due to the hydroxyl group and aromatic ring in their structure, the color of the food turns from yellow or intense red to blue (Acar and Gokmen, 2014). It is stated in various sources that *Arbutus* fruit is rich in vitamin C. Vitamin C, on the one hand, contributes significantly to the formation of a large part of blood vessels, bones, joints, teeth, and gums, and, on the other hand, it is involved in collagen production, blood vessel strengthening, hemoglobin production, adrenal hormone secretion, natural antihistamine production, and free radical neutralization (Ely, 1996; Levin et al., 2010).

Along with morphological and biochemical markers, more recently molecular markers have gained greater importance. However, the number of DNA-based molecular studies carried out on *Arbutus* species is quite low. To date, random amplified polymorphic DNA (RAPD) (Takrouni and Boussaid, 2010; Sa et al., 2011; Bertsouklis and Papafotiou, 2016; Lopes et al., 2012; Gomes et al., 2013), intersimple sequence repeat (ISSR) (Sa et al., 2011; Lopes et al., 2012), simple sequence repeat (SSR) (Gomes et al., 2013; Fazenda et al., 2019), chloroplast simple sequence repeat (cpSSR) (Ribeiro et al., 2017), and amplified fragment length polymorphism (AFLP) (Santiso et al., 2016) marker techniques have been used. In a fruit tree species with a long life cycle, selection and classical breeding can be time consuming. Thus, good characterization of wild populations and the development of molecular markers are essential to speed up the selection process. Molecular markers can also be useful for in situ and ex situ germplasm conservation, as insights about population evolution and diversity can be obtained. Studies on genetic diversity and population structure provide essential knowledge for forest management and selection of individuals and/or populations for breeding and conservation purposes (Martins et al., 2021). A microsatellite (SSR) database has been established for molecular characterization studies in many fruit species. Microsatellites are short repeat sequences of 1–6 bases and show genome specificity in higher organisms (Zhang et al., 2008). SSRs are known as PCR-based codominant markers and are used to effectively differentiate genotypes and cultivars of fruit species (Wünsch, 2009).

The aim of the present study was to determine the level of genetic diversity using morphological, biochemical, and molecular markers in some *Arbutus andrachne* L. genotypes growing naturally in Antalya, Türkiye.

2. Materials and methods

2.1. Plant materials

Fruit and leaf samples of ten promising *A. andrachne* genotypes were obtained during the development period of 2019–2020. The selection study area, Antalya Province, is located in the Mediterranean Region in Türkiye. Full maturation stage fruit samples were taken and quickly transported to the laboratory by cold chain. Fruit samples to be used for biochemical and bioactive analyses were kept in a freezer (–80 °C) until the time of analysis.

For molecular analysis, leaf samples were delivered to the laboratory under cold-chain conditions as well and stored at –86 °C. The location and coordinate information of the plant materials used in the study is given in Table 1 and the Figure.

2.2. Morphological traits

The fruit weight, fruit dimensions (width and length), fruit shape index, and fruit skin color of 10 *A. andrachne* genotypes were determined by using 100 fruit per genotype, which were divided into 5 replicates (20 fruit per replicate). Fruit weight was measured using a digital balance with a sensitivity of 0.01 g. Fruit dimensions were measured using a digital caliper once after the harvest. The fruit shape index was determined by using the fruit width-and-length ratio (Mohsenin, 1986). The skin color of the fruits was determined based on CIE units with a CR-400 chromometer (Konica Minolta, Japan).

2.3. Biochemical and bioactive content

2.3.1. Soluble solid content (SSC)

In order to measure the SSC content in fruit juice, juice of each fruit sample was diluted 1/5, crushed, and filtered and their measurements were made in a digital refractometer. The values obtained were multiplied by 5 to determine

Table 1. The location and coordinate information of strawberry tree genotypes used in the study.

Sample no.	Genotypes	Latitude (N)	Longitude (E)	Altitude (m)	Geographic location
1	07-AA-14	37°11′27″	31°10′36″	270	Köprülü Kanyon, Antalya
2	07-AA-22	37°11′43″	31°10′22″	360	Köprülü Kanyon, Antalya
3	07-AA-28	37°12′14″	31°90′54″	470	Köprülü Kanyon, Antalya
4	07-AA-29	37°12′14″	31°90′53″	540	Köprülü Kanyon, Antalya
5	07-AA-57	36°38′15″	31°57′60″	320	Kemer-Duacılar, Antalya
6	07-AA-68	36°29′59″	32°90′53″	465	Alanya, Antalya
7	07-AA-73	36°11′89″	32°25′26″	140	Alanya, Antalya
8	07-AA-81	36°20′78″	32°16′40″	284	Gazipaşa, Antalya
9	07-AA-87	36°17′31″	30°70′12″	422	Gazipaşa, Antalya
10	07-AA-109	36°15′08″	29°47′41″	437	Elmalı-Kaş, Antalya

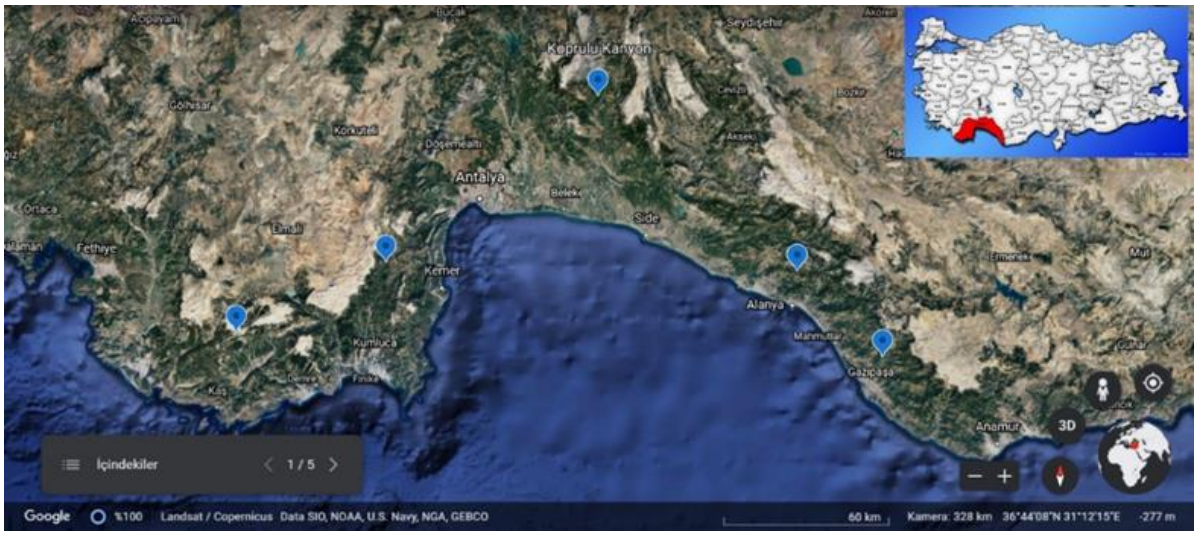


Figure. The geographic location of the strawberry tree genotypes (Google Earth 2022).

the SSC values of the genotypes in percentages (Ministry of Agriculture and Forestry, General Directorate of Food Affairs, 1983).

2.3.2. Organic acid content

Fruit samples (5 g) were transferred to centrifuge tubes and homogenized by adding 10 mL of 0.009 N 10 H_2SO_4. It was stirred for 1 h on a shaker and centrifuged at 15,000 rpm for 15 min. The aqueous fraction leaving the centrifuge was passed through coarse filter paper, a 0.45 µm membrane filter (Millipore Millex-HV Hydrophilic PVDF, Millipore, USA) twice, and a SEP-PAK C18 cartridge. The samples were analyzed in an HPLC instrument (Agilent HPLC-1100). In the HPLC system, an Aminex HPX-87H, 300 mm × 7.8 mm column (Bio-Rad Laboratories) was used and the device was controlled by computer with the software package Agilent. The detector in the system was tuned to wavelengths of 214 and 280 nm. In the study, 0.009 N H_2SO_4 passed through a 0.45 µm membrane filter was used as the mobile phase (Bevilacqua and Califano, 1989; Gundogdu et al., 2014).

2.3.3. Vitamin C

Firstly, 5 mL of the fruit juice sample was transferred to a test tube and 5 mL of 2.5% M-phosphoric acid solution was added. The mixture was centrifuged at 6500 × *g* for 10 min at +4 °C. Then 0.5 mL was taken from the clear part and made up to 10 mL with 2.5% M phosphoric solution. This mixture was filtered through a 0.45-µm Teflon filter and injected into the HPLC device. Vitamin C was determined on a C18 column (Phenomenex Luna C18, 250 × 4.60 mm, 5 µm) in HPLC. The column furnace temperature was set to 25 °C. Ultrapure water with pH level adjusted to 2.2 with H_2SO_4 at a flow rate of 1 mL min^{-1} was used as

the mobile phase in the system. Readings were obtained in a DAD detector at a wavelength of 254 nm. L-ascorbic acid (Sigma A5960) prepared at different concentrations (50, 100, 500, 1000, 2000 ppm) was used to identify and quantify the vitamin C peak (Cemeroğlu et al., 2004).

2.3.4. Sugar analysis

The method used by Melgarejo et al. (2000) was modified and used to determine the sugar contents. Five grams of homogeneous fruit samples were taken, centrifuged at 12,000 rpm for 2 min, and passed through the SEP-PAK C18 cartridge. The obtained filtrate was stored at –20 °C until analysis. The sugars in the filtered sample were determined in a HPLC instrument with a refractive index detector with the help of 85% acetonitrile liquid phase on a μbondapak-NH_2 column. Calculation of concentrations was performed according to externally supplied standards.

2.3.5. Determination of phenolic compounds and antioxidant capacity

For both analyses, 100 g of fruit sample was homogenized using a blender. The homogenized sample was extracted in tubes for 1 h using acetone, water, and acetic acid (70:29.5:0.5) solution (Ozgen et al., 2009). In the analysis of phenolic compounds by HPLC, the method determined by Rodriguez-Delgado et al. (2001) was used. Chromatographic separation was performed on the Agilent 1100 HPLC system using a DAD detector (Agilent) and a 250 × 4.6 mm, 4 μm ODS column (HiChrom). Solvent A, methanol:acetic acid:water (10:2:88), and solvent B, methanol:acetic acid:water (90:2:8), were used as mobile phase and a gradient elution program was applied. Separation was performed at 254 and 280 nm and the flow rate was determined as 1 mL min^{-1} and the injection volume was 20 μL.

The antioxidant capacity of fruits was determined using the ferric reducing antioxidant power (FRAP) method, which has been frequently used in fruit species in recent years. For FRAP analysis, first of all, 0.1 mol L^{-1} acetate (pH 3.6), 10 mmol L^{-1} TPTZ, and 20 mmol L^{-1} ferric chloride solutions were mixed in the ratio of 10:1:1 and buffer was prepared. Next, 2.97 mL of the prepared buffer was mixed with 30 μL of fruit extract and left for 10 min. It was then measured at a wavelength of 593 nm in a spectrophotometer. Obtained absorbance values were calculated with a Trolox (10–100 μmol L^{-1}) standard slope chart and were determined as μmol Trolox equivalent/g fresh fruit (Benzie and Strain, 1996).

All analyses of samples and standards for biochemical and bioactive content were performed three times.

2.4. Molecular analysis

2.4.1. Isolation and analysis of DNA

Genomic DNA was extracted from young leaf tissues using a Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) according to the instructions provided by the manufacturer. Subsequently, RNAse treatment was performed on the eluted DNA samples. The purity and concentration of the DNA were confirmed both on 1% (w/v) agarose gels and by NanoDrop ND-1000 spectrophotometer.

2.4.2. SSR-PCR analysis

SSR markers developed by Boches et al. (2005, 2006) for the *Vaccinium* species and successfully used by Gomes et al. (2013) in *Arbutus unedo* genotypes were employed in our study. The repeat motifs, primer sequences, annealing temperatures, and allele sizes of the SSR loci used in our study are given in Table 2.

A polymerase chain reaction (PCR) was conducted in a volume of 10 mL containing 15 ng of genomic DNA, 5 pmol of each primer, 0.5 mM dNTP, 0.5 units of GoTaq DNA polymerase (Promega), and 1.5 mM $MgCl_2$. The forward primers were 'labeled' with WellRED fluorescent dyes D2 (black), D3 (green), and D4 (blue) (Proligo, Paris, France). Reactions without DNA were included as negative controls. PCR amplification was performed using the Biometra PCR system. The amplification conditions consisted of an initial denaturation step of 3 min at 94

Table 2. Repeat motifs, primer sequences, annealing temperatures (Ta °C), and allele sizes of SSR loci.

SSR loci	SSR repeat motif	Primer sequences (5'→3') forward and reverse	Ta (°C)	Allele size (bp)
CA421F	$(CT)_{25}$	F: TCAAATTCAAAGCTCAAAATCAA R: GTTTAAGGATGATCCCGAAGCTCT	60	180–250
CA794F	$(GA)_{12}$	F: CGGTTGTCCCACTTCATCTT R: GTTTGAATTTGGCTTCGGATTC	60	220–290
NA741	$(TC)_9$	F: GCCGTCGCCTAGTTGTTG R: GTTTGATTTTGGGGGTTAAGTTTGC	58	240–290
NA800	$(TC)_{13}$	F: CAATCCATTCCAAGCATGTG R: GTTTCCCTAGACCAGTGCCACTTA	60	230–290
VCC_I2	$(CT)_{14}$	F: AGGCGTTTTTGAGGCTAACA R: TAAAAGTTCGGCTCGTTTGC	62	200–275

°C, followed by 1 min at 94 °C, 1 min at 52–56 °C, and 2 min at 72 °C (35 cycles) with a final extension at 72 °C for 10 min. The PCR products were run on a CEQTM 8800 XL capillary genetic analysis system (Beckman Coulter, Fullerton, CA, USA) to determine polymorphisms. The analyses were performed at least twice to ensure reproducibility of the results.

2.5. Statistical analysis

The data were subjected to Levene's homogeneity test before the statistical analysis. Differences in morphological and biochemical analysis results between Eastern strawberry tree genotypes were determined with the least significant difference (LSD) method at $p < 0.05$. The mean values and standard deviations are given (mean ± SD). The genetic analysis program IDENTITY 1.0 was used according to Paetkau et al. (1995) for calculation of the number of alleles and expected and observed heterozygosity. Genetic similarity was determined by the program MICROSAT 1.5 using proportion of shared alleles, which was calculated by using "ps (option 1 – (ps))", as described by Bowcock et al. (1994). Polymorphic information content (PIC) was calculated according to Kalinowski et al. (2007).

3. Results and discussion

3.1. Morphological measurements

In the present study, the fruit characteristics of 10 promising *A. andrachne* genotypes growing naturally were examined. There was significant variation among the genotypes in terms for all fruit characteristics ($p < 0.05$). As shown in Table 3, genotypes 07-AA-29 and 07-AA-73 had higher values of fruit weight, i.e., 2.12 g and 2.04 g, respectively. The lowest fruit weight was obtained from genotype 07-AA-28 (1.22 g). The highest average fruit width was obtained from 07-AA-73 as 15.28 mm, while the lowest value was observed in 07-AA-28 as 12.07 mm. Average fruit length was determined in the range of 10.15 mm (07-AA-28) to 14.24 mm (07-AA-29). Serce et al. (2010) recorded the mean fruit width, length, and weight values in *A. andrachne* genotypes as 12.3 mm, 10.8 mm, and 1.15 g, respectively. Celikel et al. (2008), on the other hand, determined five superior genotypes among 51 *A. unedo* genotypes that spread naturally in the Black Sea Region. The fruit weight values of the genotypes in the study were in the range of 6.17–11.08 g. As stated by Martin et al. (2021), the strawberry tree fruit (*A. unedo* L.) has spherical fruits with a diameter of about 20 mm (Piotto et al., 2001). Cakmak (2019) determined fruit weight of 2.39–4.69 g, fruit width of 16.85–20.62 mm, and fruit length of 14.52–17.29 mm in 20 *Arbutus* spp. genotypes. Fruit weight in *A. unedo* was recorded in the range of 2.28–11.00 g (Pekdemir, 2010), 1.70–9.03 g (Ersoy, 2017), 2.46–5.03 g (Toy, 2019), and 4.05–8.35 g (Kocabas, 2019). In some studies on strawberry tree (*A. unedo*) genotypes in Türkiye, while the fruit width was 16.51–28.05 mm (Pekdemir, 2010), 11.42–30.92 mm (Ersoy, 2017), and 14.14–20.59 mm (Toy, 2019), the fruit length was 13.06–22.03 mm (Pekdemir, 2010), 10.15–25.24 mm (Ersoy, 2017), and 13.03–19.40 mm (Toy, 2019). Sagbas et al. (2020) reported fruit weight between 3.86 and 6.89 g among 16 *A. unedo* genotypes in the East Black Sea Region of Türkiye.

In our study, the fruit shape index value ranged from 0.99 (07-AA-109) to 2.22 (07-AA-81), and there was variation between genotypes. In the study by Ersoy (2017), the fruit shape index was 0.75–1.33. According to a visual evaluation, the color of the fruit peel varies between light red and dark red in *Arbutus* species (Toy, 2019). In our study, according to the "a" value, which is one of the fruit

Table 3. Morphological characteristics of strawberry tree genotypes.

Genotypes	Fruit weight (g)	Fruit width (mm)	Fruit length (mm)	Fruit shape index	Fruit skin color*		
					L	a	b
07-AA-14	1.40 ± 0.14	12.61 ± 0.62	11.27 ± 0.50	1.12 ± 0.00	36.92 ± 2.02	34.07 ± 1.19	32.63 ± 4.09
07-AA-22	1.77 ± 0.78	12.74 ± 0.57	10.91 ± 0.56	1.16 ± 0.01	33.68 ± 0.37	37.68 ± 1.88	27.28 ± 0.54
07-AA-28	1.22 ± 0.20	12.07 ± 0.22	10.15 ± 0.26	1.12 ± 0.06	42.80 ± 1.59	38.08 ± 1.44	37.71 ± 1.94
07-AA-29	2.12 ± 0.21	14.55 ± 0.41	14.24 ± 0.71	1.05 ± 0.02	32.03 ± 0.84	33.80 ± 2.78	26.01 ± 0.99
07-AA-57	1.40 ± 0.34	12.89 ± 1.11	10.77 ± 0.92	1.15 ± 0.05	29.90 ± 1.25	36.44 ± 1.00	18.71 ± 0.91
07-AA-68	1.52 ± 0.20	12.44 ± 0.82	11.76 ± 0.60	1.15 ± 0.00	30.34 ± 0.93	38.60 ± 1.88	23.66 ± 2.25
07-AA-73	2.04 ± 0.23	15.28 ± 0.50	12.07 ± 0.40	1.21 ± 0.06	38.62 ± 1.87	37.74 ± 4.03	35.41 ± 2.71
07-AA-81	1.53 ± 0.17	12.10 ± 0.44	11.95 ± 0.71	2.22 ± 0.01	30.48 ± 0.23	37.50 ± 1.36	21.55 ± 0.30
07-AA-87	1.41 ± 0.20	12.14 ± 0.49	11.42 ± 0.60	1.10 ± 0.05	35.76 ± 1.31	36.76 ± 1.12	28.12 ± 1.44
07-AA-109	1.58 ± 0.26	12.20 ± 0.96	14.22 ± 1.22	0.99 ± 0.07	39.80 ± 3.40	35.71 ± 1.43	37.16 ± 4.21

* L is the brightness value (0 = black, 100 = white). Indicates + a = red, – a = green, + b = yellow, – b = blue.

color measurement criteria, 07-AA-68 (38.60) and 07-AA-28 (38.08) were more prominent in terms of fruit skin redness.

3.2. Biochemical analyses

3.2.1. Soluble solid content (SSC)

The SSC of the genotypes was between 22.17% (07-AA-68) and 27.22% (07-AA-87) (Table 4). Previously a wide variation in SSC in *Arbutus* spp. genotypes was determined (11.6%–19.6%) by Cakmak (2019). In a different study, it was 9.0%–24.6% (Toy 2019). In *Arbutus unedo* fruits, it was 24.0%–31.0% (Pekdemir, 2010), 15.0%–32.0% (Ersoy, 2017), and 25.2%–30.6% (Kocabas, 2019). Our SSC content was comparable with that of the above studies.

3.2.2. Vitamin C

According to the results presented in Table 4, the differences in genotypes in terms of vitamin C contents were statistically significant ($p < 0.05$). The lowest vitamin C value was 49.37 mg 100 g^{-1}, in 07-AA-109, and the highest value was 151.33 mg 100 g^{-1}, in 07-AA-81 (Table 4). In the literature, vitamin C varied widely among *Arbutus* species and even within same species. Vitamin C levels in *Arbutus* species were 89 mg 100 g^{-1} (Pimpão et al., 2013), 346 mg 100 g^{-1} (Alarcaoe-Silva et al., 2001), and 182 mg 100 g^{-1} (Morales et al., 2013). While the amount of vitamin C in *Arbutus unedo* was determined to be very low (6.03 mg 100 g^{-1}) by Pallauf et al. (2008), the amount of vitamin C in it was determined in the range of 98–280 mg 100 g^{-1} by Celikel et al. (2008). Gundogdu et al. (2014) determined the amount of vitamin C in *Arbutus unedo* fruits to be 59.83 mg 100 g^{-1}. In addition, vitamin C was 46.74 mg 100 g^{-1} in *Arbutus andrachne* fruit and 119.10 mg 100 g^{-1} in *Arbutus unedo* fruit obtained in the Northeast Anatolian Region of Türkiye (Aslantas et al., 2007). On the other hand, Serce et al. (2010) recorded the amount of average vitamin C as 140 mg 100 g^{-1} in a study they conducted on *A. andrachne* genotypes. In some studies in Türkiye, the amount of vitamin C in *A. unedo* was 13.53–126.60 mg 100 g^{-1} (Cakmak, 2019), 175.4 g 100 g^{-1} (Yildiz, 2014), 142–221 mg 100 g^{-1} (Kocabas, 2019), and 58.849% (Erboga, 2016). Sagbas et al. (2020) found vitamin C between 58 and 93 mg 100 g^{-1} in 16 *A. unedo* genotypes in the East Black Sea Region of Türkiye.

3.2.3. Antioxidant capacity

The differences between genotypes in terms of antioxidant capacity were statistically significant (Table 4). The antioxidant content was lowest in 07-AA-22 and 07-AA-81 (17.08 and 17.44 μmol TE g^{-1}) and highest in 07-AA-28 and 07-AA-57 (33.04 and 34.18 μmol TE g^{-1}). Similar to the values obtained in our study, Serce et al. (2010) determined that *A. andrachne* fruits have antioxidant levels in the range of 16.8–29.5 μmol TE g^{-1}. In some studies conducted in *A. unedo* in Türkiye, antioxidant capacity was 17.51–30.06 μmol TE g^{-1} (Gundogdu et al., 2014), 36.1–29.5 mmol TE g^{-1} (Yildiz, 2014), 69.18–115.22 mg Trolox g^{-1} DW (Kocak, 2014), 0.2893 TEAC (mmol g^{-1}) MeOH/H_2O (4:1, v/v) (Uysal, 2015), and 70.419 1000 μg mL^{-1} (Erboga, 2016).

3.2.4. Organic acid content

In the present study, five organic acids were detected in *A. andrachne* genotypes, namely oxalic acid, malic acid, citric acid, succinic acid, and fumaric acid. The results of the analysis for organic acid content were statistically significant in the genotypes (except succinic acid and fumaric acid) (Table 4). Among the organic acids determined

Table 4. Soluble solid content (SSC), vitamin C, total antioxidant capacity, and organic acids of the strawberry tree genotypes.

Genotypes	SSC (%)	Vitamin C (mg 100 g^{-1})	Total antioxidant capacity (μmol TE g^{-1} fw.)	Organic acids (g 100 g^{-1})				
				Oxalic acid	Malic acid	Citric acid	Succinic acid	Fumaric acid
07-AA-14	25.17 ± 0.06	68.33 ± 1.02^{c*}	23.11 ± 0.13b	0.71 ± 0.04a	4.10 ± 0.09bc	1.75 ± 0.03^{b}	0.19 ± 0.01^{NS}	0.09 ± 0.00^{NS}
07-AA-22	24.22 ± 0.15	97.15 ± 1.23^{bc}	17.08 ± 0.20c	0.55 ± 0.03bc	6.02 ± 0.08a	0.88 ± 0.02^{de}	0.23 ± 0.01	0.11 ± 0.00
07-AA-28	22.82 ± 0.15	101.19 ± 1.27^{bc}	33.04 ± 1.05a	0.70 ± 0.01a	3.48 ± 0.06bc	1.43 ± 0.01^{c}	0.20 ± 0.01	0.11 ± 0.01
07-AA-29	22.50 ± 0.10	116.32 ± 1.33^{b}	24.45 ± 0.39b	0.59 ± 0.04bc	3.01 ± 0.08c	0.96 ± 0.01^{d}	0.20 ± 0.01	0.20 ± 0.01
07-AA-57	22.22 ± 0.25	70.44 ± 1.10^{c}	34.18 ± 0.45a	0.68 ± 0.03ab	4.43 ± 0.07b	0.80 ± 0.01^{de}	0.33 ± 0.02	0.13 ± 0.01
07-AA-68	22.17 ± 0.21	80.56 ± 1.00^{bc}	22.04 ± 0.14bc	0.52 ± 0.02c	5.85 ± 0.04ab	2.30 ± 0.01^{a}	0.19 ± 0.01	0.17 ± 0.00
07-AA-73	24.22 ± 0.15	75.50 ± 0.90^{bc}	28.20 ± 0.10ab	0.68 ± 0.03ab	4.17 ± 0.01bc	0.43 ± 0.02^{e}	0.15 ± 0.00	0.09 ± 0.00
07-AA-81	24.17 ± 0.15	151.33 ± 1.45^{a}	17.44 ± 0.10c	0.41 ± 0.01d	3.59 ± 0.09bc	0.50 ± 0.01^{e}	0.22 ± 0.00	0.13 ± 0.00
07-AA-87	27.22 ± 0.06	115.10 ± 1.60^{b}	21.35 ± 0.09bc	0.44 ± 0.01cd	4.54 ± 0.07b	0.74 ± 0.01^{de}	0.28 ± 0.01	0.08 ± 0.01
07-AA-109	26.00 ± 0.20	49.37 ± 0.30^{d}	20.92 ± 0.07bc	0.61 ± 0.02b	3.34 ± 0.06bc	0.81 ± 0.01^{de}	0.19 ± 0.00	0.08 ± 0.00

Values in the same column with different lower-case letters are significantly different at $p < 0.05$. NS: Non significant.

in *A. andrachne* genotypes, malic acid was determined quantitatively more than the others. Malic acid content was recorded in the range of 3.01–6.02 g 100 g^{-1} (07-AA-29 and 07-AA-22, respectively). Malic acid content was 2.33 g 100 g^{-1} in *A. unedo* genotypes (Gundogdu et al., 2014), 0.44 g 100 g^{-1} in *A. andrachne* genotypes (Serce et al., 2010), and 0.27%–1.30% in *Arbutus* spp. genotypes (Cakmak, 2019). In our study, the highest amount of citric acid was 2.30 g 100 g^{-1}, in 07-AA-68. The citric acid content was 0.08 g 100 g^{-1} in *A. andrachne* genotypes (Serce et al., 2010), 0.87 g 100 g^{-1} in *A. unedo* genotypes (Gundogdu et al., 2014), and 0.16%–0.69% in *Arbutus* spp. genotypes (Cakmak, 2019). In our study, oxalic acid was between 0.41 g 100 g^{-1} (07-AA-81) and 0.71 and 0.70 g 100 g^{-1} (07-AA-14 and 07-AA-28, respectively). Gundogdu et al. (2014) detected a lower amount of oxalic acid in *A. unedo* genotypes (0.33 g 100 g^{-1}). In our study, succinic acid was 0.15–0.33 g 100 g^{-1} and fumaric acid 0.08–0.20 g 100 g^{-1} in *A. andrachne* fruits. Succinic acid content of 0.40 g 100 g^{-1} and fumaric acid content of 0.46 g 100 g^{-1} were determined in *A. unedo* genotypes by Gundogdu et al. (2014).

3.2.5. Sugar analysis

The results of the analysis for sugar contents were statistically significant among the ten genotypes (Table 5). In the genotypes examined in our study, the main sugar was fructose (4.55–9.05 g 100 g^{-1}). Fructose content was lowest in 07-AA-109 and highest in 07-AA-14. While glucose content was determined at the lowest value (3.30 and 3.72 g 100 g^{-1}) in 07-AA-87 and 07-AA-109, it was in the highest amount with 6.70 g 100 g^{-1} value in 07-AA-14. The sucrose determined in our study was in the range of 0.45 (07-AA-68) to 1.28 (07-AA-14) g 100 g^{-1}. Similar to the results of our study, the fructose, glucose, and sucrose values in *A. andrachne* fruits were 7.98–11.89, 4.03–6.76, and 0.43–2.06 g 100 g^{-1}, respectively, according to Serce et al. (2010). In studies conducted in *A. unedo*, fructose, glucose, and sucrose values were 3.78–6.10, 0.17–1.44, and 6.13–11.63 g 100 g^{-1}, respectively (Gundogdu et al. 2014); 25.721%, 16.409%, and 6.499%, respectively (Erboga 2016); and 4.363 g 100 g^{-1} (glucose) and 8.496 g 100 g^{-1} (fructose) (Yildiz, 2014). Furthermore, Cakmak (2019) determined the amounts of glucose, sucrose, fructose, and total sugar in *Arbutus* spp. genotypes to be 2.32–8.34, 0.02–1.93, 3.08–17.93, and 7.47–27.51 g 100 g^{-1}, respectively, showing similarities with our results.

3.2.6. Phenolic compounds

In our study, a total of 11 different phenolic compounds (gallic acid, protocatechuic acid, catechin, chlorogenic acid, vanillic acid, syringic acid, *p*-coumaric acid, ferulic acid, rutin, phloridzin, and quercetin) were determined by HPLC in the fruit juices of the promising *A. andrachne* genotypes. The results of the analysis for most of the phenolic compounds were statistically significant among the genotypes ($p < 0.05$) (Table 6). On the other hand, the differences between the genotypes in terms of *p*-coumaric acid and quercetin contents were not significant. In our study, gallic acid was the major phenolic acid and found in the range of 3.10 (07-AA-68) to 12.13 (07-AA-73) mg 100 g^{-1} among the genotypes. Considering the amount of protocatechuic acid, the lowest value was 0.44 g 100 g^{-1}, in 07-AA-109, and the highest value was 1.34 g 100 g^{-1}, in 07-AA-29. While the amount of catechin was between 0.67 (07-AA-73) and 2.40 (07-AA-57) g 100 g^{-1}, the amount of chlorogenic acid was in the range of 0.44 mg 100 g^{-1} (07-AA-68) to 3.04 mg 100 g^{-1} (07-AA-87). When the genotypes were compared in terms of vanillic acid level, the lowest value was in 07-AA-29 (0.61 g 100 g^{-1}) and the highest values were in 07-AA-68 and 07-AA-87 (4.66 and 4.81 g 100 g^{-1}, respectively). The highest syringic acid value was recorded in 07-AA-109 and 07-AA-73 (1.55 and 1.58 g 100

Table 5. Specific sugars of the ten strawberry tree genotypes (g 100 g^{-1}).

Genotypes	Fructose	Glucose	Sucrose
07-AA-14	9.05 ± 0.10a	6.70 ± 0.10a	1.28 ± 0.07a
07-AA-22	6.13 ± 0.09bc	4.35 ± 0.06abc	1.01 ± 0.04b
07-AA-28	5.33 ± 0.05bc	3.84 ± 0.05e	1.12 ± 0.03ab
07-AA-29	5.98 ± 0.09bc	4.02 ± 0.03d	0.83 ± 0.02bc
07-AA-57	6.94 ± 0.07b	5.53 ± 0.04b	0.70 ± 0.06c
07-AA-68	6.78 ± 0.03bc	4.44 ± 0.10abc	0.45 ± 0.01d
07-AA-73	5.56 ± 0.05bc	4.00 ± 0.07d	0.66 ± 0.03c
07-AA-81	7.03 ± 0.08ab	4.45 ± 0.06c	0.54 ± 0.02cd
07-AA-87	5.70 ± 0.04bc	3.30 ± 0.04e	0.52 ± 0.02cd
07-AA-109	4.55 ± 0.03c	3.72 ± 0.05def	0.73 ± 0.05bc

Values in the same column with different lower-case letters are significantly different at $p < 0.05$.

Table 6. The contents of major individual phenolics in the strawberry tree genotypes (mg 100 g^{-1}).

Genotypes	Gallic acid	Protocatechuic acid	Catechin	Chlorogenic acid	Vanillic acid	Syringic acid	*p*-Coumaric acid	Ferulic acid	Rutin	Phloridzin	Quercetin
07-AA-14	6.42 ± 0.38d	1.11 ± 0.05b	1.52 ± 0.07b	1.96 ± 0.03bc	1.15 ± 0.07c	0.78 ± 0.02bc	0.40 ± 0.02^{NS}	0.63 ± 0.02bc	1.55 ± 0.03bc	0.50 ± 0.02bc	0.30 ± 0.02^{NS}
07-AA-22	4.33 ± 0.20e	0.82 ± 0.03bc	1.94 ± 0.06ab	2.21 ± 0.05b	1.09 ± 0.08c	0.30 ± 0.01c	0.37 ± 0.03	0.21 ± 0.00cd	0.40 ± 0.04d	0.41 ± 0.02bc	0.55 ± 0.02
07-AA-28	10.13 ± 0.53b	0.67 ± 0.03c	1.85 ± 0.03ab	0.89 ± 0.02d	1.33 ± 0.08c	0.35 ± 0.01c	0.22 ± 0.01	0.14 ± 0.01d	1.71 ± 0.04b	0.77 ± 0.05bc	0.33 ± 0.01
07-AA-29	6.33 ± 0.14d	1.34 ± 0.05a	1.25 ± 0.05bc	0.59 ± 0.01de	0.61 ± 0.03d	0.29 ± 0.02c	0.19 ± 0.01	0.26 ± 0.02cd	0.30 ± 0.01d	0.40 ± 0.03bc	0.28 ± 0.04
07-AA-57	3.43 ± 0.09ef	1.29 ± 0.02ab	2.40 ± 0.06a	1.39 ± 0.03c	1.20 ± 0.03c	1.11 ± 0.03ab	0.17 ± 0.01	0.60 ± 0.02bc	0.80 ± 0.02c	0.63 ± 0.03bc	0.45 ± 0.02
07-AA-68	3.10 ± 0.10f	1.08 ± 0.03b	0.96 ± 0.01bc	0.44 ± 0.00e	4.66 ± 0.04a	0.76 ± 0.03bc	0.24 ± 0.00	0.81 ± 0.01b	2.50 ± 0.05a	0.90 ± 0.08b	0.20 ± 0.03
07-AA-73	12.13 ± 0.35a	0.89 ± 0.02bc	0.67 ± 0.02c	1.19 ± 0.04cd	3.30 ± 0.06b	1.58 ± 0.05a	0.30 ± 0.01	1.11 ± 0.04a	0.64 ± 0.01cd	0.58 ± 0.02bc	0.30 ± 0.01
07-AA-81	9.45 ± 0.16bc	1.17 ± 0.02ab	0.91 ± 0.03bc	1.36 ± 0.07c	1.22 ± 0.02c	0.94 ± 0.01b	0.41 ± 0.01	0.90 ± 0.01ab	2.35 ± 0.07ab	0.86 ± 0.06b	0.35 ± 0.01
07-AA-87	6.06 ± 0.14de	0.69 ± 0.03c	1.20 ± 0.05bc	3.04 ± 0.09a	4.81 ± 0.09a	0.80 ± 0.01bc	0.19 ± 0.01	0.95 ± 0.07ab	1.23 ± 0.04bc	1.56 ± 0.07a	0.24 ± 0.02
07-AA-109	8.84 ± 0.32c	0.44 ± 0.01d	0.87 ± 0.05bc	1.50 ± 0.06bc	1.44 ± 0.02c	1.55 ± 0.10a	0.23 ± 0.02	0.51 ± 0.03c	1.97 ± 0.06ab	0.30 ± 0.01c	0.40 ± 0.01

Values in the same column with different lower-case letters are significantly different at $p < 0.05$. NS: Nonsignificant.

Table 7. Number of alleles (Na), observed heterozygosity (Ho), expected heterozygosity (He), and polymorphic information content (PIC) of SSR loci for ten strawberry tree genotypes.

SSR loci	Na	Ho	He	PIC
CA421F	13	0.78	0.70	0.80
CA794F	6	0.75	0.65	0.70
NA741	3	0.65	0.61	0.60
NA800	7	0.70	0.67	0.72
VCC_I2	10	0.69	0.65	0.70
Mean	7.8	0.72	0.66	0.70

g^{-1}, respectively). Also in our study, *p*-coumaric acid (0.17–0.41 mg 100 g^{-1}), ferulic acid (0.14–1.11 mg 100 g^{-1}), rutin (0.30–2.50 mg 100 g^{-1}), phloridzin (0.30–1.56 mg 100 g^{-1}), and quercetin (0.20–0.55 mg 100 g^{-1}) were determined. In a study conducted by Caniyilmaz (2015) in *A. andrachne* fruit, the amounts of catechin and chlorogenic acid (0.89 and 0.20 mg g^{-1}, respectively) were higher than those of *p*-hydroxybenzoic acid, epicatechin, vanillic acid, and *p*-coumaric acid. Secondary plant-derived metabolites, including phenolic compounds, have a strong potential to clear free radicals that exist in all parts of the plant, such as the leaves, fruits, seeds, roots, and skin. Researchers think that many factors, such as water, air, soil, elevation (asl), and differences between species, cultivars, genotypes, and extraction methods affect the amounts of secondary metabolites in plants, including phenol compounds. Wild plant species in natural habitats, in addition to being ecologically compatible, can effectively synthesize secondary and active substances in environmental stress conditions (Neri-Numa et al., 2018).

3.3. Molecular characterization with SSR markers

Allele number (Na), observed heterozygosity (Ho), expected heterozygosity (He), and polymorphic information content (PIC) for the SSR loci are presented in Table 7. As a result of the analysis, a total of 39 polymorphic alleles were determined. The highest numbers of alleles were obtained from the CA421F (13 alleles) and VCC_12 (10 alleles) loci; these loci are followed by NA800 with 7 alleles, CA794F with 6 alleles, and NA741 with 3 alleles. Expected heterozygosity (He) was between 0.61 (NA741) and 0.70 (CA421F) (mean 0.66). The observed heterozygosity (Ho) ranged from 0.65 (NA741) to 0.78 (CA421F) (mean 0.72). The PIC value was between 0.60 (NA741) and 0.80 (CA421F). The number of molecular characterizations studies using SSR markers for *Arbutus* species is negligible. The usability (transferability) of a marker system developed especially for one fruit species in other fruit species has provided a great convenience in modern plant breeding. SSR loci developed for *Vaccinium* species by Boches et al. (2005, 2006) were used for *A. unedo* genotypes. Gomes et al. (2013) conducted a study with a total of 27 *A. unedo* genotypes from nine different regions of Portugal. Among the 11 SSR loci they used in the study (CA169F, CA421F, CA794F, CA855F, NA398, NA741, NA800, NA961, NA1040, VCC_K4, and VCC_12), only five (CA421F, CA794F, NA741, NA800, and VCC_12) were found to be polymorphic. The expected heterozygosity rate was 75%, the number of alleles per locus was 11.6, and the polymorphic information content (PIC) value was 71%. In our study, the expected heterozygosity rate (He) was 66%, the observed heterozygosity rate (Ho) was 0.72%, the number of alleles per locus was 7.8, and the PIC value was 70%. In addition, Fazenda et al. (2019) performed genetic diversity analysis with 40 SSR markers in 45 strawberry tree (*A. unedo*) genotypes from the Corte Veleda region of Portugal. They identified 1185 microsatellite loci for *A. unedo* using next-generation genome sequencing, and validated 40 microsatellite markers among them. The microsatellite dinucleotide motifs used in the study (AG/CT: 71.7%, AC/GT: 20.5%, AT/AT: 2.9%, and CG/CG: 0.3%) were similar to the results observed in other *Ericaceae* species. Among the 20 PCR products amplified with the 40 SSR primer pairs tested in the study, 12 PCR products (30%) were confirmed as polymorphic.

4.Conclusion

A. andrachne, which is naturally distributed in Antalya, Muğla, Adana, Mersin, İzmir, Artvin, Trabzon, Amasya, Sinop, Zonguldak, Bursa, and Çanakkale provinces in Türkiye, is a species that has the potential to be cultivated. It is important to determine the superior genotypes and to cultivate this fruit species with selection breeding studies to be planned in this country. There are many morphological and biochemical analyses and selection studies on *A. unedo* genotypes in Türkiye (Black Sea Region). However, there are only a few morphological and biochemical evaluations regarding *A. andrachne*, especially in the Mediterranean Region in Türkiye. Moreover, this study is the first molecular characterization study for the Turkish wild strawberry tree in the Mediterranean Region of southern

Türkiye. Results from this study will be an important resource in the evaluation of the germplasm resources to be planned for *A. andrachne* L. and in the variety breeding studies for Türkiye and surrounding countries where *A. andrachne* is grown.

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