Evaluation of Genetic Diversity by Morphological, Biochemical and Molecular Markers in Sour Cherry Genotypes Collected from the Kelkit Basin of Turkey

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Research Article

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Abstract

Background

Turkey's ecological and geographical diversity allows the cultivation of most plant species that have economic importance throughout the country. One of these species, sour cherry, is among the stone fruits that are very important for human health. In this study, it was aimed to determine by morphological, biochemical, and molecular marker technique (ISSR) in the genetic diversity of 21 sour cherry genotypes grown from seed in Kelkit Valley and standard Kutahya cultivars.

Methods and Results

In the results of the study, genotype 22 (especially fruit weight) in terms of fruit characteristics and genotype 20 in terms of leaf characteristics produced better results than the other genotypes. In molecular marker analysis, a total of 70 bands were obtained from 12 ISSR primers and 49 of them were polymorphic. The mean polymorphism rate in the study was 70%, and the DICE's similarity coefficient of genotypes differed between 0.81 and 0.95. Polymorphism percentages of the ISSR primers varied from 20–100%. In biochemical analyzes, G17 with 140.18 mg QE/100 g in total flavonoid content, G19 with 208.63 mg GAE/100 g in total phenolic content, G19 with 8.33 mg cyn-3-gluc /100 g in total anthocyanin content compared to other genotypes prominent genotypes.

Conclusion

The use of the obtained results in breeding studies to be carried out on this species, as well as the detection and protection of gene sources, may be beneficial for researchers.

Introduction

Turkey is the gene center of most fruit species due to its geographical and ecological diversity [1]. In this way, a wide variety of plants can be seen throughout the country, and because these species spread in the country in wild and cultural forms, plant cultivation is done naturally and economically [2]. One of these fruit species (*Prunus cerasus*) is sour cherry, sour cherry is one of the important stone fruits in the *Rosaceae* family [3]. The homeland of the sour cherry is the North Anatolian Mountains, which lie between Asia, Istanbul, and the Caspian Sea [4].

Sour cherry is generally consumed fresh and used in the jam and marmalade industry as fruit juice and liquor production [5, 6]. In addition, sour cherry is among the products beneficial to human health due to phytochemicals such as phenolic substances, antioxidants, flavonoids, and anthocyanins [7]. In this way, it reduces the risk of cancer, is used in the treatment of heart diseases and diabetes disorders [8].

Morphological, phenological and biochemical properties of plants can be affected by environmental conditions [9]. Therefore, there is a need to use techniques that do not have the effect of the environment and always give reliable results in genetic diversity studies [2]. Molecular markers are among the markers that are not affected by the environment and have been increasingly used in recent years [10]. Revealing genetic diversity in sour cherry is important in terms of the use and sustainability of genetic resources. Consequently, germplasms are highly guiding in breeding studies that enable the development of the species [11].

Molecular marker studies such as RAPD [12], SSR [13], ISSR [5], morphological marker studies [14], studies in which biochemical content is determined has been and continues to be done to determine the genetic diversity in sour cherry [15]. As in most plants, factors such as yield, flowering, disease, and drought resistance are controlled by many genes, and it is not reliable to determine them with a single method. that's why, multidisciplinary studies in which molecular marker (ISSR), morphological and biochemical features are evaluated together are required. Multidisciplinary studies of this type in the literature are very limited.

In this study, it was aimed to determine by morphological, biochemical and ISSR molecular markers of the genetic diversity of sour cherry genotypes grown from seed in the “Susehri” and “Akincilar” districts of Sivas province, located in the Kelkit valley.

Material And Method

This study was carried out on sour cherry genotypes grown from seed in in the “Susehri” and “Akincilar”of Sivas province located in Kelkit Valley in 2021 (Table 1 and Figure 1). In addition, "Kutahya" sour cherry variety was used in the study in terms of being a reference variety. At harvest time in fruit samples were taken from the genotypes used in the study and brought to the laboratory in the cold transport chain and analyzes were carried out. Leaves were taken from the same genotypes as the fruits were taken.

Table 1
Altitude, coordinate and region information of genotypes

Gen.	Coordinate	Altitude(m)	District
G1	40°10'00"N 38°05'43"E	1011	Susehri
G2	40°10'03"N 38°05'43"E	1005	Susehri
G3	40°10'05"N 38°05'43"E	1001	Susehri
G4	40°06'48"N 38°15'42"E	921	Akincilar
G5	40°06'46"N 38°15'46"E	918	Akincilar
G6	40°06'42"N 38°15'49"E	924	Akincilar
G7	40°06'41"N 38°16'19"E	901	Akincilar
G8	40°06'43"N 38°16'22"E	896	Akincilar
G9	40°04'57"N 38°20'32"E	1026	Akincilar
G10	40°05'09"N 38°20'22"E	998	Akincilar
G11	40°05'31"N 38°20'02"E	948	Akincilar
G12	40°09'29"N 38°05'51"E	1048	Susehri
G13	40°09'30"N 38°05'45"E	1049	Susehri
G14	40°09'28"N 38°05'43"E	1058	Susehri
G15	40°09'27"N 38°05'41"E	1065	Susehri
G16	40°09'27"N 38°05'35"E	1070	Susehri
G17	40°09'28"N 38°05'37"E	1061	Susehri
G18	40°09'27"N 38°05'33"E	1071	Susehri
G19	40°09'27"N 38°05'24"E	1069	Susehri
G20	40°09'28"N 38°05'20"E	1073	Susehri
G21	40°10'09"N 38°13'54"E	859	Susehri

Leaf and Fruit Analysis

In the study, analyzes were made on 20 fruits from each genotype that were harvested from different parts of the plant and 20 leaves from the middle part of the annual shoot. In fruit weight and seed weight measurements, precision scales sensitive to ± 0.01 g, fruit width and length, leaf width and leaf length values were obtained with 0.01 mm sensitive digital calipers. Color measurement (L^* , chroma and hue angle) in fruit skin was performed with Konica-Minolta, model CR-400, Japan instrument. Water soluble dry matter (SSC) values were determined with a hand refractometer, and Ph values were determined with the help of Ph meter. The formula below was used to determine the flesh/stone ratio.

Flesh/stone ratio = (Fruit weight – stone weight) / stone weight

The evaluation of the data of the biochemical content of the genotypes in the study was carried out using the SPSS 23.0 statistical package program. The difference between the means was determined according to the Duncan multiple comparison method at the 5% significance level.

ISSR analysis

Genomic DNA was extracted from approximately 100 mg of frozen sour cherry leaf tissues using a modified CTAB method [16]. The DNA concentration of sour cherry leaves was measured with 2% agarose gel followed gel electrophoresis with DNA ladder (100 bp). Following, an initial screen of 16 ISSR primers that have been used to perform PCR amplification. The amplification reactions were carried out in 15 μ l volume containing 2 μ l (10 ng/ μ l), 2.73 μ l PCR mix(buffer, taq DNA, dNTP, $MgCl_2$), 1 μ l Primer and 9.27 μ l nuclease-free water using Thermal cycle (Sense Quest) Lab Cycle programmed for an initial denaturation step at 94°C for 3 minutes, followed by 35 cycles of 1 minute at 94°C, 35 cycles of 50 seconds at the specific annealing temperature at 53°C, 35 cycles of 2 minutes at 72°C and ended with a final extension step 7 minutes at 72°C. Each reaction was repeated at least twice, and only reproducible bands were scored. The PCR products were electrophoresed using 2% (w/v) agarose gel for 4 hours at a constant voltage of 110 V, and then viewed under UV light using a MS. Major Science UV visualizing system.

ISSR markers were scored as presence (1) or absence (0) of bands in study. The sizes of bands ISSR were estimated by comparison with GENESTATM 100 bp DNA ladder. To evaluate the genetic diversity among the sour cherry genotypes NTSYS-pc (Version 2.11X, [17]) software (Numerical Taxonomy and Multi-variation Analysis System) was used to constitute the similarity index and UPGMA (Unweighted Pair Group Method with Arithmetic Mean of Cluster analysis) dendrogram [18].

Biochemical Analysis

Total flavonoid content

Total flavonoid content of sour cherry genotypes was determined according to the method used by [19]. 3.3 ml of methanol was added to 1000 µL of sample taken from genotypes and 0.1 ml of 10% AlCl_3 , H_2O and CH_3COOK were added to the prepared solution. The measurement of the samples was carried out in a spectrophotometer at a wavelength of 415 nm. Total flavonoid content is presented in the study as quercetin equivalent (QE), mg/100 g [19].

Total phenolic content

Total phenolic compound content of sour cherry genotypes was determined with the help of Folin-Ciocalteu's chemicals. referenced. 4.2 mL of distilled water was added to 500 µL of fresh fruit extract taken from the genotypes and treated with 100 µL of Folin-Ciocalteu's reagent and 2% sodium carbonate (Na_2CO_3). After the prepared solution was incubated for 2 hours, it was analyzed in a spectrophotometer at a wavelength of 760 nm. Obtained absorbance values were calculated in gallic acid and expressed as mg/100 g [20].

Total anthocyanin content

Total anthocyanin content of sour cherry genotypes was determined according to different Ph methods [21]. In the analysis, samples were diluted in buffer medium in 2 different pH environments and incubated for 30 minutes. It was then analyzed under spectrophotometer at wavelengths of 527 and 700 nm. The obtained values were calculated at a conversion rate of 26,900 and presented as mg/100 g [22].

DPPH antioxidant activity (Free radical scavenging activity)

DPPH antioxidant activity of cherry berries was determined with modifying the method by [23]. 0.26 mM DPPH (1.1-diphenyl-2-picryl-hydrazil) solution was used in the analysis. For each genotype, 2900 µL of ethyl alcohol and 1 ml of DPPH solution were added to 100 µL of fruit extract and mixed with vortex, and the mixture was incubated for 30 min. kept in the dark. The absorbance values of the genotypes were determined after incubation with a spectrophotometer at a wavelength of 517 nm. Total Antioxidant was given as % according to the method of [40].

The evaluation of the data of the biochemical content of the genotypes in the study was carried out using the SPSS 23.0 statistical package program. The difference between the means was determined according to the Duncan multiple comparison method at the 5% significance level.

Results

Leaf and Fruit Analysis

In the present study, statistically significant differences were observed between genotypes in all parameters examined in terms of fruit and leaf characteristics. The fruit weight values varying between 1.91 g and 4.81 g among genotypes were determined. The lowest and highest values in this parameter were genotypes 5 and 11, respectively. Genotype 4 (14.45 mm) and genotype 11 (19.01 mm) formed the lowest and highest values in fruit width value, as well as fruit weight. The Kutahya genotype used as a reference cultivar in the study was higher than the other genotypes in fruit size results. Genotype 14 formed the lowest fruit length value with 12.05 mm. The stone weight value is among the important quality parameters in sour cherry. In the study, the seed weight varied between 0.16 g and 0.37 g. In the study, it was determined by the researchers that the stone weight of sour cherry varies between 0.26 g and 0.42 g (Šebek, 2019). In the present study, the range of fruit flesh thickness was determined between 2.8 mm (genotype 8) and 5.04 mm (genotype 3). As in other fruit characteristics, the lowest and highest values in the flesh/stone ratio, where significant variations occur, were genotype 6 and Kutahya cultivars, respectively. While the pH values of the cherry genotypes differed between 2.91 and 3.25, the SSC values created a wide variation between 10.0 and 22.0

Leaf analysis of genotypes, the lowest and highest values in terms of leaf length were respectively genotype 13 (50.76 mm) and genotype 11 (80.30 mm). In leaf width, genotype 20 produced higher results than other genotypes. The petiole length and petiole thickness data, genotype 5 took the first place. Generally, some of the genotypes in the fruit and leaf parameters (except flesh/stone) examined in the study produced higher results than the Kutahya sour cherry variety (Table 2).

ISSR Analysis

In the molecular marker analysis performed in sour cherry genotypes, a total of 16 different ISSR primers were used and in 12 of them were obtained clear and interpretable bands. 70 scoreable bands were obtained from these primers and 49 of them were polymorphic. The average percentage of polymorphism in the study was 70%. The highest number of bands was found with 9 bands from the (CAC)3GC primer, and the lowest number of bands was found as 2 bands from the BDB(CA)7C primer. There is no primer producing monomorphic band in the study. The highest rate of polymorphism was detected in the primers BDB(CA)7C, (CT)8TG, (CA)8Y6 with 100%, while the lowest rate of polymorphism was in the primer (CA8)R with 16.6%. The mean number of bands per primer was determined as 5.8, and the mean number of polymorphic bands was determined as 4.08 (Table 3).

The expected and observed allelic frequency values (p,q) depending on the ISSR primers ranged from 0.299 to 0.921 and from 0.079 to 0.701, respectively. Number of effective alleles (N_e) ranged from 1.166 (CA8)R to 1.932 (CT)8TG (average 1.502), Shannon's information index (I) values from 0.115 (CA8)R to 0.675 ((CT)8TG) (average 0.413), expected heterozygosity (H_e) values from 0.083 ((CA8)R) to 0.482 ((CT)8TG) and unbiased expected heterozygosity (uH_e)

values from 0.085 ((CA8)R) to 0.495 ((CT)₈TG) (average 0,290) (Table 3). For all these parameters, (CT)₈TG ISSR primer gave the highest value. On the other hand (CA8)R produced the lowest values.. Cophenetic correlation between ultra-metric similarity tree and similarity matrix was found to be relatively high ($r = 0.91$, $P < 0.01$). Values of between 0.8 – 0.9 indicate a well-correlation between similarity indices and dendrogram [24]. Present value show that there was a high correlation between the similarity indices and the dendrogram. So, present dendrogram well represented the similarity index.

Table 2
Leaf and fruit characteristics of sour cherry genotypes

Gen.	Fruit Weight (g)	Fruit Width (mm)	Fruit Length (mm)	Stone Weight (g)	Flesh Thickness (mm)	Flesh/stone	Ph	SSC (%)	Leaf Length (mm)	Leaf Width (mm)	Petiole Thickness (mm)	Petiole Length (mm)
G1	2,17 hij	15,16 f-j	13,24 de	0,20 d-h	3,26 f-i	9,82 def	3,25 a	13,0 n	60,83 d-g	30,60 a-f	1,21 cde	12,10 cde
G2	2,39 f	15,65 ef	13,40 d	0,23 b-e	3,38 e-h	9,30 ef	3,08 fg	14,0 k	70,20 cde	30,86 abc	1,01 f-i	10,10 f-i
G3	3,75 b	17,28bc	17,49 b	0,27 b	5,04 a	12,57 abc	3,12 ef	14,5 i	80,03 ab	30,90 abc	1,33 abc	13,36 abc
G4	2,34 fgh	15,59 ef	13,08 def	0,23 bcd	3,80 cde	8,97 ef	3,01 hij	14,2 j	70,03 def	30,70 a-f	1,11 e-h	11,10 e-h
G5	1,91 k	14,45 k	12,50 g-j	0,18 fgh	3,13 hi	9,56 def	3,00 ij	13,0 n	60,36 gh	30,70 a-f	1,14 def	11,40 def
G6	3,46 c	17,03 c	17,60 b	0,37 a	4,22 bc	8,20 f	2,97 j	10,0 p	80,10 ab	30,70 a-f	1,38 ab	13,80 ab
G7	2,97 d	16,51 d	15,90 c	0,26 bc	3,69 d-g	10,38 c-f	2,99 ij	13,7 l	70,20 cde	30,23 def	1,23 b-e	12,36 b-e
G8	2,10 j	14,86 g-k	12,60 f-i	0,17 gh	2,80 i	11,04 cde	3,17 cd	18,0 c	50,70 l	30,33 c-f	0,83 j	8,30 j
G9	2,44 f	15,43 fg	13,32 de	0,22 c-g	3,66 d-g	10,03 def	2,91 k	22,0 a	50,90 hi	30,43 b-f	0,88 ij	8,80 ij
G10	2,60 e	15,95 e	13,04 def	0,22 c-f	3,77 c-f	10,71 c-f	3,08 fg	18,5 b	60,66 efg	40,00 ab	0,98 g-i	9,80 ghi
G11	4,81 a	19,01 a	17,66 b	0,37 a	4,52 b	12,05 bcd	3,20 bc	14,0 k	80,30 a	30,13 f	1,13 d-g	11,30 d-g
G12	2,31 f-i	15,34 fgh	12,90 efg	0,23 bcd	3,79 cde	8,86 ef	3,09 fg	14,6 l	60,50 fg	30,43 b-f	1,00 f-i	10,00 f-i
G13	2,03 jk	14,69 jk	12,17 ij	0,18 e-h	3,46 d-h	9,99 def	3,10 f	15,5 h	50,76 l	30,53 a-f	1,24 b-e	12,40 b-e
G14	2,03 jk	14,72 ijk	12,05 j	0,21 d-h	3,61 d-h	9,20 ef	3,02 hi	16,0 g	60,80 d-g	30,76 a-e	1,14 def	11,40 def
G15	2,17 hij	14,94gk	12,85 efg	0,20 d-h	3,10 hi	9,71 def	3,05 gh	14,0 k	70,30 cd	40,03 ab	1,44 a	14,43 a
G16	2,16 hij	15,24 fgh	12,31 hij	0,20 d-h	3,54 d-h	9,92 def	3,01 hij	13,8 l	70,03 def	30,90 abc	1,14 def	11,43 def
G17	2,15 ij	14,89 g-k	12,89 efg	0,21 c-g	3,53 d-h	8,98 ef	2,98 ij	17,0 d	60,63 efg	30,43 b-f	1,27 bcd	12,76 bcd
G18	2,18 hij	15,20 f-i	12,91 efg	0,19 d-h	3,43 e-h	10,33 c-f	3,15 de	11,3 o	70,13 cde	30,80 a-d	1,28 bcd	12,86 bcd
G19	2,21 g-j	15,36 fgh	12,71 fgh	0,18 e-h	3,19 ghi	10,81 cde	3,11 ef	16,5 e	40,66 j	30,16 ef	0,96 hij	9,66 hij
G20	2,36 fg	15,66 ef	12,86 efg	0,23 c-f	3,38 e-h	9,53 ef	3,05 gh	13,5 m	70,70 bc	40,10 a	1,38 ab	13,86 ab
G21	2,30 f-i	15,45 fg	13,08 def	0,16 h	3,80 cde	13,55 ab	3,22 ab	16,2 f	60,60 efg	30,90 abc	0,87 ij	8,70 ij
Kutahya	3,32 c	17,68 b	20,35 a	0,21 c-g	3,98 cd	14,41 a	3,05 gh	18,5 b	70,20 cde	40,10 a	1,09 e-h	10,96 e-h
Mean	2,55	15,73	13,95	0,22	3,64	10,36	3,07	15,08	60,84	30,67	1,14	11,40

Table 3
ISSR primers studied, total number of bands, number of polymorphic bands, rate of polymorphism, allele frequency (p and q), number of effective alleles (Ne), Shannon's information index (I), expected (He) and unbiased expected heterozygosity (uHe)

Primer	T. B. N	P. B. N	P. R. (%)	p	q	Ne	I	He	uHe
VHV(GTG) ₇	7	6	85,7	0,763	0,237	1,520	0,382	0,272	0,280
(TCC) ₅ RY	8	6	75	0,376	0,624	1,407	0,386	0,248	0,254
(GT) ₆ GG	8	7	87,5	0,378	0,622	1,753	0,609	0,421	0,432
DBDA(CA) ₇	4	1	25	0,859	0,141	1,242	0,171	0,123	0,126
(AG) ₈ T	5	1	20	0,877	0,123	1,298	0,247	0,171	0,175
(CAC) ₃ GC	9	7	77,7	0,428	0,572	1,535	0,462	0,309	0,317
BDB(CA) ₇ C	2	2	100	0,709	0,291	1,688	0,558	0,401	0,410
(CAA) ₆	7	6	85,7	0,484	0,516	1,533	0,456	0,309	0,318
(AGC) ₆ G	5	3	60	0,854	0,146	1,347	0,330	0,218	0,224
(CT) ₈ TG	4	4	100	0,544	0,456	1,932	0,675	0,482	0,495
(CA) ₈ R	6	1	16,6	0,921	0,079	1,166	0,115	0,083	0,085
(CA) ₈ Y6	5	5	100	0,299	0,701	1,681	0,566	0,385	0,396
Total	70	49	-	-	-	-	-	-	-
Mean	5,8	4,08	70	0,590	0,410	1,502	0,413	0,282	0,290

Figure. UPGMA dendrogram obtained from ISSR primers in cherry genotypes

According to the UPGMA dendrogram created in sour cherry genotypes, the similarity index of the genotypes is between 0.81 and 0.95 (Figure 2). In the dendrogram, all genotypes were separated from each other, and 2 main groups were formed among the genotypes. Genotypes in the study (except V18 and V21) were generally in group A. In group B, there are Kutahya variety, which is the reference variety, and genotype 18 and genotype 21. Genotypes 2, 20, 17 and 19 were found in group A-2, which is one of the subgroups formed in group A. In the study, genotypes closest to each other were genotype 12 and genotype 13 with a similarity index of 0.95. Besides, all genotypes collected from Akincilar district were included in group A. There are studies in which different marker systems are used in sour cherry species and the genetic relationship is determined.

Biochemical Analysis

There were wide variations in total flavonoid content, total phenolic content and total anthocyanin content obtained from sour cherry genotypes collected from Akincilar and Susehri locations, and these differences were found to be statistically significant. The locations from which genotypes were taken in the investigated parameters did not affect these parameters. The values in total flavonoid content varied between 68.14 mg QE/100 g (genotype 1) and 140.18 mg QE/100 g (genotype 17). After genotype 17, genotype 5 formed the highest value with 135.00 mg QE/100 g. Total phenolic values were determined in genotype 1 as well as the lowest total flavonoid value, which is 124.72 mg GAE/100g. The highest total phenolic value was detected in genotype 19 with 208, 63 mg GAE/100g. Genotype 20 ranked second with 180.66 mg GAE/100g.

Table 4
Total phenolic, total flavonoid, total anthocyanin contents in sour cherry genotypes

Gen.	Total flavonoids (mg QE/100 g)	Total phenolics (mg GAE/100g)	Total Antociyanin (mg cyn-3-gluc /100 g)	Antioxidant activity (%)
G1	68,14 u	124,72 u	1,23 t	13,77 n
G2	81,11 m	138,77 o	4,80 l	15,22 l
G3	94,81 k	127,96 s	2,04 p	14,13 mn
G4	108,52 e	164,04 h	4,00 k	31,16 e
G5	135,00 b	168,23 f	3,44 l	32,61 d
G6	70,55 t	125,39 t	1,49 r	14,13 mn
G7	100,74 h	134,72 p	3,10 n	15,22 l
G8	109,81 d	166,20 g	6,44 e	30,50 f
G9	113,52 c	147,82 n	2,75 o	19,57 j
G10	76,11 r	149,58 m	5,99 f	18,84 k
G11	77,40 p	124,85 u	1,49 r	13,04 o
G12	79,44 o	131,07 r	1,24 st	14,49 m
G13	101,48 g	158,91 l	7,68 b	29,35 g
G14	98,52 j	153,91 j	4,22 j	27,17 h
G15	94,81 k	152,28 l	5,89 g	24,64 i
G16	80,55 n	153,50 k	3,27 m	24,64 l
G17	140,18 a	178,23 c	5,99 f	38,04 c
G18	75,37 s	174,04 d	7,66 c	37,68 c
G19	104,07 f	208,63 a	8,33 a	44,93 a
G20	99,44 l	180,66 b	5,19 h	39,13 b
G21	94,81 k	152,28 l	1,25 s	24,64 i
Kutahya	91,85 l	172,69 e	7,21 d	37,68 c
Mean	95,28	154,02	4,30	25,49

Total anthocyanin content, which is the last biochemical content discussed in the current study, was determined as the lowest genotype 1, as well as total flavonoid and total phenolic contents. The values of the genotypes differed between 1.23 mg cyn-3-gluc /100 g and 8.33 mg cyn-3-gluc /100 g. Genotype 19 had the highest value in this parameter, followed by genotype 13 with 7.68 mg cyn-3-gluc /100 g. The lowest antioxidant value of the genotypes was determined in genotype 1 with 13,77%, while the highest antioxidant value was found in genotype 19 with 44.93% (Table 4).

Discussion

Leaf and fruit characteristics are among the important indicators in distinguishing the species. There are characterization studies in sour cherry depending on morphological data. Different researchers have reported that fruit weight values in sour cherries vary between 3.36 g and 5.01 g [14], 2.4 g and 7.8 [25]. Another study conducted with sour cherry, fruit width was found to be between 10.79 mm and 20.16 mm, and fruit length was found to be between 16.11 and 20.11 mm [26]. Researcher reported, SSC values varied between 8.80 and 10.05 [27], from 14.53 to 15.70 [28]. Leaf width values are between 34.0 mm and 47.30 mm [14], leaf length values are between 73.2 mm and 109.8 mm [29], and petiole length are between 3mm and 21.7mm [30]. Leaf and fruit characteristics investigated in the study. In general, it has similar features with the studies in the literature. The variability of the genotypes used, and the ecology can be shown as the reason for the partial differences.

Genetic diversity studies on the *Prunus cerasus* species have been very limited. ISSR markers are among the markers that produce high and reproducible bands in fruit species such as cherry, sour cherry, peach, and apricot [31]. In the genetic diversity study with ISSR markers carried out in the *Prunus* genus *Cerasus* subspecies, the average number of bands per marker was 13, the mean number of polymorphic bands was 12.58, and the mean polymorphism rate was 96.46% [32]. In another study, the average number of bands per marker was determined as 5 in the SSR analysis performed in the *Cerasus* subspecies [33]. While the average number of bands per marker was 7.78 and the mean number of polymorphic bands was 6.42 with the SSR marker method, the average polymorphism rate was 78.76% in the study [34]. Besides, researchers have reported that the similarity index in the SSR marker system varies between 0.71 and 0.96 [35]. Similarity index in cherry showed a change from 0.36 to 0.91 between genotypes in the RAPD analysis [33]. In the ISSR marker system, a similarity index varying between 0.61 and 0.79 was formed [5]. The results of the molecular marker analysis of the current study are

generally like the studies in the literature. The resulting differences can be explained by the fact that the marker systems used are different, as well as the variability in the material used.

Biochemical marker analyzes used to determine genetic diversity in sour cherry genotypes, wide variations were observed between genotypes as in other marker systems. In different studies, the total flavonoid content of sour cherry is between 138.6 mg QE/100 g [36], 60.1 mg QE/100 g and 130.3 mg QE/100 g [37]. It has been reported by researchers that the total phenolic content in the cherry type is between 74 mg GAE/100g and 754 mg GAE/100g, and in another study, it varies from 74.67 mg GAE/100g to 121.46 mg GAE/100g [15, 37]. Total anthocyanin contents ranged from 3.11 mg cyn-3-gluc /100 g to 4.93 mg cyn-3-gluc /100 g [38], 2.27 mg cyn-3 -gluc /100 g to 4.57 mg cyn-3-gluc /100 g [39]. The results of the present study have parallel results with the parameters examined with the studies in the literature.

In summary, genetic diversity was determined using morphological, molecular, and biochemical markers in 21 cherry genotypes grown from seed in Akincilar and Susehri districts of Sivas province located in the Kelkit basin. Wide variations emerged among genotypes depending on these markers. In terms of morphological characteristics, especially in terms of fruit weight, Genotype 11 produced very high results compared to the reference variety. Since morphological and biochemical markers alone will not be sufficient to determine genetic diversity, their use in combination with the molecular marker system brings reliable results. It is predicted that the results obtained will be beneficial for researchers in the breeding, protection, and creation of germplasm in this species.

Declarations

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Conflicts of Interest

The author declare that author have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Consent to participate

Author mentioned in the manuscript have agreed for authorship, read and approved the manuscript.

Consent to Publish (Ethics)

Author give the consent for the publication of identifiable details, which can include photograph(s)/tables and/or details within the text to be published in the "Molecular Biology Reports" Journal.

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Figures

Figure 1

Map of Sivas province where sour cherry genotypes were collected

Figure 2

UPGMA denrogram obtained from ISSR primers in cherry genotypes