Elucidation of Genetic Diversity as Morphological, Phytochemical and Molecular Markers in Some Hawthorn (*Crataegus*) Genotypes

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Abstract

Backround:

Thanks to its ecological and geographical location, Turkey is the homeland of many fruit species and allows many fruit species to be grown. Hawthorn, which is understood to be important in human health and nutrition, is one of these fruit types. This study was carried out to identify morphological, biochemical and molecular genetic variations of 22 hawthorn genotypes belonging to three different species collected from Kayseri province.

Methods and Results:

Morphological, biochemical and molecular marker (ISSR) techniques were used to determine genetic diversity. The fruit and leaf characteristics of the genotypes showed differences. Among the biochemical properties of the genotypes, the antioxidant activity ranged from 23.13–61.59%, the total flavonoid content ranged from 16.63 to 57.22 mg QE/100 g, and the total phenolic content ranged from 277.28 to 310.80 mg GAE/100 g. Genotypes were compared with principal component analysis according to their morphological and biochemical characteristics. In the principal component analysis, species generally formed similar clusters. In molecular marker analysis, 101 bands were obtained from 13 ISSR primers. 76 of the bands are polymorphic and the polymorphism rate was calculated as 75.24%. The similarity index in the UPGMA dendogram obtained as a result of the molecular analysis ranged between 0.71 and 0.88. In the dendrogram, genotypes did not show a dense clustering by species.

Conclusion

The results obtained may benefit researchers in the determination and protection of gene sources in breeding studies on hawthorn species.

Introduction

Since the climate in Turkey changes from subtropical to terrestrial, most plant species grow naturally and economically throughout the country [1]. Among these plants, hawthorn (*Crataegus* spp.) is widely distributed in the northern hemisphere [2]. Its tree form varies from shrub to single tree form and is widely found in Western Asia, North America, and Europe [3–4]

Turkey is an important gene center for hawthorn species. It has been reported that there are 25 hawthorn species including those recently identified in Turkey [2]. Hawthorn grows naturally in mountainous areas, bushes and rocks in our country and no cultural treatment is applied to these natural plants. While the most common hawthorn species in Turkey is *Crataegus monogyna*, *Crataegus orientalis*, *Crataegus oxyacantha*, and *Crataegus aronia* species are other common species. In different regions of Turkey, it is known by local names such as “halıç, yaban gülü, haziran, yemişen, alıç, aluç and ekşi muşmula” [5–6].

Although the hawthorn has many different uses, it has not found enough interest compared to other fruit species. Hawthorn is used as an ornamental plant due to its plant shape and flowers, and in addition, it is generally known as a wild fruit [7]. Since ancient times, its fruits were generally collected from nature and evaluated, today there is an increasing interest in the economic cultivation of the consumer and hawthorn orchards are established in Turkey. Due to its rich phytochemical content, hawthorn fruits are among the important medicinal plants in terms of both healthy nutrition and pharmacy [8]. Considering horticultural crops, hawthorn is known that it has the potential to be used as rootstock for some important pome fruit species ([1–9].

With the increasing world population, unconscious use of plant resources to meet human needs, damage to the natural cover due to land expansion, urbanization and industrialization cause the decrease and rapid loss of plant gene resources. [10]. The starting point for the effective use and protection of gene resources is the detection of genetic variation [11]. Thus, rather than those that are closely related to each other, those with a wide variety should be preferred. Morphological, molecular, phenological and phytochemical marker systems have been used for years to determine genetic diversity among plants, including hawthorn [12–13–14–15–16–17–18]. Among these marker systems, molecular techniques without the effect of environmental conditions give more precise results than others [19]. Since some features that play a role in resistance to abiotic and biotic conditions in plants are controlled by multiple genes [20], studies that combine molecular, phytochemical, and morphological markers are needed for the determination, protection, and sustainability of plant genetic diversity.

In this study, it was aimed to determine genetic variation in 22 hawthorn genotypes, which stand out in terms of different characteristics such as shape and size, in Kayseri province, by ISSR molecular marker technique, phytochemical, leaf and fruit characteristics.

Material And Method

Material

In the study, 22 different hawthorn genotypes (belonging to 3 different species) differing from each other in parameters such as fruit weight, fruit shape and fruit color were used as plant material. Genotypes were collected from different regions of Kayseri province located in the center of Anatolia (Table 1). In terms of ecological conditions, Kayseri province is generally cold and snowy in winter, hot and dry in summer, and has a soil structure that is poor in organic matter and poor water holding capacity in terms of soil characteristics (Figure 1).

Table 1
Species name, coordinate, and altitude information of hawthorn genotypes

Gen.	Species Name	Coordinate	Attitue
G1	*Crataegus azarolus* L. var. azarolus	38° 39′ 56 - 35° 31′ 28	1277
G2	*Crataegus azarolus* L. var. azarolus	38° 38′ 57 - 35° 31′ 50	1374
G3	*Crataegus azarolus* L. var. azarolus	38° 38′ 56 - 35° 33′ 11	1333
G4	*Crataegus monogyna* Jacq. var. monagyna	38° 40′ 04 - 35° 31′ 39	1261
G5	*Crataegus monogyna* Jacq. var. monagyna	38° 39′ 44 - 35° 31′ 28	1289
G6	*Crataegus monogyna* Jacq. var. monagyna	38° 39′ 12 - 35° 31′ 34	1356
G7	*Crataegus monogyna* Jacq. var. monagyna	38° 38′ 58 - 35° 31′ 45	1383
G8	*Crataegus monogyna* Jacq. var. monagyna	38° 39′ 44 - 35° 31′ 28	1289
G9	*Crataegus monogyna* Jacq. var. monagyna	38° 46′ 14 - 35° 40′ 33	1348
G10	*Crataegus azarolus* L. var. dentata	38° 47′ 04 - 35° 41′ 22	1318
G11	*Crataegus monogyna* Jacq. var. monagyna	38° 48′ 21 - 35° 42′ 34	1324
G12	*Crataegus monogyna* Jacq. var. monagyna	38° 48′ 34 - 35° 42′ 40	1322
G13	*Crataegus monogyna* Jacq. var. monagyna	38° 48′ 46 - 35° 42′ 47	1304
G14	*Crataegus monogyna* Jacq. var. monagyna	38° 48′ 45 - 35° 42′ 59	1308
G15	*Crataegus monogyna* Jacq. var. monagyna	38° 48′ 10 - 35° 42′ 28	1323
G16	*Crataegus azarolus* L. var. azarolus	38° 48′ 46 - 35° 43′ 00	1302
G17	*Crataegus monogyna* Jacq. var. monagyna	38° 47′ 33 - 35° 43′ 50	1316
G18	*Crataegus azarolus* L. var. dentata	38° 48′ 10 - 35° 42′ 28	1323
G19	*Crataegus azarolus* L. var. dentata	38° 47′ 33 - 35° 43′ 50	1316
G20	*Crataegus azarolus* L. var. dentata	38° 40′ 04 - 35° 31′ 42	1263
G21	*Crataegus monogyna* Jacq. var. monagyna	38° 39′ 17 - 35° 28′ 41	1291
G22	*Crataegus azarolus* L. var. azarolus	38° 38′ 40 - 35° 29′ 15	1395

Methods

Fruit and Leaf Characterization

For leaf and fruit characterization of genotypes, 20 leaf and fruit samples from each genotype with 3 replicates were collected at the time of fruit ripening (end of September, beginning of October). Random leaf (middle parts of the annual shoot are preferred) and fruit samples were taken from different regions of the genotype to which these samples belong. In the leaf samples, leaf width, leaf length, petiole length, petiole thickness was measured in mm with the help of a digital caliper sensitive to 0.01. In fruit samples, fruit weight and stone weight (0.01 g sensitive scale), fruit length (mm), fruit width (mm), fruit pulp/stone ratio; Flesh / stone ratio = (Fruit weight - stone weight) / stone weight was determined. The soluble solid content was determined with the help of a hand refractometer.

Phytochemical Analysis

Phytochemical properties of hawthorn genotypes were determined with 3 replicates and 20 fruits per replicate. The stones of the fruits were removed, and the extractions were carried out by making them homogeneous with a hand blender. For this, 10 g of each sample was taken, and 10 mL of 80% methanol was added. The samples were centrifuged at 6000 × g for 5 min at 4 ◦C. Supernatant was collected and used in total phenolic, anthocyanin, flavonoid, and antioxidant assay procedures.

Total monomeric anthocyanin (TMA) content

The TMA content was measured using a spectrophotometric pH differential protocol [21]. Samples taken from the extractant were incubated for 2 hours in a buffer medium, then readings were taken in the spectrophotometer at 527 and 700 nm wavelengths. Results are given as (mg cyn-3-gluc /100 g).

Total Flavonoids content

Flavonoid contents were determined according to method reported by [22]. For this purpose, 500 µL of extract was taken, 500 µL of distilled water and 100 µL of 5% $NaNO_3$ were added to it and mixed for 6 minutes. waited, then 100 µL of 10% $AlCl_3$ was added. Mixing again and 6 min. After standing, 1 mL of 1.0 M NaOH was added, and the volume was made up to 2.5 mL with distilled water. Samples were read at 510 nm wavelength and the results were presented as mg/100 g fresh weight as quercetin equivalent (QE).

DPPH antioxidant activity (Free radical scavenging activity)

Brand-Williams et al. [23]'s method was used in the determination of antioxidant activity. A 0.2 mM DPPH (1.1-diphenyl-2-picryl-hydrazil) solution was prepared for analysis. After adding 2900 µL of DPPH solution to 100 µL of fruit extract and mixing with vortex, the mixture was incubated for 30 min. was incubated in the dark. Readings were made at a wavelength of 517 nm and results presented as % according to Garcia et al. [24].

Total Phenolics Content

To determine the total phenolic content of the samples, Singleton et al. [25]'s Folin-Ciocalteu method was used with minor modifications. For this purpose, 200 µL of extract was taken and 1800 µL of distilled water and 1 mL of 1/10 diluted Folin-Ciocalteu's solution were added, and 2 mL of 2% Na2CO3 was added to the samples that were mixed and kept for 5 minutes. After the thoroughly mixed samples were kept in the dark for 1 hour, the absorbance values were measured spectrophotometrically at a wavelength of 760 nm. The results obtained were calculated in gallic acid and expressed as mg100 g (fresh weight).

ISSR Analysis

DNA isolation from young leaves in hawthorn genotypes was extracted according to the CTAB method [26]. DNA concentrations of genotypes were measured with a spectrophotometer (BioTek Instruments, Inc., Winooski, VT, United States) and DNAs were stored at -20°C. In ISSR analysis, testing was performed on 20 different primers, and band formation was observed in 13 of these primers. PCR contents 2 µl DNA (20 ng), 1.5µl 10x PCR Buffer, 0.2 µl Taq DNA polymerase (5u/µL), 1 µl dNTP (2.5mM), 1.5µl $MgCl_2$ (25 mM), 2 µl 10 mM ISSR primer, and 6.8 µl of H_2O, PCR cycles were performed according to Yaman [20].

Data Analysis

Leaf and fruit samples used in morphological characterization and phytochemical properties were analyzed using SPSS 23.0 package program. Duncan multiple comparison test was used in the evaluation of the results, and the values were presented at the 5% significance level. Principal component analysis (PCA) based on morphological and biochemical data was performed with JMP pro 14 software (SAS Institute Inc., Cary, NC, USA). In ISSR analyzes, images obtained after agarose gel electrophoresis and imaging process (Kodak) were scored in the presence of band (1), if there is no band (0), and if amplification did not occur (9). The data obtained were evaluated by using the NTSYS (Version 2.11X) computer program [27]. By creating a similarity matrix with the Dice method, dendrograms were formed in hawthorn genotypes according to the UPGMA (Unweighted Pair Group Method with Arithmetic Mean of Cluster analysis) method [28]. In addition, the total number of bands, the number of polymorphic bands and the rate of polymorphism were determined for each marker used in the study. While calculating the polymorphism ratio, the formula (Number of Polymorphic Bands X 100 / Total Number of Bands) was used.

Results And Discussion

Morphological Data

In all the morphological (leaf and fruit) parameters examined in the study, wide variations occurred between genotypes, and these results were found to be statistically significant (Table 2). While the G18 genotype with 48.53 mm and the G7 genotype with 46.40 mm produced high results in leaf length values of the genotypes, the lowest leaf length was found in the G10 genotype with 20.93 mm. In leaf width, the G7 genotype took the first place with 46.33 mm, and the average leaf width of the genotypes was determined as 27.39 mm. Leaf characteristics are among the parameters used to distinguish genotypes, and there may be differences in the results depending on the plant material used [29]. It has been reported

that the leaf length parameter varies between 20.4 mm and 130.3 mm, the leaf width parameter varies from 13.3 mm to 45.7 mm, and the petiole value varies between 6.2 mm and 20.0 mm in different hawthorn genotypes [30]. Petiole length and petiole thickness values of 19.67 mm (G7 genotype) and 0.97 mm (G3 genotype), respectively, are the highest results. In the leaf parameters examined in general, the G7 genotype formed higher values compared to the other genotypes. As in our study, it has been reported in the literature that there are differences in leaf parameters between hawthorn genotypes [31].

Fruit characteristics are among the important morphological features in hawthorn as in most fruit species. Fruit length values examined in the present study differed between 10.77 mm (G15 genotype) and 17.32 mm (G3 genotype), and the average fruit length was determined as 12.88 mm. Various studies have been carried out to determine fruit characteristics in hawthorn species. It has been reported that fruit length values in different genotypes vary between 13.00 mm and 18.20 mm in Iran [32], and between 23.90 mm and 27.00 mm in Turkey [33]. In our study, the highest fruit width was determined at 20.64 mm in the G3 genotype, while the lowest was 10.33 mm in the G11 genotype. In previous studies, it has been reported that the variation in fruit width values ranges from 6.56 mm to 20.78 mm [34] and 10.52 mm to 29.48 mm [35]. In the fruit weight results, while the average value of the genotypes was 1.42 g, the highest value was 3.13 g and the G3 genotype formed. The most prominent genotypes compared to other genotypes in stone weight and meat core ratio were G7 genotype with 0.32 g and G2 genotype with 16.59 values, respectiv. In a study, it was determined that the fruit weight values ranged from 0.65 g to 4.19 g [36], and the stone weight was between 0.19-0.38 g [37]. In the current study, it was determined that the SÇKM contents of the genotypes varied between 9.00% and 18.00%. In general, our findings on fruit characteristics showed similar features with the findings in the literature, as well as partial differences. The reason for this may be the different genetic material used.

Table 2. Leaf and fruit characteristics of hawthorn genotypes

Gen.	L.L (mm)	L.W (mm)	P.L (mm)	P.T (mm)	F.L (mm)	F.WH (mm)	F.W (g)	S.W (g)	Flesh/ Stone	SSC
G1	28,43de	23,87gh	3,33ij	0,57bc	14,69bc	18,98b	2,58c	0,18e-i	12,96bc	13,00cd
G2	30,87cde	37,47abc	6,40hij	0,90a	16,00b	16,71c	2,61c	0,15i	16,59a	11,00ef
G3	37,43bc	42,50ab	7,87j	0,97a	17,32a	20,64a	3,13a	0,20d-i	15,33ab	13,00cd
G4	34,73bcd	25,10e-h	8,07f-i	0,47cde	12,27d-j	13,05h-k	1,34f	0,21c-i	5,48ef	14,00c
G5	36,27bc	35,53bcd	12,97b-f	0,50cd	13,45cd	13,24hij	1,33f	0,30ab	3,33gh	16,00b
G6	36,57bc	31,03c-g	17,67ab	0,40c-f	12,13d-j	11,78j-m	1,11hi	0,22b-i	4,17gh	17,00ab
G7	46,40a	46,33a	19,67a	0,47cde	13,58cd	12,68ijk	1,16hi	0,32a	2,64gh	14,00c
G8	25,43ef	18,80hi	11,73c-g	0,17g	11,65e-j	10,69m	0,81kl	0,24a-i	2,51gh	10,00fg
G9	27,77de	25,33e-h	13,90bcd	0,47cde	12,88def	13,66ghi	1,20fg	0,30abc	3,08gh	14,00c
G10	20,93f	13,07i	2,73j	0,30efg	12,67d-g	15,03def	1,74d	0,22b-i	7,12e	12,00de
G11	28,63de	20,97hi	9,10d-h	0,40c-f	10,83ij	10,33m	0,69l	0,19e-i	2,75gh	14,00c
G12	28,00de	19,80hi	7,43g-i	0,30efg	12,06d-j	10,92lm	0,80kl	0,26a-g	2,12g	11,00ef
G13	33,10bcd	24,23fgh	13,57b-e	0,43c-f	12,47d-h	12,49i-l	1,02ij	0,26a-g	3,06gh	12,00de
G14	36,03bc	34,10b-e	16,57abc	0,37def	11,23g-j	11,89j-m	0,82kl	0,28a-e	2,04g	13,00cd
G15	25,33ef	18,53hi	9,63d-h	0,27fg	10,77j	10,58m	0,79kl	0,18e-i	3,58gh	9,00g
G16	28,63de	17,20hi	8,43e-i	0,43c-f	12,90def	15,40cde	1,73d	0,16f-i	10,60cd	12,00de
G17	32,20b-e	33,70b-f	13,23b-e	0,47cde	11,10hij	11,41klm	0,86k	0,28a-d	2,11g	13,00cd
G18	48,53a	38,23abc	19,63a	0,73b	12,90 def	16,36cd	0,91jk	0,25a-h	2,83gh	9,00g
G19	38,90b	25,77e-h	6,90g-i	0,50cd	12,34d-i	14,61efg	1,51e	0,19d-i	7,06e	16,00b
G20	32,40b-e	23,27gh	7,30g-i	0,57bc	11,37f-j	13,60ghi	1,05hij	0,31a	2,38gh	18,00a
G21	32,47b-e	26,53d-h	17,20ab	0,27fg	13,00de	13,10h-k	1,17hi	0,27a-f	3,48gh	14,00c
G22	39,00b	21,27hi	5,53hij	0,53cd	15,73b	20,90a	2,80b	0,31a	7,97de	14,00c
Mean	**33,09**	**27,39**	**10,86**	**0,48**	**12,88**	**14,00**	**1,42**	**0,24**	**5,60**	**13,14**

L.L: Leaf Length, LW: Leaf Width, P.L: Petiole Length, P.T: Petiole Thickness, F.L: Fruit Length, F.WH: Fruit Width, F.W: Fruit Weight, S.W: Stone Weight, SSC: Soluble Solid Content

Different lower case letters show statistically significant differences between genotypes in column ($p < 0.05$

Phytochemical Data

Phytochemicals are very important for human health. Studies have been carried out in different fruit species, especially on antioxidants, phenolics, anthocyanins and flavonoids that constitute phytochemicals. In all these traits examined in the present study, wide variations emerged between genotypes, and these results were found to be statistically significant (Table 3).

Table 3. Phytochemical characteristics in hawthorn genotypes

Gen.	Antioxidant activity (% inhibition)	Total flavonoids (mg QE/100 g)	Total phenolics (mg GAE/100 g)	Total Monomeric Anthocyanin (mg cyn-3-gluc /100 g)
G1	51,45g	26,48ij	290,26j	0,85n
G2	61,59a	36,48cd	286,74k	1,42k
G3	58,70bc	35,00de	277,28l	1,38kl
G4	39,13m	20,55k	302,69h	3,27e
G5	55,80de	15,74l	302,42h	2,84fg
G6	34,06no	32,77ef	303,23gh	2,95f
G7	53,62f	25,37j	308,91ab	2,71gh
G8	23,13r	25,74j	306,47b-e	2,55hi
G9	48,70h	30,18gh	305,39c-g	2,76fgh
G10	56,52d	32,03fg	308,09a-d	2,40ij
G11	29,71p	31,66fg	306,74b-e	4,69a
G12	39,86lm	25,74j	306,47b-e	2,26j
G13	43,48ij	47,59b	307,28bcd	4,07b
G14	42,03jk	28,70hi	306,20b-f	3,19e
G15	34,78n	46,85b	309,45ab	3,95bc
G16	32,61o	57,22a	304,04fgh	1,11m
G17	44,20i	26,48ij	308,36abc	3,64d
G18	58,70bc	22,40k	298,09i	1,19lm
G19	54,35ef	37,96c	310,80a	1,55k
G20	60,14ab	36,85cd	307,55bcd	3,93bc
G21	57,25cd	14,63l	304,85d-g	3,82cd
G22	41,30kl	28,33hi	279,72l	1,35kl
Mean	46,41	31,12	301,87	2,63

Different lower case letters show statistically significant differences between genotypes in column ($p < 0.05$)

In the study, the highest value in % antioxidant activity was the G2 genotype with 61.59%, and the G20 genotype took the second place with 60.14%. The lowest value was G8 genotype with 23.13%. The lowest and highest values in total flavonoid values were between 14.63 mg QE/100 g (G21 genotype) and 57.22 mg QE/100 g (G16 genotype), while the average total flavonoid value was determined as 31.12 mg QE/100 g. 10 genotypes (G2, G3, G6, G10, G11, G13, G15, G16, G19, G20) of the 22 different hawthorn genotypes used in the study showed flavonoid content above the average value. In a previous study, the total amount of flavonoids in different hawthorn genotypes was determined as 78.2 and 272.6 mg/100 g in terms of catechins [38]. The variation of genotypes in total phenolic content in the study ranged from 277.28 mg GAE/100 g to 310.80 mg GAE/100 g. The lowest value was G3 genotype, and the highest value was G19 genotype. It was determined that the genotypes included in the study had an average total phenolic content of 300 mg GAE/100 g. In a study, it was reported that the phenolic content of different hawthorn genotypes ranged from 21.19 to 69.12 mg GAE/g [39]. In the current study, the G1 genotype had the lowest value with 0.85 mg cyn-3-gluc /100 g, and the G11 genotype with 4.69 mg cyn-3-gluc/100 had the highest value among the total monomeric anthocyanin values of the genotypes. Salmanian et al. [40]

determined the total anthocyanin value in hawthorn fruit as 1.94 mg CE g/l. In general, the results obtained from the study are like the studies in the literature. The reason for the differences may be the differences in the genetic material used in the study as well as the differences in the methods used.

Principal Component Analysis (PCA) of Morphological and Biochemical Characteristics

Principal component analysis values based on morphological and biochemical data of hawthorn genotypes are given in Table 4. According to principal component analysis, the first three components can explain most of the total variation (72.16%). The first (PC1), second (PC2) and third (PC3) principal components represent 42.14%, 20.40% and 9.62% of the total variance, respectively. The contribution rates of morphological and biochemical properties to the first three main components differ. Among the basic components, the highest contribution to PC1 is made mainly by fruit weight, fruit width, fruit length, total phenolic substance content, flesh/stone ratio and petiole thickness. Leaf length, leaf width, stem length and total flavonoid properties made the highest contribution to PC2, while SSC, stone weight and petiole length properties made the highest contribution to PC3.

The graph of the positions corresponding to the correlation values of the hawthorn genotypes is given in Figure 2. In the decomposition made according to the first two basic components, genotypes belonging to the species were grouped within themselves in general terms. However, although they are different species according to their morphological and biochemical characteristics, G16 genotype was clustered with *C. azarolus var. dentate* species and G20 genotype was clustered with *C. monogyna var.* species. We can show the effect of the environment on morphological features as the reason for this difference. Our finding of the cumulative variance value for the first two components was similar to that of Moghadam et al. [41] and Olvera et al. [42] results, Su et al. [43] results was found to be higher. Principal component analysis reduces complex data with a small number of variables by reducing the number of associated variables. The high PC1, PC2 and PC3 variance values obtained from the study results show that the observed traits are successful in classifying the hawthorn genotypes.

Tablo 4. Principal component analysis and contribution ration based on morphological and biochemical data of hawthorn genotypes

Parameters	PC1	% Contribution	PC2	% Contribution	PC3	% Contribution
Fruit Width	0.3766	14.18	-0.0146	0.02	0.1348	1.81
Fruit Length	0.3660	13.39	0.0513	0.26	0.0517	0.26
Fruit Wight	0.3861	14.90	-0.1108	1.22	0.1577	2.48
Stone Wight	-0.1087	1.18	0.3155	9.95	0.4903	24.03
Flesh/Stone	0.3574	12.77	-0.2001	4.00	-0.1267	1.60
Soluble Solid Content	-0.0124	0.01	0.1982	3.92	0.5041	25.41
Leaf Length	0.1091	1.19	0.4608	21.23	-0.2620	6.86
Leaf Width	0.1446	2.09	0.4592	21.08	-0.2653	7.03
Petiole Length	-0.1423	2.02	0.4090	16.72	-0.3925	15.40
Petiole Thickness	0.3246	10.53	0.1576	2.48	-0.1170	1.36
Total Phenolics Content	-0.3634	13.20	0.0004	0.00	-0.0311	0.09
Total Monomeric Anthocyanin	-0.3151	9.92	0.0641	0.41	0.1087	1.18
Total Flavonoids Content	0.0201	0.04	-0.3447	11.87	-0.2569	6.59
DPPH antioxidant activity	0.2130	4.53	0.2604	6.78	0.2413	5.82
Eigen Value	5.90		2.86		1.35	
Variance (%)	42.14		20.40		9.62	
Cumulative Variance (%)	42.14		62.54		72.16	

ISSR Data

ISSR marker analysis results on hawthorn genotypes are presented in Table 5. As seen in the table, 101 bands were obtained from 13 different ISSR primers. While 76 of these bands were polymorphic, the average polymorphism rate was determined as 75.24%. While the number of scoreable bands varying between 4 and 13 were obtained from the primers, the average number of scoreable bands per primer was determined as 7.76. The (TCC)5RY primer produced a completely monomorphic band, and the percentage of polymorphism related to this was the lowest (0%) in this primer. The highest value in terms of polymorphic band numbers is 10 and these bands were obtained from HVH(TCC)7, (CA)6AC and (CAA)6 primers. In addition, all bands obtained from primer VHV(GTG)7 and (AGC)6G were determined as polymorphic. In the study, the average number of polymorphic bands per primer is 5.84. To determine the genetic relationship in hawthorn species, studies were carried out using different marker systems. Among these studies, in a study using the SSR marker system, scoreable bands ranging from 2 to 21 were obtained from the primers. [44]. In another study, the average number of bands per marker was determined as 8.53 in ISSR analysis in hawthorn genotypes [45]. A total of 79 scoreable bands were obtained from 6 different ISSR primers used in the study carried out to determine genetic variation in 5 different hawthorn species grown in Iran, and the average polymorphism rate was determined as 89.9%, 71 of these bands are polymorphic [46].

Table 5. Sequence and polymorphism information of ISSR primers

Primers	Sequence (5'>3')	T.B.N	P.B.N	P.R (%)
$(AGC)_6G$	AGCAGCAGCAGCAGCAGCG	6	6	100
$(CAC)_3GC$	CACCACCACGC	8	7	87,5
$(CA)_6AC$	CACACACACACAAC	13	10	76,92
$(AG)_8T$	AGAGAGAGAGAGAGAGT	4	1	25
$(CT)_8TG$	CTCTCTCTCTCTCTCTG	9	8	88,88
$(TCC)_5RY$	TCCTCCTCCTCCTCCRY	6	0	0
$(CAA)_6$	CAACAACAACAACAACAA	12	10	83,33
$(GACA)_4$	GACAGACAGACAGACA	4	3	75
$VHV(GTG)_7$	VHVGTGGTGGTGGTGGTGGTGG	9	9	100
$BDB(CA)_7C$	BDBCACACACACACACAC	6	2	33,33
$(GA)_8YG$	GAGAGAGAGAGAGAGAYG	5	4	80
$DBDA(CA)_7$	DBDACACACACACACACA	8	6	75
$HVH(TCC)_7$	HVHTCCTCCTCCTCCTCCTCCTCC	11	10	90,90
Mean	**-**	**7,76**	**5,84**	**75,24**
Total	**-**	**101**	**76**	**-**

T.B.N: Total Bant Number, P.B.N: Polymorphic Band Number, P.R: Polymorphism Rate

As a result of the study, the similarity index of 22 hawthorn genotypes in the dendrogram created using the UPGMA method varied between 0.71 and 0.88 (Figure 3). In the dendrogram, 2 main groups were formed among the genotypes, while only G5 was in group A, all other genotypes used in the study were in group B. In the dendrogram, group B is divided into 2 subgroups, and there is only G12 in the B-I subgroup. In the study, the genetically closest individuals were the G9 genotype and the G16 genotype with a similarity index of 0.88. In addition, while genotypes of the same species were generally grouped together in the dendrogram, some genotypes were distributed independently of the species. Gene exchange that occurs with foreign pollination and the targeting of the ISSR marker system to random regions in the genome can be shown as the cause of this difference [47]. In a study, 92 different genotypes of the *Crataegus songorica* species were used and it was reported that the similarity index values in the genotypes varied between 0.53 and 0.87 in the ISSR marker analysis [48].

In our current study, 22 different hawthorn genotypes belonging to 3 different species were used, and it was aimed to determine genetic variation between genotypes by molecular, morphological, and phytochemical analyzes. Wide variations were detected between genotypes in all the examined marker systems, and G3 genotype produced better results than other genotypes in terms of fruit characteristics. In phytochemical analyzes, it was determined that phenolic compounds which are very important for human health, are intensely present in hawthorn genotypes. On the other hand, in the molecular marker analyzes we conducted, genotypes were generally grouped according to their species, and 2 main groups were formed in the dendrogram. As a result of the study, molecular and morphological data did not fully overlap. In molecular studies, the G9 and G16 with a similarity ratio of 0.88 were determined as the closest

individuals to each other. Although these two individuals showed similarity in molecular analysis, some differences emerged in morphological analysis. This situation can be attributed to the effect of the environment on morphological features and gene exchanges caused by foreign pollination and fertilization that have occurred over the years. While it is determined by the results of the study that ISSR markers are among the marker systems that can be used to determine genetic diversity among genotypes, it is thought that all the data obtained can guide researchers in the protection and development of this species.

Declarations

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There is no funding for this study.

Conflicts of Interest

The authors 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

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

Consent to Publish (Ethics)

Authors 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

Location of Kayseri province on the map of Turkey (source: wikipedia.org)

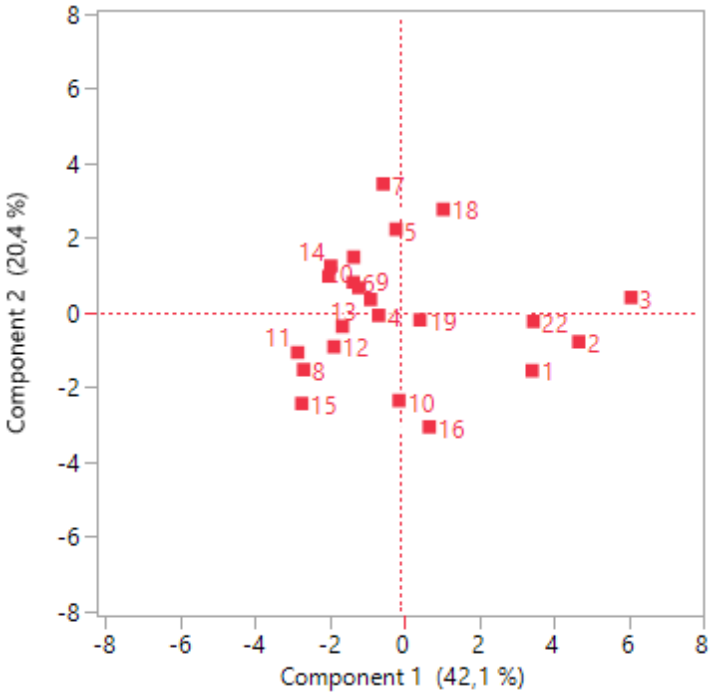

Figure 2

Principal Component Analysis plot estimated of variables observed on hawthorn genotypes

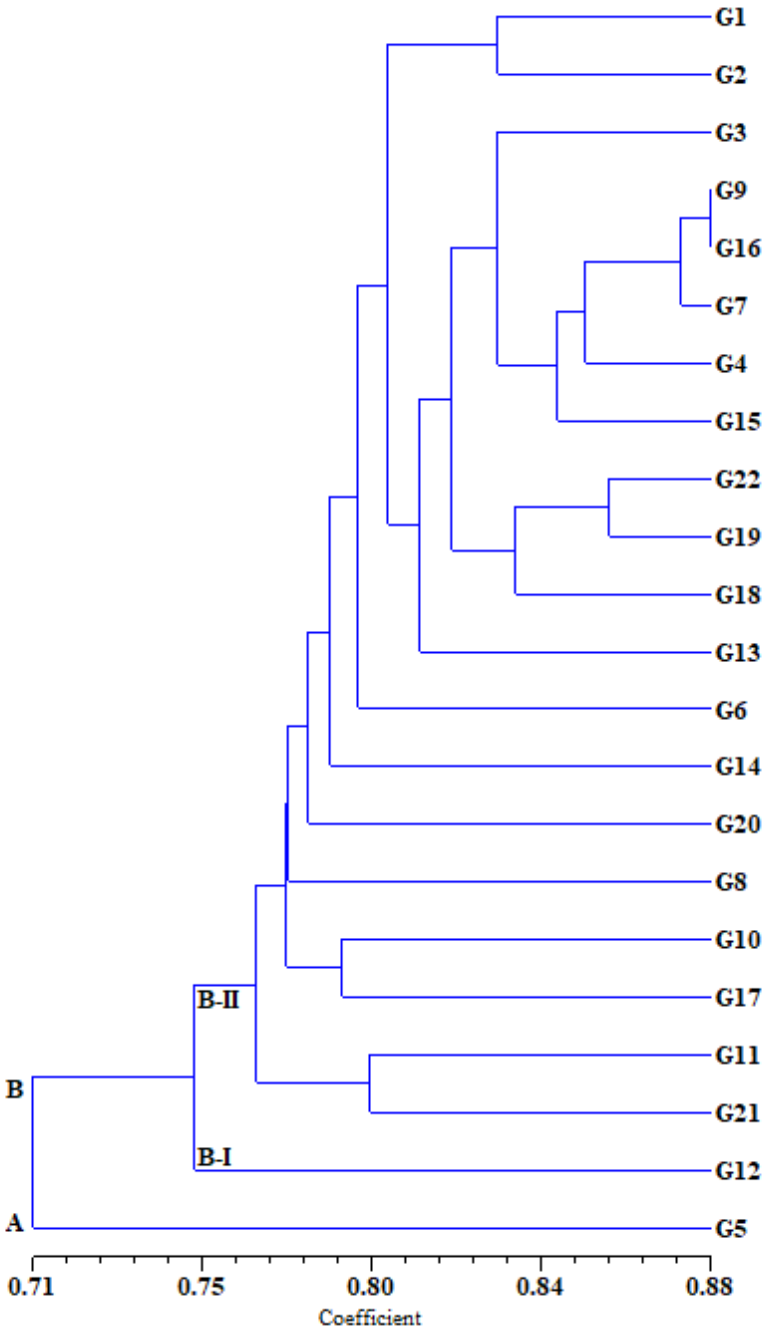

Figure 3

UPGMA dendrogram of hawthorn genotypes