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Research Article

Multivariate Analysis Revealed the Morphological Variability Among *Crataegus* Species**

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Abstract: The *Crataegus* genus has many species growing as wild that have positive effects on human health. The information about the phenotypic diversity of this species is rather inadequate. Phenotypic diversity among forty-four genotypes belonging to four *Crataegus* species (*C. monogyna*, *C. orientalis*, *C. astrosanguinea*, and *C. meyeri*) was determined based on morphological and physicochemical characteristics. Significant differences were observed among the species in terms of studied characteristics. Fruit weight ranges from 1.60 to 2.50 g for *C. orientalis*, 1.53 to 2.33 g for *C. meyeri*, 1.26 to 2.41 g for *C. astrosanguinea*, and 0.38 to 1.98 g for *C. monogyna*. The highest fruit length (15.19 mm) and width (17.58 mm) were determined in *C. orientalis*. The heights color values, L*, a*, and hue° values were highest in *C. meyeri*, while b* value was highest in *C. monogyna* and Chroma* was in C. orientalis. The highest TSS and pH were found in *C. monogyna* as 3.99% and 4.33%, respectively. TA was highest in *C. monogyna* with 1.83 mg L^{-1}. Seed size ranged from 1.36 (*C. monogyna*) to 4.33 (*C. orientalis*), while seed weight ranged from 0.23 g to 0.45 g in *C. mongyna* and *C. astrosanguinea*, respectively. The correlation analysis indicated significant correlations between morphological characters. The principal component and clustering analyses revealed high phenotypic variety among and within the species, showing that the studied characters were useful for describing *Crataegus* species. The results provided valuable insights for morphological and colorimetric characterization of hawthorn species and their utilization.

Crataegus Türleri Arasındaki Morfolojik Değişkenliğin Çok Değişkenli Analizle Belirlenmesi

Makale bilgileri

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Anahtar kelimeler

Kümeleme analizi,
Renk çeşitliliği,
Crataegus,
Morfolojik çeşitlilik,
Çoklu değişkenli analiz.

Öz: İnsan sağlığı üzerinde olumlu etkileri olan ve yabani olarak yetişen *Crataegus* cinsinin birçok türü mevcuttur. Bu türlerin fenotipik değişkenlikleri ile ilgili bilgiler oldukça yetersizdir. Morfolojik ve fizikokimyasal özellikler bakımında dört *Crataegus* türüne (*C. monogyna, C. Orientalis, C. astrosanguinea* ve *C. meyeri*) ait kırk dört genotipte fenotipik değişkenlikler incelenmiştir. İncelenen özellikler açısından türler arasında önemli farklılıklar gözlenmiştir. İncelenen türlerin meyve ağırlığı 1.60-2.50 g *(C. orientalis)*, 1.53-2.33 g *(C. meyeri)*, 1.26-2.41 g (*C. astrosanguinea)* ve 0.38-1.98 g (*C. monogyna)* arasında değişmiştir. En yüksek meyve boyu (15.19 mm) ve genişliği (17.58 mm) *C. orientalis* türünde tespit edilmiştir. En yüksek L*, a*, ve hue° değerleri *C. meyeri* türünde bulunurken, en yüksek b* değeri *C. monogyna* ve Chroma* değeri ise *C. orientalis* türünde belirlenmiştir. En yüksek SÇKM ve pH sırasıyla %3.99 ve 4.33

ile *C. monogyna*'da tespit edilmiştir. Toplam Asitlik ise 1.83 mg L^{-1} ile *C. monogyna'*da en yüksek belirlenmiştir. Tohum sayısı 1.36 adet (*C. monogyna*) ile 4.33 adet (*C. orientalis*) arasında değişirken, tohum ağırlığı sırasıyla *C. mongyna* ve *C. astrosanguinea'*da 0.23 g ile 0.45 g arasında belirlenmiştir. Morfolojik karakterler arasında önemli ilişkilerin olduğu korelasyon analiziyle belirlenmiştir. Temel bileşen ve küme analizi, çalışılan tür içinde ve türler arasında yüksek fenotipik çeşitliliğin olduğu ve bu karakterlerin *Crataegus* türlerinin tanımlanması için yararlı olduğunu göstermiştir. Çalışma sonuçları, alıç türlerinin morfolojik karakterleri, renk karakterizasyonun belirlenmesinin ve kullanımları hakkında faydalı bilgiler ortaya çıkarmıştır.

** This article was produced from some part of the MSc thesis of Saime Gürsoy.

1. Introduction

The consumption of colorful and native species increases day by day thanks to beneficial effects on health. Hawthorn is one of these species that contain high amounts of bioactive compounds such as organic acid, sugars, antioxidants, phenolic constituents, and vitamin C (Edwards et al., 2012; Gonzalez-Jimenez et al. 2018; Alirezalu et al., 2018; Muradoğlu et al., 2019). Its fruits are rich in vitamins, carbohydrates, and sugar. Besides, hawthorn fruits consist of energy, cellulose, oil, ash, acidity, and TSS (Özcan et al., 2005; Edwards et al., 2012). Therefore, various parts of hawthorn species, including flowers, leaves, and fruits, have been used in many traditional and modern medical applications such as resisting oxidation, promoting digestion, constipation, stomachache anti-inflammatory properties, reducing blood pressure, preventing cardiovascular diseases, and against type II diabetes (Zhang et al. 2002; Chang et al. 2006; Tassell et al., 2010; Aierken et al., 2017).

Crataegus species belongs to the *Rosaceae* family and are widely spread primarily in Central Asia, East Asia, North America, and Europe. Hawthorn is a deciduous tree and naturally grows as multibranched large shrubs or small trees that can reach a height of 10 m, while the average height is between 2 to 5 m. The bushes or trees produce dense clusters of white flowers often with a characteristic trimethylamine scent (Zhao and Tian 1996; El-Sayed 2011; Bor et al. 2012). The hawthorn grows not only in forests, hedges, edge of railways, and roads but also in parks and gardens (Phipps, 1998). Hawthorns are used for very different purposes. Plants are used as ornamentals, windbreakers, rootstock for pome fruit species, and forestation zone in the arid regions, while the fruits are usually eaten fresh or processed to vinegar, marmalades, jams, desserts, candies, and canned fruits. Its leaves are used both in folk medicine and as a tea replacement (Baytop, 1984; Cao et al., 1995; Phipps et al. 2003; Hummer and Janick, 2009).

Crataegus species bears small fruits that ripen during harvest with fruit colors ranging from yellow to bright red and black (Phipps et al., 2003). Browicz (1972) described fruit colors of *Crataegus* species as yellow facing with red (*C. tanacetifolia*), reddish-orange (*C. orientalis*), black or blackish-purple (*C. pentagyna*), dark purple (*C. curvisepala*), yellow to orange (*C. pontica*), red (*C. stevenii*), bright red (*C. microphylla*), dark red (*C. atrosanguinea*) and red or brownish-red (*C. monogyna*). The high diversity of fruit colors of *Crataegus* species distributed in Turkey represents that Turkey is one of the gene centers of Crataegus (Christensen, 1992) resulting in very rich hawthorn genetic resources spontaneously grown and extensively spread.

Some *Crataegus* genotypes or species were identified in different countries (Bektaş et al., 2017; Khadivi et al., 2019; Stoenescu and Cosmulescu, 2020; Yalçın-Dokumacı et al., 2021). But the studies generally focused on the determination of morphological features of genotypes or only one species of the *Crataegus* genus. Currently, the studies on characterization and revealing the differences among species are insufficient. This research aimed to identify and evaluate the morphological, physio-chemical, and color characteristics of fruits of four *Crataegus* species.

2. Material and Methods

2.1. Plant Material

This research was conducted on 44 hawthorn accessions belong to four species: *C. monogyna* subsp. Monogyna Jocq (24 accessions), *C. orientalis* var. Pallasex Bieb (8 accessions) *C. astrosanguinea* Pojark (9 accessions) and *C. meyeri* pojark (3 accessions). The fruits and seeds of studied species are presented in Figure 1. Samples were taken from native hawthorn population of Bahçesaray region from Eastern Turkey (Figure 2).

Figure 1. The appearance of whole fruit, fruit flesh, and seeds of the hawthorn species.

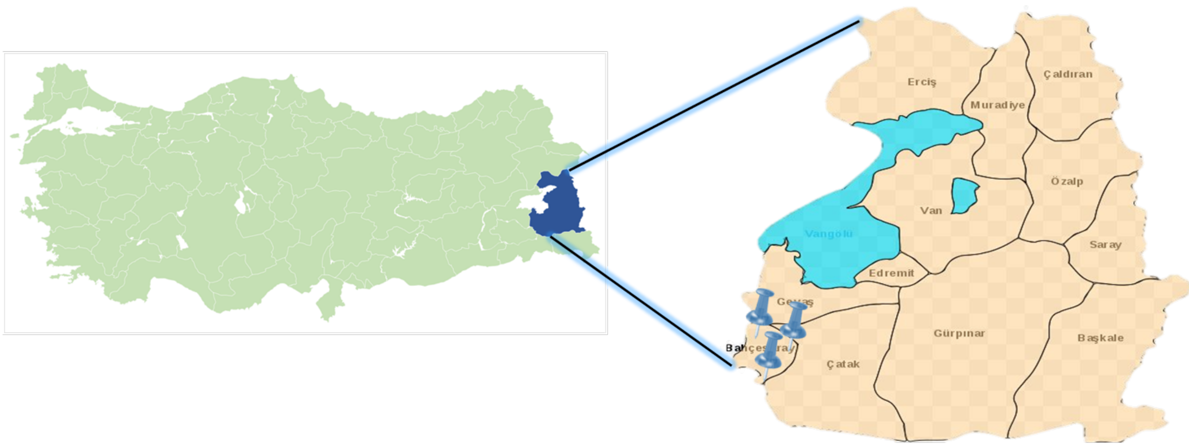


Figure 2. A visual presentation of the research area and sampling territory.

2.2. Method

Samples were randomly taken from hawthorn trees of different populations in the middle and late September. In total, twelve morphological characters were evaluated for two consecutive years. Thirty fruits were measured for each accession in each year. Fruit size characters such as fruit length (FL) and fruit width (FWD) were measured using a caliper with an accuracy of 0.1 mm. Fruit weight (FWT) and seed weight (SW) were measured with an electronic scale with a 0.01 g precision. The color parameters (L*, a*, b* Chrome* and hue°) were determined by using a hand-type colorimeter (Minolta Co., model CR-400, Tokyo, Japan). pH was determined by pH a table type pH-meter (Thermo Science, Orion star A 111). Total soluble solids (TSS) was determined using a hand reflectometer (ATC, NTRM01). TA was measured by titration method as adding 0.1 N NaOH to fruit juice (5 ml) until final pH reaches 8.10.

2.3. Statistical analysis

The data were subjected to analysis of variance using SPSS 23.0 statistical software. The minimum (Min.), maximum (Max.), mean, standard deviation values (SD), and coefficient of variations (CV%; SD/mean×100) of the properties were determined. Moreover, the relationship among characteristics was determined by Pearson's correlation analysis using R Studio software by the package of 'corrplot' (Wei et al., 2017). The principal component analysis (PCA) was performed to clarify the relationships of features with each other and accessions using R Studio software by the package of 'ggplot2' (Wickham, 2016). Clustering analysis (CA) was utilized to create a dendrogram showing the hierarchical distribution of accessions in terms of studied characteristics.

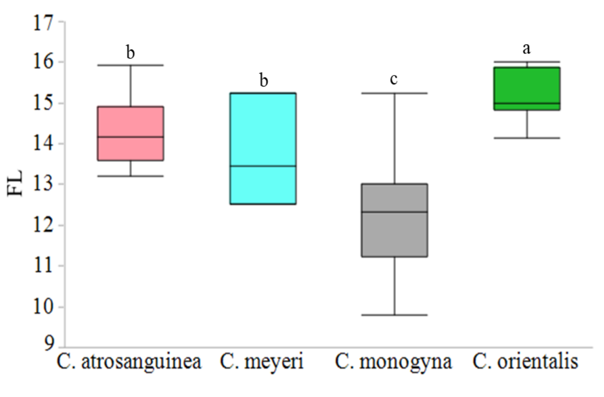
3. Results and Discussions

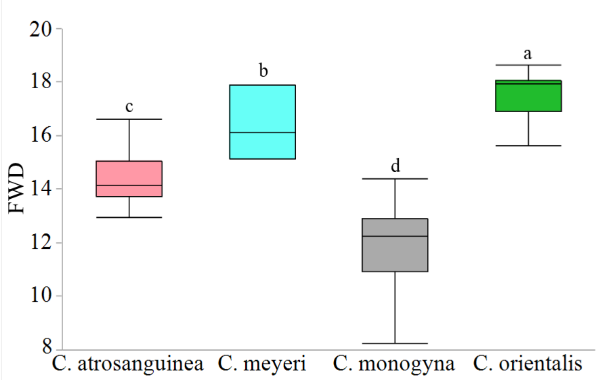
A wide variation was determined amongst the studied characters and all variations were highly significant ($p< 0.05$). The b* and L* showed the highest CVs (68.55 and 65.48%, respectively), while pH (3.51%) and total soluble solids (12.39%) had the lowest CVs. Nine out of thirteen features had higher than 30.00% CVs (Table 1). The high CV of traits is the indicator of dissemblance. Therefore, there is no significant difference between the species in FL, FWD, and pH, which show very low CVs. Using these features as separators are considered not appropriate in the differentiation of the 4 studied species.

Table 1. Overall descriptive statistics of the hawthorn species

Characters	Abbreviations	Unit	Min	Max	Mean	SE	CV %
Fruit Length	FL	mm	9.79	16.02	13.29	0.25	12.61
Fruit Width	FWD	mm	8.23	18.62	13.82	0.40	19.44
Fruit Weight	FWT	g	0.38	2.50	1.45	0.08	40.60
Seed Size	SS	Piece	1.00	4.83	2.35	0.19	53.55
Seed Weight	SV	g	0.10	0.61	0.33	0.01	39.32
L*	L	Lightness	19.83	75.43	40.13	2.23	36.87
a*	a	Greene/redness	-1.78	44.95	31.68	2.04	42.76
b*	b	Blueness/yellowness	2.12	50.43	22.65	2.34	68.55
Chroma	C	Chroma	14.27	61.97	41.43	2.21	35.51
hue°	h°	Lightness angle	8.22	92.23	31.70	3.13	65.48
Total Soluble Solids	TSS	%	2.8	4.16	3.73	0.06	12.39
pH	pH	H^+ ions	3.89	4.53	4.23	0.02	3.51
Titratable Acidity	TA	%	0.76	2.23	1.15	0.06	34.66

High fruit dimensions and weight are among the most desirable fruit characteristics for *Crataegus* species in breeding programs. These characters mostly depend on the genotype and ecological conditions. Significant variations were observed in fruit dimensions between species and within species (Figure 3). Fruit length ranged from 9.79 mm to15.25 mm in *C. monogyna*, 13.21 mm to 15.94 mm in *C. astrosanguinea*, 14.15 mm to 16.02 mm in *C. orientalis*, and 12.53 mm to 15.25 mm in *C. meyeri*. The highest mean fruit width was determined in *C. orientalis* (17.58 mm) followed by *C. meyeri* (16.39 mm), *C. astrosanguinea* (14.44 mm), and *C. monogyna* (12.01 mm). Fruit weights of species were determined 2.13 g in *C. orientalis*, 1.89 g in *C. meyeri*, 1.69 g in *C. astrosanguinea*, and 1.01 g in *C. monogyna* as mean. Yanar et al., (2011) reported fruit dimensions (length and width) and weights of *Crataegus* species between 10.06-18.07 mm, 9.88-20.39 mm, and 0.65-4.19 g, respectively. In another study carried out in Romania, fruit length, width, and weight of *C. monogyna* have been reported from 6.41 to12.64, from 5.75 to 13.94, and 0.18 to 1.15 g, respectively (Stoenescu and Cosmulescu, 2020). Khavidi et al. (2019) reported fruit length of *C. monogyna* between 8.29-15.4 mm and *C. pentagyna* between 8.52-12.69 mm, while ten-fruit weight ranged from 3.08 g to12.80 g and 2.75 to 7.80 g, respectively. Bektaş et al. (2017) studied Crataegus genotypes to identify their fruit characteristics and reported fruit length, width, and weight as 8.27-19.56 mm, 8.27-19.56 mm, and 0.98-6.76 g, respectively. When the differences and similarities with the previous studies are examined, the results of the studies in the same longitudinal zone in which this study took place are similar, but the fruit sizes in different zones are different. This shows that different hawthorn species spread naturally in different longitudes, and the ecology of the territory is one of the main factors determining the spreading of species.





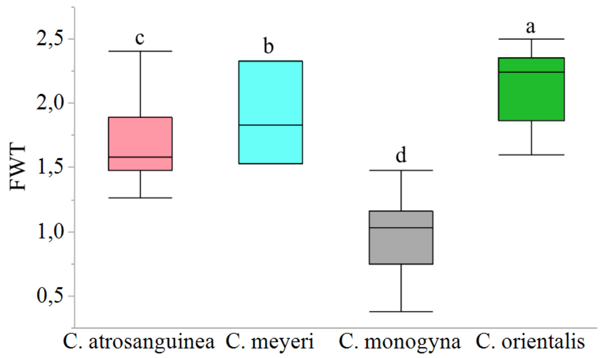


Figure 3. Fruit dimension features of *Createagus* species.

Hawthorn seeds are often thought of as a discarded material, like many other fruit seeds, in the food industry. But recent studies showed hawthorn seeds have a variety of pharmacological properties on analgesic, hypolipidemic, anti-inflammatory, and antioxidant with rich flavonoids (Can et al., 2010; Peng et al., 2016). Therefore, the determination of seed characteristics became more crucial in breeding programs. Seed size of hawthorn species ranged from 2.99 to 4.33. The highest mean seed size was determined as 4.33 in C. *orientalis* followed by 3.07 (*C. meyeri*), 1.36 (*C. monogyna*), and 2.99 (*C. astrosanguinea*). The highest seed weight was 0.45 g in both C. *orientalis* and C. *astrosanguinea*, while the lowest was ranged from 0.20 g to 0.38 g in *C. monogyna* and *C. meyeri* respectively (Fig. 4). The seed size and seed weight of hawthorn species were previously reported ranging from 2.40 to 2.50 and 0.6 g to 0.8 g, respectively (Çalışkan, et al., 2018). Khavidi et al. (2019) also declared the seed number and 10 seed weight of *C. pentegyna* and *C. monogyna* as between 4.17-1.00 g and 0.42 g - 1.74 g, respectively. Besides the results of the present study supporting some of the previous researches, some substantial phenomenon were also discovered. One of the remarkable inferences was the high variation of the SWT, although there were very low CVs of fruit size characteristics. This circumstance clearly indicates SWT is one of the foremost distinctive traits for hawthorns and should not be omitted in future selection studies. The other striking result was the discordance of SWT and SS. While the SWTs of *C. astrosanguinea* and *C. orientalis* were completely equal, there was a clear separation of SS. This proves the fact that hawthorns have unique seed density, which can be used as a good identifier in future studies.

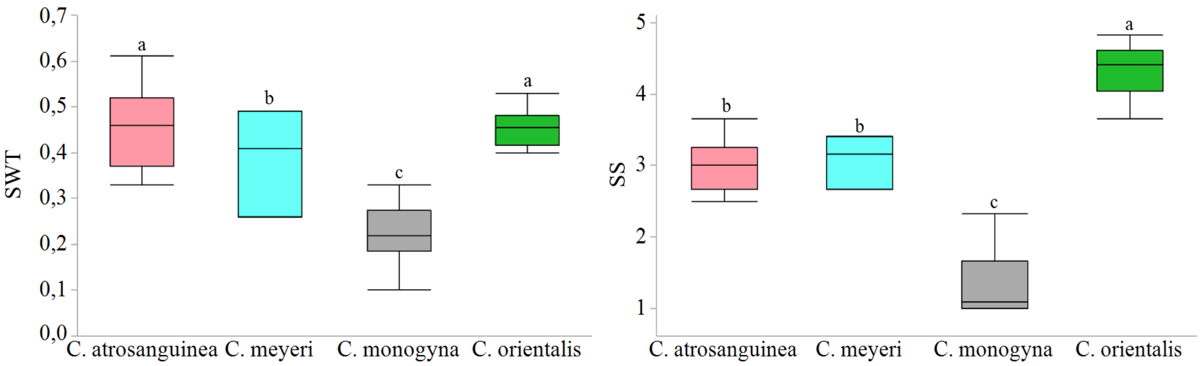


Figure 4. Seed weight and size features of the hawthorn species.

Color is one of the important qualities features that prefer consumer's choice in fresh consumption and food processing technology. Besides, peel color is related to the antioxidant compounds and their levels. Therefore, describing the peel color of the fruit is highly significant for breeding programs. Peel colors of species showed significant variability among species. The color of t varied from yellow to black (yellow, orange, red, and black). The highest L*, b*, and hue^{o}* were determined from 73.96 to 75.43, from 47.43 to 50.43, and from 88.44 to 92.23, respectively in *C. meyeri*, while the highest a* and Chroma* were determined from 28.84 to 44.95 and from 54.95 to 61.96, respectively in *C. orientalis* (Figure 5). In similar studies, the color values (L*, a* b*, hue^{o}*, and Chroma*) of *C. monogynma* were determined as 29.27, 27.76, 8.19, 16.19, and 28.94, respectively (Yalçın-Dokumacı et al., 2021). Bektaş et al. (2017) also mentioned that the peel color of *Crataegus spp*. was observed as yellow, orange, and red in the Malatya region. Yanar et al., (2011) suggested that the colors also could be seen as yellow, orange, light orange, light green, red, and dark red, observing *Crataegus* species of the same region. Color characterization of fifteen *Crataegus* species from seven provinces of Iran reported by Alirezalu et al., (2020) ranging from yellow to black (yellow, yellow-orange, red, orange-red, purple, purple-black, and black). They also reported the highest a* was found in *C. atrosanguinea*, while b*, L*, Chroma*, and hue^{o} were highest in the extracts of *C. azarolus var. aronia.* The results of the present study mostly contradict previous reports. C. *atrosanguinea* had the least a* values, and it was the darkest species being almost pure black. Discordant reports may be due to early harvesting of the species preventing it from reaching the final color and the difference in sunning duration period of the studied territories. Sunning duration and period also affect anthocyanin contents, and being one of the anthocyanins, proanthocyanins are mostly responsible for the occurrence of black color in plants (Khoo et al., 2017).

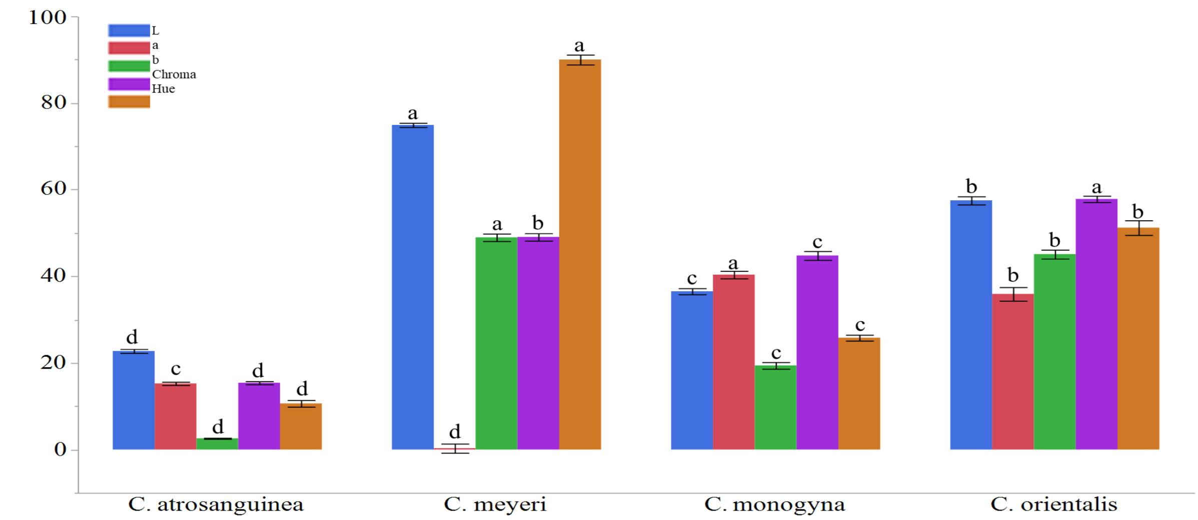


Figure 5. Peel color features of four hawthorn species.

The pH levels were recorded in the range of 4.07 – 4.33. the pH was at the highest level in *C. monogyna*, while the lowest was in *C. meyeri*. The highest (3.99 Brix) and the lowest (2.90 Brix) TSS were observed in *C. monogyna* and *C. meyeri*, respectively. Both the lowest and highest TA were observed in red and black-fruited hawthorns. The TA was at its lowest (0.95 mg L^{-1}) in *C. monogyna*, whereas the highest value (1.83) was observed in *C. astrosangueina* (Figure 6). Yalçın-Dokumacı et al. (2021) reported the pH and acidity of hawthorn fruits (*C. monogyna*) were 4.08 and 1.56%, respectively. Alirezulea et al., (2020) reported the pH (3.03-4.35), TA (0.75-1.17%), and TSS (15.15-23.43 °Brix) features of *Crataegus* species of Iran. In a study from Turkey, pH and TSS were stated between 2.82-6.40 and 6.40-16.00 % (Yanar et al., 2011). Our results were in line with the previous findings, but little differences may be caused by especially lighting, maturity, harvest time, soil, and climatic conditions.

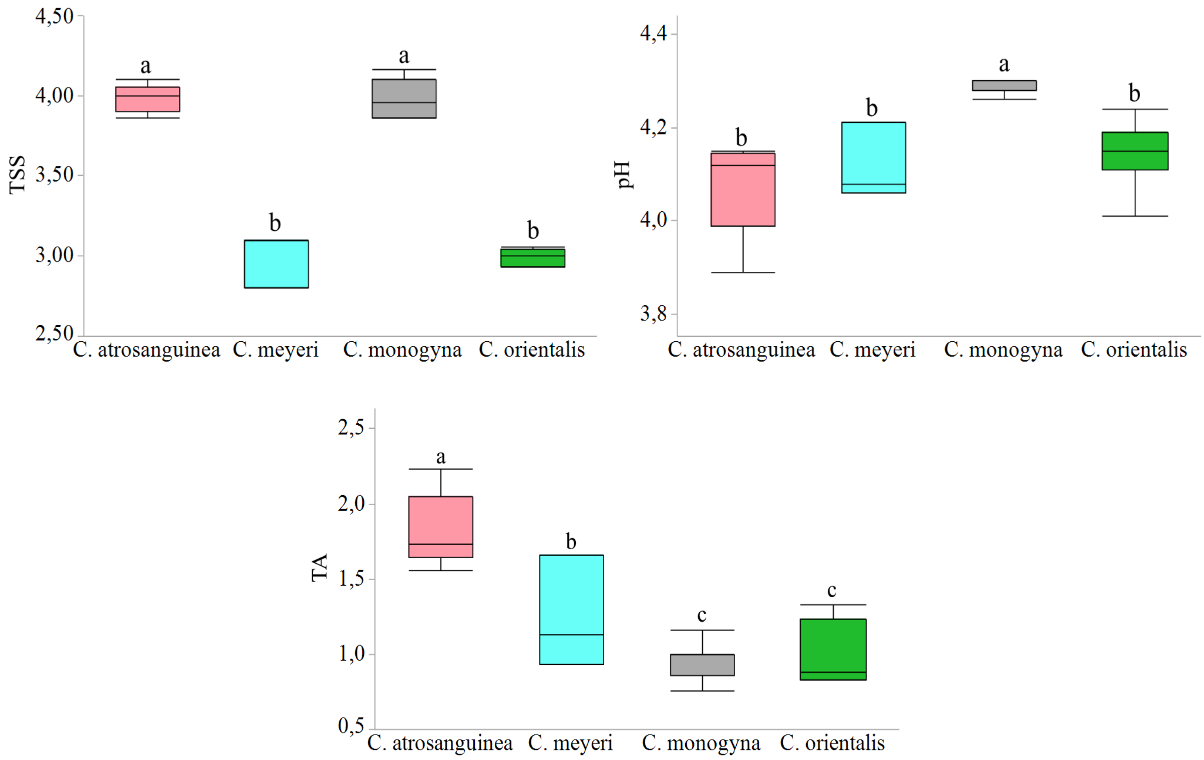


Figure 6. Some chemical features of *Createagus* species.

Strong correlations (P<0.05) were monitored amongst studied characteristics (Figure 7). The high correlations among fruit characters such as FL, FWD, FWT, SS, SW, TSS, pH, TA, and chromatic parameters were correlated with each other and fruit traits and agreed with previous reports about hawthorn (Khadivi et al., 2019; Stoenescu and Cosmulescu, 2020). On the contrary, a* and TSS were negative with FL, FWD, FWT, SS, and SW, indicating that big-fruited hawthorns have less TSS and red color tones. A completely negative relationship between pH and TA both with each other and in their effects on other properties were proof of a well-known phenomenon. Both pH and TA measure total H^+ ions in the medium, and the change of their values is related to the alteration of these ions. If the concentration of H^+ ions in the medium rises pH decreases, while TA increases. The h° was highly and positively correlated with L*, b*, and C*.

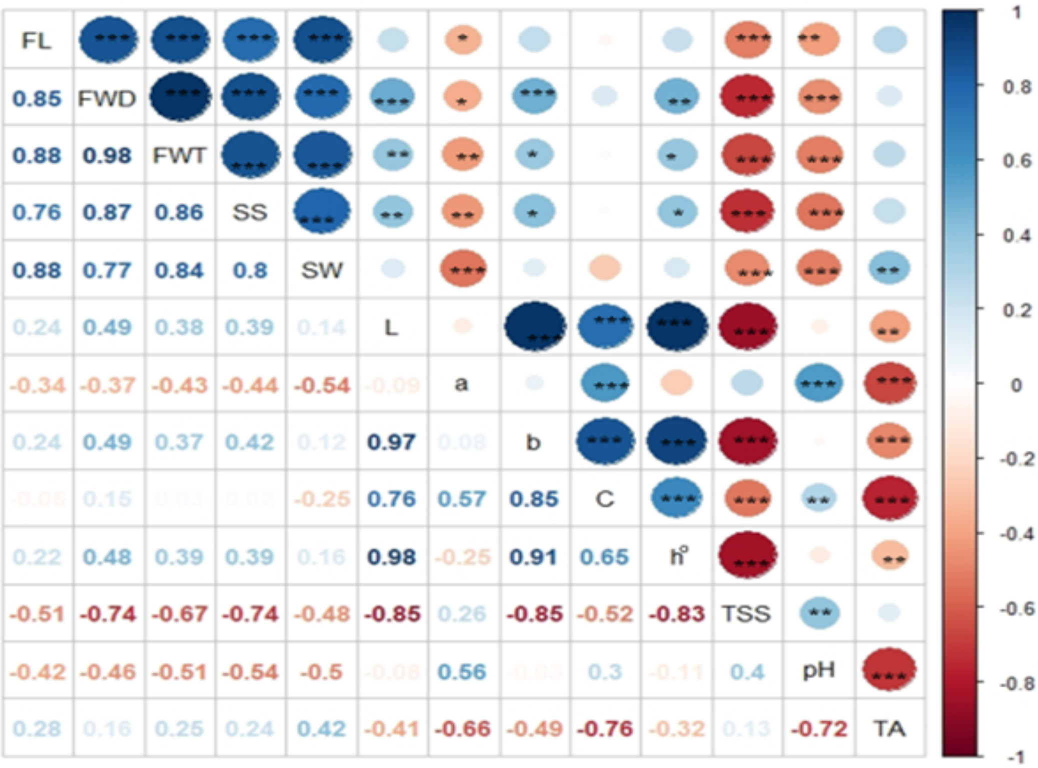


Figure 7. Pearson's pairwise correlations among studied characteristics of the hawthorn species.

The principal component analysis (PCA) and cluster analyses (CA) were grouped the hawthorns into four depending on their species. As a result of PCA that performed on all variables studied to assess and distinguish each of the four hawthorn species, nine principal components were significant (p<0.05), and 99.80% of the total variance was explained by them. The correlation between studied variables and the first and second principal components is shown in Figure 7. The first component (PC1) constituted 49.4% of the total variance and was positively correlated with FL, FWD, FWT, SS, and SW. The second component (PC2) constituted 31.95% of the total variance and was positively correlated with chroma, while was negatively correlated with TA. The third component (PC3) accounted for 9.17% of the total variance and had positive correlations with a* and pH. The remaining components also contained other variables and explained less variability (9.46% of total variance), and corresponded with other results (Alirezalu et al., 2018; Khadivi et al., 2019; Stoenescu and Cosmulescu, 2020).

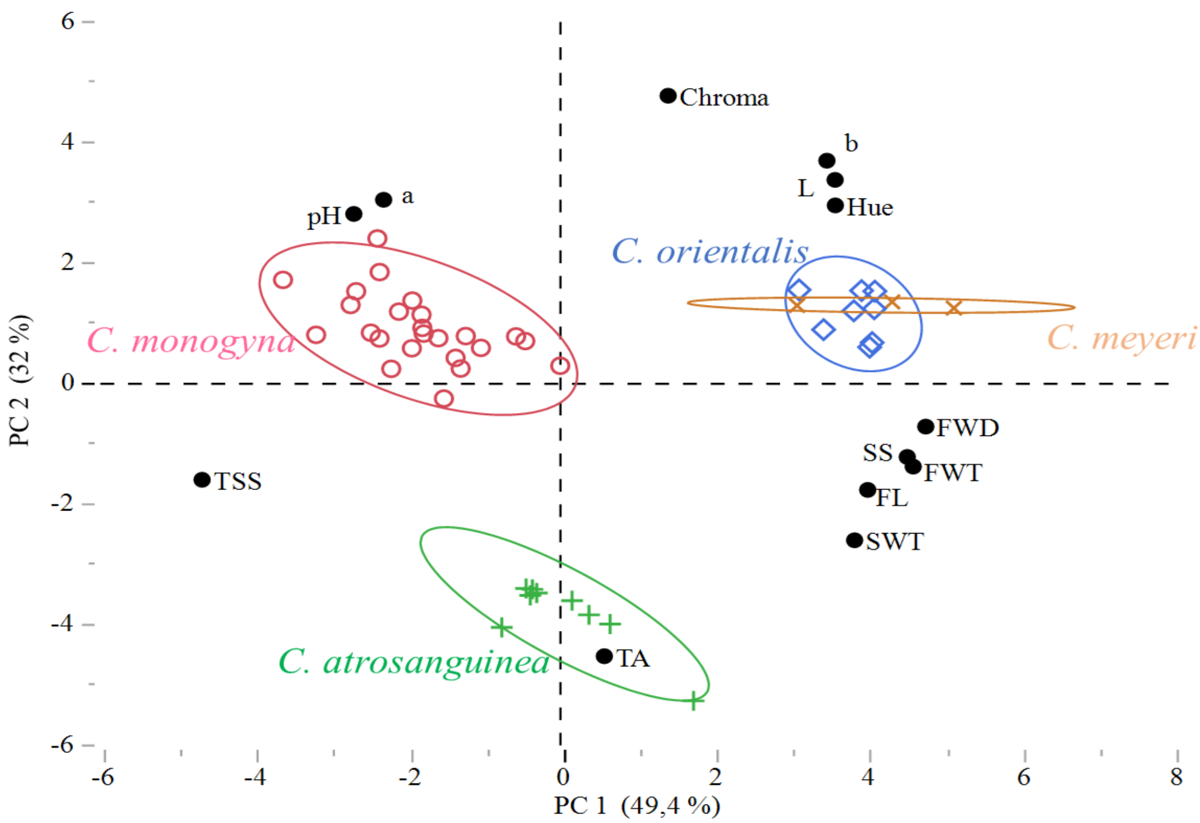


Figure 7. Distribution of the species and traits on the biplot.

Group ellipses were created by a significance level of $p<0.05$.

Clustering analysis was used to define the similarity degree of genotypes that belong to hawthorn species, and shown in Fig 9. Accordingly, the dendrogram was separated into four main clusters. The highest similarity was between 01 and 03 genotypes included in *C. meyer*i species, while the farthest distance was observed between 01 numbered genotypes of *C. monogyna* and *C. orientalis*. Cluster I included *C. monogyna* and was characterized by low values of a*, total soluble solids, and pH. Cluster II included *C. astrosanguinea* that was characterized by high values of color parameters. Cluster III and IV also included respectively *C. orientalis* and *C. meyeri* and characterized by FL, FWD, FWT, and SS for *C. orientalis* while *C. meyeri* was characterized by low values of L*, b*, and hue°*. The distribution of the genotypes and species was like PCA, and these evaluation methods were proven to support each other. Similar results obtained from these methods were reported previously (Güler et al., 2021; Macit, 2021).

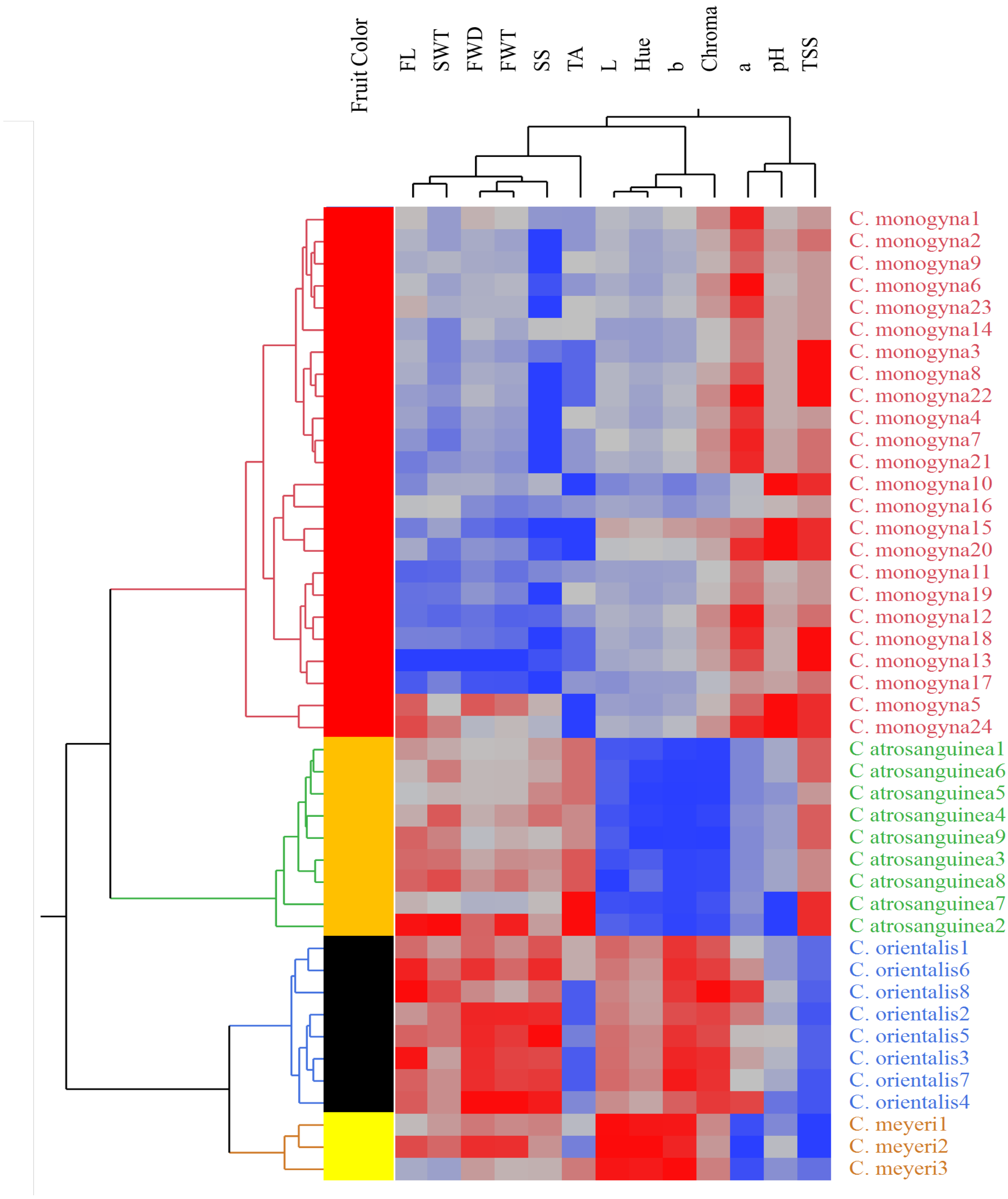


Figure 9. Dendrogram of the four *Crataegus* species based on morphological variables obtained by Hierarchical Clustering Analysis according to Ward's method.

4. Conclusions

The phenotypic variability among species/genotypes is a crucial indicator of classifications and utilization of genetic resources being key factors for breeding. In the present study, phenotypic variability among *Crataegus* species was investigated. Our findings exhibited that hawthorn species have their own fruit and seed characteristics that can effectively be used to classify *Crataegus* species. *C. monogyna* was identified with high fruit and seed size and weight, while *C. orientalis* separated from the others with the lowest values of these characteristics. The most important feature that separates *C. meyeri* from *C. orientalis* was the redness (a* value) of peel, while it was quite similar in terms of other traits. Multivariate analysis was very effective on the distinction of the species and was evaluated that it can be used successfully in future studies.

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