Jalali *et al. BMC Plant Biology* (2024) 24:899
https://doi.org/10.1186/s12870-024-05608-1

BMC Plant Biology

RESEARCH

Open Access

Morphological and biochemical characteristics of wild red-fleshed apples (*Malus sieversii* f. niedzwetzkyana) in the North and Northeast of Iran

Majid Jalali[1], Mehdi Abedi[1*], Mehdi Tabarsa[2] and Diego A. Moreno[3]

Abstract

Background Red-flesh apples (*Malus sieversii* f. niedzwetzkyana) have attracted attention from consumers and researchers due to their pleasant appearance and taste. These exotic apples are rich sources of nutrients and health-promoting polyphenols and phenolics. This study aimed to investigate morphological (40 quantitative and 13 qualitative traits) and biochemical (5 traits) characteristics of four socioeconomically important red-fleshed apple populations in North and Northeast region of Iran, which are understudied and under serious extinction risk.

Results The examined characters exhibited wide and statistically significant variations, especially in leaf color (68.86%) and the number of seeds per fruit (61.61%), and more dramatically in the total flavonoids (146.64%) and total phenolics contents (105.81%). There were also strong variations in fresh fruit weight and fruit length, diameter, and flesh thickness. Red, with 34 accessions, was the dominant ripe fruit skin color. All biochemical traits also showed high variations, particularly in total flavonoid content. Red-fleshed Gavramak and Kalateh Khij apples contained the highest biochemical and morphological values, respectively. Principal component analysis (PCA) revealed that the first five principal components together accounted for more than 60.83% variation of the total observed variations. Moreover, the cluster dendrogram analysis based on Ward's method indicated three different clusters based on the characters measured, indicating high variation among the accessions.

Conclusion red-flesh apples can be considered suitable sources of natural antioxidants with great potential as healthy foods and nutraceutical applications. Based on the commercial characters, Red-fleshed Gavramak and Kalateh Khij apples showed the highest fruit quality with proper size and thus can be suggested as superior for cultivation or use in breeding programs due to having higher quality fruits.

Keywords Morphological trait, Biochemical traits, Flavonoid, Anthocyanin, Functional ecology, Antioxidant activity, Functional food

*Correspondence:
Mehdi Abedi
mehdi.abedi@modares.ac.ir; abedimail@gmail.com
[1]Department of Range Management, Faculty of Natural Resources and Marine Sciences, Tarbiat Modares University, Noor, Mazandaran Province, Iran
[2]Department of Seafood Processing, Faculty of Marine Sciences, Tarbiat Modares University, Noor, Mazandaran Province, Iran
[3]Laboratorio de Fitoquímica y Alimentos Saludables (LabFAS), CEBAS, CSIC, Campus Universitario de Espinardo –25, Murcia 30100, Spain

© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

Background

Apple is a fruit classified within the genus Malus of the Rosaceae family, specifically in the Maloideae subfamily. Apples are small deciduous shrubs or trees extensively cultivated globally, especially in temperate zones, and have significant economic significance [1]. Central Asia, China, the Caucasus, and Asia Minor are the primary sources of apple biodiversity [2]. Iran is a crucial hub of apple genetic diversity in Central Asia, especially for red-fleshed apple varieties [3, 4]. Unfortunately, only a few small populations of wild red-fleshed apples exist in the North, North-East, and North-West regions of Iran, and they are highly endangered [5]. All red-fleshed apples, including those cultivars already domesticated and available hybrids, are believed to have originated from *M. sieversii* f.niedzwetzkyana [6]. Red-fleshed apples have attracted considerable attention from consumers and researchers due to their appealing appearance and flavor. Numerous research studies have been undertaken on the components of red-fleshed apples [8–11]. These apples also have value due to the presence of anthocyanins in other non-commercial components of the plant, such as flowers, leaves, and branches [12–14]. Red-fleshed apples exhibit superior nutritive value and health-promoting properties compared to commonly available white-fleshed cultivars, being richer in nutrients such as fructose, glucose, citric acid, malic acid, vitamin C, and essential minerals, as well as valuable phytochemicals including flavonoids, anthocyanins, glycosylated flavonols, and dihydrochalcones [10, 14, 15]. In general, the products obtained from red-fleshed apples are considered promising for new product developments [16], diversifying the choices of healthy products for the increasing market demands worldwide for healthy and safe foods [17–20].

Table 1 Geographical coordination, sampling information, and climatic data of the study areas in the growing season of red-fleshed apple populations from Semnan and Mazandaran Province, Iran

Collection site	Bekran	Kalateh	Kalateh-Khij	Gavramak
Abbreviation	Bek	Kal	KaK	Gav
Province	Semnan	Semnan	Semnan	Mazandaran
Latitude (N)	36°34′	36°31′	36°41′	36°53′
Longitude (E)	55°51′	56°05′	55°18′	50°36′
Altitude (m)	986	1250	1490	347
Mean-P (mm)	155.3	161.4	322.8	1373.7
Mean-T (°C)	15.25	13.95	11.05	15.15
Max-T (°C)	33.85	33.25	27.35	25.45
Min-T (°C)	-3.05	-4.85	-5.35	3.95
AI	0.11	0.12	0.30	1.91
AI category	Arid	Arid	Semi arid-dry	Humid

Note: **Mean-P**: Mean annual precipitation, **Mean-T**: Mean annual temperature, **Max-T**: Maximum temperature of the warmest month, Min-T: Minimum temperature of the coldest month, **AI**: Aridity index

The characterization of plant genetic resources is an essential step in developing conservation and breeding strategies for plant improvement programs and germplasms. Morphological evaluation and characterization create the basic information for managing genetic resources [21], and quantitative and qualitative phenotypic traits aid in identifying differences between individuals of a species [22]. Assessing the morphological and biochemical characteristics of wild germplasm is essential for domestication and breeding plant species [23]. Morphological diversity has been evaluated for *M. orientalis* in Iran [24, 25]. However, despite a few reports about red-fleshed apple species in two locations of Semnan province [5, 26, 27], we still do not have a study about the phenotypic diversity of red-fleshed species to select desirable accessions with suitable agronomic and biochemical traits for direct production, breeding, and conservation in the north and northeast of Iran. Therefore, this study aimed to investigate (1) what is the degree of variations of traits among accessions of *M. sieversii*; (2) what is the degree of variation of the bioactive composition and associated and antioxidant capacity in *M. sieversii* in the different habitats; (3) what are the major sources of variations among traits and the selection of superior traits; and (4) how the similarities in accessions of red-fleshed apples may be related to environmental (climatic) and geographical conditions.

Materials and methods

Plant material

In the present study, morphological diversity of red-fleshed apple accessions (*M. sieversii* f. niedzwetzkyana) collected from four distinct populations studied in the northeast of Iran in Semnan province, including 'Bekran', 'Kalateh', and 'Kalateh-Khij', and the 'Gavramak' populations in Mazandaran province in the north of Iran. The above populations were selected from the few available populations in Iran. Red-flesh apples are limited and scattered in the study area. However; however, an attempt has been made to identify the maximum number of apple trees in the target area so that samples can be collected. Climatic variables related to the study populations were provided in Table 1. These variables included the geographical coordinates, altitude, and climate data obtained and recorded for the four populations from chelsa-climate data.

The traits evaluated

In total, 53 morphological and five biochemical characters were used for evaluations (Table 2). Plant samples were randomly collected from different parts of the trees. It is broadly divided into quantitative and qualitative traits, which include fruit-related traits (32 traits), leaf-related traits (14 traits), seed-related traits (6 traits),

Table 2 Statistical description of morphological and biochemical traits of the studied red-flesh apples from Semnan province and Mazandaran Province, Iran

NO.	Trait	Abbreviation	Unit	Min.	Max.	Mean	SD	CV (%)
Traits related to fruit (32 traits)								
V1	Fruit fresh weight	FfW	g	12.53	70.62	29.27	14.30	48.87
V2	Fruit dry weight	Fdw	g	2.12	12.02	5.13	2.30	44.96
V3	Fruit dry matter	Fdm	%	12.77	23.68	17.84	2.43	13.63
V4	Fresh weight/Dry weight	Frw/ Drw	Ratio	4.22	7.83	5.71	0.73	12.93
V5	Fruit length	Frl	mm	26.10	51.71	35.60	5.94	16.70
V6	Fruit diameter	Frd	mm	29.29	57.46	40.67	7.06	17.37
V7	Length/ diameter	Frl/ Frd	Ratio	0.76	1.01	0.87	0.05	6.67
V8	Thickness of skin	Ths	mm	0.31	1.94	1.21	0.31	25.59
V9	Tail thickness	Tat	mm	1.11	2.30	1.60	0.29	18.25
V10	Tail length	Tal	mm	5.07	40.01	20.64	6.48	31.42
V11	Depth of eye basin	Deb	mm	0.23	18.19	5.54	2.93	52.88
V12	Width of eye basin	Web	mm	2.80	17.01	10.95	3.11	28.44
V13	Width of stalk cavity	Wsc	mm	5.76	16.41	11.09	2.19	19.81
V14	Depth of stalk cavity	Dsc	mm	4.27	12.71	7.75	1.84	23.77
V15	Length of core	Lec	mm	10.61	21.03	14.26	2.44	17.12
V16	Diameter of core	Dic	mm	14.63	32.88	21.01	4.44	21.15
V17	Number of seed in fruit	Nsf	Number	1	11	4.88	3.008	61.61
V18	Fresh peel weight	Fpw	g	2.16	10.81	4.60	1.90	41.45
V19	Dry peel weight	Dpw	g	0.48	2.004	1.08	0.40	37.40
V20	Fresh flesh weight	Ffw	g	7.38	52.95	20.79	11.13	53.52
V21	Dry flesh weight	Dfw	g	1.10	8.46	3.43	1.72	50.28
V22	Center weight + tail	Cwt	g	1.18	8.45	3.51	1.60	45.68
V23	Flesh thickness	Flt	mm	3.1	18.27	9.36	2.60	27.79
V24	Fruit shape	Frs	Code	1	13	4.26	2.52	59.14
V25	Flesh color	Flc	Code	1	11	7.83	2.58	33.003
V26	Color of ripe fruit skin	Cfs	Code	1	7	2.70	1.36	50.70
V27	Rust in fruit	Rif	Code	1	3	1.30	0.72	55.39
V28	Fruit texture	Frt	Code	1	5	2.60	1.31	50.67
V29	Fruit firmness	Ffi	Code	1	7	4.26	1.51	35.54
V30	Fruit aroma	Fra	Code	1	5	2.33	1.36	58.34
V31	Fruit flavor	Frf	Code	1	13	8.03	4.09	50.93
V32	Flesh flavor	Flf	Code	1	5	4.26	1.16	27.24
Traits related to leaf (14 traits)								
V33	Leaf length	Lel	mm	47.01	103.06	75.52	12.25	16.23
V34	Leaf width	Lew	mm	25.57	57.69	40.40	6.58	16.29
V35	Leaf length/Leaf width	Lel/ Lew	Ratio	1.33	2.69	1.89	0.32	17.05
V36	Leaf thickness	Let	mm	0.20	0.47	0.31	0.06	19.02
V37	Petiole thickness	Pet	mm	0.81	1.99	1.31	0.24	18.53
V38	Leaf fresh weight	Lfw	g	0.22	0.99	0.46	0.15	33.64
V39	Dry weight Leaf	DwL	g	0.09	0.51	0.24	0.08	33.60
V40	Leaf dry matter	Ldm	%	39.43	80.43	52.23	7.54	14.44
V41	Leaf area	Lea	mm^2	1150.78	3872.20	2340.41	621.44	26.55
V42	Petiole length	Pel	mm	13.92	40.72	26.38	6.18	23.44
V43	Petiole length/Leaf length	Pel/ Lel	Ratio	0.23	0.48	0.34	0.05	16.16
V44	Leaf color	LeC	Code	1	9	4.33	2.98	68.86
V45	Leaf shape	Les	Code	1	5	2.73	1.66	60.94
V46	Leaf apex shape	Las	Code	1	3	1.83	0.99	54.23
Traits related to seed (6 traits)								
V47	Seed weight	Sew	g	0.04	0.50	0.24	0.13	55.40
V48	Seed length	Sel	Mm	6.48	9.21	7.55	0.63	8.35
V49	Seed diameter	Sed	Mm	1.57	5.03	2.83	0.58	20.76

Table 2 (continued)

NO.	Trait	Abbreviation	Unit	Min.	Max.	Mean	SD	CV (%)
V50	Seed length/ Seed diameter	Sel/ Sed	Ratio	1.44	5.38	2.79	0.75	27.04
V51	Seed shape	Ses	Code	1	3	1.66	0.95	57.04
V52	Seed color	Sec	Code	1	3	2.30	0.96	41.82
Traits related to tree (1 trait)								
V53	Tree height	Trh	M	2.14	5.17	4.35	0.83	19.05
Traits biochemical (5 traits)								
V54	Total phenolic content	TPC	mg/kgFW	1325.18	12475.9	4496.40	4757.95	105.81
V55	Total flavonoid content	TFC	mg/kgFW	51.21	1227.56	333.12	488.52	146.64
V56	Total anthocyanin content	TAC	mg/g FW	2.10	18.93	7.32	6.68	91.35
V57	Radical scavenging activity	DPPH	%	63.87	99.45	83.48	13.91	16.66
V58	Radical scavenging activity	ABTS	%	64.52	98.31	79.20	11.88	15.00

Note: **CV** = (SD/Mean) × 100

Table 3 Frequency distribution for the measured qualitative morphological characters in the studied red-fleshed apple accessions

Character	Frequency (no. of accessions)						
	1	**3**	**5**	**7**	**9**	**11**	**13**
Fruit Quality Traits							
Fruit shape	Flat globular (6)	Globular (30)	Globular to long globular (13)	Globular conic (6)	Medium conic (2)	Long conic (2)	Cylindrical (1)
Flesh color	White, pink around of carpel (1)	White pink (2)	Pink (12)	Reddish (7)	Red (26)	Dark red (12)	-
Color of ripe fruit skin	Light red (18)	Red (34)	Dark red (7)	Brown red (1)	-	-	-
Rust in fruit	Low (51)	Medium (9)	-	-	-	-	-
Fruit texture	Low juicy (20)	Relatively juicy (32)	Juicy (8)	-	-	-	-
Fruit firmness	Soft (3)	Medium (23)	stiff (27)	Very stiff (7)	-	-	-
Fruit aroma	Mild (27)	Intermediate (26)	Strong (7)	-	-	-	-
Fruit flavor	Slightly bitter (11)	Bitter-astringent (1)	Sour (6)	Slightly sour to sour-sweet (5)	Sour-sweet to sour (14)	Sour-sweet (14)	Relatively sweet (9)
Flesh flavor	Bitter (3)	Sour (16)	Sour–sweet (41)	-	-	-	-
Leaf Quality Traits							
Mature leaf color	Light green (16)	Green (18)	Dark green (9)	Red (4)	Brown red (13)	-	-
Mature leaf shape	Wide oval (25)	Elongated oval (18)	Lanceolate Elongated oval (17)	-	-	-	-
Leaf apex shape	Bend to side (35)	Lanceolate (25)	-	-	-	-	-
Seed Quality Traits							
Seed shape	Short Conic (40)	Pyramid (20)	-	-	-	-	-
Seed color	Red (21)	Light brown (39)	-	-	-	-	-

and tree-related traits (1 trait). The morphological traits were evaluated using 15 repetitions in each part (leaf and mature fruit) for each population. To measure the weight of fruits using a digital scale with an accuracy of 0.001 g (Sartorius, TE153S, d=0.001) and the dimensions of different organs with a digital caliper (Model 10-754-500, Mitutoyo Cooperation, d=0.01 d), leaves thickness with digital micrometer((Mitutoyo, MDC-25SB, d=0.001 mm) and all the fresh leaves were scanned with a scanner (HP Scanjet G4050) and the area of all of them was obtained using the ImageJ software. In addition, qualitative traits were measured based on ranking and coding (Table 3). The most utilized morphological descriptors are derived from international guidelines such as the International Board for Plant Genetic Resources (IBPGR) [28], and the International Union for the Protection of New Varieties of Plants [29].

Biochemical analysis

Preparation of phenolic extracts

The hydroalcoholic extracts were prepared to analyze the total polyphenol content, total anthocyanins, and antioxidant capacity of formulations of red-fleshed apples following the protocol described by Gironés-Vilaplana et al. [30]. In short, raw materials and formulations were mixed with 5 volumes of methanol/formic acid/water (25:1:24).

Colorimetric assays for reducing and antioxidant capacity

Total phenolics content (TPC)

The total phenolics content (TPC) of red-fleshed apple fruit samples collected from the studied populations in the north and northeast of Iran was determined by the Folin-Ciocalteu colorimetric method using gallic acid as standard [31]. Briefly, 100 µl of the extract was diluted in 400 µl D.W., and then 250 µl of diluted Folin solution was added. It was placed in the dark at room temperature for 1 min. Then 1250 µl 12.5% sodium carbonate was added, and the samples were left again for 40 min in the dark at room temperature. Then, the absorbance of the extract was read at a wavelength of 750 nm using an Elisa microplate reader (BioTek 800 TS). Results were expressed as mg gallic acid equivalents on a fresh weight (FW) basis (GAE mg/kg FW).

Total flavonoids content (TFC)

Total flavonoids content (TFC) was measured by aluminum chloride colorimetric method and using quercetin as a standard. First, standard solutions were prepared in ethanol [32]. Then, 3500 µl D.W and 300 µl of 5% sodium nitrite were added to 200 µl extract. After 5 min, 300 µl of 10% aluminum chloride and 200 µl of 4% sodium hydroxide were added, and the final solution was kept for 30 min at room temperature in the dark. This process was performed for all samples in triplicate. The absorbance of the samples was read at a wavelength of 510 nm in a spectrophotometer. Finally, total flavonoids were calculated using the quercetin standard curve and expressed as mg quercetin per kg of fresh weight (QU mg/kg FW).

Total anthocyanins content (TAC)

The extracts total anthocyanin content (TAC) was measured by the pH differential method [33]. Samples were diluted with two different solutions: potassium chloride (0.25 M), pH: 1.0, and sodium acetate (0.4 M), pH: 4.5. The pH was adjusted with concentrated hydrochloric acid. Samples were diluted to be analyzed at an absorbance of 520 nm. Diluted samples were kept for 20 min before measurement. The absorbance was measured at 520 nm and 700 nm, and a set was kept with distilled water as a blank. The absorbance difference between the pH-1.0 and pH-4.5 samples was calculated:

$$A = (A_{520} - A_{700})_{PH\ 1.0} - (A_{520} - A_{700})_{PH\ 4.5}$$

$$TAC = (A{*}MW{*}DF{*}V_e{*}$$

1000)/ (ϵ*1*M)Where MW is the molecular weight of cyanidin-3-glucoside (449 g/mol), DF is the dilution factor, and Ve is the extract volume, ε is the molar extinction coefficient of cyanidin-3-glucoside (29,600), l - the path length, and M is the mass of the fruits extracted. The total anthocyanins were reported on the basis of mg/g fw (C3GE mg/g FW).

DPPH

DPPH free radical scavenging activity was determined using Brand-Williams et al. [34] method with minor modifications. Diluted samples (100 µl) were added to 0.1 mM DPPH solution (100 µl) in three replicates in a 96-well microplate. Ascorbic acid was used as standard. After 30 30-minute reactions under darkness at RT, absorbance at 515 nm was measured with a microplate reader. DPPH free radical scavenging activity was calculated by the following equation:

DPPH scavenging activity (%) = (Ac-As) /Ac × 100)

In this formula, Ac is the absorbance of the control (100 µl ethanol with 100 µl DPPH) and A**s** is the absorbance of the sample.

ABTS

ABTS radical scavenging activity was measured by the Re et al. [35] method with slight modifications. The radical cation of ABTS (ABTS•+) was produced by reacting 7 mM ABTS solution with 2.45 mM potassium per sulfate, then it was diluted until its absorbance at 734 nm were 0.7. Diluted samples (40 µl) were added to ABTS solution (120 µl) in three replicates in a 96-well microplate. In this test, ascorbic acid was used as standard. After 30 min in the dark microplate absorbance in a microplate reader was read.

ABTS scavenging activity (%) = (Ac-As) /Ac × 100)

In this formula, Ac is the absorbance of the control (100 µl ethanol. with 100 µl of ABTS) and, As is the absorbance of the sample.

Statistical analysis

Mean and CV% are the most used indices for assessing morphological diversity [24]. Results of analyses were subjected to one-way analysis of variance (ANOVA) at a confidence level of 95% to determine whether there was any significant difference between the means of morphological and biochemical characteristics of red-fleshed apples from four populations. Values were reported as means ± standard error (SE) from triple replicates. Principle component analysis (PCA) of the 52 morphological traits was used to evaluate the diversity of morphological traits and correlations between the traits. Hierarchical clustering analysis (HCA) based on Ward's method and Euclidean distance was applied and creating a biplot based on Dim1 and Dim2 was done using the R software package FactoMineR [36] and heatmap using Rsoftware package pheatmap [61]. R software version 4.2.3 was used to perform all statistical calculations.

Results and discussion

This study considered morphological and biochemical parameters for assessing red-fleshed apples in the north and northeast of Iran. The results indicated significant differences between red-fleshed apples among the accessions and study sites for all the variables tested.

Variations of traits

Morphological descriptions of accessions

There were significant differences among the accessions investigated (ANOVA, $p<0.01$). Coefficient of variation (CV) was more than 20.00% in many measured characters (39 out of 58 characters), indicating high diversity among the accessions. In general, if the coefficient of variation was greater than 10%, indicating that the trait varies among different germplasm individuals were diversified [37]. The range of CV was from 6.64 (in length/diameter) to 146.64% (in total flavonoid content), with an average of 37.28 (Table 2). The total phenolic content (105.81%), total anthocyanin content (91.35%), leaf color (68.86%), number of seeds in fruit (61.61%), leaf shape(60.94%), fruit shape (59.14%), fruit aroma(58.34%), seed shape (57.04%), and seed weight (55.40%), depth of eye basin (52.88), fresh flesh weight (53.52), dry flesh weight (50.28), Color of ripe fruit skin (50.79), rust in fruit (55.39), fruit texture (50.67), fruit flavor (50.93) had CV higher than 50%. The high coefficient of variation suggests significant variability in these traits among the different populations. In contrast, length/diameter (6.67%), seed length (8.35%), fresh weight/dry weight (12.93%), fruit dry matter (13.63%), leaf dry matter (14.44%), and petiole length/leaf length (16.16) shown low the CV indicating less variation among the populations. Moradi et al. [25] reported that fruit symmetry (190.91%) and petiole color (72.61%) had the highest coefficient of variation in several oriental apples (*Malus orientalis* Uglitzk.). The coefficient of variation (CV) quantifies the dispersion or variability among the accessions for each trait. Higher CV values indicate greater variability, whereas lower values reflect greater homogeneity in traits among the accessions. Homogeneous characteristics can be reliable and uniform for identification or comparative analysis. Conversely, a coefficient of variation over 20% in characteristics suggests a higher level of diversity among accessions, which can be beneficial in breeding programs or selection processes. The CV enables researchers and breeders to identify stable and uniform traits and those with greater variability, helping inform decisions about selection, breeding, and differentiation among accessions. Table 2 presents data recorded on 40 quantitative, 13 qualitative, and 5 biochemical traits in four red-fleshed apples.

Morphometrical analysis of fruits and leaves

A wide range of quantitative traits related to fruit was as follows: The CV of fruit fresh weight and dry weight were 48.87% and 44.96% and changed from 12.53 to 70.62 g and from 2.12 to 12.02 g, respectively. Whereas, fruit length and diameter showed a CV coefficient of 16.70% and 17.37%, varying from 26.10 to 51.71 and 29.29 to 54.46 mm, respectively. Also, the range of fruit-related traits was as follows: fruit dry matter: 12.77 to 23.68%, fresh weight/dry weight: 4.22 to 7.83 ratio, length/diameter: 0.76 to 1.01 ratio, the range of thickness of skin, tail thickness, and tail length was as follows: 0.31 to 1.94 mm, 1.11 to 2.30 mm, 5.07 to 40.01 mm, respectively.

We determined the depth of the stalk cavity in the red-fleshed apples from 4.27 mm to 12.71 mm and the depth of the eye basin from 0.23 to 18.19 mm. Also, the range of the width of the eye basin, width of the stalk cavity, length of the core, diameter of the core, and flesh thickness was as follows: 2.80 to 17.01 mm, 5.76 to 16.41 mm, 10.61 to 21.03 mm, 14.63 to 32.88 mm, 3.10 to 18.27 mm, respectively. Meanwhile, the number of seeds in fruit ranged from 1 to 11. An important diagnostic feature is the depth of the stalk cavity and depth of the eye basin because the measured features may have a specific range for each variety and genotype [38]. The range of fresh peel weight, dry peel weight, fresh flesh weight, dry flesh weight, center weight+tail was as follows: 2.16 to 10.81 g, 0.48 to 2.004 g, 7.38 to 52.95 g, 1.10 to 8.46 g, 1.18 to 8.45 g, respectively, (Table 2). For comparison, Faramarzi et al. [27] reported a mean of 48.15 mm for fruit length, 53.17 mm for fruit diameter, 0.87 for Length/diameter, and 45.88 g for fruit weight fresh in a red-fleshed apple from 'Bekran', Iran. In addition, Abedi et al. [5] reported the mean fruit weight as 40.25 g for fruit weight in a red-fleshed apple from 'Bekran', Iran.

Seven types of fruit shapes were observed. The shape of the fruits in many accessions was globular (30 accessions), globular to long globular (13), globular conic (6), flat globular (6), medium conic (2), long conic (2), and cylindrical (1) (Table 3). Damyar et al. [26] reported globular for the fruits of red-fleshed apples. The color of the ripe fruit skin was red in 34 accessions. Also, the color of the flesh of the fruit showed a few variations, which were mainly red (26), white, and pink around the carpel (1), white pink (2), pink (12), reddish (7), and dark red (12) was also observed. For comparison, Damyar et al. [26] reported six flesh colors: white pink, red, pink white, red skin around, white, pink around the carpel, dark red, pink white, and darker around the skin for fruits red-fleshed apple germplasm from Iran. The fruits were mostly free of rust. Rust was low (51 accessions), and medium (9). Fruit color is one of the important qualitative parameters of consumers in choosing their favorite apple fruit [39]. The red color observed in red-fleshed apples results from a high accumulation of anthocyanin [9, 11]. On the other hand, the red color is an important quality parameter of fruit consumers evaluate as it highly affects their marketability [19]. The texture of the fruit was relatively juicy (32 accessions), low juicy (20), and juicy (8). At the same time, the firmness of the fruit was soft (3 accessions), medium (23), stiff (27), and very stiff (7). For comparison, Faramarzi et al. [27] reported a range of 4.22–5.65 n/mm2 for fruit firmness in red-fleshed apples from Iran. Damyar et al. [26] reported a range of 3.11–6.22 n/mm^2 for fruit firmness in red-fleshed apples from Iran. The fruits had mainly intermediate (26 accessions), mild (27), and strong (7) aromas. The flavor of the fruit was sour-sweet and sour-sweet to sour in most accessions (14 accessions), slightly bitter (11), relatively sweet (9), sour (6), slightly sour to sour-sweet (5), and bitter-astringent (1) was observed. In the study of a red- fleshed apple collection from Iran, fruit flavor showed strong diversity, ranging from slightly bitter to relatively sweet [26]. The flavor of the flesh was also bitter (3 accessions), sour (16), and sweet-sour (41) (Table 3). Although most of the red-fleshed apples in the world are not very pleasant in terms of taste because they are of wild origin and because they have a very sour or bitter taste, they are unusable and not widely known in the market [26, 40]. Wild apples in Iran, such as the red-flesh genotypes in Shahroud and Gavramak, have excellent taste, proper fruit size and weight, red color indicating high antioxidants, a sweet-sour taste, considerable fruit firmness, and no bitterness. They are well-suited for making jam, juice, and fresh food due to their high capacity [26, 41].

A wide range of leaf-related traits were as follows: leaf length: 47.01 to 103.06 mm with an average of 75.52 mm, leaf width: 25.57 to 57.69 mm with an average of 40.40 mm, and leaf thickness: 0.20 to 0.47 mm with an average of 0.31 mm, petiole thickness: 0.81 to 1.99 mm with an average of 1.31 mm, leaf area: 1150.78 to 3872.41 mm^2 with an average of 2340.41 mm^2 and petiole length: 13.92 to 40.72 mm with an average of 26.38 mm. also, leaf fresh weight: 0.22 to 0.99 g with an average of 0.46 g and dry weight leaf: 0.09 to 0.51 g with an average of 0.24 g. Whereas leaf length/leaf width is from 1.33 to 2.69 ratio with an average of 1.89, and petiole length/leaf length is from 0.23 to 0.48 ratio with an average of 0.34 (Table 2). For comparison, Moradi et al. [25] reported a range of 46.45 to 83.25 mm for leaf length, 25.73 to 46.69 mm for leaf width, 1358.66–3815.51 mm^2 for leaf area, and 15.65 to 35.62 mm for petiole length in *M. orientalis* accessions from the Sistan-va-Baluchestan province from the southern part of Iran. Additionally, Khadivi et al. [24] reported a range of 38.56 to101.36 mm for leaf length, 20.27 to 45.31 mm for leaf width, 899.05 to 4043.25 mm^2 for leaf area, 12.77 to 46.30 mm for petiole length.

The red-fleshed apples selected in the study showed considerable variability for qualitative traits related to leaf attributes. Three types of leaf shapes were observed, including wide oval (25 accessions), elongated oval (18), and lanceolate elongated oval (17). The leaf color is light green in 16 accessions, green in 18, dark green in 9, red in 4, and brown red in 13. The lanceolate shape of the leaf was present in 25 accessions, while the shape of the leaf tip was bent to the side in 35 accessions (Table 3). The presence of secondary metabolites, including anthocyanins, has been reported in the leaves of Iranian red-flesh apple varieties [42]. The red color observed in red-fleshed apples results from a high accumulation of anthocyanins [11]. There were no reports of red-flashed apple leaves in Iran for comparison.

1 to 11 seeds were observed in each fruit, and the characteristics related to the seed are as follows: seed weight 0.04 to 0.50 g, seed length 6.48 to 9.21 mm, seed diameter 1.57 to 5.03 mm, and ratio Seed length/Seed diameter 1.44 to 5.38 were observed (Table 2). While the seed color was red (21 accessions) and light brown (39), the seed shape is short conic in 40 accessions and pyramid in 20 accessions (Table 3). Moradi et al. [25] reported a range of 4.15 to 7.30 mm for seed length, 2.63 to 4.36 mm for seed width, and 1.05 to 2.46 mm for seed thickness in *M. orientalis* accessions from the Sistan-va-Baluchestan province from the southern part of Iran. There were no reports for comparisons of red-flashed apple seeds in Iran. Finally, tree height varied from 2.14 to 6.17 m, averaging 4.35 m (Table 2). Damyar et al. [26] reported that tree height ranged from 425 to 305 cm.

Several studies have demonstrated that red-fleshed apples collected in Iran [5, 26, 27] showed high variability in morphological traits. Fig 1 shows the leaf and fruit pictures of four accessions studied of red-fleshed apples. Generally, both qualitative and quantitative morphological characteristics are useful. Variation in qualitative characters is useful for varietal identification, whereas quantitative characters would be of direct interest to plant breeders [43].

Biochemical descriptions

The minimum, maximum, and mean values of biochemical variables were shown in Table 2. A high level of variability was observed in the measured biochemical characteristics. Total flavonoid content and ABTS changed from 51.21 to 1227.56 mg/kg fresh weight and 64.52 to 98.31%, respectively. The highest CV was 146.64%, and the lowest was 16.66%. On average, the total amount of anthocyanin in fresh weight was 7.32 mg/g, ranging from 2.10 to 18.93 mg/g. (Table 2).

Biochemical descriptions of four red-fleshed apples were presented in Table 4. In our study, among red-fleshed apples (whole fruit), total phenolic

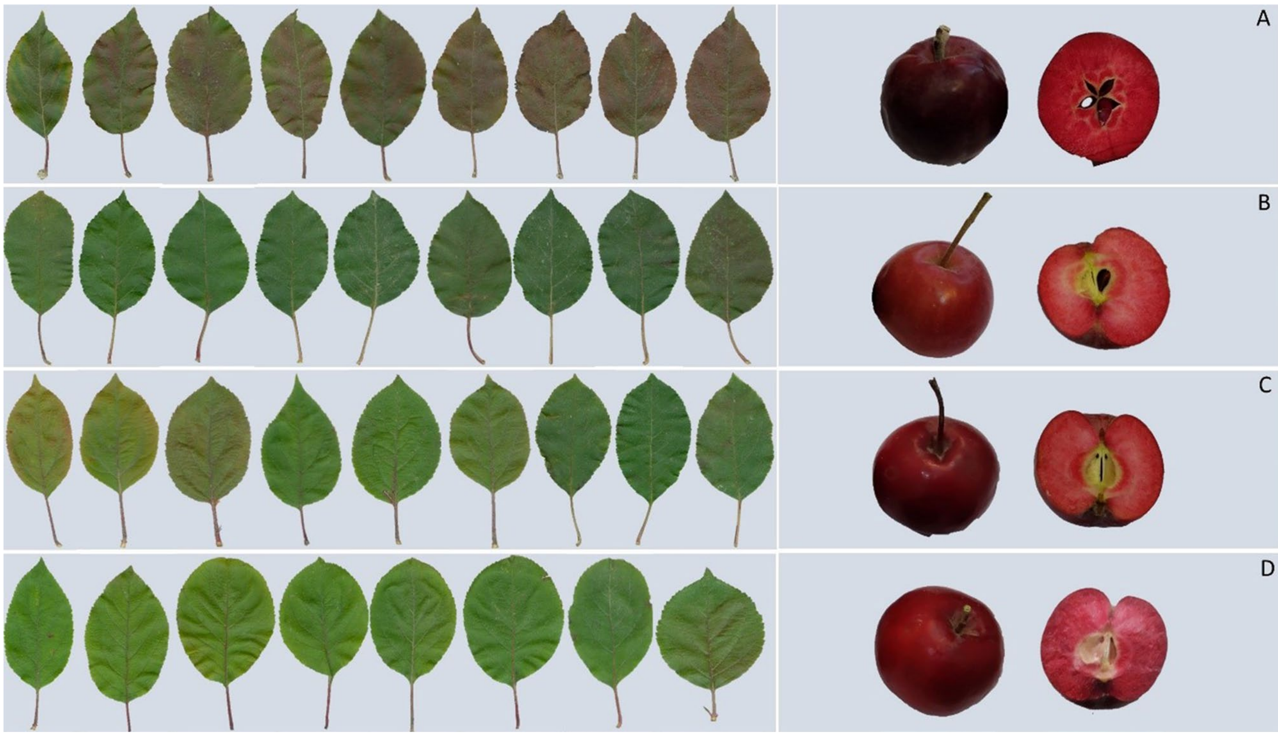

Fig. 1 The leaves and fruits pictures of four accessions studied in red-fleshed apple (**A**: 'Kalateh', **B**: 'Bekran', **C**: 'Kalateh-Khij', and **D**: 'Gavramak')

Table 4 Contents of total phenolics (TPC), flavonoids (TFC), anthocyanins (TAC), and antioxidant activity (was determined by DPPH and ABTS assays) from the whole fruit of red-fleshed apple

Collection site	Bekran	Kalateh	Kalateh-Khij	Gavramak	F-value	*P*-value
TPC (GAE mg/kg FW)	1460.98 ± 64.60d	2377.03 ± 56.60b	1783.21 ± 8.61c	12364.40 ± 63.79a	6401	< 0.001
TFC (QU mg/kg FW)	53.48 ± 1.07b	79.49 ± 2.03b	60.30 ± 0.35b	1139.21 ± 52.62a	277.6	< 0.001
TAC (C3GE mg/g FW)	2.30 ± 0.08c	5.04 ± 0.55b	3.70 ± 0.28bc	18.23 ± 0.33a	282.4	< 0.001
DPPH (%)	64.11 ± 0.11c	78.46 ± 1.08b	97.10 ± 0.96a	94.26 ± 0.74a	232.2	< 0.001
ABTS (%)	76.53 ± 0.40b	74.22 ± 0.05b	68.04 ± 1.50c	98.01 ± 0.16a	184.2	< 0.001

Note: **GAE**; gallic acid equivalent, **QU**; quercetin equivalent, **C3GE**; cyanidin 3-O-glucoside equivalents, **FW**, fresh weight. Values were reported as means ± SE values of three replicates. Different letters (a, b, c and d) within the same column indicate significant difference at $p < 0.05$ by ANOVA test

content (TPC), total flavonoid content (TFC), totalanthocyanin content(TAC), and antioxidant activity (DPPH and ABTS) varied significantly ($p < 0.05$). The total phenolic content (TPC) ranged from 1460.98 to 12364.40 mg GAE/kg FW in the whole Fruit. Red-fleshed 'Gavramak' apples contained the highest TPC (12364.40 mg/kg FW) in fruit, respectively. In contrast, the lowest values were measured from the red-fleshed 'Bekran' (1460.98 mg/kg FW) (Table 4). For comparison, Katiyo et al. [43] reported the total phenolic content in whole apples (3467.47 mg GAE/kg). Phenolic compounds are one of the most common and widespread groups of secondary metabolites in plants [44]. Wang et al. [45] reported that the value of TPC in red-fleshed apples ranged from 2062.3 to 4727.8 mg GAE/kg FW in the peel and from 342.9 to 1056.0 mg GAE/kg FW in the flesh. A significant difference was observed between the examined apples regarding the amount of total flavonoids. TFC varied from 53.48 to 1139.21 mg Qu/kg FW. The highest amounts of TFC were observed in the 'Gavramak' apples (1139.21 mg per 100 g of fresh weight) and the 'Kalateh' red-fleshed apple (79.49 mg/100 g FW), and the lowest amount of TFC in 'Bekran' red-fleshed apple (53.48 mg/100 g of FW) was observed. Studies have shown that flavonoids can play a crucial role in improving human health [4, 46]. TAC varied from 18.23 to 2.30 mg/g FW (Table 4). The highest amount of TAC was observed in the red-fleshed apple of 'Gavramak' (18.23 mg/g of fresh weight), and the lowest amount was observed in the red-fleshed apple of 'Bekran' (2.30 mg/g FW). In the previous study of a 'Bekran' red-fleshed apple genotype, the amount of anthocyanin (88.50 mg/100 g FW) was reported [5]. Also, Bars-Cortina et al. [10] reported the range of the TAC in the red-fleshed from 9.34 to 49.3 mg/kg. Various anthocyanins have been identified and measured in red-fleshed apples in skin and flesh [47–49]. The red color observed in red-fleshed apples results from a high accumulation of anthocyanins, which consumers can notice [9, 11, 12, 50]. Anthocyanins are natural products that structurally belong to flavonoid compounds, and they are a group of plant pigments that exist widely in nature [13]. Overall, the red-fleshed apples from 'Gavramak' had the highest values for TPC, TFC, and TAC, which is enormously higher than previous results of red-fleshed apples in 'Bekran', which shows the high potential of red-fleshed apples as functional foods [5].

In this study, two antioxidant assays (DPPH and ABTS method) were applied to obtain more accurate evaluations of antioxidant activities (%) (Table 4). When the DPPH assay was conducted, 'Kalateh-Khij' exhibited the strongest antioxidant capacity at 97.10%. In contrast, red-fleshed 'Gavramak' in the ABTS measurement demonstrated the strongest antioxidant effect at 98.01%. There was no significant difference ($p > 0.05$) between the ABTS of the 'Bekran' and 'Kalateh'. For comparison, Katiyo et al. [43], in the DPPH assay, the activity was (1098.62 mg/kg); also, in the ABTS assay, (2122.89 mg/kg) was observed in the whole apples. Abedi et al. [5] reported that the range of antioxidant power was 93.46%. Whereas Wang et al. [45], evaluating the phenolic composition and antioxidant capacity of three red-fleshed varieties from Xinjiang, China and one from the USA, showed that DPPH ranged from 17.1 to 73.0 mg/ml in the peel and 94.6 to 465.4 mg/ml in the flesh mg/ ml equivalents; and for ABTS, ranged from 35.2 to 72.1 μmol/g FW in the peel and 7.4 to 22.4 μmol/g FW. The antioxidant capacity of red-fleshed apples has been previously confirmed [8, 11, 13, 15, 20, 43, 45, 51, 52]. The type and concentration of polyphenol compounds in apple extract are decisive for their relative antioxidant effectiveness [8]. Research has shown that red-fleshed apples are a rich source of natural chemical compounds due to their high content of phenolic compounds and antioxidants in the skin and flesh. The phenolic extract in their skin and flesh can have antiproliferative effects in inhibiting breast cancer cells in humans [11, 18].

Correlations between the quantitative traits

The correlation coefficients with positive and negative significant correlations among the 39 quantitative morphological traits were reported in Table 5. Most of those characteristics were significantly correlated with one another. Fruit fresh weight showed significant positive correlations fruit dry weight (r=0.96), depth of eye basin (r=0.54), width of eye basin (r=0.55), depth of stalk cavity (r=0.58), length of core (r=0.55), diameter of core (r=0.92), fresh peel weight (r=0.97), dry peel weight (r=0.71), fresh flesh weight (r=1.00), dry flesh weight (r=0.81), center weight+tail (r=0.94), flesh thickness (r=0.80), leaf length/ leaf width (r=0.44), and seed diameter (r=0.49), while observed negative correlations with length/diameter (-0.35) and seed length/seed diameter (-0.52). A positive and significant correlation was observed between fruit weight with fruit length (r=0.93), and fruit diameter (r=0.95), and agreed with the previous results in red-fleshed apples [41]. Also, Khadivi et al. [24] reported a positive and significant correlation between fruit weight, fruit width, and fruit length in *M. orientalis*. Also, length of core was positively correlated with fruit dry weight (r=0.53), fruit length (r=0.55), fruit diameter (r=0.47), tail length (r=0.45), and leaf length was positively correlated with fruit fresh weight (r=0.39), fruit dry weight (r=0.38), fruit length (r=0.45), fruit diameter (r=0.42), diameter of core (r=0.35), fresh peel weight (r=0.40), dry peel weight (r=0.47), fresh flesh weight (r=0.39), center weight+tail (r=0.38). Larger leaves provide structural support and allow leaves to be spaced out, reducing shading among foliage and maximizing light interception for photosynthesis. Therefore, it provides more resources to increase the number of inflorescences and fruits. These data can be exploited by breeding programs or by facilitating the identification of apple genotypes during field surveys.

Principal component analysis (PCA)

Principal Component Analysis (PCA), a multivariate statistical technique, was used to identify the most significant traits within the data. The PCA findings indicated significant diversity across the specimens based on morphological characteristics (Fig. 2). Five components (Table 6) were identified, accounting for 60.83% of the total variance, with the first three principal components explaining 50.56% of the variance. PC1 and PC2 were significant in differentiating the examined accessions. Moradi et al. [25] indicated that his first 13 components represented 83.30% of the overall variance, whereas the first three principal components explained 32.76% of the entire variance in *M. orientalis* accessions from the Sistan-va-Baluchestan province in southern Iran.

The PC1 explained 30.27% of the total variance. It was represented by fruit fresh weight (0.94), fruit dry weight (0.94), fruit length (0.91), fruit diameter (0.95), depth of eye basin (0.50), width of eye basin (0.56), depth of stalk cavity (0.58), diameter of core (0.86), fresh peel weight (0.91), dry peel weight (0.80), fresh flesh weight (0.94), dry flesh weight (0.86), Center weight+tail (0.89), flesh thickness (0.81), fruit flavor (0.77), flesh flavor (0.71), leaf length (0.52), leaf length/leaf width (0.51), petiole length (0.62), and Seed diameter (0.62), fresh weight/dry weight (0.63), tail length (0.64), number of seed in fruit (0.76), seed weight (0.66), seed length (0.59), and seed length/seed diameter (0.53) with positive correlations. These traits are associated with increasing the yield of fruit biomass, which is very important for fruit quality

Table 5 The correlation coefficient between 39 quantitative traits measured in red-fleshed apples. * $P<0.1$, *$P<0.05$, **$P<0.01$, ***$P<0.0001$

Trait	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20
V1	1																			
V2	0.96***	1																		
V3	-0.24	0.04	1																	
V4	0.25	-0.03	-0.98***	1																
V5	0.93***	0.91***	-0.18	0.18	1															
V6	0.95***	0.94***	-0.13	0.15	0.88***	1														
V7	-0.35***	-0.33***	0.08	-0.13	-0.19	-0.45***	1													
V8	0.17	0.24	0.14	-0.13	0.20	0.27*	-0.31*	1												
V9	0.27*	0.32*	0.07	-0.03	0.31*	0.26*	-0.12	0.06	1											
V10	0.10	-0.01	-0.38***	0.33*	0.07	0.01	0.11	-0.25	-0.42***	1										
V11	0.54***	0.55***	-0.04	0.03	0.46***	0.51***	-0.20	0.10	0.21	-0.08	1									
V12	0.55***	0.56***	-0.06	0.10	0.53***	0.55***	-0.31*	0.27*	0.22	0.00	0.06	1								
V13	0.28*	0.31*	0.08	-0.07	0.31*	0.25	-0.12	0.11	0.23	-0.07	0.13	0.16	1							
V14	0.58***	0.55***	-0.13	0.17	0.56***	0.58***	-0.32*	0.10	0.08*	0.01	0.34**	0.39**	0.17	1						
V15	0.55***	0.53***	-0.16	0.13	0.55***	0.47***	-0.06	-0.11	0.17	0.45***	0.18	0.23	0.11	0.23	1					
V16	0.92***	0.85***	-0.33**	0.33**	0.83***	0.85***	-0.39**	0.13	0.17	0.14	0.45***	0.48***	0.27*	0.64***	0.48***	1				
V17	-0.03	-0.18	-0.47***	0.43***	-0.12	-0.20	0.18	-0.31*	0.27*	0.39**	0.03	-0.26*	0.05	0.01	0.04	0.14	1			
V18	0.97***	0.93***	-0.21	0.23	0.90***	0.90***	-0.33**	0.19	0.26*	0.13	0.49***	0.54***	0.22	0.53***	0.60***	0.90***	-0.02	1		
V19	0.71***	0.76***	0.14	-0.10	0.71***	0.75***	-0.33**	0.41**	0.16	-0.04	0.41**	0.40**	0.27*	0.46***	0.26*	0.70***	-0.16	0.68***	1	
V20	1.00***	0.96***	-0.23	0.25	0.93***	0.95***	-0.36**	0.18	0.28*	0.08	0.55***	0.55***	0.29*	0.59***	0.53***	0.92***	-0.05	0.95***	0.70***	1
V21	0.81***	0.86***	0.11	-0.09	0.79***	0.83***	-0.36**	0.28*	0.30*	-0.13	0.49***	0.45***	0.28*	0.44***	0.35**	0.74***	-0.32*	0.79***	0.80***	0.81***
V22	0.94***	0.90***	-0.18	0.19	0.88***	0.91***	-0.31*	0.18	0.21	0.07	0.41**	0.54***	0.24	0.54***	0.48***	0.84***	-0.09	0.88***	0.70***	0.92***
V23	0.80***	0.82***	0.00	0.03	0.76***	0.82***	-0.38**	0.17	0.19	-0.06	0.43***	0.45***	0.34**	0.52***	0.38**	0.72***	-0.22	0.75***	0.60***	0.82***
V33	0.39**	0.38**	-0.10	0.09	0.45***	0.42***	-0.14	0.18	0.10	0.19	0.16	0.19	0.12	0.14	0.22	0.35**	-0.02	0.40**	0.47***	0.39**
V34	-0.09	-0.13	-0.14	0.13	-0.08	-0.10	0.07	0.08	-0.04	-0.02	0.13	-0.12	-0.14	0.03	-0.21	-0.10	0.15	-0.11	-0.04	-0.09
V35	0.44***	0.46***	0.05	-0.05	0.47***	0.46***	− 0.020	0.05	0.09	0.22	0.01	0.26*	0.23	0.07	0.40**	0.41**	-0.13	0.47***	0.46***	0.43***
V36	-0.29*	-0.24	0.22	-0.21	-0.35**	-0.18	-0.04	0.21	0.01	-0.19	-0.21	-0.10	-0.00	-0.18	-0.23	-0.30*	-0.12	-0.31*	-0.19	-0.28*
V37	-0.17	-0.12	0.13	-0.13	-0.12	-0.05	-0.05	0.47***	0.07	-0.23	-0.09	-0.05	-0.09	-0.01	-0.21	-0.16	-0.22	-0.19	0.05	-0.15
V38	0.17	0.10	-0.27*	0.25	0.19	0.16	0.06	0.13	-0.02	0.17	0.11	0.04	-0.02	0.13	0.02	0.22	0.26*	0.15	0.22	0.18
V39	0.24	0.21	-0.17	0.16	0.28*	0.25	-0.00	0.31*	0.05	0.08	0.13	0.14	0.07	0.13	0.03	0.26*	0.12	0.21	0.35**	0.25
V40	0.17	0.27*	0.29*	-0.26*	0.20	0.23	-0.21	0.44***	0.16	-0.19	0.07	0.25	0.17	-0.01	0.03	0.09	-0.37***	0.17	0.37***	0.17
V41	0.26*	0.25	-0.12	0.11	0.35**	0.32*	-0.04	0.39**	0.15	0.07	0.14	0.15	0.04	0.16	0.04	0.23	-0.09	0.25	0.33*	0.27*
V42	0.48***	0.48***	-0.05	0.03	0.48***	0.50***	-0.24	0.18	0.08	0.24	0.27*	0.20	0.29*	0.14	0.27*	0.42***	-0.08	0.45***	0.47***	0.49***
V43	0.30*	0.32*	0.04	-0.07	0.23	0.30*	-0.23	0.10	0.02	0.17	0.22	0.12	0.30*	0.05	0.19	0.25	-0.11	0.25	0.21	0.32*
V47	-0.04	-0.15	-0.29*	0.27*	-0.14	-0.17	0.22	-0.27*	-0.22	0.24	0.01	-0.24	0.12	-0.03	-0.01	0.08	0.80***	-0.04	-0.10	-0.05
V48	-0.32*	-0.40**	-0.28*	0.25	-0.32*	-0.45***	0.35**	-0.40**	-0.23	0.44***	-0.30*	-0.23	-0.00	-0.23	0.11	-0.24	0.47***	-0.25	-0.43***	-0.34**
V49	0.49***	0.59***	0.23	-0.22	0.54***	0.56***	-0.25	0.41**	0.30*	-0.18	0.25*	0.37**	-0.03	0.35**	0.23	0.35**	-0.46***	0.51***	0.53***	0.49***
V50	-0.52***	-0.60***	-0.22	0.19	-0.55***	-0.63***	0.35*	-0.50***	-0.31*	0.33**	-0.36**	-0.41**	0.01	-0.43***	-0.11	-0.40**	0.55***	-0.49***	-0.57***	-0.53***
V53	0.21	0.33**	0.38**	-0.35**	0.28*	0.35**	-0.21	0.48***	0.18	-0.51***	0.21	0.20	0.02	0.14	-0.23	0.10	-0.63***	0.16	0.41**	0.22

Table 5 (continued)

Trait	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20
Trait	**V21**	**V22**	**V23**	**V33**	**V34**	**V35**	**V36**	**V37**	**V38**	**V39**	**V40**	**V41**	**V42**	**V43**	**V47**	**V48**	**V49**	**V50**	**V53**	
V1																				
V2																				
V3																				
V4																				
V5																				
V6																				
V7																				
V8																				
V9																				
V10																				
V11																				
V12																				
V13																				
V14																				
V15																				
V16																				
V17																				
V18																				
V19																				
V20																				
V21	1																			
V22	0.78***	1																		
V23	0.73***	0.73***	1																	
V33	0.29*	0.38**	0.27*	1																
V34	-0.16	-0.13	-0.16	0.48***	1															
V35	0.41**	0.48***	0.38**	0.50***	-0.50***	1														
V36	-0.21	-0.31*	-0.26*	-0.12	0.14	-0.28*	1													
V37	-0.11	-0.15	-0.05	0.30*	0.45***	-0.19	0.30*	1												
V38	0.00	0.14	0.03	0.74***	0.80***	-0.07	0.03	0.46***	1											
V39	0.15	0.22	0.11	0.76***	0.71***	0.02	0.05	0.54***	0.92***	1										
V40	0.40**	0.19	0.17	0.13	-0.13	0.23	0.04	0.19	-0.12	0.26*	1									
V41	0.18	0.24	0.17	0.71***	0.43***	0.20	0.09	0.47***	0.66***	0.69***	0.05	1								
V42	0.37**	0.43***	0.42***	0.73***	0.11	0.57***	-0.06	0.17	0.40**	0.52***	0.33**	0.55***	1							
V43	0.24	0.24	0.33*	0.09	-0.29*	0.33*	0.07	-0.01	-0.12	0.03	0.38**	0.09	0.73***	1						
V47	-0.28*	-0.12	-0.20	-0.16	-0.01	-0.13	0.11	-0.29*	0.11	-0.02	-0.35**	-0.16	-0.11	-0.02	1					
V48	-0.44***	-0.34**	-0.50***	-0.32*	-0.18	-0.10	-0.00	-0.37**	-0.17	-0.24	-0.16	-0.32*	-0.30*	-0.11	0.47***	1				
V49	0.57***	0.51***	0.36***	0.25	-0.10	0.29*	-0.06	0.19	-0.04	0.11	0.38**	0.32*	0.26*	0.11	-0.45***	-0.42***	1			
V50	-0.62***	-0.53***	-0.54***	-0.28*	0.01	-0.22	0.04	-0.26*	-0.04	-0.19	-0.35**	-0.43***	-0.31*	-0.14	0.52***	0.75***	-0.84***	1		

Table 5 (continued)

Trait	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20
V53	0.43***	0.28*	0.34**	0.20	-0.02	0.16	0.07	0.34**	-0.06	0.09	0.39**	0.25	0.23	0.13	-0.56***	-0.63***	0.51***	-0.62***	1	
Abbreviation	FfW	Fdw	Fdm	Frw/Drw	Frl	Frd	Frl/Frd	Ths	Tat	Tal	Deb	Web	Wsc	Dsc	Lec	Dic	Nsf	Fpw	Dpw	Ffw
	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20
	Dfw	Cwt	Flt	Lel	Lew	Lel/Lew	Let	Pet	Lfw	DwL	Ldm	Lea	Pel	Pel/Lel	Sew	Sel	Sed	Sel/Sed	Trh	
	V21	V22	V23	V33	V34	V35	V36	V37	V38	V39	V40	V41	V42	V43	V47	V48	V49	V50	V53	

and yield. Also, fresh weight/dry weight (0.63), tail length (0.64), number of seeds in fruit (0.76), seed weight (0.66), seed length (0.59), and seed length/seed diameter (0.53) showed positive and significant correlations with PC2. These traits are associated with increasing the yield of seed and reproductive biomass. The PC3 was correlated with leaf length (0.62), leaf width (0.87), petiole thickness (0.63), leaf fresh weight (0.92), dry weight Leaf (0.86) and leaf area (0.68), leaf length/leaf width (0.52), petiole length (0.61), petiole length/leaf length (0.60), leaf shape (0.65). These traits are associated with increasing the yield of vegetative biomass. Finally, leaf shape (0.61) was found to be influential on PC4, andPC5 showed no significant correlations. PCA has been previously used to investigate the diversity of most plants [25], and red-fleshed apple genotypes [41, 53], and their conclusions were similar to the present results.

Cluster analysis and scatter plot

Cluster analysis uses multiple variables to categorize samples according to a set of measured characteristics into distinct groups, ensuring that similar subjects are grouped. The cluster and heatmap derived from morphological variables revealed two main clusters (Fig. 2a). The first cluster included a population from the 'Kalateh-Khij' habitat, which exhibited superior in various traits, including fruit fresh, flesh, peel weight, flesh thickness, fruit aroma, fruit length, and diameter, as well as leaf size-related attributes. These characteristics directly influence the enhancement of fruit biomass yields. Size, weight, and flavor are the primary criteria for marketing. Consequently, populations demonstrating superiority in these characteristics may be utilized in breeding projects. The 'Kalateh-Khij' habitat's climatic conditions in Iran, with 300 mm precipitations, favor the cultivation of red-fleshed apples. This indicates that the species grows and performs under the particular environmental conditions present in that location.

The second cluster was subdivided into two sub-clusters. The first sub-cluster contained a population from the 'Gavramak' population. This population exhibited superior in certain characteristics, including seed length, seed shape, seed weight, and seed quantity per fruit, while conversely demonstrating minimal values in fruit size and weight characteristics such as FfW, Fdw, Frl, and Frd. Seed number and size are crucial for plant reproduction, particularly for rare red-fleshed apple varieties, of which few populations remain alive. Consequently, populations demonstrating superior seed qualities may be used for breeding and propagation actions. The second sub-cluster included two populations from 'Bekran' and 'Kalateh,' which exhibited similarities in several characteristics, including fruit dry matter, fruit firmness, leaf and skin thickness, leaf dry matter, and the colors of skin

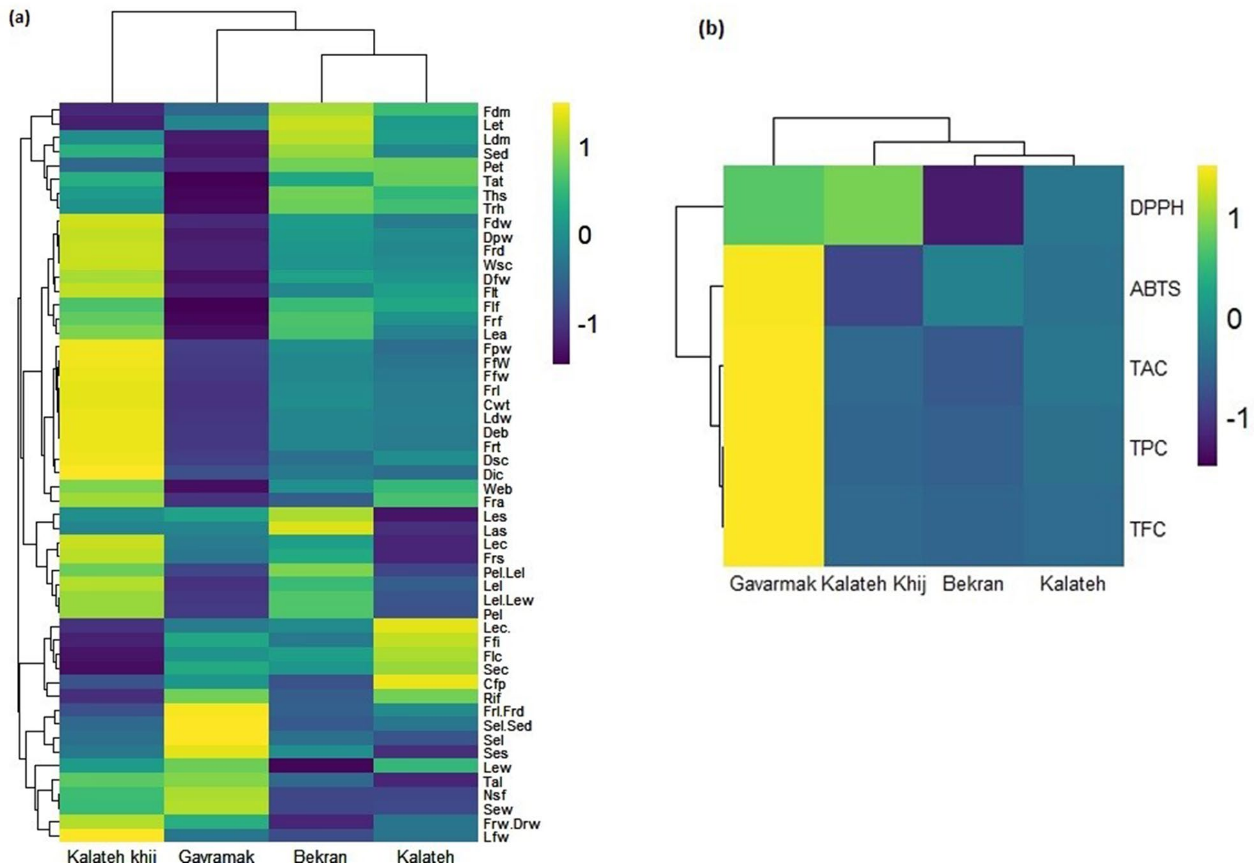

Fig. 2 Heat map for the four studied populations using morphological traits (a) and biochemical traits (b). Mean values refer to colors from minimum displayed in dark blue to maximum represented in yellow

and leaves. The two populations of this group were nearly geographically close. The results obtained align with prior studies demonstrating the impact of geographical conditions and ecological factors on population grouping [24].

A study on red-fleshed apples revealed that plants grown at lower altitudes in the 'Bekran' and 'Kalateh' regions exhibited smaller, thicker leaves and skin with high leaves and fruits dry mass. This indicates that *M. sieversii* individuals at lower altitudes in arid environments typically invest greater resources towards adaptations for dry and hotter conditions. Conversely, species from higher altitudes in the Kalateh Khij had larger leaves and fruits. An examination of the morphological variability of the Fuji apple tree, collected from various altitudes, revealed that accessions collected at an elevation of 1715 m demonstrated the highest values for fruit and leaf morphological traits [55]. This suggests that *M. sieversii* individuals from higher altitudes may have adapted to colder and mountainous environments by developing larger leaf and fruit dimensions. Furthermore, individuals from the Hyrcanian region within 'Gavramak' populations had reduced leaf and fruit sizes, which could be due to the region's high rainfall. These findings highlight the significance of accounting for ecological factors, including altitude and geographical location, in examining red-fleshed apples' growth and metabolic responses. Environmental influences can profoundly influence plant physiology and biochemistry, resulting in differences in growth patterns and the synthesis of secondary metabolites such as phenolic compounds.

The cluster analysis of biochemical characteristics revealed two distinct clusters (Fig. 2b). The first cluster included a population from the 'Gavramak' population exhibiting the highest concentrations of all biochemical components in comparison to the populations within the second cluster which included populations from Semnan province ('Kalateh-khij' 'Bekran' and 'Kalateh'). This group was determined to be superior in bioactive chemicals. High levels of total phenolic content total flavonoid content and antioxidant activity suggest the possible existence of bioactive compounds with beneficial properties. In this regard 'Gavramak' and more broadly the Hyrcanian region exhibit significant potential for developing red-fleshed varieties rich in bioactive chemicals.

The variations in morphological and biochemical traits within different populations of>M. sieversii directly impact breeding efforts to improve desirable

Table 6 Eigenvalues of principal component axes from principal component analysis (PCA) of morphological characters in the studied accessions of red-fleshed apple. *$P < 0.05$, **$P < 0.01$

Character	Principal component				
	Dim. 1	Dim. 2	Dim. 3	Dim. 4	Dim. 5
FfW	**0.94****	0.24	-0.09	-0.16	0.01
Fdw	**0.94****	0.04	-0.16	-0.08	0.05
Fdm	-0.08	-0.67	-0.27	0.26	0.03
Frw/Drw	0.10	**0.63****	0.25	-0.29	-0.06
Frl	**0.91****	0.16	-0.05	-0.12	0.17
Frd	**0.95****	0.04	-0.07	-0.12	-0.02
Frl/Frd	-0.44	0.15	0.08	0.04	0.24
Ths	0.36	-0.48	0.23	0.13	-0.16
Tat	0.28	-0.22	-0.09	-0.16	0.24
Tal	0.00	**0.64****	0.07	0.28	0.24
Deb	**0.50****	0.04	0.01	-0.24	-0.09
Web	**0.56****	-0.08	-0.11	-0.14	0.13
Wsc	0.29	0.09	-0.13	0.16	-0.30
Dsc	**0.58****	0.11	0.00	-0.36	-0.12
Lec	0.47	0.40	-0.22	0.13	0.47
Dic	**0.86****	0.35	-0.04	-0.19	-0.12
Nsf	-0.22	**0.76****	0.22	-0.10	-0.21
Fpw	**0.91****	0.25	-0.12	-0.12	0.09
Dpw	**0.80****	-0.09	0.03	0.05	-0.08
Ffw	**0.94****	0.22	-0.08	-0.16	-0.01
Dfw	**0.86****	-0.14	-0.21	-0.10	0.003
Cwt	**0.89****	0.16	-0.11	-0.14	0.06
Flt	**0.81****	-0.02	-0.17	-0.15	-0.10
Frs	0.44	0.27	0.004	-0.01	0.35
Flc	-0.43	-0.51	-0.22	-0.11	0.07
Cfp	-0.18	-0.25	-0.03	-0.15	-0.23
Rif	-0.30	-0.02	0.08	-0.29	0.29
Frt	**0.64****	0.11	0.13	-0.11	-0.24
Ffi	-0.48	-0.38	0.03	-0.10	-0.05
Fra	0.43	0.01	0.04	-0.06	-0.45
Frf	**0.77****	-0.36	0.04	0.08	-0.15
Flf	**0.71****	-0.34	-0.03	0.12	-0.18
Lel	**0.52****	0.06	**0.62****	0.33	0.25
Lew	-0.06	-0.04	**0.87****	-0.20	0.04
Lel/Lew	**0.51****	0.13	-0.26	**0.52****	0.21
Let	-0.22	-0.29	0.18	0.18	-0.36
Pet	0.01	-0.49	**0.63****	0.11	-0.03
Lfw	0.21	0.16	**0.92****	-0.01	0.10
DwL	0.35	0.002	**0.86****	0.14	0.07
Ldm	0.34	-0.42	-0.10	0.40	-0.05
Lea	0.41	-0.10	**0.68****	0.23	0.14
Pel	**0.62****	0.10	0.29	**0.61****	-0.06
Pel/Lel	0.38	0.07	-0.18	**0.60****	-0.32
LeC	-0.22	-0.41	0.13	-0.29	0.39
Les	0.11	0.22	-0.26	**0.65****	0.37
Las	0.03	0.09	-0.13	0.37	-0.18
Sew	-0.23	**0.66****	0.06	-0.04	-0.40
Sel	-0.50	**0.59****	-0.19	0.15	0.14
Sed	**0.62****	-0.40	-0.08	0.00	0.29
Sel/Sed	-0.70	**0.53****	-0.03	0.11	-0.07
Ses	-0.29	0.37	0.02	0.29	-0.24

Table 6 (continued)

Character	Principal component				
	Dim. 1	**Dim. 2**	**Dim. 3**	**Dim. 4**	**Dim. 5**
Sec	-0.30	-0.32	-0.06	-0.03	0.28
Trh	0.41	-0.70	0.01	0.04	-0.11
% of Variance	30.27	11.82	8.47	5.81	4.45
Cumulative %	30.27	42.09	50.56	56.38	60.83

Note: ** coordinations ≥ 0.05 are significant (which are indicated by bold letters)

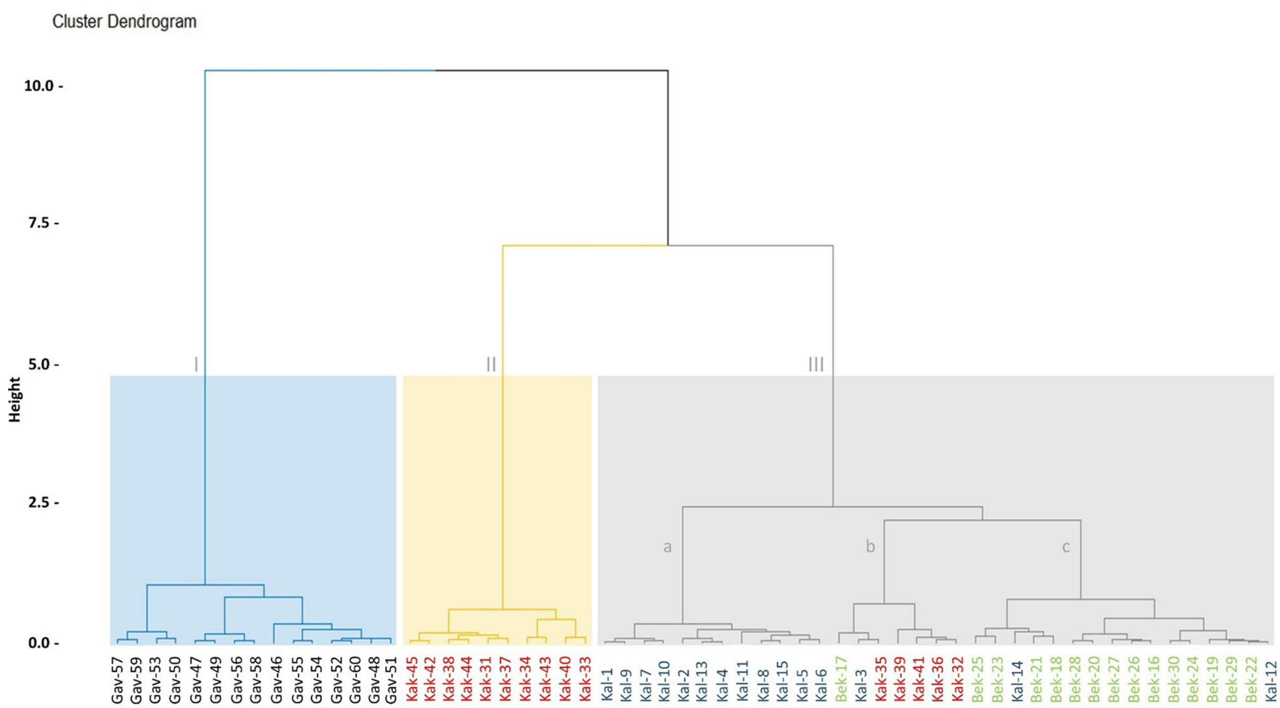

Fig. 3 Ward dendrogram of cluster analysis for the studied specimens of red-fleshed apples accessions based on morphological traits using Euclidean distances

characteristics. Identifying populations with unique biochemical profiles can help choose those with higher production of secondary metabolites often associated with therapeutic properties. Selective breeding of individuals with superior biochemical profiles can lead to new varieties with enhanced therapeutic qualities and higher yields. Additionally recognizing the morphological diversity can assist in selecting individuals who exhibit favorable growth patterns disease resistance and other essential qualities for cultivation.

Figure 3illustrates a dendrogram derived from all morphological variables utilizing Euclidean distance for accessions from four populations of red-fleshed apples ('Gavermak' 'Kalateh-Khij' 'Kalateh' and 'Bekran') demonstrating significant variance among the accessions. Cluster analysis identified three main clusters I IIand III which categorize apple groups exhibiting similar traits. Cluster I contained 15 accessions Cluster II included ten accessions and Cluster III was subdivided into three sub-clusters. Sub-cluster III-a contained 12 accessions Sub-cluster III-b included seven accessions and Sub-cluster III-c included 16 accessions. Gavermak's population is grouped in cluster (I) The Kalateh-Khij site was included in cluster (II) Finally, 'Kalateh' and 'Bekran' populations were grouped in Cluster III.

A scatter plot was created based on Dim1 and Dim2, representing 42.09% of the total variance. It indicated a relationship between morphological characteristics(Fig. 4). The accessions nearby were more similar regarding effective traits in PC1 and PC2 and were grouped together. A good correlation was observed between morphological variation and geographical distribution. The highest interregional similarity was observed between 'Bekran' and 'Kalateh' due to low geographical distance and similarity in climate conditions. Geographical distribution showed significant differences between the Mazandaran population and Semnan province due to the large geographical distance and varying climate conditions (humid compared to arid and semi-arid).

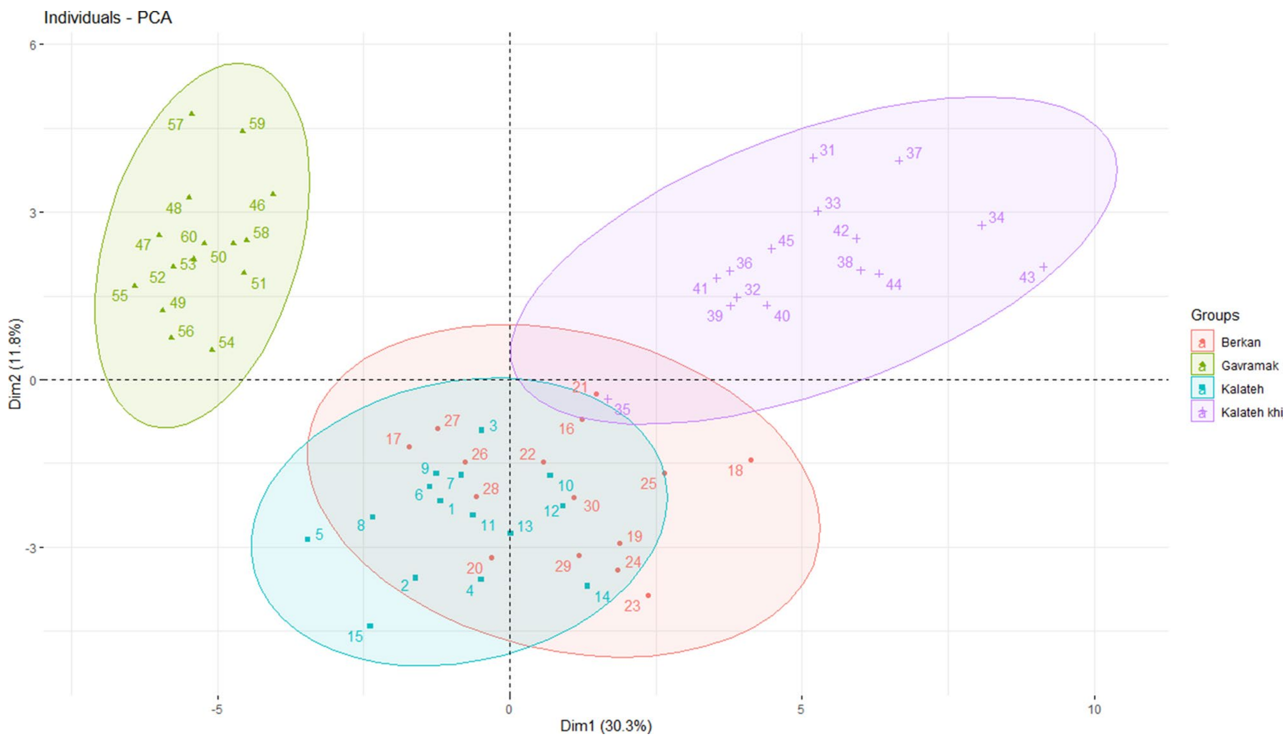

Fig. 4 Individual plots for 45 accession in study sites

These results were consistent with the results of Ward's dendrogram.

Biplot analysis of red-fleshed apples

The biplot of the examined accessions, based on PC1 and PC2, classified them into four groups, potentially correlated with their geographical proximity. Consequently, certain populations from neighboring habitats were positioned near together on the biplot, perhaps due to similar environmental conditions and gene flow between these groups. The Biplot results supported the results of the cluster analysis. The first group consisted of two 'Kalateh' and 'Bekran' correlated to Flc, Ffi, Sec, Fdm, Trh, Ths, Pet, Let, Lec.1, Frf, and Flf. The second group includes accessions collected from the 'Kalateh-Khij,' relating to Dic, Fpw, FfW, Frl, Gwt, and Lec, while the last group consists of accessions collected from the Ghavarmak population, correlated with Sed, Sel, Sew, Nsf, Fri, and Frd (Fig. 5).

The Gavramak population had the most significant values for the Simpson, Shannon, Margalef, and Menhinick indices. However, the highest values of the McIntosh index were recorded for both the Gavramak and Kalateh Khij populations (Table 7). The examined accessions showed considerable variation for the majority of the observed parameters. The observed phenotypic variability in the current study, which included 53 accessions from various natural regions, exceeded previous reports on red-fleshed apples based on morphological traits, such as those by Damyar et al. [26], Faramarzi et al. [41], Faramarzi et al. [27], and Abedi et al. [5], which documented 26, 17, 6, and 3 accessions of red-fleshed apples based on morphological variables, respectively. Consequently, the increased morphological variation observed in this study may be attributed to a larger sample size, diverse natural environments, and gene flow [54].

Climate change and management factors emphasize the preservation of red-fleshed apples. Drylands have significant trait diversity, demonstrating extensive plant adaptations to arid and grazed ecosystems [56]. There is currently no data regarding the impact of climate change on wild red-fleshed apples. Due to climate change scenarios, *Malus orientalis*, a wild apple species in Western Asia, would experience habitat loss in warmer low-altitude regions. Furthermore, Hyrcanian's western and central regions will constitute a vital area of the species distribution, characterized by appropriate habitats and significant connections among *M. orientalis* populations. They may serve as significant refugia for wild apples [57]. Consequently, red-fleshed apples in Semnan, specifically in 'Bekran' and 'Kalateh,' situated in arid regions, are predicted to decline suitable habitats markedly due to future climate change. Habitats located at high altitudes and in the Hyrcanian region provide significant potential for breeding projects in response to climate change. Moreover, increasing human activities and habitat management practices, including agriculture, grazing, and deforestation, result in the deterioration and fragmentation of natural ecosystems. Habitat

Fig. 5 Two-dimensional bi-plot for Dim1/Dim2 (42.1% of total variance) among the studied accessions of red-fleshed apples

Table 7 Diversity indices of wild-growing red-fleshed apples populations from in the north and northeast of Iran

Population	Simpson	Shannon	Margalef	Menhinick	McIntosh
Kalateh	1.47	1.02	6.57	1.01	0.17
Berkan	1.46	1.005	6.49	0.97	0.17
Kalateh-Khij	1.52	1.07	6.46	0.95	0.19
Gavramak	1.55	1.10	6.72	1.11	0.19
Min	1.46	1.005	6.46	0.95	0.17
Max	1.55	1.10	6.72	1.11	0.19

loss and fragmentation can adversely affect plant species' population size and genetic diversity, including *M. sieversii*, particularly in environments with higher stress levels [58]. Wild fruit trees are a vital element of crop wild relatives. Nonetheless, the in situ conservation of wild fruit trees has been overlooked [59]. Wild edible plants, including red-fleshed apples, have socioeconomic values [60]. Therefore, conservation plans are essential due to the limited habitats of wild red-fleshed apples, including a few existing individuals.

Evaluating the morphological and biochemical variation of *M. sieversii* can yield significant information into the present condition of its populations and their prospective resilience to habitat degradation. Populations of *M. sieversii* may have variations in growth patterns, disease resistance, secondary metabolite synthesis, and other significant characteristics. Examining these variances allows us to find superior genotypes for cultivation and breeding objectives. This may result in superior cultivars with augmented medicinal attributes, increased yields, and enhanced resilience to environmental stresses [24]. Evaluating the morphological and biochemical diversity of *M. sieversii* establishes a basis for focused breeding initiatives to satisfy the increasing demand for this medicinal plant. Cluster analysis utilized all morphological data and included the complete variability. The scatter plot was generated using the cumulative variance of PC1 and PC2, which was notable at 42.09%. The chosen accessions exhibiting fruit color, increased weight, texture, and flavor are suitable for future breeding programs. The findings of the current investigation Research indicated that wild red-fleshed apples (*M. sieversii* f. niedzwetzkyana) in northern and northeastern Iran may be advantageous for identifying candidate genotypes with superior growth performance and increased yield.

Conclusions

The current study demonstrated significant variety in Iran's morphological and biochemical properties of naturally occurring red-fleshed apple accessions. The accessions obtained from the Kalateh Khij location had the most significant values for the assessed morphological attributes of leaves and fruits. The Gavramak population exhibited more significant levels of total phenolic content, total flavonoid content, and antioxidant activity, applicable in multiple industries such as pharmaceuticals, cosmetics, and food. This species is a significant source of natural antioxidants, with substantial potential to be developed as a functional food. Moreover, these findings can be employed to develop and introduce novel red-fleshed apple varieties. Traits include fruit weight, firmness, juiciness, flavor, and color, which are essential factors to consider in breeding efforts for red-fleshed apples. In red-fleshed apple breeding projects, desirable features include fruit weight, firmness, juiciness, flavor, and color quality. The current findings indicate that the most suitable places for cultivating *M. sieversii*, producing substantial phenolic and flavonoid concentrations, are Kalateh Khij and the Hyrcanian areas. The current findings may provide a foundation for conserving the genetic resources of red-fleshed apples. It is advisable to utilize the optimal accessions identified in breeding operations. The preservation of the diversified population of Iranian wild red-fleshed apples is advised.

Abbreviations

ABTS: 2	azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt
CV	Coefficient of Variation
TPC	Total Phenol Content
TFC	Total Flavonoid Content
TAC	Total Anthocyanin Content
DPPH	2,2-diphenyl-1-picrylhydrazyl
FW	Fresh weight
PCA	Principal component analysis
SD	Standard deviation
SE	Standard Error

Acknowledgements
We thank Dr Bahram Naseri and Caspian forest seed center for their cooperation in populations selections and Tarbiat Modares University for the support.

Author contributions
MJ performed the experiments and collected data, MT guided biochemical analysis, DAM, MA conceived of the research idea. MA guided all aspects of the research project, analyzed data and wrote the manuscript. All authors discussed the results, commented on the manuscript and approved the final manuscript.

Funding
Not applicable.

Data availability
The data were used in current study, when reasonable request, are available from the corresponding author.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Informed consent
Not applicable.

Statement specifying permissions
For this study, we acquired permission to collect the red-fleshes apple issued by the Agricultural and Natural Resources Ministry of Iran.

Statement on experimental research and field studies on plants
All methods performed on plants, including the collection of plant material, comply with relevant institutional, national, and international guidelines and domestic legislation of Iran.

Conflict of interest
The authors declare no competing interests.

Received: 23 June 2024 / Accepted: 19 September 2024
Published online: 30 September 2024

References

1. Phipps JB, Robertson KR, Rohrer JR, Smith PG. Origins and evolution of subfam. Maloideae (Rosaceae). Syst Bot. 1991;1:303–32.
2. Forsline PL, Aldwinckle HS, Dickson EE, Luby JJ, Hokanson SC. Collection, maintenance, characterization, and utilization of wild apples of Central Asia. Hortic Rev (Am Soc Hortic Sic). 2003;29:1–62.
3. Faramarzi S, Yadollahi A, Soltani BM. Preliminary evaluation of genetic diversity among Iranian red fleshed apples using microsatellite markers. J Agric Sci Technol. 2014;16:373–84.
4. Wang N, Jiang S, Zhang Z, Fang H, Xu H, Wang Y, et al. *Malus sieversii*: the origin, flavonoid synthesis mechanism, and breeding of red-skinned and red-fleshed apples. Hortic Res. 2018;5:70.
5. Abedi B, Parvaneh T, Ardakani E. Evaluation of physical properties of fruit, secondary metabolites, and browning index of Bekran red flesh apple genotype and some spring apple cultivars. J Hortic Sci. 2019;33(4):609–22.
6. Van Nocker S, Berry G, Najdowski J, Michelutti R, Luffman M, Forsline P, Alsmairat N, Beaudry R, Nair MG, Ordidge M. Genetic diversity of red-fleshed apples (*Malus*). Euphytica. 2012;185(2):281–93.
7. Zhao P, Yang Y, Wang X, Guo Y. Evolution of typical aromas and phenolic compounds of a red-fleshed apple throughout different fruit developmental periods in Xinjiang, China. Food Res Int. 2021;148:110635.
8. Faramarzi S, Pacifico S, Yadollahi A, Lettieri A, Nocera P, Piccolella S. Red-fleshed apples: old autochthonous fruits as a novel source of anthocyanin antioxidants. Plant Foods Hum Nutr. 2015;70(3):324–30.
9. Sato H, Otagaki S, Saelai P, Kondo S, Shiratake K, Matsumoto S. Varietal differences in phenolic compounds metabolism of type 2 red-fleshed apples. Sci Hortic. 2017;219:1–9.
10. Bars-Cortina D, Macia A, Iglesias I, Romero MP, Motilva MJ. Phytochemical profiles of new red-fleshed apple varieties compared with traditional and new white-fleshed varieties. J Agric Food chem. 2017;65(8):1684–96.
11. Li CX, Lin ZX, Zhao XH, Zuo WF, Wang N, Zhang ZY, et al. Differential effects of phenolic extracts from red-fleshed apple peels and flesh induced G1 cell cycle arrest and apoptosis in human breast cancer MDA-MB-231 cells. J Food Sci. 2021;86(9):4209–22.
12. Espley RV, Bovy A, Bava C, Jaeger SR, Tomes S, Norling C et al. (2013). Analysis of genetically modified red-fleshed apples reveals effects on growth and consumer attributes. J Plant Biotechnol. 2013; 11(4):408–419.
13. Zhang X, Xu J, Xu Z, Sun X, Zhu J, Zhang Y. Analysis of antioxidant activity and flavonoids metabolites in peel and flesh of red-fleshed apple varieties. Molecules. 2020;25(8):1968.
14. Juhart J, Medic A, Veberic R, Hudina M, Jakopic J, Stampar F. Phytochemical composition of red-fleshed apple cultivar 'Baya Marisa'compared to traditional, white-fleshed apple cultivar 'Golden Delicious'. Hortic. 2022;8(9):811.
15. Contessa C, Botta R. Comparison of physicochemical traits of red-fleshed, commercial and ancient apple cultivars. Hortic Sci. 2016;43(4):159–66.
16. Wojdyło A, Lech K, Nowicka P. Effects of different drying methods on the retention of bioactive compounds, on-line antioxidant capacity and color of the novel snack from red-fleshed apples. Molecules. 202;25(23):55–21.
17. Erbil N, Arslan M, Murathan ZT, Ilcim A, Sayin Borekci B. Some biological effects of the fruits and leaves of different apple cultivars, including red-fleshed apples, grown in a microclimatic region of Turkey: part I. Erwerbs-Obstbau. 2020;62(4):399–410.
18. Li CX, Zhao XH, Zuo WF, Zhang TL, Zhang ZY, Chen X. Phytochemical profiles, antioxidant, and antiproliferative activities of four red-fleshed apple varieties in China. J Food Sci. 2020;85(3):718–26.
19. Zanetti M, Samoggia A, Young J. Fruit sector strategic management: an exploration of agro-food chain actors' perception of market sustainability of apple innovation. Sustainability. 2020;12(16):6542.

20. Zhang X, Huo H, Sun X, Zhu J, Dai H, Zhang Y. Nanocrystallization of anthocyanin extract from red-fleshed apple' QN-5' improved its antioxidant effect through enhanced stability and activity under stressful conditions. Molecules. 2019;24(7):1421.
21. Wang M, Zhang J, Guo Z, Guan Y, Qu G, Liu J, Yan X. Morphological variation in *Cynodon dactylon* (L.) Pers., and its relationship with the environment along a longitudinal gradient. Hereditas. 2020;157:1–11.
22. Achigan-Dako EG, Avohou ES, Linsoussi C, Ahanchede A, Vodouhe RS, Blattner FR. Phenetic characterization of *Citrullus* spp. (Cucurbitaceae) and differentiation of egusi-type (*C. Mucosospermus*). Genet Resour Crop Evol. 2015;62:1159–79.
23. Zare A, Khorshidi J, Vafaee Y. Natural biochemical and morphological diversity of oleaster (*Elaeagnus angustifolia* L.) genotypes from the west of Iran: applicable for conservation, domestication, and breeding. Genet Resour Crop Evol. 2023;70(8):2575–92.
24. Khadivi A, Mirheidari F, Moradi Y, Paryan S. *Malus orientalis* Uglitzk., an important genetic resource to improve domestic apcharacterizationzation and selection of the promising accessions. Euphytica. 2020;216:1–21.
25. Moradi Y, Khadivi A, Mirheidari F. Multivariate analysis of oriental apple (*Malus orientalis* Uglitzk.) Based on phenotypic and pomological characterizations. Food Sci Nutr. 2022;10:2532–41.
26. Damyar S, Hassani D, Parvaneh T. Evaluation of some characteristics of native red-fleshed apple genotypes of Iran. Seed Plant J. 2013;29(3):483–501.
27. Faramarzi S, Yadollahi A, Barzegar M, Karimzadeh G. Comparison of the important quality characteristics of red flesh apples and some Golab cultivars. Crop Improv. 2015;17(3):729–41.
28. Watkins R, Smith RA. Descriptors List for Apple *(Malus)*; International Board for Plant Genetic Resources (IBPGR); Commission of the European Communities (CEC): Wageningen. The Netherlands. 1982.
29. UPOV. Apple (*Malus domestica Borkh*). Guidelines for the Conduct of tests for distinctness, Uniformity and Stability; International Union for the Protection of New Varieties of Plants (UPOV). Geneva, Switzerland; 2005. p. 25.
30. Gironés-Vilaplana A, Baenas N, Villaño D, Speisky H, García-Viguera C, Moreno DA. Evaluation of latin-american fruits rich in phytochemicals with biological effects. J Funct Foods. 2014;7:599–608.
31. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In Methods in enzymology. Elsevier;1999: Vol. 299, pp. 152–78.
32. Akkol EK, Göger F, Koşar M, Başer KHC. Phenolic composition and biological activities of *Salvia halophila* and *Salvia virgata* from Turkey. Food Chem. 2008;108(3):942–9.
33. Rodriguez-Saona LE, Wrolstad RE. Extraction, isolation, and purification of anthocyanins. Curr Protoc Food Anal Chem. 2001;(1):F1–1.
34. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Tech. 1995;28(1):25–30.
35. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Bio Med. 1999;26(9–10):1231–7.
36. Lê S, Josse J, Husson F. FactoMineR: an R package for multivariate analysis. J Stat Softw. 2008;25:1–18.
37. Shiqie B. Ecological characteristics and morphological variations of centipedegrass in different populations. J Beijing for Univ. 2002;24(4):97–101.
38. Sedláčková VH, Hulin M, Vinogradova Y, Goncharovska I, Brindza J. Comparison of old and local apple varieties and seedlings (*Malus domestica* Borkh.) in the variability of some morphological characters of fruits and seeds. Agrobiodiversity for Improving Nutrition, Health and Life Quality. 2021;5(1).
39. Drkenda P, Ćulah A, Spaho N, Akagić A, Hudina M. How do consumers perceive sensory attributes of apple? Foods. 2021;10(11):2667.
40. Chagné D, Carlisle CM, Blond C, Volz RK, Whitworth CJ, Oraguzie NC, et al. Mapping a candidate gene (MdMYB10) for red flesh and foliage colour in apple. BMC Genom. 2007;8(1):1–11.
41. Faramarzi S, Yadollahi A, Hajnajari H, Shojaeean A, Damyar S. Study of morphological characteristics of Iranian red-fleshed apples vs. some Iranian landraces and commercial cultivars. Crops Improv. 2014;16(1):1–10.
42. Parvaneh T, Abedi B, Davarynejad GH, Moghadam EH. Enzyme activity, phenolic and flavonoid compounds in leaves of Iranian red flesh apple cultivars grown on different rootstocks. Sci Hortic. 2019;264:862–70.
43. Luitel BP, Ro NY, Ko HC, Sung JS, Rhee JH, Hur OS. Phenotypic variation in a germplasm collection of pepper (*Capsicum chinense* Jacq.) From Korea. J Crop Sci Biotechnol. 2018;21:499–506.
43. Katiyo W, Yang R, Zhao W. Phenolic composition and antioxidant activity of Chinese red-fleshed apples (*Malus pumila* Niedzwetzkyana (Dieck) and effect of different pasteurization treatments on the cloudy juice. Int Food Res J. 2018;25(5):2185–94.
44. Delgado AM, Issaoui M, Chammem N. Analysis of main and healthy phenolic compounds in foods. JAOAC Int. 2019;102(5):1356–64.
45. Wang X, Li C, Liang D, Zou Y, Li P, Ma F. Phenolic compounds and antioxidant activity in red-fleshed apples. J Funct Foods. 2015;18:1086–94.
46. Maleki SJ, Crespo JF, Cabanillas B. Anti-inflammatory effects of flavonoids. Food Chem. 2019;299:125–124.
47. Zhang Y, Zhu J. Morphological characteristics and pollination compatibility of a new red flesh apple. Hongxun 1 Res Crop. 2013;14(1):199–204.
48. Ceci AT, Bassi M, Guerra W, Oberhuber M, Robatscher P, Mattivi F, et al. Metabolomic characterization of commercial, old, and red-fleshed apple varieties. Metabolites. 2021;11(6):378.
49. Yang C, Sun N, Qin X, Liu Y, Sui M, Zhang Y, et al. Analysis of flavonoid metabolism of compounds in succulent fruits and leaves of three different colors of Rosaceae. Sci Rep. 2024;14(1):49–33.
50. Keller-Przybylkowicz S, Oskiera M, Liu X, Song L, Zhao L, Du X, et al. Transcriptome analysis of white-and red-fleshed apple fruits uncovered novel genes related to the regulation of anthocyanin biosynthesis. Int J Mol Sci. 2024;25(3):1778.
51. Rupasinghe HPV, Huber GM, Embree C, Forsline PL. Red-fleshed apple as a source for functional beverages. Can J Plant Sci. 2010;90(1):95–100.
52. Angeli L, Populin F, Morozova K, Ding Y, Asma U, Bolchini S et al. Comparative analysis of antioxidant activity and capacity in apple varieties: insights from stopped flow DPPH• kinetics, mass spectrometry and electrochemistry. Food Biosci. 2024;103729.
53. Sharifani MM, Jabari M, Yamchi A, Darzi Ramandi H. Genetic differences and heritability using morphological and phytochemical traits of some Iranian apples. J Plant Prod Res. 2020;27(3):131–47.
54. Sefc KM, Lopes MS, Lefort F, Botta R, Roubelakis-Angelakis KA, Ibanez J, et al. Microsatellite variability in grapevine cultivars from different European regions and evaluation of assignment testing to assess the geographic origin of cultivars. Theor Appl Genet. 2000;100:498–505.
55. Luo WW, Gao CX, Zhang D, et al. Effects of environmental factors at different altitudes on leaves and fruit quality of Fuji apple. Ying Yong Sheng Tai Xue Bao. 2014;25(8):2243–50.
56. Gross N, Maestre FT, Liancourt P, et al. Unforeseen plant phenotypic diversity in a dry and grazed world. Nature. 2024;632(8026):808–14.
57. Walas Ł, Alipour S, Haq SM, et al. The potential range of west Asian apple species *Malus orientalis* Uglitzk. Under climate change. BMC Plant Biol. 2024;24(1):381.
58. Ha YH, Oh SH, Lee SR. Genetic admixture in the Population of Wild Apple (*Malus Sieversii*) from the Tien Shan Mountains, Kazakhstan. Genes (Basel). 2021;12(1):104.
59. Tian Zhongping H, Song et al. 2022. Wild Apples Are Not That Wild: Conservation Status and Potential Threats of *Malus sieversii* in the Mountains of Central Asia Biodiversity Hotspot *Diversity* 14, no. 6: 489.
60. Jalali M, Abedi M, Memariani F, Ghorbani AB. Ethnobotanical study of wild edible plants in the mountainous regions of Semnan Province, Iran. J Ethnobiol Ethnomed. 2024.
61. Kolde R, Kolde MR. Package pheatmap R package. 2015; 1:790.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.