RESEARCH

Investigate the Genetic Relationships and Morphological Diversity of Some Afghan and Iranian Melon Cultivars for Breeding Purposes

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Abstract

To investigate the genetic diversity of 40 melon and cantaloupe cultivars, which were collected from the central regions of Iran and southwestern Afghanistan, inter-simple sequence repeat (ISSR) primers and several morphological traits were used. The results demonstrate that 12 selected ISSR markers generated polymorphic bands with a distinct band pattern. There were a total of 150 bands created, with 95 bands exhibiting polymorphism (62.44% diversity). Based on shape and characteristics, cluster analysis and principal coordinate analysis (PCoA) classified the cultivars into five groups: winter and late ripening, medium ripening, sweet, and early melons, including cantaloupe and melon. In the majority of instances, PCoA was consistent with cluster analysis, whereas the molecular data did not perfectly match the morphological results. The results of the analysis classified the morphological characteristics of individuals into five groups: the first group comprised nine Iranian and Afghan melon genotypes with long, thin-skinned forms and intermediate medium ripening, and the second group comprised five Afghan melon genotypes with late ripening, winter ripe, and thick skin and flesh. The third group of 11 Iranian and Afghan melon cultivars was typically medium-sized, elliptical-shaped melons with thin skin. The fourth group consists of two Iranian melons with small seeds, small fruit, rapid maturation, and no flavor. The final group possessed dense and tender flesh, a spherical shape, and large seeds. In conclusion, morphological traits and ISSR analysis could be useful tools for classifying melon germplasm for future breeding applications. The morphological and molecular similarities between Afghan and Iranian cultivars suggest their origin.

Keywords ISSR · Genetic diversity · Melon · Morphology

Introduction

Melon (*Cucumis melo* L.) is an annual outcrossing plant belonging to the Cucurbitaceae family (Chikh-Rouhou et al. 2021), which yields a variety of nutritious, flavorful, and colorful crops. In recent decades, advances in agricultural technologies, such as breeding and hybridization between varieties, have increased to meet the increasing demand for nutrient-dense and diverse vegetables. Melons originated in Central Asia, beyond the Caucasus, Iran, Afghanistan, India, Turkmenistan, Tajikistan, and Uzbekistan (Renner et al. 2007). The selection of high-yielding and disease-resistant varieties based on the genetic diversity of populations is one of the most important topics studied by breeders (Gepts 2006). On the other hand, environmental challenges threaten the preservation of landraces, particularly the conservation of wild and endemic varieties in third-world nations with a high potential for landrace genotype.

Previously, genetic diversity was evaluated primarily based on phenological and morphological traits, whereas DNA molecular markers are a powerful tool for genetic diversity evaluation (Ren and Timko 2001). ISSR (inter-simple sequence repeat) markers are widely used by scientists to analyze genetic diversity. ISSR is a random marker with high reproducibility and polymorphism that is utilized in a variety of plant species. This marker has efficient characteristics, including simultaneous analysis of multiple gene loci, high precision, high diversity, low cost, and rapid and simple implementation (Reddy et al. 2002). Landraces play a crucial role in the evolution of melons, as detailed in Indian, Israeli, Greek, American, and Chinese

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sources (Singh et al. 2020). Iran is one of the global centers of melons' diversity and domestication, with five distinct and well-known types, including melon, cantaloupe, honeydew melon, and Armenian cucumber (Zamyad et al. 2004). High genetic diversity is described at the molecular and morphological levels among 30 cultivars of melon species (Salehi Najafabadi et al. 2010). Using ISSR markers, Dhillon et al. (2009) examined the genetic diversity of 54 melons from India and found a high rate of diversity. These researchers demonstrated that these markers are effective for fingerprinting and distinguishing melon accessions. Comparable research uncovered a great deal of variation among the 30 melon stands indigenous to Khorasan (northeast Iran) (Kouhpayagani and Behbahani 2008). In another study of 32 Iranian melon accessions with 15 microsatellite markers, a high level of genetic diversity and average heterozygosity of 0.51 were observed (Soltani et al. 2010). Using SSR markers on 50 melon accessions planted in different regions of India, a second study demonstrated a high level of genetic diversity in the accessions and reported a mean heterozygosity of 0.23 for the accessions (Chikh-Rouhou et al. 2021). Using 20 ISSR primers, Rahmanpour et al. (2014) assessed the genetic diversity of 11 native accessions and 12 melon hybrid cultivars. The results indicated that ISSR markers are exceptionally useful for analyzing genetic diversity and melon cultivars.

Identifying, preserving, and maintaining the desired genes of compatible populations and preventing the genetic erosion of melon populations are advantageous due to their adaptability to a particular region over thousands of years. There is no research regarding the evaluation and comparison of Iranian and Afghan cultivars, and there is no information about Afghan cultivars, according to the available literature. This study employed a set of morphophysiological traits and ISSR markers to determine the genetic relationship between Afghan and Iranian melon cultivars belonging to different groups of melons and to compare their characteristics, thereby determining the degree of genetic diversity between and within populations of melons in the Afghan and Iranian populations.

Materials and Methods

Field Experiment

In 2019, 14 cultivars from central Iran and 26 cultivars from the southwest of Afghanistan, as described in Table 1 (Fig. 1), were collected. The collected seeds were sown in a greenhouse, and the seedlings were transplanted to the Isfahan University of Technology Research Farm, Iran, using a completely randomized block design with three replications. In each replication, three plants of each cultivar were planted.

The following morphophysiological characteristics were evaluated and described using descriptors: The European Cooperative Programme for Plant Genetic Resources (ECPGR), (Díez et al. 2008): leaf length and width (cm), fruit length and width (cm), inside fruit length and width (cm), fruit weight (g), fruit skin thickness (mm), flesh fruit thickness (mm), tail length and diameter (mm), fruit dry weight (kg), fruit tissue firmness (kN), groove width, length and width of seed cavity (cm), 1000-seed weight (g), fruit color before and after ripening, fruit shape, fruit tail changes after ripening, skin pattern surface of the fruit, total soluble solids (TSS) (%) and pH of fruit juice, taste quality, and the number of fruit per plant.

Furthermore, a variety of melons were observed for the timing of fruit harvesting, germination, the two-leaf stage, and the appearance of the first male and female flowers (days). The diameter, width, and length were measured with a ruler. The fruit's weight was measured using a digital scale. A penetrometer (OSK-I-10576) was used to gauge the fruit's hardness. A digital refractometer was used to calculate fruit TSS. The colors of flesh and skin were noted using the Royal Horticultural Society Color Chart. Cultivars were categorized into smooth, wrinkled, and wavy groups based on the fruit index's skin pattern surface, as per the descriptor ECPGR (Díez et al. 2008). The cultivars were categorized as aromatic sweet, sweet, medium sweet, and non-aromatic sweet based on the fruit's flesh flavor quality index (Stepansky et al. 1999). Furthermore, according to the fruit's flesh color index, cultivars were divided into eight categories: green, yellow, pink, white, orange, green, and white ECPGR (Díez et al. 2008).

DNA Extraction and PCR Amplification

Total genomic DNA was extracted from the young leaves of 40 cultivars using a modified Murray and Thompson's (1980) method containing 2% cetyltrimethylammonium bromide (CTAB). The genomic DNA was extracted from young leaves samples. One hundred and fifty milligrams of the leaf sample mixed with pre-heated (65 °C) CTAB buffer (100 mM Tris pH 8.0, 1.4 M NaCl, 20 mM EDTA, 2.0% CTAB, 0.5% sodium bisulfate, and 1.0% mercaptoethanol). The tubes were incubated at 65 °C for 1 h in the water bath. Further, chloroform/iso-amyl alcohol (24:1 v/v) was added, and the contents were mixed with a shaker for 5 min. Contents were centrifuged (10 min, 13,000 rpm). The supernatant was transferred in a new centrifuge tube and 2/3rd volume of chilled iso-propanol was added, stored at −20 °C for 2 h and centrifuged (at 13,000 rpm for 10 min) to precipitate nucleic acid. The pellet of genomic DNA was washed with ethanol (70% v/v) twice, centrifuged (13,000 rpm, 3 min), and dissolved in deionized water. The extracted DNA's quality was determined using agarose gel electrophoresis (0.7%) and ethidium bromide staining.

To analyze the samples, 12 different ISSR primers (Table 2) were used. Metabion Company synthesized the

Table 1 Studied samples and their collection places

Accession number	Native name	Species	Place of collection	Latitude
1	Naki	*Cucumis melo* L.	Afghanistan-Badghis	35° 63′ N
2	Ghatori	*Cucumis melo* L.	Afghanistan-Badghis	35° 63′ N
3	Potk-e Ablagh	*Cucumis melo* L.	Afghanistan-Badghis	35° 63′ N
4	Barg-e Ney	*Cucumis melo* L.	Afghanistan-Badghis	35° 63′ N
5	Ghandak-e Zard	*Cucumis melo* L.	Afghanistan-Badghis	35° 63′ N
6	Murchaghi	*Cucumis melo* L.	Afghanistan-Badghis	35° 63′ N
7	Saderati	*Cucumis melo* L.	Iran-Isfahan	32° 51′ N
8	Bandi Pirzali	*Cucumis melo* L.	Afghanistan-Badghis	35° 63′ N
9	Zanki	*Cucumis melo* L.	Afghanistan-Badghis	35° 63′ N
10	Kale gorgi doroneh	*Cucumis melo* L.	Afghanistan-Badghis	35° 63′ N
11	Habibabadi cantaloupe	*Cucumis melo* L.	Iran-Isfahan	32° 51′ N
12	Zardak	*Cucumis melo* L.	Afghanistan-Badghis	35° 63′ N
13	Gorgi from Shirdan	*Cucumis melo* L.	Iran-Isfahan	32° 51′ N
14	Isfahan melon	*Cucumis melo* L.	Iran-Isfahan	32° 51′ N
15	Nazokch-e Nasvari	*Cucumis melo* L.	Afghanistan-Badghis	35° 63′ N
16	Kadouei	*Cucumis melo* L.	Afghanistan-Badghis	35° 63′ N
17	Garmeh Afghanistan	*Cucumis melo* L.	Afghanistan-Badghis	35° 63′ N
18	Torkamani	*Cucumis melo* L.	Afghanistan-Badghis	35° 63′ N
19	Ghandak Tanabi-e Sefid	*Cucumis melo* L.	Afghanistan-Badghis	35° 63′ N
20	Gorgi in Ivan	*Cucumis melo* L.	Iran-Isfahan	32° 51′ N
21	Gorgab	*Cucumis melo* L.	Iran-Isfahan	32° 51′ N
22	Shahabadi melon	*Cucumis melo* L.	Iran-Isfahan	32° 51′ N
23	Bandi Bobak	*Cucumis melo* L.	Afghanistan-Badghis	35° 63′ N
24	Naki Johari	*Cucumis melo* L.	Afghanistan-Badghis	35° 63′ N
25	Chini	*Cucumis melo* L.	Afghanistan-Badghis	35° 63′ N
26	Mashhadi	*Cucumis melo* L.	Iran-Isfahan	32° 51′ N
27	Varamini honeydew melon	*Cucumis melo* L.	Iran-Isfahan	32° 51′ N
28	Darounak melon	*Cucumis melo* L.	Afghanistan-Badghis	35° 63′ N
29	Golzardak	*Cucumis melo* L.	Afghanistan-Badghis	35° 63′ N
30	Saveh honeydew melon	*Cucumis melo* L.	Iran-Isfahan	32° 51′ N
31	Abasi	*Cucumis melo* L.	Iran-Isfahan	32° 51′ N
32	Max Tanbal honeydew melon	*Cucumis melo* L.	Iran-Isfahan	32° 51′ N
33	Gazgi	*Cucumis melo* L.	Iran-Isfahan	32° 51′ N
34	Potk-e Johari	*Cucumis melo* L.	Afghanistan-Badghis	35° 63′ N
35	Hachkeh Johari	*Cucumis melo* L.	Afghanistan-Badghis	35° 63′ N
36	Kale Gorgi	*Cucumis melo* L.	Afghanistan-Badghis	35° 63′ N
37	Tanabi-e Bandi	*Cucumis melo* L.	Afghanistan-Badghis	35° 63′ N
38	Hachkeh Darouneh	*Cucumis melo* L.	Afghanistan-Badghis	35° 63′ N
39	Bandi Siah	*Cucumis melo* L.	Afghanistan-Badghis	35° 63′ N
40	Eivanaki zard	*Cucumis melo* L.	Iran-Isfahan	32° 51′ N

primers (Germany). A 15 µl reaction mixture was used for DNA amplification. The PCR mixture contained 2 µl of genomic DNA, 7.5 µl of Ampliqon's Master Mix Red (Finland), 2 µl of each primer, and distilled water to the final volume. The PCR amplification program included 2 min of initial denaturation at 94 °C, 40 cycles of 1 min at 94 °C, 1 min of annealing at 50–57 °C, 2 min of elongation at 72 °C, and a final extension 10-min stage at 72 °C. DNA fragments were separated on agarose gel (1.5% w/v) electrophoresis and stained with ethidium bromide for 2 h at 90 V.

Fig. 1 Geographical distribution of studied melon cultivars

Data Analysis

The obtained morphological data were subjected to ANOVA using a completely randomized block design and the SAS v9 software (SAS Institute 2002). The least significant difference (LSD, $p \leq 0.05$) test was used to compare means. For all traits measured by SAS software and using Euclidean distances, a dendrogram derived from morphological data was plotted. The STATGRAPHICS, ver.6, was used to draw a biplot diagram, principal component analysis (PCA), and cluster the cultivars for all morphological characteristics using the Ward method.

The presence (1) or absence (0) of the bands was scored for each ISSR marker. To determine genetic similarity, Jaccard's similarity coefficient was used, and cluster analysis was designed using NTSYS software's UPGMA algorithm method (V. 2.21). The polymorphic information content (PIC) was calculated as $\text{PIC} = 1 - \sum P2_{ij}$, where P_{ij} is the frequency of the jth allele for the ith marker. NTSYS software (V. 2.21) calculated the cophenetic correlation coefficient

Table 2 Nucleotide sequence and binding temperature of selected primers

Temperature (°C)	Primer
50	$5' - (TG)8 - G - 3'$
50	$5' - (TC)8 - C3 - 3'$
50	$5' - (GA)8 - G - 3'$
53	$5' - T - (AG)'9 - 3'$
50	$5' - (CT)8 - T - 3'$
50	$5' - (AC)8 - C - 3'$
55	$5' - CCA - (CT)8 - 3'$
54	$5' - (AG)8 - SG - 3'$
53	$5' - T - (AG)9 - 3'$
57	$5' - (AG)10 - 3'$
54	$5' - (CAC)9 - C - 3'$
52	$5' - (TC)9 - C' - 3'$

to test the goodness of fit between the clustering and the similarity coefficient matrix. STATGRAPHICS was used for biplot analysis (V. 18).

Results

Morphological Analysis

The analysis of variance (ANOVA) revealed that genotype had a significant effect on all studied morphological traits (Table 3). Chini (67.33 and 15.73 cm), Ghatori (3.97 cm), Kadoi (6.36), and Torkamani (0.77 kN) genotypes had the most fruit tail length and diameter, fruit dry weight, fruit juice pH, and fruit flesh firmness. The genotype "Naki" had the highest values for fruit weight (3.650 kg), length of seed cavity (29.83 cm), inside fruit length (36.78 cm), and TSS of fruit (22.78%).

Furthermore, the melon genotype "Habibabadi" had the greatest amount of fruit width (56.77 cm), fruit groove width (7.50 cm), seed cavity width (10.13 cm), and inside fruit width (16.47 cm). The genotype "Bandi Bobak" had the thickest flesh fruit (4.2 cm) and the highest seed weight (1000 seeds) (80.74 g). The number of fruits per plant ranged from 1.33 (Kale gorgi doroneh, Torkamani, and Bandi Bobak) to 5.33 (Kale gorgi doroneh, Torkamani, and Bandi Bobak) (Gorgi from Shirdan and Varamini cantaloupe). Skin fruit thickness ranged from 0.12 mm in the "Chini" genotype to 8.67 mm in the "Abasi" genotype. The Isfahan melon had the longest leaf length (19.03 cm) and the widest leaf width (26.23 cm).

Comparison of Phenological Traits

A comparison of mean data showed that germination time in different cultivars ranged from 5 days (Potk-e Ablagh and Garmeh Afghanistan) to 9 days (Gorgab) in Afghan and Iranian cultivars, respectively. Ghatori, Zanki, Bandi Siah, and Bandi Bobak cultivars took the longest to germinate (9 days), while Gorgi in Shirdan, Gorgi in Ivan, and Varamini germinated the quickest (4 days). In Afghan and Iranian cultivars, the 2-leaf stage time ranges from 6 (Garmeh Afghanistan) to 11 days and 5 to 9 (Habibabadi cantaloupe and Mashhadi) days after seed germination. Bandi Siah and Bandi Bobak had the longest 2-leaf stage time (11 days), while Gorgi in Shirdan and Gorgi in Ivan had the shortest (2-leaf stage time) (5 days). The emergence of the first male flowers in various melon cultivars ranges from 25 (Potk-e Ablagh) to 30 days and 24 to 28 (Mashhadi) days in Afghan and Iranian cultivars, respectively. As a result, Zanki (30 days) and Gorgi in Shirdan and Gorgi in Ivan (24 days) had the longest and shortest

Table 3 Results of analysis of variance for the morphological traits in Afghan and Iranian melon cultivars

Source of variation	df	Germination time	2-leaf stage time	Emergence time of the first male flowers	Emergence time of the first female flowers	Fruit length	Fruit width	Groove width	Fruit weight	Flesh fruit thickness
Block	2	0.05^{ns}	0.49^{ns}	0.12^{ns}	0.003^{ns}	7.59^{ns}	36.20^{*}	0.13^{ns}	0.26^{*}	0.19^{ns}
Cultivar	39	5.65^{**}	6.38^{**}	7.76^{**}	6.900^{**}	98.15^{**}	145.80^{**}	16.77^{**}	1.68^{**}	1.53^{**}
Error	78	0.17	0.24	0.40	0.093	7.39	10.09	0.05	0.08	0.08
CV		6.53	5.74	2.31	3.50	10.14	8.38	12.01	14.44	10.06

Source of variation	df	Length of seed cavity	Width of seed cavity	Fruit skin thickness	1000-seed weight	Open fruit length	Open fruit width	Leaf length	Leaf width	Fruit color before ripening
Block	2	3.44^{ns}	1.65^{ns}	0.06^{ns}	0.0018^{ns}	14.89^{ns}	6.550^{ns}	1.38^{*}	5.62^{**}	0.0085^{**}
Cultivar	39	106.61^{**}	6.32^{**}	14.02^{**}	1.49^{**}	139.15^{**}	14.06^{**}	18.85^{**}	50.00^{**}	2.15^{**}
Error	78	3.50	0.76	0.14	0.0058	6.54	2.08	0.27	2.15	0.02
CV		12.48	13.67	9.53	2.91	12.88	11.77	3.91	3.94	10.87

Source of variation	df	Fruit shape	Fruit tail length	Fruit tail diameter	Fruit tail in ripening time	Fruit dry weight	Total soluble solids	Fruit PH	Fruit harvesting time	Fruit tissue firmness	Skin surface of fruit
Block	2	0.008^{ns}	9.82^{ns}	1.08^{*}	0.02^{ns}	0.01^{ns}	0.005^{ns}	0.003^{ns}	0.16^{ns}	0.001^{ns}	0.07^{ns}
Cultivar	39	1.61^{**}	458.44^{**}	16.72^{**}	0.69^{**}	1.19^{**}	116.44^{**}	0.59^{**}	88.15^{**}	0.10^{**}	4.82^{**}
Error	78	0.03	7.33	0.31	0.03	0.01	0.12	0.01	0.09	0.0001	0.07
CV		9.30	7.43	7.86	12.73	3.81	2.63	2.22	0.71	7.96	13.69

* and ** indicate significance at $P<0.05$ and $P<0.01$, respectively, and ns means non-significant

time to the emergence of the first male flowers, respectively. The time it takes for the first female flowers to emerge in different melon cultivars ranges from 7 (Potk-e Ablagh and Garmeh Afghanistan) to 12 days and 6 to 9 (Gorgi in Ivan and Gorgab) days in Afghan and Iranian cultivars, respectively. Ghatori, Pirzali, and Zanki cultivars took the longest (12 days), while Gorgi in Shirdan and Gorgi in Ivan took the shortest (6 days). The first harvest of fruit in various melon cultivars occurred between 34 (Garmeh Afghanistan) and 51.67 (Gazgi) days after the first emergence of female flowers in Afghan and Iranian cultivars, respectively. Ghatori had the longest time to the first fruit harvest (51.67 days), while Gorgi in Shirdan and Gorgi in Ivan had the shortest (33 days).

Comparison of Fruit Characterization

Cantaloupe and the majority of melons had green fruit before ripening. Melons such as Kale Gorgi, on the other hand, were white before ripening, whereas Ghatori, Ghandak-e Zard, and Saderati were yellow. After ripening, the Kale Gorgi and Ghandak Tanabi-e Sefid were white, while the Barg-e Ney and Hachkeh Johari were green. Melon clusters can be spherical, elliptical, or slender. Ghatori, Saderati, Potk-e Johari, and Kale Gorgi were elliptical, while Naki, Mashhadi, Naki Johari, Tanabi, and Bag-e Ney were spherical. When it came to the fruit tail after ripening, late matures and long-shaped cultivars were accompanied by fruit. However, when the fruit ripened, the tail became separated from the fruit. The cultivars of Zanki, Saderati, Kale Gorgi, and Garmeh Afghanistan had smooth skin surfaces; Saveh honeydew melon, Shahabadi melon, Darounak melon, and Esfahan were wrinkled smooth skin surface; and Gorgab, Bandi Pirzali, Chini, and Torkamani were wavy smooth skin surface.

The cultivars of Zanki, Gorgab, Barg-e Ney, and Mashhadi were aromatic sweet; Max Tanbal, melon, Potk-e Ablagh, Varamini, and Bandi Pirzali were non-aromatic sweet; Potk-e Johari, Isfahan cantaloupe, and Shahabadi were moderately sweet; and Kale Gorgi, Kadouei, Gorgi in Shirdan, and Gorgi in Ivan were the slightest taste cultivars. Based on the results, the fruit flesh of Varamini melon, Barg-e Ney, Hachkeh Johari, and Hachkeh Darouneh were green; Isfahan cantaloupe, Habibabadi cantaloupe, Zanki, Ghandak-e Zard, and Bandi Pirzali were yellow; Abasi, Gorgi in Ivan, Kadouei, Potk-e Ablagh, and Garmeh Afghanistan were pink;, Naki, Max Tanbal melon, Kale Gorgi were white; and Shahabadi melon and Saderati were orange.

Cluster Analysis of Morphological Traits

The morphological traits were used to perform cluster analysis. The Ward method was used to divide all of the cultivars studied into five groups (Fig. 2). Cluster 1 was

distinguished by middle-ripening flesh (average of fruit harvesting time = 41.63 day) that was sweet and aromatic, as well as an elongated fruit shape (average of open fruit length = 22.85 cm). Cluster 2 was made up of five winter cultivars that had no aroma and ripened late (average of fruit harvesting time = 50 day). The third group of cultivars is distinguished by their sweet fruit and thin skin (average of skin fruit thickness = 3.52 cm). Cluster 4 was distinguished by its small seeds and fruits (average of open fruit length = 11.6 cm), delicate aroma, and early ripening (average of fruit harvesting time = 33 day). Cluster 5 included cultivars with a spherical shape, middle-ripening flesh (average of fruit harvesting time = 40.02 day), and thick flesh (average of flesh fruit thickness = 3.2 cm).

Principal Component Analysis of Morphological Data

The clustering analysis was confirmed by the PCA results. The first two PCA axes confirmed 43.33% of the total variation among the studied cultivars, according to the results of the PCA analysis (Fig. 3). The minor difference in classification between these two analyses could be attributed to the nature of the modification to the modified principal component.

Correlation Analysis of Morphological Data

Correlation analyses (Fig. 4) also revealed that increasing the germination time increased the fruit harvesting time. Yield and quality in melon fruits are important parameters for selection. The correlation analysis revealed that accessions with more fruit per plant had lower TSS, flesh taste quality, 1000-seed weight, and fruit weight per plant. Therefore, it is necessary to select varieties that have a balanced level of yield and quality.

Molecular Variation

ISSR Amplification and Levels of Polymorphism

The 12 ISSR markers were used to investigate the genetic diversity of the 40 melon cultivars, yielding a total of 88 polymorphic bands, with an average of 7.9 bands per primer. The average polymorphism information content (PIC) value was 0.48 (Table 4). Primers 18 and 6 had the highest (0.57) and lowest (0.44) PIC values, respectively (Fig. 5).

Phylogenetic Relationships of Molecular Data

The UPGMA method of clustering molecular data classified the cultivars into five major groups (Fig. 6). Seven cultivars
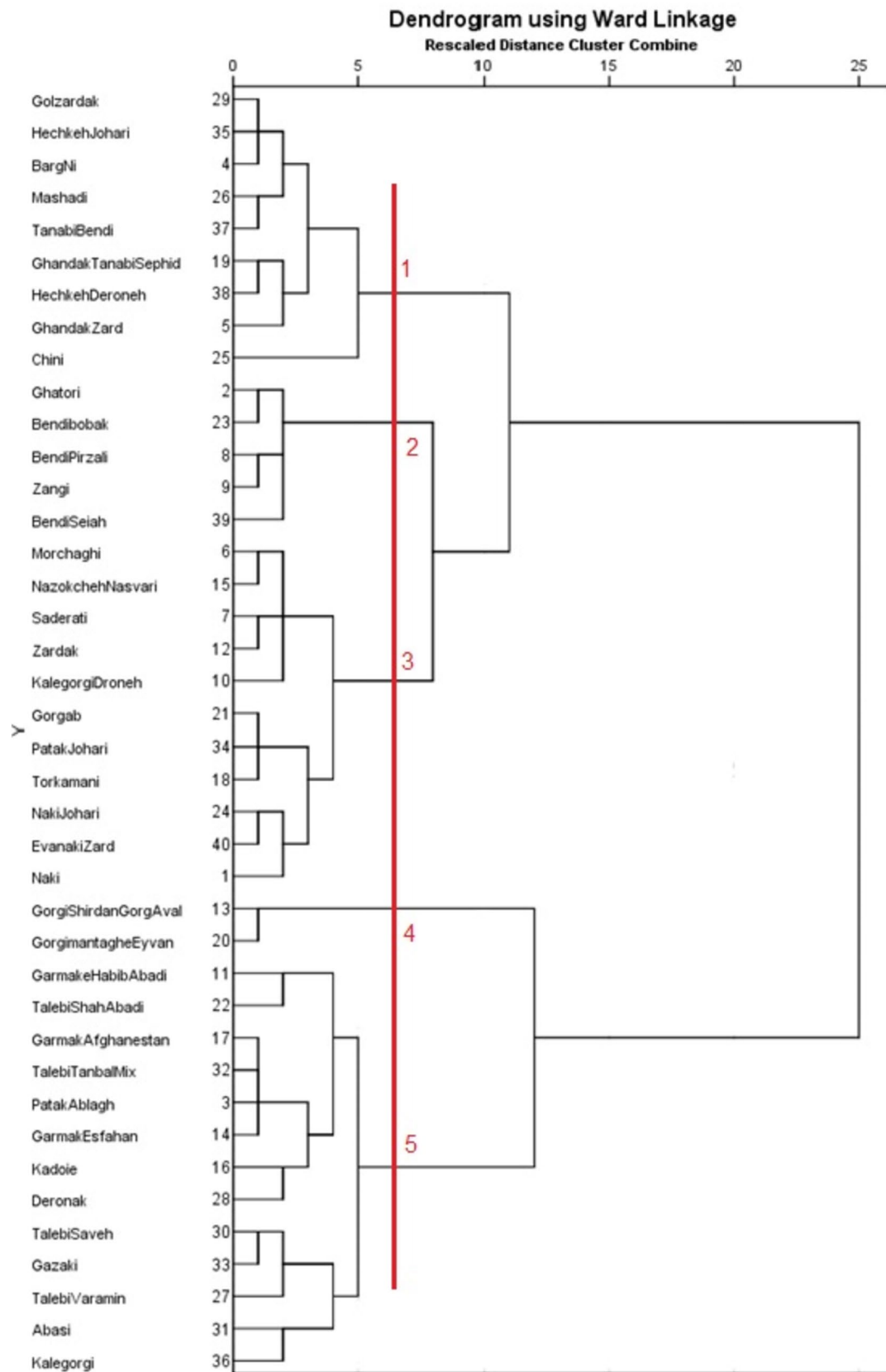
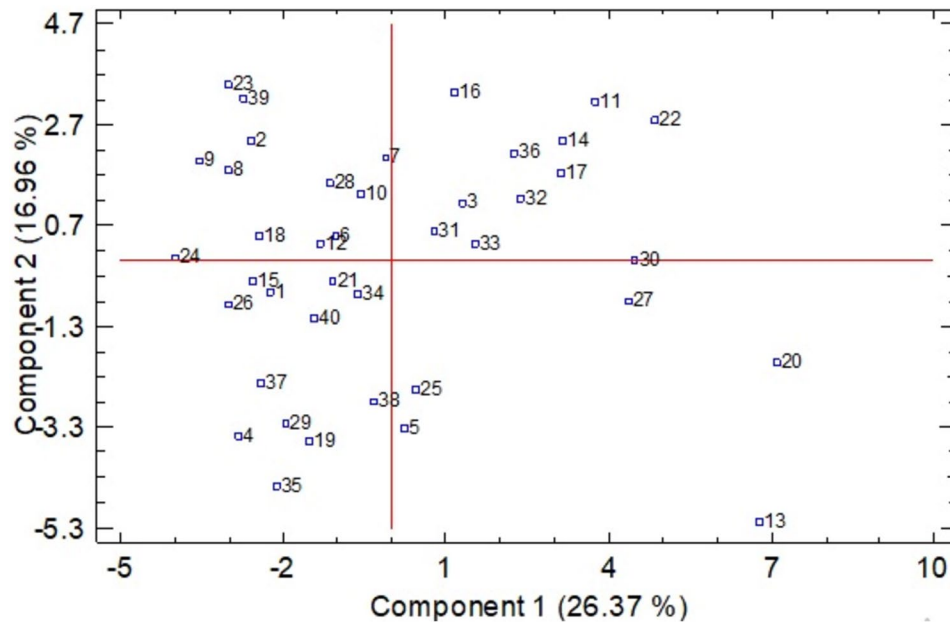**Fig. 2** Grouping of studied melon cultivars using morphological traits based on the Ward method

Fig. 3 PCA analysis in studied melon cultivars. TG, germination time; Tb, 2 leaf stage time; FW, fruit weight; S50, 1000-seed weight; FA, skin pattern surface of the fruit; pH, acidity; PT, fruit skin thickness; LL, leaf length; LW, leaf width; DDF, tail diameter; LDF, tail length; TFGM, emergence time of the first female flowers; TFGN, emergence time of the first female flowers; MT, fruit length; TSS, total soluble solids; CFA, fruit color after ripening; CFB, fruit color before ripening; LFB, inside fruit length; WFB, inside fruit width; FT, fruit tissue firmness; LHS, length of seed cavity; WHS, width of seed cavity; FSh, fruit shape; WF, fruit width; CG, fruit flesh color; DFH, changes of fruit tail after ripening; TamG, taste quality of flesh fruit; GT, flesh fruit thickness; HT, fruit harvesting time; WSH, groove width; FWa, fruit water (difference between fresh and dried fruits); FN, fruit number per plant

were included in the first group. Cluster 2 had four cultivars, while Clusters 3 and 4 had four genotypes each. Cluster 5 was made up of 15 genotypes.

Group 1 consisted of seven cultivars, including six Afghan landraces and one Iranian landrace (Naki, Naki Johari, Tanabi-e bandi, Golzardak, Barg-e Ney, Hachkeh Johari, and Mashhadi). Group 1 landraces shared characteristics such as being mid-maturing (average of fruit harvesting time = 41.66 day), long in shape (average of fruit length = 29.31 cm), very watery and sweet, thin skin (average of fruit skin thickness = 2.58 cm), and aromatic

fruit flesh. Group 2 contained four landraces, two of which were related to Afghanistan and the rest to Iran (Ghandak-e Zard, Zardak, Abasi, and Saderati). Group 3 contains seven landraces, six of which are from Afghanistan and one from Iran. Group 3 landraces shared characteristics such as thick flesh (average of flesh fruit thickness = 2.9 cm), sweetness, and lack of aroma (Ghatori, Bandi Pirzali, Darounak, Ghandak Tanabi-e Sefid, Potk-e Johari, Hachkeh Darouneh, and Eivanaki zard). Group 4 consists of seven landraces from Afghanistan (6 landraces) and Iran (1 landrace) that share characteristics such as wintry and non-aromatic traits,

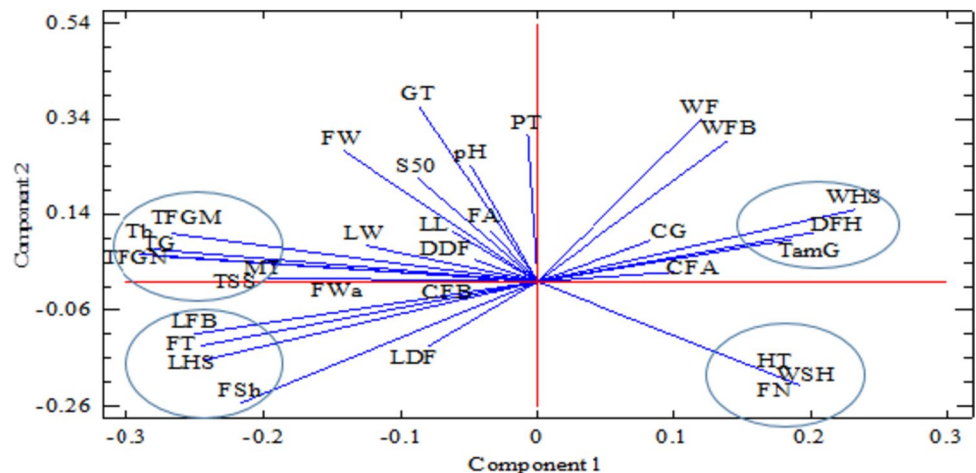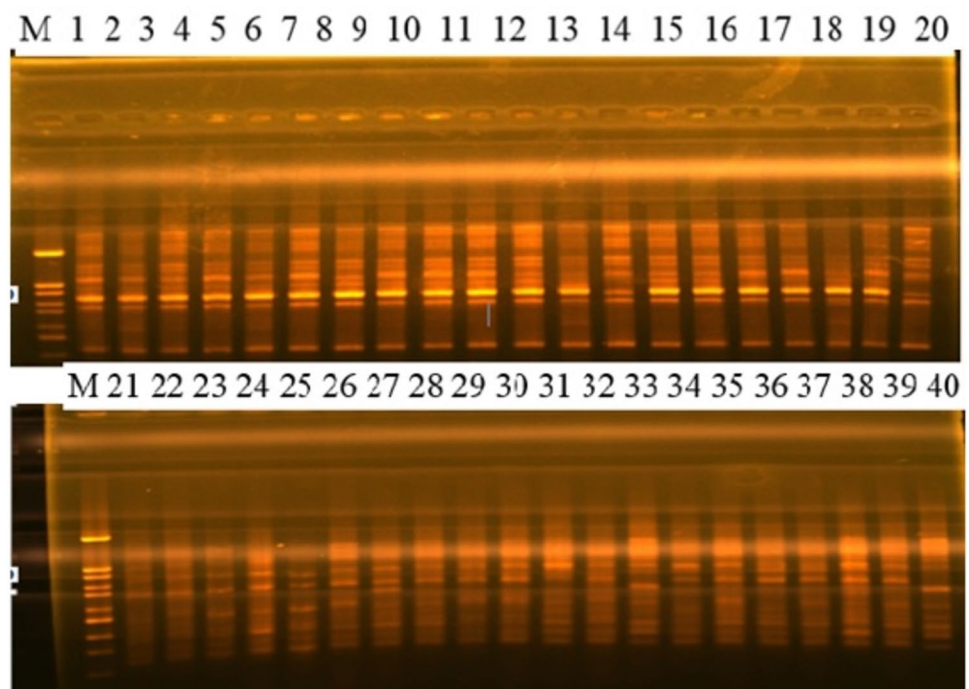**Fig. 4** Biplot graph of melon trait correlations

Table 4 The number of total and polymorphic fragments along with polymorphic information content (PIC) per ISSR primer

Primer	Number of alleles	Number of polymorphic alleles	Percentage of polymorphism	PIC
(TG)8-G	12	7	58.33	0.47
(TC)8-C	14	6	86.42	0.44
(GA)8-G	10	6	60.00	0.46
T-(AG)	15	10	67.66	0.48
(CT)8-T	9	5	55.56	0.57
(AC)8-C	12	7	58.33	0.45
CCA-(CT)8	15	12	80.00	0.46
(AG)8-SG	12	6	50.00	0.49
T-(AG)9	11	7	63.64	0.50
(AG)10	13	8	61.56	0.48
(CAC)9-C	17	14	82.35	0.50
(TC)9-C	10	7	70.00	0.49

late maturing (average of fruit harvesting time = 46.19), thick skin and flesh (average of skin and flesh fruit thickness = 5.09, 3.2 cm), firmer flesh, and elliptical shape (Murchaghi, Nazokch-e Nasvari, Bandi Siah, Torkamani, Zanki, Bandi Bobak, Max Tanbal honeydew melon). Group 5 had 15 landraces, while Afghanistan and Iran had nine and six landraces, respectively (Habibabadi cantaloupe, Shahabadi honeydew melon, Varamini cantaloupe, Saveh honeydew melon, Gorgab, Gorgi in Shirdan, Isfahan melon, Gorgi in Ivan, Gazgi, Potk-e Ablagh, Garmeh Afghanistan, Kale gorgi doroneh, Kadouei, Kale Gorgi, and Chini).

Melon, cantaloupe, and similar melons in group 5 shared characteristics such as spherical shape, thick flesh (average of flesh fruit thickness = 2.88 cm), soft flesh, low sweetness, and early maturing (average of fruit harvesting time = 39.06).

Principal Coordinate Analysis (PCoA) of the Molecular Data

PCoA was used to show the genetic relationships between melon cultivars. The PCoA analysis based on the ISSR marker revealed significant genetic diversity among the cultivars studied (Fig. 7). Based on the PCoA, the 40 melon cultivars can be divided into 5 clusters. The first three principal coordinates explained 69.62% of the total variation, according to the PCoA. PCoA also confirmed the cluster analysis results. Group 1 consisted of mid and long landraces, group 2 of yellow and elliptical cultivars, group 3 of thick and non-aromatic flesh, and group 4 of wintry and late maturing cultivars. Honeydew melons, cantaloupe, and melons were included in group 5.

Discussion

Genetic analysis is an effective method for estimating genetic diversity and phenotypic relationships between cultivars (Gharibi et al. 2011). In this study, the genetic diversity of Afghan and Iranian melon cultivars was investigated using morphological traits as well as ISSR markers. Previous research found that ISSR primers with AC and AG repeats resulted in higher polymorphism than other repeats

Fig. 5 Band patterns of melon cultivars revealed by the primer 3

Fig. 6 Dendrogram of studied melon cultivars using ISSR data
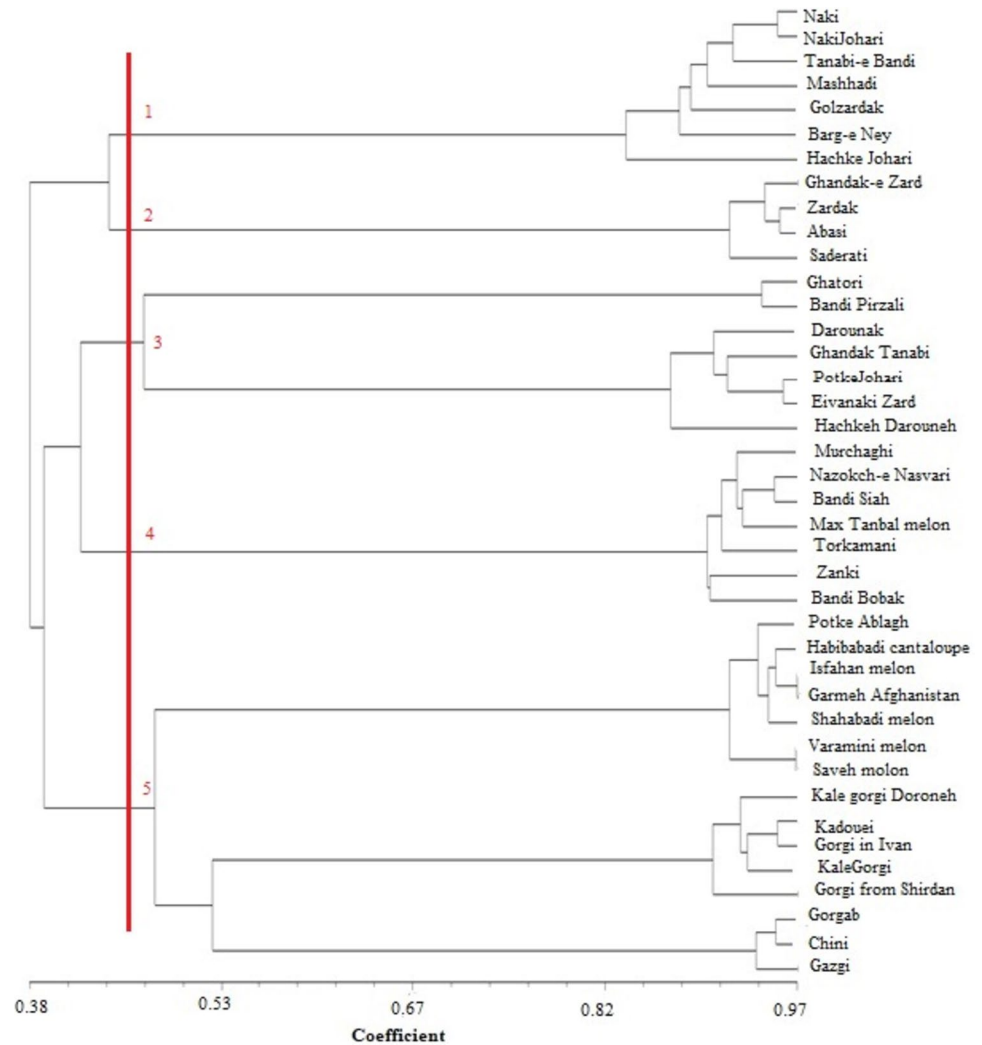
(Monforte et al. 2003). These findings agreed with those of the current study. In some cases, the inconsistency may be due to differences in the frequency of duplicate sequences in the genomes of different plants. Melon's primary diversity center is also in Central Asia, such as Iran and Afghanistan. As a result, it is expected that these areas will be rich in genetic variation. The PIC index is affected by some factors, including the number of alleles per locus; the content of nucleotides, such as G and T, in replicated regions; and the number of landraces (Nei 1978). In the current study, all primers had a high PIC value, demonstrating their effectiveness in distinguishing the studied cultivars.

There is a wide range of phenotypic variation in melon fruit traits because the Iranian cultivars are open-pollinated and not the result of controlled crosses (Kishor et al. 2020). Soltani et al. (2022) assessed the genetic diversity of 45 Iranian and 15 foreign melons based on phenotypic traits and discovered excellent genetic variation for breeding program improvement. The relationship between genetic diversity and

geographical distribution in various squash family species was also investigated. Maleki et al. (2017), for example, examined local melon landraces using 11 ISSR primers and discovered that 31% of total diversity was related to inter-population diversity and 13% to intra-population diversity. Because the degree of difference in the genomes of these populations decreases with their intersection over time, this low diversity among populations can be attributed to the plant's heterozygosity. However, because of the differentiation of individuals within the population, there is a high level of diversity in the population. Overall, ISSR markers were effective in studying the genetic diversity of native cantaloupe populations. Yildiz et al. (2011) found very high levels of polymorphism in Turkish genotypes using ISSR, RAPD, and sequence-related amplified polymorphism markers. Rahmanpour et al. (2014) used 20 ISSR primers to study native (11 landraces) and hybrid (12 landraces) melons. The highest genetic distance in their study was between Dargzi and Durango hybrids, and the

Fig. 7 PCoA diagram in studied melon cultivars
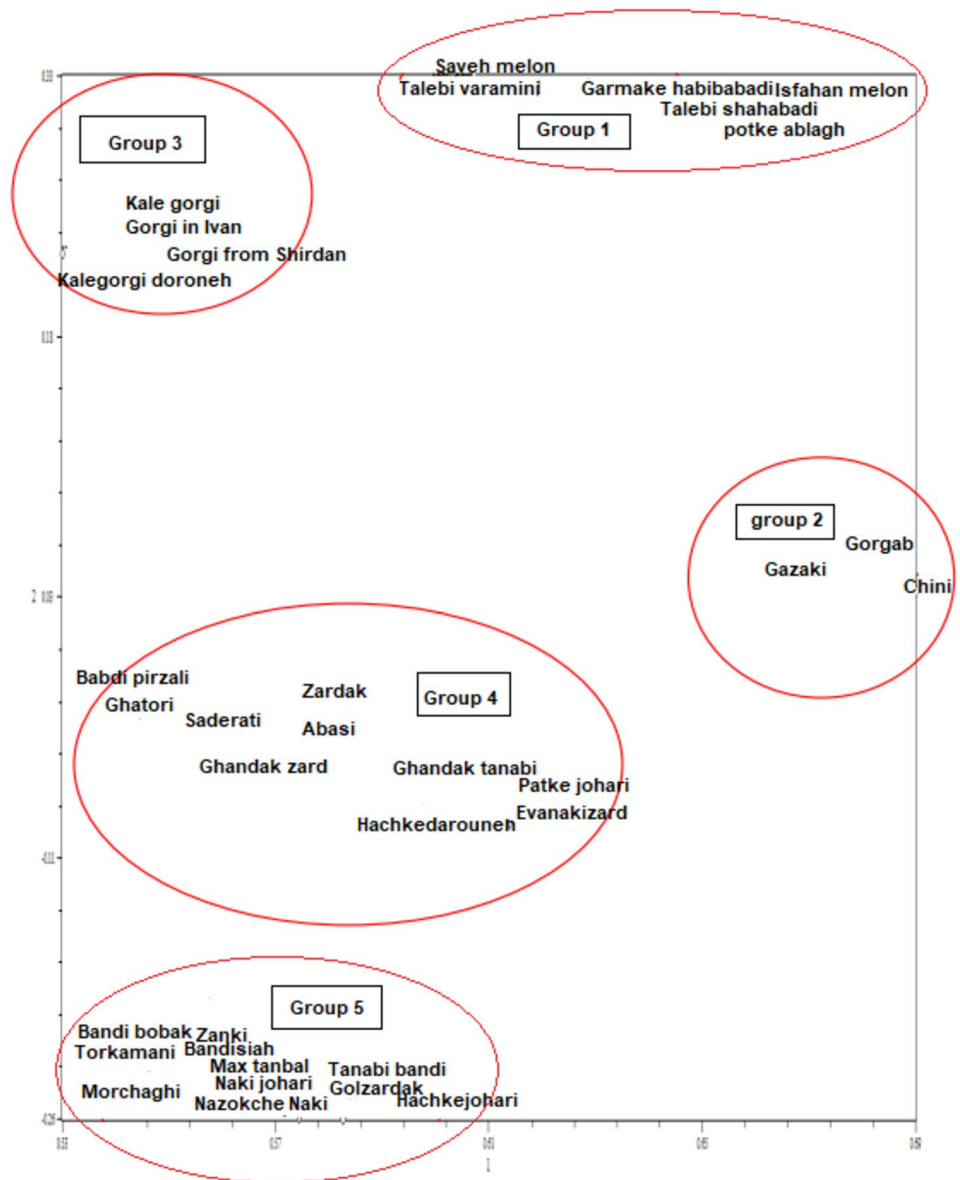
lowest genetic distance was between Dargzi and Khatouni. Using the RAPD marker, Feyzian et al. (2007) examined 30 native landraces of melons to assess the diversity and affinity of different groups. The use of molecular markers in their study was unable to distinguish different groups, indicating that the genomes of these plants are very similar to one another. Salehi Najafabadi et al. (2010) investigated Iranian melon germplasm by amplifying 12 RAPD primers and grouping the accessions, revealing 7 genetically distinct groups with 82% similarity.

As a result, the RAPD molecular markers can, to some extent, distinguish between different groups of melons. Nonetheless, the closeness of these groups is due to the high similarity of the genomes of these samples. Raghami

et al. (2014) used microsatellite markers to assess the genetic diversity between and within Iranian melon landraces, as well as their relationship with germplasm from other regions. They divided the Iranian landraces into two groups, one for honeydew melon and cantaloupe and another for the gourd. The first group in Raghami et al. (2014), including cantaloupe, honeydew melon, and melon, had a high rate of consistency with the fifth molecular group in the current study, which included cantaloupe, honeydew melon, and some other melon accessions. Maleki et al. (2017) investigated the genetic diversity of 27 melon accessions in Iran using ISSR molecular markers. The results showed that the ISSR markers could distinguish between the sweet and non-sweet melon groups. Sestili et al. (2011) used ISSR

primers and morphological traits to assess genetic diversity in 13 Italian melon populations. They discovered that almost all of the melon genotypes could be clustered based on the skin color of the fruits and demonstrated the effectiveness of ISSR markers in distinguishing between genetic resources with a limited genetic background.

The results of the morphological trait analysis were not entirely consistent with the results of the molecular data. The pollination system is responsible for the slight difference between molecular and morphological groups. Melon is a cross-pollinated plant, and pollen grain transport between cultivars can be attributed to environmental factors such as climate, soil, and altitude (Kumar et al. 2014; Jain et al. 2017). Furthermore, environmental factors influence morphological traits, and molecular bands may be found in positions unrelated to important phenotypic traits (Yildiz et al. 2011). The color of fruit flesh is influenced by the particular carotenoid type and concentrations which are affected by genetic and environmental factors (Fergany et al. 2011).

The cluster analysis in this study revealed no significant clustering pattern based on geographic regions. It could be attributed to melon farmers dispersing seeds or sharing breeding seeds, which resulted in low diversity between cultivars collected from close geographic locations (Kumar et al. 2014; Guliyev et al. 2018). Similarly, Trimech et al. (2013) could not separate some melon groups, which may be due to the high heterogeneity and introgression of characters. This confirms the results of Silberstein et al. (1999) and may indicate that these cultivars are close, despite the important morphological differences between them. To better understand of melon intra-specific relationships, it is necessary to study more accessions of each cultivar group. Also, it should be considered that morphological variation used to classify melon resulted from an intensive selection over a relatively short time and may be the result of very few genetic modifications in developmental regulator genes, with little diversity in DNA sequence. Other studies showed that there is no differentiation between group inodorous and cantaloupe which may be attributed to their genetic similarities. The lack of distinction in the clustering of accessions from the same botanical groups was also reported by Soltani et al. (2010) and Aragão et al. (2013). This evidence shows the inevitable outcrossing between melon genotypes. As a result, outcrossing between Iranian and Afghan cultivars is a possibility. Although the studied morphophysiological traits showed a high genetic diversity between accessions, similar levels of genetic variation indicate that climate and cultivation conditions allowed for extensive germplasm conservation. Classification studies are also the first steps for the management of germplasm banks, contributing to the selection of melons for breeding programs.

Conclusions

Cucumis melo L. has a diverse genetic profile and large germplasm. ISSR primers and morphological characteristics could both be useful resources for germplasm identification. Significant phenotypic and molecular differences were found among the cultivars studied. Using the UPGMA algorithm, cluster analysis classified the cultivars studied into five groups. Cluster analysis of morphophysiological traits classified the cultivars into five groups as well. Finally, group 1, which includes sweet, fragrant, watery, and more marketable melons, is recommended for greenhouse cultivation. Winter-cultivated melons, non-aromatic, and late maturing are classified as group 4 (molecular) and group 2 (morphological). Honeydew melon, cantaloupe, and some early maturing melons from group 5 (morphological and molecular parts) are recommended for early crop cultivation.

Declarations

Ethics Approval Not applicable.

Competing Interests The authors declare no competing interests.

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