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Genetic diversity among melon (*Cucumis melo* L.) accessions revealed by morphological traits and ISSR markers

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Abstract: The genetic relationships among 48 melon (*Cucumis melo* L.) genotypes collected from various parts of Azerbaijan were determined by comparing their phenotypic and molecular traits. Eleven agromorphological traits and 10 polymorphic inter-simple sequence repeat (ISSR) primers were used to define the genetic diversity. Principal component analysis grouped the agromorphological traits into the first four axes, describing 78% of the total variations. The highest genetic variation coefficient was found for yield per hectare (20.32%) and for fruit length (17.35%). Calculated heritability for yield was 0.96. The analysis of morphological traits grouped the accessions into four clusters. The 10 ISSR primers yielded 35 polymorphic alleles, representing 85.4% of all the amplified loci. The average genetic diversity index determined was 0.70. The highest and the lowest similarity indexes were equal to 0.97 and 0.36, respectively. The 48 accessions were grouped into 10 clusters based on ISSR markers. Correlation between distance matrices based on agromorphological traits and ISSR markers was not statistically significant (r = 0.012).

Key words: Melon, genetic diversity, markers, yield, accessions, trait

1. Introduction

The cultivated melon (*Cucumis melo* L., 2n = 2x = 24) is an important crop widely distributed in the warmest areas of the world and grown extensively in countries with temperate climate (Pech et al., 2007). Melon is one of the most polymorphic species that is very variable in plant, leaf, flower, and fruit characteristics. Because significant morphological variation exists in fruit characteristics and composition of *C. melo* genotypes, this species is thought to contain the most diverse varieties in the genus *Cucumis* (Stepansky et al., 1999).

An understanding of the extent of genetic diversity and relationships among different local genotypes is beneficial both for the identification and effective conservation of genetic resources, and for the success of breeding programs (Solmaz et al., 2016). Genetic diversity in melon has been analyzed using different molecular markers, ranging from phenotypic (Escribano and Lázaro, 2009; Szamosi et al., 2010) and isozymic (McCreight et al., 2004) to molecular DNA markers, including random amplification of polymorphic DNA (Sensoy et al., 2007; Soltani et al., 2010), amplified fragment length polymorphism (Frary et al., 2013; Shamasbi et al., 2014), simple sequence repeat primers (Monforte et al., 2003; Tzitzikas et al., 2009; Kaçar et al., 2016), inter-simple sequence repeat (ISSR) primers (Parvathaneni et al., 2011; Sestili et al., 2011), and other DNA markers.

Valuable melon genetic resources with distinct morphological differences exist in Azerbaijan. Nevertheless, not a single study has been conducted to illustrate the genetic variability of the local melon genotypes. In the present study, 48 *C. melo* L. genotypes from different geographical areas of Azerbaijan were collected and screened for genetic diversity based on both some agromorphological traits and ISSR markers. The results obtained could be useful to conserve genetic variability and to encourage their use for genetic improvement in breeding programs.

2. Materials and methods

The research material consisted of 48 melon genotypes that represented the main accessions cultivated in various regions of Azerbaijan. The names and geographical locations of collection site of accessions are presented in Table 1.

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2.1. Morphological diversity analysis

The seed materials of all samples collected from different regions were used in the experiment (Table 1). Fifteen seeds for each 3 replicates were sown in a greenhouse and seedlings were transplanted into an open field on 5 May at the Absheron experimental station of the Institute of Genetic Resources in Azerbaijan. Genotypes were evaluated in a randomized complete block design

Table 1. Melon (*Cucumis melo* L.) germplasm used for genetic diversity comparisons.

No.	Accession name	Collection region	Latitude	Longitude	Altitude
1	Kurdamir №1	Kurdamir	40°28.879′N	47°48.216′E	3
2	Kurdamir №2	Kurdamir	40°28.879′N	47°48.216′E	3
3	Kurdamir №3	Kurdamir	40°28.879′N	47°48.216′E	3
4	Kurdamir №4	Kurdamir	40°19.999′N	48°09.621′E	5
5	Kurdamir №5	Kurdamir	40°19.999′N	48°09.621′E	5
6	Kurdamir №6	Kurdamir	40°19.999′N	48°09.621′E	5
7	Kurdamir №7	Kurdamir	40°19.528′N	48°11.212′E	4
8	Kurdamir №8	Kurdamir	40°19.528′N	48°11.212′E	4
9	Kurdamir №9	Kurdamir	40°19.528′N	48°11.212′E	4
10	Kurdamir №10	Kurdamir	40°19.528′N	48°11.212′E	4
11	Saatly №11	Saatly	39°54.300′N	48°20.258′E	–15
12	Saatly №12	Saatly	39°54.300′N	48°20.258′E	–15
13	Kurdamir №13	Kurdamir	40°19.528′N	48°11.212′E	4
14	Sabirabad №14	Sabirabad	30°58.001′N	48°27.000′E	–12
15	Sabirabad №15	Sabirabad	30°58.001′N	48°27.000′E	–12
16	Saatly №16	Saatly	39°54.300′N	48°20.258′E	–15
17	Sabirabad №17	Sabirabad	30°58.001′N	48°27.000′E	–12
18	Saatly №4	Saatly	39°54.300′N	48°20.258′E	–15
19	Saatly №5	Saatly	39°54.300′N	48°20.258′E	–15
20	Sabirabad №4	Sabirabad	30°58.001′N	48°27.000′E	–12
21	Saatly №6	Saatly	39°54.300′N	48°20.258′E	–15
22	Absheron №1	Absheron, Chayli	40°31.001′N	49°29.000′E	71
23	Masally №1	Masally	39°04.051′N	48°38.433′E	5
24	Lankaran №2	Lankaran, Shulavur	38°45.314′N	48°50.037′E	–12
25	Lankaran №3	Lankaran, Shulavur	38°45.314′N	48°50.037′E	–12
26	Astara №4	Astara	38°28.032′N	48°52.236′E	–23
27	Masallı (uzun)	Masallı	39°04.051′N	48°38.433′E	5
28	Absheron №2	Absheron, Chayli	40°31.001′N	49°29.000′E	71
29	Absheron №3	Absheron, Chayli	40°31.001′N	49°29.000′E	71
30	Absheron №4	Absheron, Chayli	40°31.001′N	49°29.000′E	71
31	Saray №4	Absheron, Saray	40°32.001′N	49°44.000′E	41
32	Saray №5	Absheron, Saray	40°32.001′N	49°44.000′E	41
33	Saray №6	Absheron, Saray	40°32.001′N	49°44.000′E	41
34	Nazly	Absheron	40°24.001′N	49°49.001′E	50
35	Kolchoznick	Absheron	40°24.001′N	49°49.001′E	49
36	Timsah	Absheron, Chayli	40°31.001′N	49°29.000′E	71
37	Ghizil yemish	Nakhchıvan	39°12.001′N	45°29.000′E	807
38	Saray №1	Absheron, Saray	40°31.001′N	46°44.001′E	36
39	Saray №2	Absheron, Saray	40°31.001′N	46°44.001′E	36
40	Saray №3	Absheron	40°31.001′N	46°44.001′E	36
41	Sapharaliyev	Ganja-Yevlakh road	41°41.378′N	46°29.835′E	382
42	Barda №1	Barda, Shirvanly	40°20.855′N	47°07.744′E	84
43	Barda №2	Barda, Shirvanly	40°20.855′N	47°07.744′E	84
44	Agstafa №1	Agstafa	41°06.811′N	47°27.584′E	362
45	Agstafa №2	Agstafa, Giraq Kasaman	41°13.155′N	45°27.905′E	221
46	Agstafa №3	Agstafa,Poylu	41°11.098′N	45°20.976′E	307
47	Gazakh	Gazakh	41°05.890′N	45°20.976′E	371
48	Tovuz №1	Tovuz, Ashagi Eyyublu	40°55.787′N	45°48.641′E	391

with three replications. Plants were spaced 1.0 m apart on raised beds in rows spaced 1.5 m apart. Five plants of each replication were examined for a set of 11 agromorphological characters based on *Descriptors for Melon* (Kerje, 2003). Measured traits included main stem length, lateral stem number, leaf length, leaf width, seed length, seed width, stem diameter, fruit length, fruit width, flesh thickness, and yield. Measurements of vegetative parts were performed prior to fruit ripening, while all others were recorded during harvesting, starting from the middle of July and continuing through the end of August.

The similarity matrix, principal component analysis (PCA), and other measurements for morphological diversity analysis were generated using SPSS 14.0 (SPSS Inc., Chicago, IL, USA). Morphological diversity analysis among the studied genotypes was evaluated using the Ward method (Johnson and Wichern, 2002). Heritability in the broad sense was calculated as the ratio of the total genetic variance to the phenotypic variance (Falconer et al., 1996).

2.2. PCR reaction

The leaves were ground to powder using liquid nitrogen in microfuge tubes. Genomic DNA from the leaves was extracted by CTAB protocol (Doyle and Doyle, 1987). The quality and concentration of isolated DNA was checked using a NanoDrop 2000 spectrophotometer and 1% agarose gel.

A total of 10 ISSR primers synthesized by IDT (https://www.idtdna.com) were used for PCR amplification. PCR reactions were performed in a final volume of 20 µL, containing 1X AMS PCR buffer, 1.5 mM $MgCl_2$, 25 mM of each deoxynucleotide (dATP, dTTP, dGTP, and dCTP), 0.5 mM of primer, 0.6 U of Taq DNA polymerase enzyme, and 50 ng of DNA. All of the reagents used for PCR reactions were obtained from Sinaclon (http://www.sinaclon.com/). A Verity Thermo Cycler (Applied Biosystems) was used for PCR amplification.

Amplification reactions were performed in 35 cycles, each consisting of a denaturation step at 94 °C for 1 min, annealing step at primer-dependent temperatures (5 °C below T_m) for 1 min, and extension step at 72 °C for 2 min, followed by a final extension at 72 °C for 7 min. The initial denaturation step was performed for 4 min at 94 °C.

The PCR products were separated by electrophoresis using a 1.2% (w/v) agarose gel in 1X TBE buffer for 1.15 h. A 100-bp DNA ladder (Sinaclon) was used for approximate fragment size calculations. Gel stained with ethidium bromide solution was recorded with the Molecular Imager Gel Doc XR System (Bio-Rad).

Amplified alleles were analyzed by scoring them as present (1) or absent (0). The genetic diversity index for each primer was calculated according to Nei (1973):

$H = 1 - \Sigma P_i^2$, where H is the genetic diversity index and P_i is the frequency of each pattern.

The genetic relationships among accessions were evaluated by Jaccard's similarity coefficient for pairwise comparisons based on the genotyping data using the PAST statistic program package (Hammer et al., 2001). A dendrogram based on ISSR data was constructed by the unweighted pair-group method (UPGMA). The similarity between matrices based on different marker systems (agromorphological data and ISSR) were calculated using the standardized Mantel coefficient (Mantel, 1967).

3. Results and discussion

3.1. Morphological evaluation

Eleven agromorphological quantitative traits were assessed to characterize and estimate genetic diversity among local melon accessions. A significant difference with respect to all traits (results not shown) among the studied melon genotypes was detected based on variance analysis. PCA grouped the 11 agromorphological traits into the first four axes, describing 78% of the total variation. The percentages of total variation accounted for by each of the four principal components were 40.7%, 16.98%, 11.56%, and 9.46%, respectively (Table 2).

Traits that had a correlation with the first component include main stem length (r = 0.464**), leaf length (r = 0.571**), leaf width (r = 0.755**), stem diameter (r = 0.484**), fruit length (r = 0.741**), fruit width (r = 0.658**), and yield per hectare (r = 0.87). High correlation between the first component and yield with the other traits associated was observed. Masally №1, Lankaran №2, Lankaran №3, Astara №4, Absheron №4, Saray №6, Nazly (Absheron), Kolchoznick, and Ghizil yemish (Nakhchivan) genotypes differed from all other genotypes for the aforementioned component (Figure 1).

Cluster analysis using the Ward method was carried out and genotypes were divided into 4 groups (Figure 2). Clusters 3 and 4 mainly consisted of accessions that differed based on principal components.

The values of genetic variance, broad-sense heritability, coefficient of variation, and minimum and maximum values of the studied traits are shown in Table 3. Calculated heritability for yield in our study was 0.96. Most probably, high genetic variance for this trait has led to calculating its heritability as more than its actual value. The high heritability percentage reflects the large heritable variance, which may offer the possibility of improvement through selection (Eshghi and Akhundova, 2010). Although the highest broad-sense heritability was observed for traits of plant height (0.98), fruit length (0.97), flesh thickness (0.88), and leaf length (0.77), the broad-sense heritability of seed width, fruit width, fruit length, stem diameter, and leaf width was also relatively high. The highest genetic variation coefficient and the highest diversity among the genotypes were found for yield per hectare (20.32%) and for fruit length (17.35%).

Table 2. Studies on principal component for 48 varieties and 11 traits in melon.

Traits	PC1	PC2	PC3	PC4
Main stem length	0.747	–0.165	0.110	0.143
Lateral stem number	0.091	–0.394	0.481	0.603
Leaf length	0.813	–0.009	–0.332	0.162
Leaf width	0.844	–0.022	–0.319	0.120
Seed length	0.028	0.875	0.328	0.025
Seed width	–0.119	0.876	0.063	0.341
Stem diameter	0.621	–0.096	0.269	0.410
Fruit length	0.819	0.205	0.268	–0.217
Fruit width	0.795	0.115	0.276	–0.431
Flesh thickness	0.209	0.295	–0.694	0.268
Yield	0.890	0.004	–0.056	–0.163
Individual percentage	40.71	16.98	11.56	9.46
Cumulative percentage	40.71	57.69	69.25	78.71

Figure 1. Diversity of fruit size, color, shape, and texture of some melon accessions collected from different regions of Azerbaijan.

3.2. Molecular diversity analysis using ISSR markers

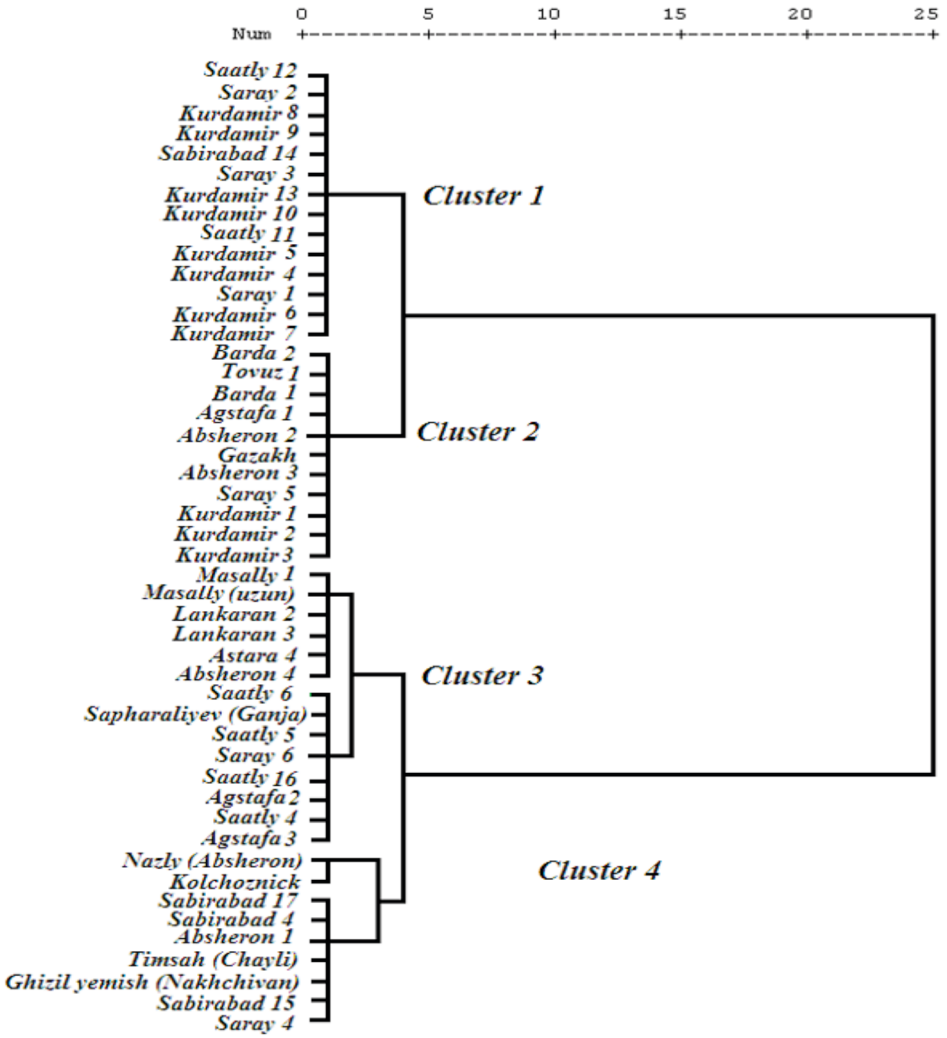
Characterization of the genetic relationship between different melon (*C. melo* L.) genotypes was conducted with 10 ISSR primers. The reproducible alleles amplified were scored as 1 for presence or 0 for absence and imported into the PAST3 statistic program. The 10 ISSR primers yielded a total of 41 scorable bands, 35 of which (85.4%) were polymorphic. The details of fragments amplified by the ISSR primers among the 48 genotypes are given in Table 4. The highest (7) and the lowest (2) number of bands was produced by UBC860 and ISSR35, respectively. The percentage of polymorphism ranged from 50% to 100%, with an average of 85.6% polymorphism per primer. Primers UBC857 and UBC860 generated the greatest diversity indexes with values of 0.94 and 0.85, respectively. The details of markers amplified by primer UBC857 are given in Figure 3. The lowest diversity was identified by primer UBC834, with an index of 0.52. The average genetic diversity index calculated was equal to 0.70.

3.3. Genetic relationship and clustering analysis

Binary data were used for computing Jaccard's similarity indices. The similarity coefficients based on 10 ISSR alleles ranged from 0.36 to 0.97. The highest similarity index (0.97) was observed between Kurdamir №9 and Saatly №16, while the lowest similarity (0.36) was noted between Kurdamir №4 and Saray №1 (Figures 4 and 5). A high genetic similarity was also found between Kurdamir №6 and Saatly №6 (0.94), Kurdamir №6 and Kurdamir №10, Sabirabad №15 and Absheron №2, Kurdamir №9 and Kurdamir №7, and Masally №1, Lankaran №3, and Masally (uzun) (0.91) accessions.

The similarity values obtained for each pairwise comparison of ISSR markers data were used to construct a dendrogram (Figure 6).

At 0.70 similarity level, 48 genotypes were grouped into ten clusters. Each of clusters 1, 2, 6, 7, 8, and 10 was homogeneous and composed of only one accession. Similarly, both clusters 3 and 9 contained only two accessions. Cluster 4 consisted of 4 accessions. Cluster 5 was highly heterogeneous and could be further divided into two subclusters, 5a and 5b. Although there were clear morphological differences among them, all other 32



2. Dendrogram of 48 *Cucumis melo* L. genotypes based on agromorphological data.

Table 3. Estimates of maximum, minimum, mean, genetic variance, environmental, and genetic coefficient of variation (CV) and broad sense heritability (h^2bs) for 11 agromorphological traits.

Traits	Min.	Max.	Mean	Genetic variance	Environmental CV%	Genetic CV (%)	h^2bs
Main stem length (cm)	125.89	159.50	138.34	121	6.38	7.95	0.98
Lateral stem number (no.)	4	5	4.16	0.282	13.24	12.76	0.48
Leaf length (cm)	7.12	12.7	8.89	0.412	3.98	7.22	0.77
Leaf width (cm)	8.28	13.48	9.94	0.284	5.14	5.36	0. 52
Seed length (mm)	10.85	12.4	11.42	0.082	2.1	2.51	0.58
Seed width (mm)	4.75	6	5.29	0.054	2.8	4.39	0.71
Stem diameter (mm)	9.87	12.68	11.44	0.167	3.04	3.57	0.58
Fruit length (cm)	15.28	29.87	20.33	12.44	2.65	17.35	0.97
Fruit width (cm)	14.88	24.2	18.36	4.135	7.85	11.1	0.65
Flesh thickness (cm)	3.3	4.75	4.06	0.092	2.8	7.47	0.88
Yield (t/ha)	9.98	23.32	13.93	8.01	3.97	20.32	0.96

Table 4. Genetic diversity index exhibited by ISSR primers.

Primers	Sequence (5'-3')	Melting temperature (T_m)	Total no. of bands	Number of polymorphic bands	Polymorphism (%)	Genetic diversity index
UBC840	$(GA)_8T$	47.4	5	5	100	0.77
UBC888	$TAC(CA)_7$	47.0	3	2	67	0.54
UBC825	$(AC)_5T$	51.4	5	4	80	0.77
UBC834	$(AG)_5YT$	49.2	4	3	75	0.52
ISSR857	$(AC)_8YT$	53.1	5	5	100	0.85
UBC860	$(TG)_5RA$	53.1	7	7	100	0.94
UBC818	$(CA)_8G$	52.1	3	2	67	0.58
UBC848	$(CA)_8RG$	50.6	3	2	67	0.67
ISSR-3	$TGTA(CA)_7$	43.0	4	4	100	0.76
ISSR35	$TCGA(CA)_7$	54.7	2	1	50	0.56
Mean			4.1	3.5	85.6	0.70

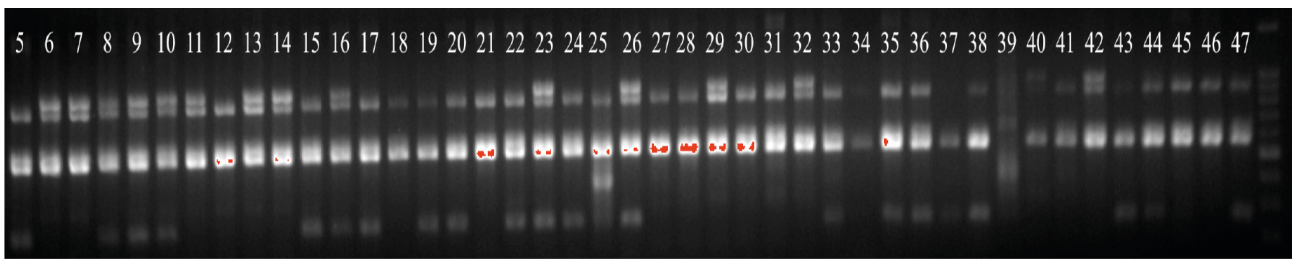


Figure 3. ISSR profile amplified by primer UBC857.

Figure 4. Kurdamir №4.

Figure 5. Saray №1 (Absheron, Saray).

genotypes collected from different regions were grouped in the same subcluster. This indicates that they are closely related and we have to use more polymorphic markers to differentiate their genetic relationship more clearly. The other subcluster holds only two genotypes.

The Mantel test to provide a comparison between similarity matrices extracted from agromorphological traits and ISSR markers was performed and the correlation between distance matrices was not found to be statistically significant (r = 0.012). Some researchers also found

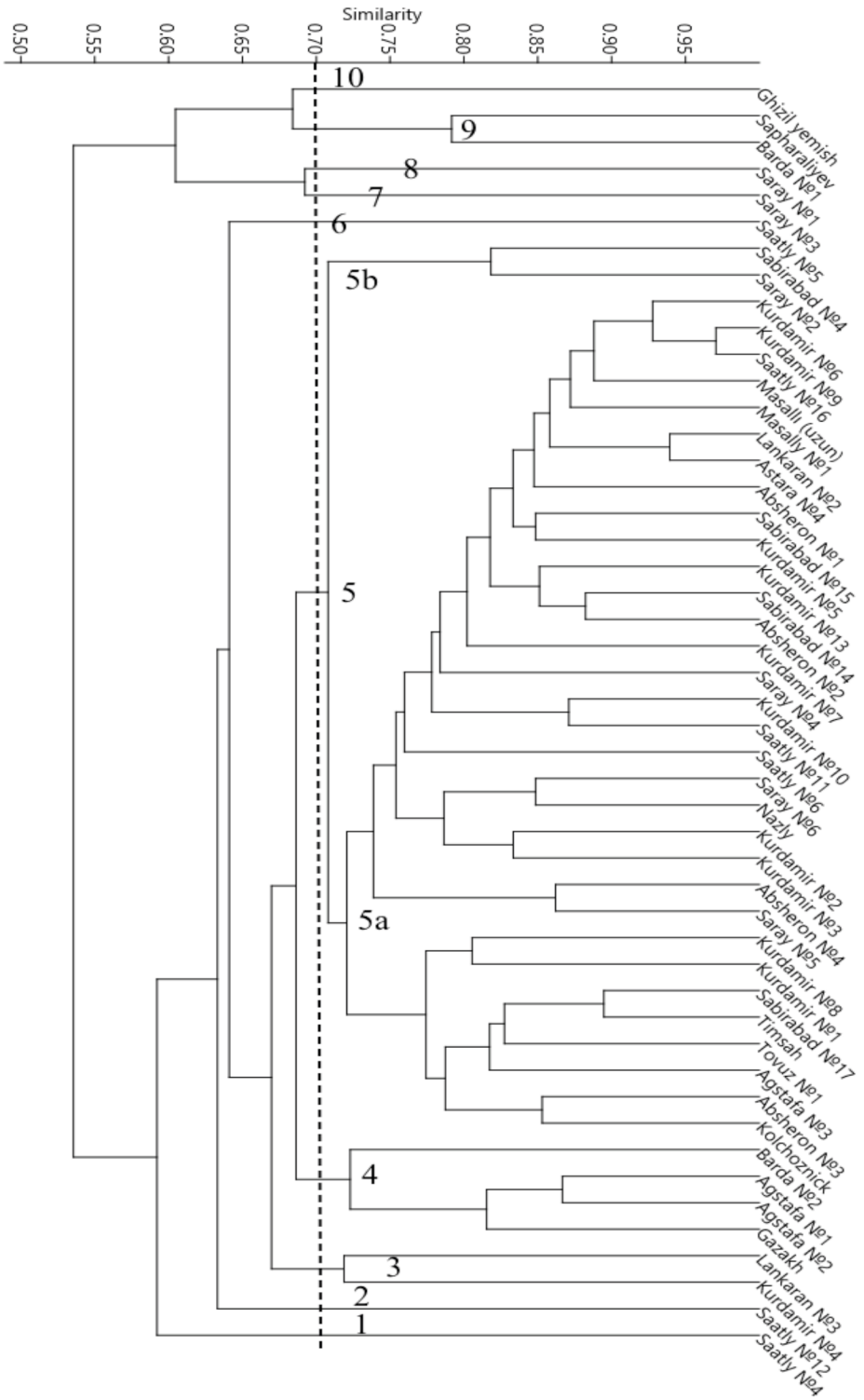


Figure 6. Dendrogram of 48 melon accessions generated by the data from 10 ISSR primers.

different results from the comparative analysis of genetic diversity among melon genotypes using morphological and molecular markers. *Cucumis* genotypes from different geographical areas of India were screened for genetic diversity using 19 morphological traits and 15 ISSR primers by Parvathaneni et al. (2011). They found that phenotypic

variation existed among the 13 *Cucumis* accessions studied and ISSR markers produced a clear difference between the North Indian genotypes. In other research, the genetic relationships among 13 melon inodorus populations were assessed using 100 ISSR primers and 15 morphological traits and good correlation between the morphological and molecular data was revealed. The authors confirmed the effectiveness of such a comparative approach (Sestili et al., 2011).

In our experiment the dendrogram based on ISSR analysis did not show any significant pattern of clustering according to geographic location. Some authors suggest that lack of association of genetic diversity with geographic location may be attributed to the substitution of seed materials by growers from different zones (Kumar et al., 2014). We also believe that the sharing of breeding materials could be the main reason for low diversity between genotypes collected from distant geographic locations in our experiment. On the other hand, ISSR markers did not show a significant correlation with agromorphological data, which can be explained by the environmental factors as reported for several other crops (Kumar et al., 2014; Jain et al., 2017). However, overall, our results displayed a sufficient amount of morphological and genetic variability within the local melon germplasm (Figure 6). This kind of morphological and genetic diversity has been commonly reported in different horticultural plants (Kamiloglu et al., 2009; Tosun et al., 2009; Ercisli et al., 2012; Dogan et al., 2014; Alp et al., 2016). The results of this study should be useful both for identification of distinct accessions and establishment of core collections in gene banks, as well as utilization of genotypes in breeding programs. Nevertheless, further comprehensive assessment using different types and more polymorphic molecular markers could be necessary for elucidating genetic relationships among those 32 accessions grouped into the same highly heterogeneous cluster based on Jaccard's similarity indices.

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