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Original scientific article

ANATOMICAL VARIABILITY AND POPULATION STRUCTURE OF *Cucumis melo* L. ACCESSIONS COLLECTED FROM IRAN

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Melon (*Cucumis melo*) is one of the most important cultivated cucurbits. In this project, the genetic variability among 14 accessions of melon collected by the authors has been studied using 23 anatomical characteristics across 2 consecutive years as well as 146 random amplified polymorphic DNA (RAPD) markers. Combined analysis of variance revealed significant effects on genotype and year × genotype effects. Among examined anatomical characters, the highest CV% values across two years belonged to fruit store at room temperature (88.96%, 103.6%) and total fruit weight (42.2%, 40.00%), while the lowest CV% values were observed in flower petal width (0.31%, 0.21%), flower petal length (0.28%, 0.49%), and peduncle length (0.40%, 0.19%). Classification of melon

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accessions based on anatomical characteristics using the Ward method produced three groups. The highest Jaccard's similarity coefficient (0.76) was observed between accessions "Atashi koluche" and "Atashi miyaneh" and the lowest value (0.49) was found between accessions "Bakermellon" and "Mashhadi" with the mean value of 0.59. In this study, OPA06 and OPB13 primers possessed greater efficiency in the genetic evaluation of the studied germplasm. Analysis of population structure, which imply on the existence of admixture in the studied melon germplasm, revealing three subpopulations. Accession "Sabzevari" identified as mixed subgroups. From the breeder's view, the introduced heterotic groups can be utilized in parental selection for the construction of mapping population and the identified population structure can prevent any false positive output in marker-trait association studies of melon.

Keywords: melon, RAPD, heterotic groups, population structure

INTRODUCTION

Melon (*Cucumis melo* L.) with a chromosome number of 2n =2x=24 is belonged to the family Cucurbitaceae, and as an important horticultural crop has been included in wild-types and numerous varieties, either consumed as a dessert fruit or a vegetable in tropical and subtropical regions. Central Asia, Afghanistan, India, Transcaucasia, Turkmenistan, Tajikistan, Uzbekistan, China and Iran are considered the primary diversity centers for melon (TZITZIKAS *et al.*, 2009). Globally, more than 31 million tons of melons were produced in 2017, where China, Turkey and Iran were the major producers, respectively (FAO, 2019). In Iran, farmers grew almost 79379 ha of melon with a total production of 1915735 tons (ANONYMOUS, 2018). Both varieties *Cantalupensis* and *Dudaim* originated from Persia and were cultivated in various areas of Iran (NESOM, 2011). Iranian melon cultivars differ widely in fruit size, morphology and taste, as well as in vegetative traits and climatic adaptation (LOTFI and KASHI, 1999). Breeders have attempted to evaluate these cultivars to discard identical accessions. A comparison of the plant phenotype is the simplest approach for the detection of mislabeled genotypes and the assessment of genetic diversity. Estimating and determining relationships among morphological variables in the melon germplasm can enhance the efficiency of its management and support effective genetic improvement efforts. Multivariate analysis methods such as cluster analysis and principal component analysis are useful approaches within this context (MOHAMMADI and PRASANNA, 2003). These methods have been used frequently for genetic diversity analysis in melon, *Cucumis melo* L. (SENSOY *et al.,* 2007; TRIMECH *et al.,* 2013; ABDEL-GHANI and MAHADEEN, 2014). However, phenotypic evaluation could be influenced by environmental conditions and might not distinguish between closely related genotypes (SENSOY *et al.,* 2007). Morphological markers have been known for a very long time and these visually observed markers are few in number and might have epistatic effects (MEGLIC and STAUB, 1996). While molecular DNA marker analyses, which are not affected by the environment have been suggested for the determination of genetic similarity among genotypes (GILBERT *et al.,* 1999). Markers aided by the polymorphisms in DNA structures have become popular in order to compensate for the disadvantages of morphological markers (SENSOY *et al.,* 2007). Molecular DNA marker methods have been increasingly employed in melon genetic studies (GARCIA-MAS *et al.,* 2000; NAKATA *et al.,* 2005; TZITZIKAS *et al.,* 2009). In most studies, random amplified polymorphism DNA

(RAPD) markers proved generally effective in the determination of genetic similarity among melon genotypes (GARCIA-MAS *et al.,* 2000; MLIKI *et al.,* 2001; STAUB *et al.,* 2004; TANAKA *et al.,* 2007; RAGHAMI *et al.,* 2014). Both analyses (phenotypic and molecular) indicated that non-sweet melon types were dissimilar from sweet types and the diversity of Turkish melon genotypes was higher than that of sweet foreign cultivars examined, but similar to that of the reference accessions employed (SENSOY *et al.,* 2007). In relation to Iranian melons, FEYZIAN *et al.* (2007) examined 38 melon accessions using RAPD analysis; however, they could not separate horticultural groups of melons. SOLTANI *et al.* (2010) studied melon accessions using RAPD markers and observed a high level of genetic diversity in var. *flexuosus* and a genetic similarity with reference accessions of large seed type (vars. *inodorus* and *cantalupensis*). This report showed a large variability in the Iranian melon germplasm, but it focused mainly on var. *flexuosus*.

The main objective of the present study was the simultaneous utilization of both molecular RAPD markers and phenotypic characteristics to identify genetic variability among melon accessions collected by authors from several districts in Iran as well as to determine the genetic structure of the studied melon germplasm.

MATERIAL AND METHODS

Plant material and experimental methodology

Seeds of fourteen Iranian melon accessions were collected by the authors from geographically diverse regions (Table 1) and cultivated in Miyaneh agriculture research station during 2019 and 2020. This station is located in the East-Azerbaijan province of Iran (longitude 47°42' E and latitude 37°20' N, altitude of 110 masl).

Table 1. Accession number, origin as well as membership percentage (Q values) of studied accessions to identified subgroups using STRUCTURE software

Code		Origin	Q-matrix			Subgroup
	Accession		Q1	Q2	Q3	
G01	Ananasi	Gorgan	0.03	0.91	0.07	Green
G02	Sefidkesh	Hamedan	0.03	0.96	0.02	Green
G03	Balo	Urmia	0.97	0.02	0.01	Red
G04	Keshavarz	Urmia	0.97	0.03	0.01	Red
G05	Sabzevari	Sabzevari	0.40	0.58	0.01	Mixed
G06	Harati	Khorasan	0.92	0.06	0.03	Red
G07	Khatooni	Khorasan	0.03	0.92	0.05	Green
G08	Tashkandi	Khorasan	0.01	0.98	0.01	Green
G09	Achachi	Miyaneh	0.03	0.93	0.04	Green
G10	Nikabadchae	Miyaneh	0.89	0.06	0.05	Red
G11	Bakermellon	Gorgan	0.86	0.03	0.11	Red
G12	Mashhadi	Khorasan	0.03	0.87	0.11	Green
G13	AtashiKolucheh	Miyaneh	0.03	0.01	0.96	Blue
G14	Atashimiyaneh	Miyaneh	0.01	0.08	0.91	Blue

Field preparation including semi-deep plowing, disc and other operations were performed according to the custom of the region in the middle and each experimental plot consisted of four rows. The dimensions of each experimental plot were considered 6 × 3 and about 50 cm from the beginning and end of each plot were considered the margin. The experiment was carried out in a randomized complete block design with three replications and to measure each morphological character according to the melon plant cultivation instructions, 9 plants were randomly selected from each plot.

Morphological characteristics

Based on standard phenotypic characteristics described for melon in biodiversity international and UPOV (1999), twenty three variables including: DF: Day to flowering, FPL: Flower petal length, FPW: Flower petal width, % 50 DF: Day to 50% flowering, LT: Leaf tail, FRT: Fruit ripening time, LL: Leaf length, LW: leaf width, ID: Internode distance, NAS: Number of arrows in stem, PL: Peduncle length, PD: Peduncle diameter, PS: Plant size, FFW: Fruit fresh weight, TFW: Total fruit fresh weight, FSRT: Fruit store at room temperature, TFF: Thickness of fruit flesh, NSPF: Number of seed per fruit, 100SW: 100-seed weight, SW: seed width, SL: seed length, FL: Fruit length, FW: Fruit width were measured in each year. Three fruits per genotype were hand harvested in each plot at the ripening stage and were transferred to the lab for further analysis.

Genomic DNA extraction and RAPD fingerprinting

Leaf tissues from at least 5 plants of each accession at the two-to-three leaf stages were sampled and mixed for employment in the CTAB protocol of DNA extraction (DOYLE and DOYLE, 1987). The concentration of extracted DNA was determined by spectrophotometer (BioPhotometer 6131; Eppendorf, Hamburg, Germany) at 260 nm. The quality of the extracted DNA was checked by running 1 µl DNA on 0.8% (w/v) agarose gel in 0.5× TBE buffer (45 mM Tris base, 45 mM boric acid, and 1 mM ethylenediamine tetraacetic acid (EDTA) pH 8.0). DNA samples with a smear on the gel were rejected.

In this study, 12 RAPD primers from Operon Technologies were chosen according to previous melon diversity analyses (MLIKI *et al.*, 2001; LOPEZ-SESE *et al.*, 2002; STAUB *et al.*, 2004) and were used for DNA fingerprinting (Table 3). PCR was performed in a 96-well Eppendorf Mastercycler Gradient (Type 5331, Eppendorf AG, Hamburg, Germany) with the following program comprising 5 min of heating at 94°C, 40 cycles of 60 s at 94°C, 63s at 36°C, 59 s ramps, 120 s 72°C a final extension reaction of 10 min at 72°C. Each PCR reaction included 30 ng DNA, 0.2 µM primer, 100 µM M dNTPs, 1 U Taq DNA Polymerase (Fermentas), 100 mM Tris– HCl, 1.5 mM MgCl2, and 50 mM KCl, pH 8.8, in a 15-µl final volume. The PCR products were mixed with dyes and resolved on a 2% (w/v) agarose gel in 0.5× TBE buffer. The gel was stained with ethidium bromide. Gels were photographed under UV light, using a Gel-Doc image analysis system (Gel Logic 212 PRO, USA).

Data analysis

For the field data, after the determination of outliers and normalization tests, a combined analysis of variance was done using SAS software. Also, descriptive statistics including mean,

standard deviation, and coefficient of variation (CV) were calculated for each studied character. Cluster analysis was applied to classify the studied melon accessions based on squared Euclidean distance and the Ward method using Minitab software. To avoid scaling errors, each morphological character was standardized using the Z-score.

A binary data matrix was obtained from scoring polymorphic RAPD bands (1 for presence and 0 for absence of bands) and then used for the calculation of the Jaccard (JACCARD, 1908) similarity coefficient between studied melon accessions. Then, the unweighted pair-group method using arithmetic average (UPGMA) along with principal coordinate analysis was employed in NTSYpc version 2.02 (ROHLF, 1997) to classify accessions. Total alleles (n_a), effective alleles (n_e), percentage of polymorphic loci, and Shannon index (I) were calculated by Popgene 1.13 (YEH *et al.*, 1999) software. Population structure was analyzed using a model-based Bayesian approach in the software package STRUCTURE 2.3.4 (PRITCHARD *et al.*, 2000). Five independent runs were performed setting the number of subpopulations (K) from 1 to 10, the Markov Chain Monte Carlo (MCMC) replication number both to 100,000, and a model for admixture and correlated allele frequencies. The K value was determined by the log-likelihood for each K; Ln P(D) = L(K) (ROSENBERG *et al.*, 2002). Since the distribution of Ln P(D) did not show a clear number of the true K value, delta K (ΔK) based on the second-order rate of change in the likelihood (ΔK) (EVANNO *et al.*, 2005) was used alternatively to identify a clear peak to represent the true K value. Inferred ancestry estimates of individuals (Q-matrix) were derived for the selected subpopulation (PRITCHARD *et al.*, 2000).

RESULTS AND DISCUSSION

Melon (*Cucumis melo* L.) has a high export value in the country of Iran and therefore from breeder's view the production of quality and market-friendly cultivars is vital. Accordingly, it is important to evaluate genetic variability among melon accession by either morphological or molecular markers. In this study, a high level of genetic diversity was seen in the studied melon germplasm was seen which is in accordance with previous studies in the species *C. melo* (BATES and ROBINSON, 1995). Results pertaining to the combined analysis of variance (Table 2) revealed significant genotype × year interaction for days to 50% flowering, fruit fresh weight, number of seeds per fruit, and fruit width in the %1 probability level and day to flowering, leaf tail, leaf length, thickness of fruit flesh, and 100-seed weight in the %5 probability level. Besides genotype × year interaction, there are significant differences among studied melon accessions based on morphological characters (Table 2) and these findings propose the existence of diverse melon germplasm that has possessed also differeing morphological reactions across years. According to the literature, significant differences were reported for fruit length, fruit width, fruit weight, seed weight and yield by NAROOEIRAD *et al*. (2009) and for number of fruits per plant, number of days to ripening, and yield by FEYZIAN *et al*. (2007). Descriptive statistics related to the studied characters were calculated in Table 3. Among characters examined in two consecutive years, the highest coefficient of variation belonged to fruit store at room temperature (88.96%, 103.6 %) and total fruit weight (42.2%, 40.00 %), while the lowest CV values were observed in flower petal width (0.31%, 0.21%), flower petal length (0.28%, 0.49 %), and peduncle length (0.40, 0.19 %) (Table 3). In this study, fruit ripening time as an effective characteristic of melon differed significantly among 14 genotypes studied and varied between 81

to 104 in the year 2019 and between 86 to 118 in the year 2020 (Table 3). ALI-SHTAYEH *et al.*, (2015) also reported the mean and CV values of 8.32 mm and 14.04% for stem thickness, 115.96 g and 32.99% for fruit size, as well as 3.6 g and 15.6 % for seed weight in melon accessions collected from Palestine.

Table 2. Combined analysis of variance for morphological traits of studied melon accessions

		MS											
Source	DF	DF	FPL	FPW	%50DF	LT	FRT	LL	LW	ID	NAS	PL	PD
Year	1	160.19**	3.687	1.31250*	443.440**	287.002**	3936.01**	75.052**	307.052**	13.681	654.646**	1.23993	1.4405
Rep(Year)	4	8.81	0.585	0.05607	8.810	4.573	22.83	1.194	5.181	1.109	0.628	0.03649	1.2738
Gen	13	22.47*	0.145	0.15045*	60.913	4.966	354.19**	4.035	7.534	3.456	20.132	0.10652	2.1438**
Year*Gen	13	8.03*	0.114	0.05404	35.697**	7.562*	39.11	3.467*	4.879	3.895	9.498	0.08428	0.1584
Error	52	3.84	0.141	0.05505	3.758	3.705	22.71	1.496	3.390	2.624	10.862	0.10145	1.3379

DF: Day to flowering, FPL: Flower petal length, FPW: Flower petal width, %50DF: Day to 50% flowering, LT: Leaf tail, FRT: Fruit ripening time, LL: Leaf length, LW: leaf width, ID: Internode distance, NAS: Number of arrows in stem, PL: Peduncle length, PD: Peduncle diameter

Table 2. Continued

		MS									
Source	DF	PS	FFW	TFW	FSRT	TFFF	NSPF	100SW	SW	SL	FL	FW
Year	1	215369**	51.3768**	75.6772**	13.29	20.8802**	1721884**	24.9501**	0.6696	48.7619*	932.301**	3325.15**
Rep(Year)	4	9278	0.0461	1.1753	73.30	0.5234	9078	0.3194	0.2679	1.2262	1.582	4.61
Gen	13	2353	0.7738	1.1302	2214.84**	0.2652	27901	2.2305	0.4819	2.1712**	64.187*	19.96
Year*Gen	13	1068	0.7729**	0.8400	146.53	0.3225*	30326**	1.0702*	0.4325	0.3132	20.432	26.64**
Error	52	835	0.2357	0.4691	170.21	0.1737	9085	0.5663	0.2903	0.8480	12.998	4.28

PS: Plant size, FFW: Fruit fresh weight, TFW: Total fruit fresh weight, FSRT: Fruit store at room temperature, TFF: Thickness of fruit flesh, NSPF: Number of seed per fruit, 100SW: 100-seed weight, SW: seed width, SL: seed length, FL: Fruit length, FW: Fruit width

Classification of studied melon accessions based on two years of data of anatomical characteristics by using the Ward clustering method and squared Euclidean distance has been separated into three main groups (Figure 1) named Green ("Ananasi", "Sefidkesh", "Achachi" and "AtashiKolucheh"), Red ("Keshavarz", "Atashimiyaneh" "Bakermellon") and Blue (other accessions). Herein, the classification pattern of accessions was not coincidental with geographical distribution and this is parallel with the findings of FABRIKI-OURANG *et al.,* (2009). The above-identified heterotic groups could be effective in future breeding programs of for melons through a hybrid breeding approaches as well as parental selection for the construction of mapping populations for the identification of genomic regions controlling anatomical characteristics. In this project, the genetic diversity among 14 studied melon accessions was evaluated using 12 RAPD primers (Table 4). In total, 146 alleles were detected and the number of alleles per locus varied from 10 to 14.

Table 3. Descriptive statistics related to studied morphological traits in 2 consecutive years

Trait	Year	Min.	Max.	Mean	St.Dev.	C.V	Trait	Year	Min.	Max.	Mean	St.Dev.	C.V
DF	2019	35.00	45.00	39.41	2.38	6.03	PS	2019	95.50	327.00	197.09	49.91	25.32
	2020	39.00	51.00	42.17	3.12	7.39		2020	59.00	155.00	97.26	21.68	22.29
FPL	2019	1.10	2.40	1.80	0.28	15.55	FW	2019	0.90	3.91	2.28	0.86	37.71
	2020	1.30	3.00	2.22	0.49	22.07		2020	0.27	1.35	0.70	0.21	30.00
FPW	2019	0.50	1.90	1.05	0.31	29.52	TFW	2019	0.90	4.85	2.64	1.12	42.42
	2020	0.40	1.40	0.80	0.21	26.25		2020	0.29	1.51	0.65	0.26	40.00
%50DF	2019	41.00	47.00	43.60	1.45	3.32	FSRT	2019	4.00	72.00	22.39	19.92	88.96
	2020	43.00	61.00	48.19	5.84	12.11		2020	3.00	90.00	23.33	24.17	103.60
LT	2019	12.30	19.60	15.77	1.65	10.46	TFF	2019	1.70	4.40	3.25	0.52	16.00
	2020	6.60	17.00	12.01	2.53	21.06		2020	1.50	3.40	2.25	0.43	19.11
FRT	2019	81.00	104.00	90.67	7.76	8.55	NSPF	2019	422.00	1002.00	645.20	147.60	22.87
	2020	86.00	118.00	104.36	9.77	9.36		2020	154.00	581.00	361.90	94.80	26.19
LL	2019	7.60	15.30	11.89	1.61	13.54	100SW	2019	3.15	8.62	5.29	1.16	21.92
	2020	7.50	12.80	10.00	1.34	13.4		2020	2.72	5.69	4.20	0.67	15.95
LW	2019	10.10	20.60	16.71	2.36	14.12	SW	2019	3.50	6.00	4.64	0.59	12.71
	2020	9.80	16.60	12.89	1.78	13.80		2020	4.00	6.00	4.82	0.58	12.03
ID	2019	2.90	8.90	5.33	1.49	27.95	SL	2019	11.50	16.00	13.46	0.90	6.68
	2020	3.30	10.40	6.14	1.88	30.61		2020	9.50	14.00	11.94	1.08	9.045
NAS	2019	7.00	16.00	11.99	2.23	18.59	FL	2019	11.40	30.70	22.27	4.95	22.22
	2020	8.00	24.00	17.57	4.27	24.30		2020	5.40	23.90	15.42	4.37	28.33
PL	2019	0.60	2.20	1.07	0.40	37.38	FW	2019	9.30	29.60	21.58	4.36	20.20
	2020	0.40	1.30	0.81	0.19	23.45		2020	5.70	10.90	8.99	1.30	14.46
PD	2019	3.00	8.00	5.24	1.12	21.37							
	2020	2.00	7.00	4.98	1.14	22.89							

DF: Day to flowering, FPL: Flower petal length, FPW: Flower petal width, %50DF: Day to 50% flowering, LT: Leaf tail, FRT: Fruit ripening time, LL: Leaf length, LW: leaf width, ID: Internode distance, NAS: Number of arrows in stem, PL: Peduncle length, PD: Peduncle diameter, PS: Plant size, FW: Fruit weight, TFW: Total fruit weight, FSRT: Fruit store at room temperature, TFF: Thickness of fruit flesh, NSPF: Number of seed per fruit, 100SW: 100-seed weight, SW: seed width, SL: seed length, FL: Fruit length, FW: Fruit width

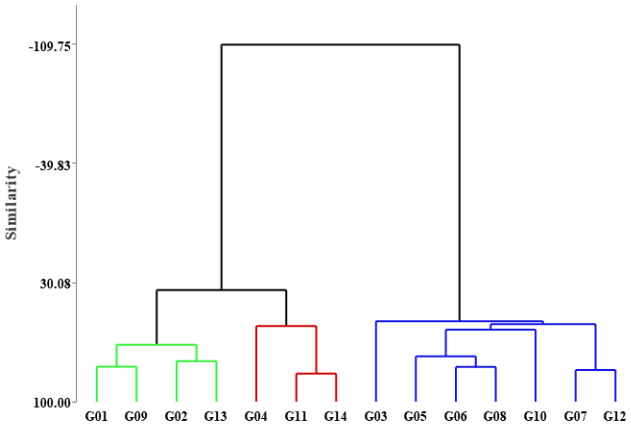

Figure 1. Classification of melon accessions based on morphological traits using Ward clustering method and squared Euclidean distances

The mean value for polymorphic bands (%) was 81.62%, Shannon index (I) was 0.38, number of alleles (na) was 1.81, and number of effective alleles (ne) was 1.34 (Table 4). The highest and lowest values of polymorphic bands (%) belonged to OPA06, OPB13 (100 %), and OPF10 (54.55%) (Table 4).

Table 4. Name, sequence, and characteristics of RAPD primers

Primer name	Sequence (5′-3′)	Number of bands	Number of polymorphic bands	Polymorphism (b/a × 100)	n_a	n_e	I
OPA06	GGTCCCTGAC	13	13	100	2.00	1.55	0.35
OPB13	TTCCCCCGCT	13	13	100	2.00	1.53	0.49
OPD10	GGTCTACACC	11	9	81.82	1.81	1.50	0.42
OPD20	ACCCGGTCAC	14	12	85.71	1.85	1.54	0.45
OPE14	TGCGGCTGAG	11	10	90.91	1.90	1.55	0.49
OPF10	GGAAGCTTGG	11	6	54.55	1.54	1.28	0.28
OPG02	GGCACTGAGG	15	12	80	1.8	1.49	0.41
OPG10	AGGGCCGTCT	13	9	69.23	1.69	1.25	0.27
OPG12	CAGCTCACGA	12	9	75	1.75	1.43	0.38
OPJ16	CTGCTTAGGG	10	9	90	1.90	1.42	0.42
OPJ18	TGGTCGCAG A	13	12	92.30	1.92	1.43	0.41
OPM12	GGGACGTTGG	10	6	60	1.6	1.32	0.30
Sum		146	120	-	-	-	-
Mean		12.16	-	81.62	1.81	1.34	0.38

n_a: number of alleles; n_e: effective alleles, I: Shanon index

In previous reports, statistical measures of genetic variation as measured by RAPD markers for Turkish and non-Turkish melon accessions revealed that the number of alleles in

each accession (na), Shannon's information index (I) as well as percentage of polymorphic loci are 58, 0.43, 89.9 %, respectively for Turkish accessions and 21, 0.47 and 94.5% respectively for non-Turkish accessions (SENSOY *et al.,* 2007). The polymorphism percentage, amplified alleles, and polymorphism information content was reported 74%, 84% and 94% in Iranian melon accessions (FABRIKI-OURANG *et al.,* 2009). Based on Jaccard's similarity coefficients (Table 5), the highest similarity coefficient (0.76) was observed between "Atashikoluche" and "Atashimiyaneh" and the lowest value (0.49) was found between "Bakermellon" and "Mashhadi" with the mean value of 0.59. The classification of melon accessions by using RAPD marker data and the method of principle coordinate analysis (Figure 2) also located into groups that were identified by cluster analysis. In agreement with anatomical classification, PCoA analysis depicted that some of the studied accessions could not be grouped exactly according to their geographical distribution (Figure 2). In agreement with our findings, DE AMORIM *et al.,* (2016) in the study of 15 Brazilian melon accessions SALEHI NAJAFBADI *et al.* (2010) and SOLTANI *et al.* (2010) in the study of 30 and 35 Iranian accessions had manifested that RAPD molecular marker data classification of melon accessions did not pursue their anatomical grouping as well as geographical distribution.

Table 5. Jaccard similarity coefficient between studied melon accessions

	Ananasi	Sefidkesh	Balo	Keshavarz	Sabzevari	Harati	Khatooni	Tashkandi	Achachi	Nikabadchae	Bakermellan	Mashhadi	AtashiKoluc	Atashimiyan
Ananasi	1.00													
Sefidkesh	0.69	1.00												
Balo	0.60	0.58	1.00											
Keshavarz	0.51	0.60	0.73	1.00										
Sabzevari	0.64	0.63	0.67	0.60	1.00									
Harati	0.56	0.56	0.71	0.66	0.73	1.00								
Khatooni	0.58	0.58	0.54	0.51	0.60	0.61	1.00							
Tashkandi	0.60	0.63	0.58	0.54	0.65	0.59	0.64	1.00						
Achachi	0.67	0.69	0.61	0.61	0.69	0.62	0.61	0.72	1.00					
Nikabadchae	0.56	0.55	0.64	0.59	0.56	0.66	0.53	0.57	0.66	1.00				
Bakermellan	0.54	0.53	0.58	0.61	0.53	0.57	0.51	0.47	0.55	0.64	1.00			
Mashhadi	0.50	0.55	0.54	0.50	0.56	0.55	0.59	0.59	0.58	0.50	**0.49**	1.00		
AtashiKoluc	0.57	0.51	0.59	0.51	0.56	0.61	0.51	0.50	0.59	0.57	0.58	0.55	1.00	
Atashimiyan	0.59	0.57	0.53	0.50	0.57	0.57	0.60	0.53	0.65	0.56	0.54	0.51	**0.76**	1.00

In order to understand the genetic structure of the studied melon panel, a model-based Bayesian approach in the STRUCTURE software was used to assign each accession to the corresponding subgroup. Following the EVANNO method in the STRUCTURE Harvester software, the optimal number of subpopulations was 3 (Figure 3A). The three subpopulations (Green, Blue and Red) are constructed based on their Q-values (Table 1) and depicted in Figure 3B. Considering Q-values (Table 1), each accession with Q-values below 0.7 was assigned as mixed. Regarding Table 1, these subgroups are "Balo", "Keshavarz", "Harati", "Nikabadchae", and "Bakermellon" as Red subgroup, "Ananasi", "Sefidkesh", "Khatooni", "Tashkandi", "Achachi",

and “Mashhadi” as Green subgroup and finally “Atashikolucheh”, and “Atashimiyaneh” as Blue subgroup. In this research, the “Sabzevari” accession was categorized as mixed based on their Q value (Table 1). It is inferred from population structure analysis that melon admixture information must be considered in any marker-traits association studies of melon in order to inhibit false positive associations (YU and BUCKLER, 2006; ZHANG *et al.*, 2012). This issue was proven in previous studies in several plants such as tobacco (BASIRNIA *et al.*, 2014) and wheat (GHAVAMI *et al.*, 2011).

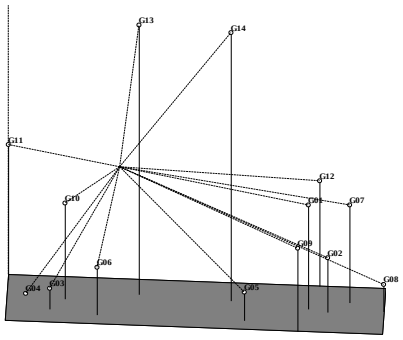

Figure 2. Illustration of melon accessions classification by principal coordinate analysis using RAPD data

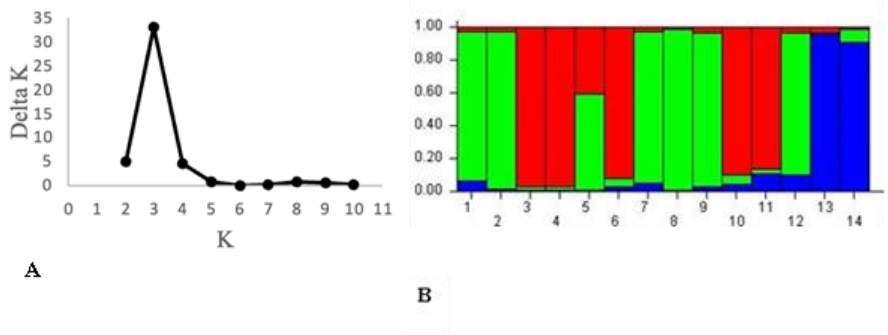

Figure 3. (A) Bilateral chart to determine the optimal number of K, (B) Genetic relatedness of 14 Iranian melon accessions as analyzed by the STRUCTURE program. The color of the bar indicates the three groups identified through the STRUCTURE program. Numbers on the y-axis indicate the membership coefficient and, on the x-axis, indicate the accession code. Accessions with the same color belong to the same subgroup.

CONCLUSION

To sum up, there is a vast amount of genetic variability among melon accessions collected by authors from different districts of Iran. Fluctuation of anatomical characteristics across two years, as well as significant genotype × year interaction, imply the existence of environmental variance and quantitative genetic control for most of the melon`s anatomical characteristics. Here, the RAPD marker technique along with anatomical study has been implemented for the evaluation of genetic variability and population structure analysis. OPA06 and OPB13 out of implemented RAPD primers were more suitable for the evaluation and depiction of genetic variability. Also, both anatomical and RAPD markers could efficiently distinguish and classify the studied melon germplasm into three groups that had no coincidental with their geographical distributions. Population structure analysis of studied melon germplasm depicted three subpopulations with "Sabzevari" as a mixed subpopulation. Totally, information about heterotic groups and subpopulations identified in this project can be utilized in future melon breeding programs like the QTL mapping approaches through bi-parental or genome-wide association analyses.

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ANATOMSKA VARIJABILNOST I STRUKTURA POPULACIJE *Cucumis melo* L. UZORAKA PRIKUPLJENIH IZ IRANA

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Izvod

Dinja (*Cucumis melo*) je jedna od najvažnijih gajenih biljaka iz porodice tikava. U ovom projektu, proučavana je genetska varijabilnost 14 vrsta dinje koje su sakupili autori koristeći 23 anatomske karakteristike tokom 2 uzastopne godine, kao i 146 DNK (RAPD) markera. Kombinovana analiza varijanse pokazala je značajne efekte genotipa i interakcije godina × genotip. Među ispitivanim anatomskim osobinama, najveće vrednosti CV% u toku dve godine imale su skladište voća na sobnoj temperaturi (88,96%, 103,6%) i ukupna masa ploda (42,2%, 40,00%), dok su najniže vrednosti CV% zabeležene kod širine latice cveta (0,31%, 0,21%), dužine cvetnih latica (0,28%, 0,49%) i dužine peteljke (0,40%, 0,19%). Klasifikacija uzoraka dinje na osnovu anatomskih karakteristika po *Ward* metodi dala je tri grupe. Najveći *Jaccard*-ov koeficijent sličnosti (0,76) zabeležen je između uzoraka „Atashi koluche" i „Atashi miianeh", a najniža vrednost (0,49) je utvrđena između uzoraka „Bakermellon" i „Mashhadi" sa srednjom vrednošću od 0,59. U ovoj studiji, OPA06 i OPB13 prajmeri su imali veću efikasnost u genetskoj proceni ispitivane germplazme. Analizom populacione strukture, koja ukazuje na postojanje primesa u proučavanoj germplazmi dinje, otkrivene su tri subpopulacije. Uzorci "Sabzevari" identifikovani kao mešovite podgrupe. Sa stanovišta oplemenjivača, uvedene heterotične grupe mogu se koristiti u roditeljskoj selekciji za mapiranje populacije, a identifikovana struktura populacije može sprečiti bilo kakav lažno pozitivan rezultat u asocijativnom proučavanju markera i osobina dinje.

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