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Original Article

Population structure, morphological and genetic diversity within and among melon (*Cucumis melo* L.) landraces in IranMasoud Maleki^a, Abdolali Shojaeiyan^{a,*}, Sajad Rashidi Monfared^b^a Department of Horticultural Science, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran^b Department of Agricultural Biotechnology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran

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ABSTRACT

ISSR markers were applied to evaluate the genetic diversity and differentiation of 270 individuals of 27 Iranian *C. melo* landraces of various varietal groups include vars. *inodorous*, *cantalupensis*, *reticulatus*, *ameri*, *dudaim*. Genetic diversity among the studied genotypes obtained by GeneAlix analysis ($H = 0.08$, $I = 0.12$, $N_a = 0.77$, $PPL = 22.6\%$). Cluster analysis divided Iranian melon landraces into two main cluster. Non-sweet genotype (*dudaim* group) was well separated from sweet genotypes (*inodorous*, *ameri*, *reticulatus*, *cantalupensis*). The most similar genotypes were BANI and TONI (0.95) and the most dissimilar ones were GER and TS (0.58). AMOVA result showed that the percentage of genetic variation among and within Iranian melon is 69% and 31%, respectively. All landraces evaluated based on 10 morphological traits which revealed the diversity of melon varietal groups. Bayesian analysis assigned ten landraces to Pop 1, eight landraces to Pop 2 and nine melon landraces to Pop 3. Bayesian and UPGMA cluster analyses demonstrated the almost related results. Our results indicated that ISSR markers technique alongside polyacrylamide gel analysis could be helpful to discriminate varieties of melon.

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1. Introduction

The genus *Cucumis* belongs to Cucurbitaceae family. Two commercially important species of the genus are melon (*C. melo*) and cucumber (*C. sativus*) [1]. Melon is a diploid species with $2n = 2x = 24$ chromosomes [2]. According to Kerje and Grum [3] Africa and Asia have been proposed as possible regions of origin. Besides the domestication of melon might have occurred independently or in parallel in Asia and Africa [3]. But according to Garg et al. [1] the center of diversity is Asia. Melon domestication has occurred around 3000 B.C. and it was cultivated in Egypt at 2000 B.C. and in India at 1000 B.C [1]. Melon spread from the Middle East (perhaps Iran) to Turkey, China, and Afghanistan (secondary centers of diversity) [4]. The earliest melon seeds were excavated in Iran and China (at 3000 B.C.). Iran (also known as Persia) was proposed as one of the main origin of melon [5], which through it and Caucasus melon were imported to Europe [3].

The world production of melon was estimated at 31.36 million t from 1.24 million ha of land in 2016, and it was 30.15 and 29.97 million t in 2014 and 2015 years, respectively. China alone accounted for half of the world's production of melon (51%). Iran and Turkey are in the third and second places with 5 and 6 percent of world's production, respectively [6].

So far, melon classification has been progressively evolving by adopting the propositions of many researchers [7–9]. Furthermore, many investigations have been carried out to assess genetic diversity in germplasm throughout of the world [10–13]. Pitrat et al. [9] in an overview of infra-specific nomenclature proposed 16 varieties (synonym. Groups), five of which can be assigned to subsp. *agrestis* and 11 to subsp. *melo*, include: *conomon*, *makuwa*, *chinensis*, *acidulus* and *momordica* within the subsp. *agrestis*, and *cantalupensis*, *reticulatus*, *adana*, *chandalak*, *ameri*, *inodorus*, *flexuosus*, *chate*, *tibish*, *dudaim* and *chito* within subsp. *melo*.

Genetic erosion or losing the endemic germplasms often occur due to the presentation of new cultivars and ignoring landraces [14]. Study of genetic variation not only is used for organization and protection plant materials but also is important for heterosis and hybrid seed production. Organization of plant genetic resources involves creation, enrichment, evaluation, documentation, and conservation of plant collections [15]. Rao and Hodgkin

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[16] noted that the elucidation of the vastness and dispensation of the different aspects of genetic diversity in a species is one of the main necessities to detect what, where and how to conserve. They are among the most essential of the world's natural resources, so during the last 2–3 decades major improvement has been made in conserving plant genetic resources.

Molecular markers have been functional tools for plant breeding and activities associated with the conservation of genetic resources. Various molecular and morphological markers are used to assess diversity among the landraces. Up to now, some markers have been used to characterize genetic diversity in Iranian melons, include Amplified Fragment Length Polymorphism [17] Simple Sequence Repeat [18,19], Random Amplified Polymorphic DNA [20,21] and morphological marker [20–22]. This study aimed to identify and collect melon landraces from all over Iran, as a primary diversity center, to assess some endemic Iranian melon landraces which most of them have not yet evaluated, regarding population structure, genetic distance, and morphological traits.

2. Materials and methods

2.1. Plant material

This study was conducted at Faculty of Agriculture of Tarbiat Modares University, Tehran, Iran during 2012–2013 years. Ten individuals of each of 27 Iranian melon landraces of various regions (from seven provinces) were evaluated for intra-landraces variation (Table 1, Figs. 1 and 2). All the landraces were open-pollinated which gathered from local farmers. Based on Pitrat et al. [9] botanical classification, all of the landraces were classified into subsp. *melo*, involving 5 varieties (vars. *inodorus*, *cantalupensis*, *reticulatus*, *ameri*, *dudaim*) and one intermediate variety (IV) (Table 1).

2.2. Morphologic traits

The experiment was carried out in the Randomized Complete Block design with three replications. Five plants per replication were grown such that row and within row spacing were 2.6 m and 0.5 m, respectively. Qualitative and quantitative traits selected according to the descriptor of the International Plant Genetic Resources Institute (IPGRI) have shown in Table 2. The qualitative traits include plant growth habit, fruit shape, primary color of immature fruit, main color of flesh, fruit ribbing. The quantitative traits were days to first mature fruit (DT), average fruit weight per bush (AF), skin thickness (ST), total soluble solid (TSS) and flesh thickness (FT).

2.3. DNA extraction

Fifteen to 20 seeds of each landrace were germinated at 30 °C in Petri dishes containing a wet filter paper for 6–7 days at incubator. Germinated seeds were planted in pots containing standard pot mixture and transferred into the greenhouse until seedling developed two to three true leaves. Fresh and juvenile leaves were put in sealed plastic bags containing 50–75 g silica gel to the accelerate desiccation process. Leaf tissues of 10 individual plants of each landrace were used for genomic DNA extraction according to CTAB modified method [23]. The concentration and quality of extracted DNA were determined by reading at 230, 260 and 280 nm using spectrophotometer (Epoch Microplate Spectrophotometer, Biotek, USA). DNA samples were diluted to a final concentration of 10 ng/μl by 1X TE buffer and stored at –20 °C prior to PCR amplification.

2.4. ISSR analysis

A total of 23 Inter-simple sequence repeat (ISSR) primers were initially screened in 10 representative samples from the 27 lan-

Table 1
Details of 27 melon landraces in the present study.

No.	Code	Local name	Origin City	Horticultural variety (group)	Latitude	Longitude
1	GER	Gergah	Gilan-e Gharb	var. <i>dudaim</i>	34°10'N	46°00'E
2	MIKE	Mirpanj	Kermanshah	var. <i>inodorus</i>	34°18'N	47°04'E
3	GES	Garmak	Esfahan	var. <i>reticulatus</i>	32°38'N	51°39'E
4	SHA	Talebi Shahabadi	Esfahan	var. <i>reticulatus</i>	32°38'N	51°39'E
5	GOR	Gorgab	Esfahan	var. <i>inodorus</i>	32°38'N	51°39'E
6	KONA	Komboze	Najafabad	var. <i>inodorus</i>	32°45'N	51°15'E
7	AHAR	Ahar	Ahar	var. <i>ameri</i>	38°28'N	47°04'E
8	TSAV	Talebi Saveh	Saveh	var. <i>reticulatus</i>	35°01'N	50°21'E
9	BANI	Barge Ney	Mahallat	var. <i>inodorus</i>	33°54'N	50°27'E
10	TONI	Tozard	Mahallat	var. <i>inodorus</i>	33°54'N	50°27'E
11	ZANI	Zard	Mahallat	var. <i>inodorus</i>	33°54'N	50°27'E
12	BONI	Bodagh	Mahallat	var. <i>inodorus</i>	33°54'N	50°27'E
13	AISI	Alashalta Sijaval	Bandar Torkaman	IV*	36°54'N	54°04'E
14	KSI	Khiyari Sijaval	Bandar Torkaman	IV	36°54'N	54°04'E
15	KHM	Khatoni	Nishapur	var. <i>inodorus</i>	36°12'N	58°47'E
16	NB	Nishbaba	Nishapur	var. <i>ameri</i>	36°12'N	58°47'E
17	SANI	Sabz	Nishapur	var. <i>inodorus</i>	36°12'N	58°47'E
18	GNI	Garmak	Nishapur	var. <i>reticulatus</i>	36°12'N	58°47'E
19	GB	Ghanat Boshroye	Nishapur	var. <i>inodorus</i>	36°12'N	58°47'E
20	TS	Tashkandi	Nishapur	var. <i>inodorus</i>	36°12'N	58°47'E
21	TISA	Til Sabz	Nishapur	var. <i>cantalupensis</i>	36°12'N	58°47'E
22	TA	Til Atashi	Nishapur	var. <i>cantalupensis</i>	36°12'N	58°47'E
23	TM	Til Magasi	Nishapur	var. <i>cantalupensis</i>	36°12'N	58°47'E
24	KN	Khaghani	Nishapur	var. <i>inodorus</i>	36°12'N	58°47'E
25	AV	Eyvanaki	Garmsar	var. <i>inodorus</i>	35°13'N	52°20'E
26	HM	Hajmoshalahi	Gonabad	var. <i>inodorus</i>	34°21'N	58°41'E
27	CP	Chahpaliz	Gonabad	var. <i>inodorus</i>	34°21'N	58°41'E

* IV = Intermediate Varieties.

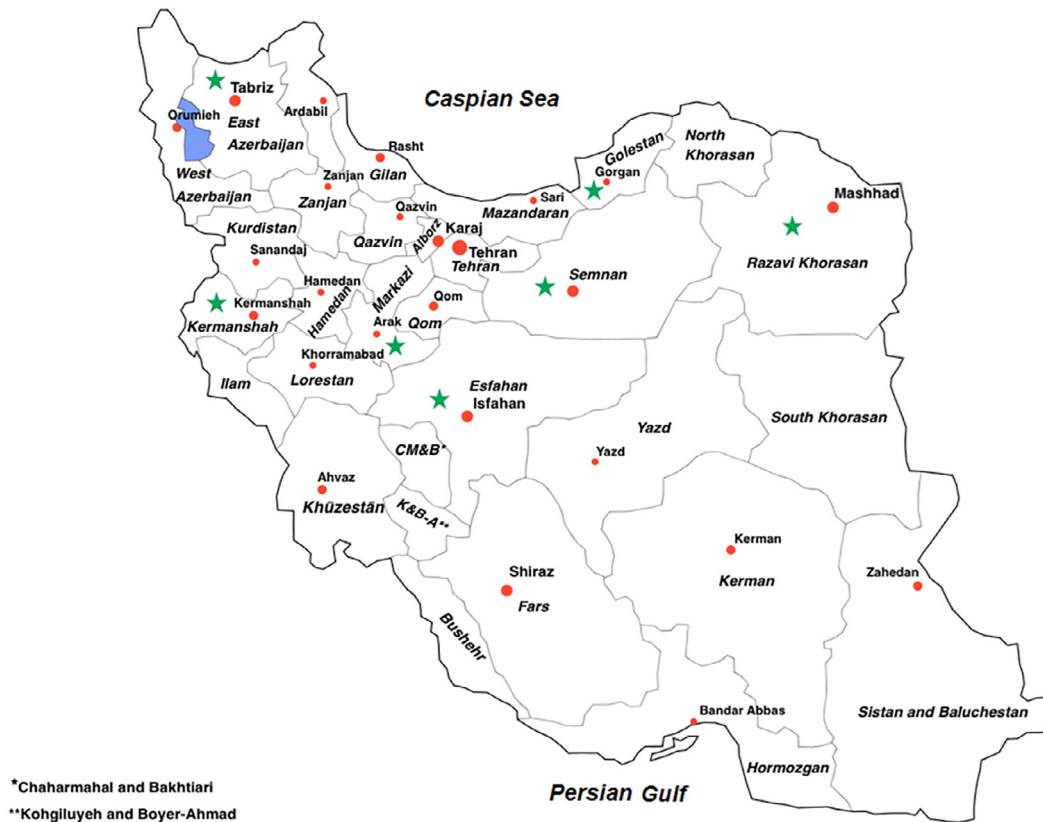


Fig. 1. Map of Iran showing provinces where landraces have been collected (shown with a green asterisk). These provinces including Esfahan (Esfahan & Najafabad cities), Markazi (Saveh & Mahalat cities), Kermanshah (Kermanshah & Gilan-e Gharb cities), East Azerbaijan (Ahar city), Golestan (Bandar Torkaman city), Razavi Khorasan (Gonabad & Nishapur cities), Semnan (Garmsar city).

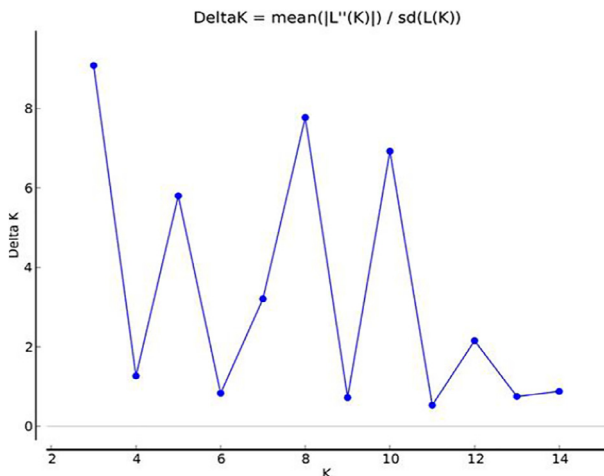


Fig. 2. For the studied melon landraces based on the ISSR marker data, value of ΔK from structure analysis as a function of K , calculated following the ΔK methods as proposed by Evanno et al. [33]. The modal value of these distributions indicates the true K or the highest level of structure, here three subgroups.

draces. Ten primers which produced clear, reproducible and polymorph fragments were selected (Table 3). Polymerase chain reaction (PCR) mixture contained: 20 ng DNA, 1.15 mM MgCl_2 , 0.77 μM primer, 0.15 mM dNTPs, 1X PCR buffer, 0.5 unit of Taq DNA polymerase (Smart taq DNA polymerase, Sinaclon™, Iran) in a total volume of 13 μL . The ISSR PCR was performed as follow: initially, 3 min of denaturing at 95 °C, 1 min of denaturing at 95 °C,

1 min annealing at 30–55 °C (depending on primer) and 2 min of elongation at 72 °C for 34 cycles. A final cycle was allowed at 72 °C for 10 min. The PCR of ISSR were carried out in a Thermocycler (C 1000TM Thermal Cycler, BIORAD, USA). The PCR products were analyzed on 10% polyacrylamide gels in 1x TBE buffer running at 400 v for 1 h and then silver stained according to the reported protocol [24]. Inter-microsatellite bands sizes were estimated comparing their migration with DNA size marker (GeneRuler 100 bp Plus DNA Ladder 100–3000 bp, Fermentas, USA).

2.5. Statistical analysis

2.5.1. Morphological traits

Means and SE means of all quantitative traits were calculated by Minitab 17 (Minitab Inc., State College, PA, U.S.A.). SAS statistical software (V. 9.0) was applied to reveal relationship among varieties with regard to metric traits (Table 4). Orthogonal contrasts analysis was used to compare melon groups with respect to morphological traits.

2.5.2. ISSR genotyping

A binary data matrix (presence (1)/absence (0)) obtained from scoring polymorphic bands. Nei's genetic distance applied to draw Polar dendrogram based on UPGMA (Unweighted pair-group method using arithmetic average) by PowerMarker software [25] and visualized with FigTree v1.4.3 software [26]. The computer program GeneAlex 6.501 [27] was used to calculate statistical measures of genetic variation (i.e., Nei's gene diversity [28]), Shannon's information index and percentage of polymorphic loci (PPL) for Iranian melon genotypes. In addition, Analysis of molecular variance

Table 2
Qualitative and quantitative traits evaluated on 27 melon landraces collected from seven provinces of Iran.

Landraces	PGH [*]	FSH [*]	PCIF [*]	MCF [*]	FRI [*]	DT [*] (day)	AF [*] (kg)	RT [*] (mm)	TSS [*] (°Brix)	FT [*] (mm)
MIKE	Indeterminate	Acorn	Light green	White	Not present	108	2.81	3.21	11.77	41.41
KHM	Indeterminate	Elliptical	Light green	White	Intermediate	100.5	1.92	2.6	11.62	30.9
GOR	Indeterminate	Ovate	Light green	White	Not present	105	2.08	1.5	7.4	37.7
BONI	Indeterminate	Acorn	Intermediate	White	Not present	103	3.16	4.89	8.38	25.86
KONA	Indeterminate	Ovate	Light green	White	Not present	89.67	1.98	2.35	10.03	28.72
BANI	Indeterminate	Elongate	Light green	White	Not present	101.3	1.7	3.38	10.48	26.8
TONI	Indeterminate	Acorn	Intermediate	Salmon	Not present	96.7	2.7	4.12	10.19	34.15
ZANI	Indeterminate	Acorn	Light green	White	Not present	103.58	2.01	3.99	10.07	30.86
SANI	Indeterminate	Elongate	Dark green	Pale green	Intermediate	100.93	1.98	1.21	11.96	26.96
AV	Indeterminate	Elliptical	Light green	White	Not present	100.83	2.29	2.74	11.94	31.59
TS	Indeterminate	Elliptical	Intermediate	Pale orange	Intermediate	78.47	1.46	4.27	11.6	28.43
KN	Indeterminate	Elliptical	Intermediate	Pale green	Superficial	104	2.48	2.22	11.7	31.85
CP	Indeterminate	Elliptical	Light green	Pale orange	Not present	103	1.48	1.95	10.67	25.62
GB	Indeterminate	Elongate	Light green	White	Superficial	107.67	2.34	2.3	9.5	30.07
HM	Indeterminate	Elongate	Intermediate	Pale green	Superficial	100.5	2.06	3	9.63	24.65
TSAV	Compact	Globular	Light green	Green	Intermediate	86	1.52	3.11	8	33.53
GES	Compact	Oblate	Light green	Pale orange	Superficial	65.63	1.19	0.83	6.75	26.05
SHA	Dwarf	Oblate	Light green	Pale orange	Deep	78.83	1.92	0.78	5.8	26.96
GNI	Compact	Oblate	Intermediate	Pale orange	Superficial	89.33	1.1	0.57	5.57	31.04
TISA	Indeterminate	Globular	Light green	Green	Superficial	84.55	1.29	2.48	7.23	29.28
TA	Indeterminate	Globular	Light green	Salmon	Superficial	91.53	2.09	1.88	8.02	33.68
TM	Indeterminate	Ovate	Light green	Pale green	Superficial	89.1	1.58	2.31	9.37	27.85
AHAR	Determinate	Ovate	Dark green	Pale orange	Not present	86.42	0.8	1.27	11.34	24.35
NB	Determinate	Ovate	Dark green	Pale orange	Not present	85.17	1.02	2.26	11.4	28.4
GER	Dwarf	Oblate	Intermediate	Pale green	Not present	63.11	0.34	0.71	6.05	16.4
ALSI	Determinate	Globular	Intermediate	Pale green	Superficial	71.67	1.19	2.47	10.09	31.66
KSI	Determinate	Elongate	Dark green	White	Not present	89.33	1.43	1.72	11.46	28.69
Max	Indeterminate	ND ^{**}	Light green	White	Not present	108	3.16	4.89	11.96	43.90
Min	Dwarf	ND ^{**}	Dark green	Salmon & Green	Deep	63.11	0.34	0.57	5.57	15.80
Mean						91.99	1.78	2.37	9.55	29.678
SE Mean						1.43	0.07	0.13	0.24	0.55

* PGH: plant growth habit, FSH: fruit shape, PCIF: primary color of immature fruit, RT: rind thickness, MCF: main color of flesh, FRI: fruit ribbing, DT: days to first mature fruit, AF: average fruit weight per bush, RT: skin thickness, TSS: total soluble solid, FT: Flesh thickness.

** ND: Not Dominate.

Table 3
Genetic features of 10 ISSR primers used for the study.

ISSR	Primer sequence	Tm (°C)	Total bands	Polymorphism (%)	PIC
1	5'-(TG) ₈ G-3'	41.9	20	45.00	0.20
2	5'-(AG) ₈ T-3'	29.9	26	73.08	0.36
3	5'-(GA) ₈ T-3'	30.3	21	80.95	0.25
4	5'-(GA) ₈ YC-3'	35.8	17	29.41	0.15
5	5'-(AC) ₈ YG-3'	43.6	20	10.00	0.27
6	5'-(CA) ₈ A-3'	52.7	14	35.71	0.36
7	5'-(AC) ₈ G-3'	54.9	10	40.00	0.15
8	5'-(GAA) ₆ -3'	47.8	16	62.50	0.29
9	5'-(GACA) ₄ -3'	48	13	76.92	0.30
10	5'-(GA) ₈ A-3'	50	16	37.50	0.31
Mean			17.30	50.29	0.26

Table 4
Mean contrast of five metric traits in five horticultural groups and one intermediate group (IV) of Iranian melon base on orthogonal contrasts analysis.

	N	DT [*]	RT [*]	AF [*]	TSS [*]	FT [*]
<i>inodorus</i>	15	100.21 a	2.92 a	2.17 a	10.46 a	91.11 a
<i>reticulatus</i>	4	79.95 c	1.32 d	1.44 bc	6.53 c	94.07 a
<i>cantalupensis</i>	3	88.39 b	2.22 b	1.65 b	8.21 b	90.82 a
<i>ameri</i>	2	85.79 bc	1.77 c	0.91 cd	11.37 a	79.12 b
<i>dudaim</i>	1	63.11 d	0.71 e	0.34 d	6.05 c	49.20 c
IV	2	80.50 c	2.09 bc	1.31 bc	10.78 a	90.51 a

*: Significant at 0.05 level.

DT: days to first mature fruit, AF: average fruit weight per bush, RT: skin thickness, TSS: total soluble solid, FT: Flesh thickness.

(AMOVA) procedure was used to calculate the partitioning of genetic variance among and within populations.

Polymorphism information content (PIC) of the ISSR markers was calculated in an Excel file by following formula: $PIC_i = 2 f_i (1 - f_i)$, where the PIC_i is the polymorphic information content of mar-

ker 'i', f_i is the frequency of the amplified fragments (band present) and $1 - f_i$ is the frequency of non-amplified fragments (band absent) [29,30] (Table 3). PIC for dominant markers (like ISSR) ranged from 0 to 0.5 but ranged from 0 to 1 for co-dominant markers (such as SSR) [31].

2.5.3. Population structure

To detect population structure, estimating the number of populations (K) and to assign individuals to one or more of these populations (K) the Bayesian analysis of population structure was carried out on the entire data set using the program STRUCTURE version 2.3 [32]. The number of genetically distinct clusters (K) was adjusted to range from 1 to 15. The model was run for 10 independent simulations for each K, used a burn-in length of 10,000 and a run length of 100,000 iterations. Next, the program's dominant marker settings, the "no admixture" model was used, and uncorrelated allele frequencies among populations were assumed. Since the distribution of log likelihood L(K) often did not indicate a mode for the true K, an *ad hoc* measure ΔK proposed by Evanno et al. [33] was used to ascertain the true K present in the ISSR marker data.

3. Results

3.1. Morphological comparisons and field observations

Based on 10 morphological characteristics are provided in Table 2, almost most of the landraces (66.7%) had indeterminate growth habit, rest of them had determinate (14.8%), compact (11.1%) and dwarf (7.4%) growth habit. Six fruits shape were observed in the studied landraces (Table 2) including; elongate, elliptical and ovate fruits, with the same frequencies (18.5%). Similarly, globular, acorn and oblate fruits with the same and low abundances (17.8%). There were three different primary colors for immature fruits: light green (55.5%), intermediate (29.6%) and dark green (14.8%). White, pale orange, pale green and salmon colors for flesh were observed at 37.0, 25.9, 22.2 and 7.7 percent of fruits, respectively. Fruit ribbing wasn't observed at 48.1% of landraces in the present study, the remained landraces presented intermediated (14.8%), superficial (33.3%) and deep (3.7%) fruit ribbing (Table 2).

Horticultural groups (vars. *inodorus*, *cantalupensis*, *reticulatus*, *ameri*, *dudaim*, and an intermediate variety of melon) indicated various values of quantitative characteristics which all were related to fruits. Mean days to mature fruits differed significantly among landraces and ranged from 63.11 days (for GER landrace) to 108 days (for MIKE landrace). Time of maturity according to the descriptor of IPGRI divided into four main classes: early (<70 days), intermediate (70–90 days), late (91–110 days) and very late (>110 days). This trait was significantly different among the six groups with the *inodorus* > *cantalupensis* > *ameri* > IV > *reticulatus* > *dudaim* (Table 4). Therefore, the *inodorus* variety had the latest mature fruit among the horticultural groups. The *cantalupensis*, *ameri* and *reticulatus* varieties had intermediate mature fruit. The earliest mature fruits among horticultural groups were founded in *dudaim* variety with 63.11 days to the first mature fruit. The very late mature fruit wasn't observed among the landraces.

Rind thickness was different among the horticultural groups with the *inodorus* > *cantalupensis* > IV > *ameri* > *reticulatus* > *dudaim*. Means ranged from 0.71 (*dudaim*) to 2.92 (*inodorus*) mm among the Iranian melon varieties. Although differences between *cantalupensis* and IG, *ameri* and IG were not significant statistically (Table 4).

Average fruit weight per bush differed among the horticultural groups: *inodorus* > *cantalupensis* > *reticulatus* > IV > *ameri* > *dudaim*. Means ranged from 0.34 (*dudaim*) to 2.17 (*inodorus*) kg. There was no significant difference among *cantalupensis*, IV and *reticulatus* groups. In addition, there were no significant differences among *reticulatus*, *ameri* and IV groups (Table 4).

TSS differed among the groups with *ameri* > IV > *inodorus* > *cantalupensis* > *reticulatus* > *dudaim*. Mean of TSS ranged from 6.05

(*dudaim*) to 11.37 (*ameri*) °Brix. Among *ameri*, IV groups and *inodorus* and between *reticulatus* and *dudaim* varieties there were no significant differences based on orthogonal contrasts analysis (Table 4).

Flesh thickness means ranged from 49.20 (*dudaim*) to 94.07 (*reticulatus*) mm. It differed among horticultural groups with *reticulatus* > *inodorus* > *cantalupensis* > IV > *dudaim*. Though there were no significant differences among *inodorus*, *reticulatus*, *cantalupensis* and IV groups (Table 4).

3.2. Population structure & molecular characterization using ISSR markers

Up to 25 ISSR primers were evaluated in the present study. Consequently, 10 ISSR primers which produced high levels of polymorphism and clear fragments were selected to DNA fingerprinting of 270 individuals of melon landraces. A total of 173 markers were detected among all subjected melon genotypes which 87 markers were polymorph. The average numbers of generated ISSR markers were 17.30 markers per primer and the level of polymorphism was 50.29%. The number of polymorphic markers detected by each primer varied from 2 (5'-(AC)₈G-3') to 17 (5'-(GA)₈T-3'). The highest polymorphism percent was related to P3 ((GA)₈T). PIC for each primer ranged from 0.15 to 0.36 with a mean value 0.26 (Table 3). High value of PIC was related to (AG)₈T primer which resulting in more efficiency as compared to the other primers. Two of these ISSR primers were very informative (PIC > 0.35) with the highest PIC values recorded for (AG)₈T and (CA)₈A.

Based on the statistical variation measures, the PPL ranged from 12.5% (MIKE landrace) to 35.23% (TONI and ZANI landraces), with an average of 22.6%. The average effective number of alleles per locus was 1.1740. Nei's gene diversity (H) varied from 0.044 (HM and MIKE landraces) to 0.140 (TONI landrace), with an average of 0.08, and Shannon's information index (I) ranged from 0.067 (HM landrace) to 0.186 (ZANI landrace), with an average of 0.121.

The UPGMA tree based on Nei's genetic distance was depicted in Fig. 3(B) which the 27 populations were separated to two main clusters (C1 and C2). According to the dendrogram, GER was the most distant landrace and was alone in cluster 1 (White highlight) (Fig. 3B). Within C2 two main branches were defined, subcluster 1 (SC1) and subcluster 2 (SC2). Within each of the SC1 and SC2 subclusters, two groups, G1, G2 in SC1 and G3 and G4 in SC2, respectively, were ascertained (Fig. 3B). In SC1 all landraces had sweet and edible fruits. The G1 includes 17 landraces, all from the vars. *inodorus* (15 landraces, Pink highlight) and *ameri* (two landraces, Blue highlight). In this group, TONI, BANI, BONI and ZANI landraces had a similar geographic origin. In the G2, KSI and ALSI landraces were belong to the same region. According to morphological traits, ALSI landrace had characteristics such as smooth (special trait for group *cantalupensis*) and without ribs skin (special trait for group *reticulatus*) which indicated it is an intermediate variety, but according to molecular analysis ALSI landrace alongside KSI landrace were placed in the same group (G2, Purple highlight) (Fig. 3B). Subcluster 2 includes seven landraces which divided into two groups (G3 and G4, Red and Yellow highlight) (Fig. 3B). *cantalupensis* and *reticulatus* varieties genetically well separated from each other as evidenced at the dendrogram. All landraces in group 3 were belonged to var. *cantalupensis*. The G4 had three landraces which all belong to var. *reticulatus*.

Based on Nei's genetic distance matrix (data not were shown), the most similar genotypes were BANI and TONI (0.95) followed by BANI and ZANI (0.94), AHAR and NB (0.94); the most dissimilar ones were followed by GER and TS (0.58), GER and GOR (0.59). GER as a separate landrace in clustering had the most similarity (0.72) with GES landrace. The cophenetic correlation coefficient estimated by Mantel's test and revealed a high and significant cophenetic

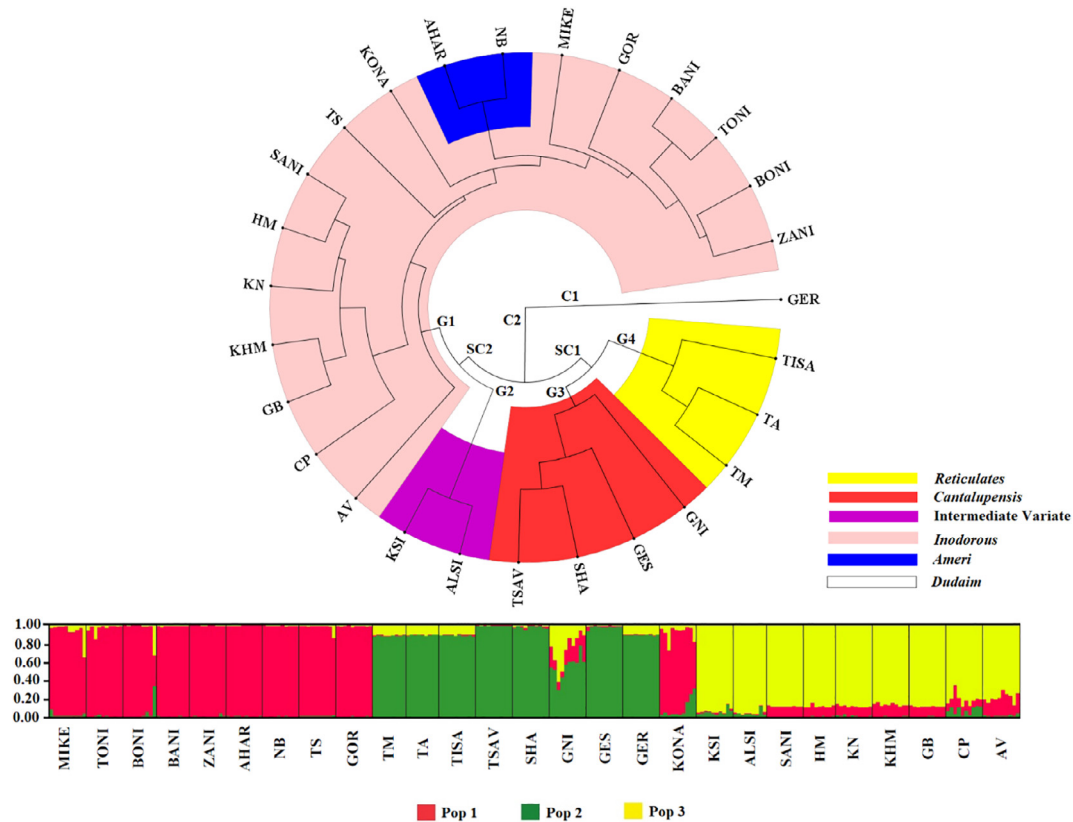


Fig. 3. (A) Polar dendrogram based on UPGMA method and Nei [28] genetic distances among landraces. (B) Results based on $K = 3$ using a Bayesian framework implemented in the STRUCTURE program across individuals from the studied landraces.

netic correlation coefficient ($r = 0.863$) between cluster analysis and the similarity coefficient matrix.

Analysis of molecular variance (AMOVA) showed that the percentage of genetic variation among and within Iranian melon landraces are 31% and 69%, respectively. A high significant ($P < 0.001$) genetic difference was found among and within populations.

The $L(K)$ obtained by STRUCTURE did not show a clear cutoff for the true subgroups with K ranging from 1 to 15 (data not shown), and thus the *ad hoc* measure ΔK was used to infer the subgroups. For the entire melon landraces herein, an obvious optimum regarding ΔK was observed at $K = 3$ (Fig. 2). Ten melon landraces were assigned to Pop 1, eight landraces to Pop 2 and nine landraces to Pop 3 (Fig. 3B).

4. Discussion

Various studies have investigated the molecular and morphological variation of melon genotypes in Iran. In previous studies, Raghmi et al. [19] used SSR markers to assess genetic diversity among Iranian melon landraces and their relationships with melon landraces of diverse origins. They used three horticultural groups (*inodorus*, *dudaim*, and *cantalupensis*) which were known as endemic commercial melon landraces in Iran. They emphasize on the importance of Iranian melon landraces for studying of origin and diversification of vars. *inodorus* and *dudaim*. Therefore, the present study involved assessment genetic diversity of more extent horticultural groups of Iranian melon landraces including; *inodorus*, *ameri*, *reticulatus*, *cantalupensis*, and *dudaim*, which all of them were known as the commercial landraces. The present study evaluated some landraces that had not been studied before, such as 'Alashalta Sijaval', 'Khyiyari Sijaval' and 'Barge Ney' landraces. Our

results indicated that ISSR technique alongside polyacrylamide gel analysis would be the appropriate method to classify genotypes in horticultural groups of melon. However, according to Garcia-Mas et al. [10], if more data is provided, any marker method would be optimal for genetic similarity studies in melon germplasm.

In the present study, statistical variation measures ($H = 0.08$ and $I = 0.121$, and 22.6% of polymorphic loci) was less than Raghmi et al. [19] results. Results of AMOVA based upon ISSR data is consistent with previous studies by different molecular markers on Iranian melon landraces [19,21], where genetic variation within populations was more than among populations.

Non-sweet genotype (specific characteristics of *dudaim* group) was well separated from sweet genotypes (*inodorus*, *ameri*, *reticulatus*, *cantalupensis*) which was in agreement with some previous studies [10,34–36]. In another hand, a distinction between genotypes of the *inodorus* and *cantalupensis* groups was also observed that was in accordance with some researchers achievements [19,36,37]. Inconsistent with Raghmi et al. [19], despite the significant differences between vars. *cantalupensis* and *inodorus* in morphology and physiology, the molecular resolution between them was slight. Therefore, comparatively small number of genes might be responsible for the difference between horticultural groups [35].

UPGMA cluster analysis indicated some Iranian genotypes that are belonging to the same geographical region were clustered in the common group (i.e. 'TONI', 'BANI', 'BONI' and 'ZANI' landraces from Mahallat city, ALSI and KSI landraces from Bandar Torkaman city). The result was confirmed by Roy et al. [11] on wild Indian melon genotypes, Erdinc et al. [38] on Turkish melon landraces and Raghmi et al. [19] on Iranian melon landraces. Although morphological characteristics of 'KSI' and 'ALSI' landraces were close to *inodorus* variety (for KSI landrace) and *cantalupensis* and *reticulatus*

(ALSI) varieties, they separated from G1 and formed a separated group (G2) together. According to Bayesian analysis, these two landraces placed in Pop3 which involving genotypes such as 'SANI', 'HM', 'KN', 'KHM', 'GB', 'CP' and 'AV'. Intermediate forms might have been formed among horticultural groups due to traditional farming practices employed by some local small-scale melon growers by centuries of production. Intra-specific hybrids among melon genotypes are rather common in Iran. Thus, it is no strange to observe such a wide genetic variation among Iranian melon genotypes.

Inodorus variety is an extensive horticultural group. In the present study, all of the Iranian *inodorus* genotypes had characteristics such as indeterminate growth habit, different shapes of fruits, and mostly white flesh, with or without fruit ribbing. Regarding quantitative traits, the *inodorus* group had the highest values in compare to other groups except flesh thickness and as well as total soluble solid (TSS). Most of the landraces used in the present study were belonged to this horticultural group and had such an important commercial place in Iran. Based on UPGMA cluster analysis, *inodorus* group divided to two parts, part 1 including 'MIKE', 'TONI', 'BANI', 'BONI', 'ZANI', 'GOR', 'KONA' genotypes, and part 2 including 'TS', 'SANI', 'HM', 'KN', 'KHM', 'GB', 'CP' and 'AV' genotypes (Fig. 3B). Our results revealed that *ameri* group was genetically very close to *inodorus* variety and placed at the part 2. In addition, Bayesian analysis indicated that all genotypes of part 2 couple with intermediate varieties ('ALSI' and 'KSI' landraces) form Pop 3 apart from 'TS' genotype. The *ameri* is one of the horticultural group which has not been extensively investigated in previous studies. 'NB' and 'AHAR' are landraces that their morphological traits were so matched to group *ameri*. Regarding morphological traits, the *ameri* had the maximum values at TSS. All of the *inodorus* genotypes had indeterminate growth habit, while in *ameri* group, determinate growth habit was observed. Nevertheless, results of UPGMA dendrogram and Bayesian analyses showed that *ameri* group did not separate in a distinct group and clustered in the common group with *inodorus*. Since 'NB' and 'AHAR' landraces were very similar to commercial cultivar of 'Ananas', so probably this cultivar was imported to Iran and gradually was replaced with poor economically endemic landraces by farmers. Hence, it can be an alarming sign for extinction of indigenous Iranian melon landraces. There is a genotype suggested by Pitrat [2] as a member of *ameri* group, named 'Khatoni'. Raghani et al. [19] noted that 'Khatoni' (*inodorus* type of Iranian landraces) possesses some characteristics like netted skin surface, crisp flesh and various fruit shape, no climacteric, and long shelf life which does not quite match with vars. *inodorus* and var. *ameri*. Thus, they suggested a specific group called *iranian-sis* group, as previously suggested by Lotfi and Kashi [39]. Despite the facts that mentioned above, it can be recommended that *ameri* is very close to *inodorus* and can be merged into this particular group. Aierken et al. [40] also proposed *ameri* group should be classified as *inodorus*.

Raghani et al. [19] noted that there are five known melon groups (in Persian word) consisting 'Kharboze', 'Talebi', 'Garmak', 'Dastanbou' and 'Khiarchanbar' in Iran. They stated that "based on classification proposed by Pitrat [2] characters of 'Talebi', 'Garmak', 'Dastanbou' and 'Khiarchanbar' is close to vars. *cantalupensis*, *reticulatus*, *dudaim* and *flexuosus*, respectively". Given that *reticulatus* group was mainly differed from *cantalupensis* group in having a netted surface [41] and also based on UPGMA cluster analysis, we suggest classifying 'Talebi' genotype as *reticulatus* variety. Moreover, Ritschel et al. [42] quote from Robinson and Decker-Walters that they suggested the *reticulatus* group should be part of the *cantalupensis* group.

Bayesian analysis assigned ten landraces to Pop 1, eight landraces to Pop 2 and nine melon landraces to Pop 3. Pop 1 and Pop 2 included all genotypes placed in SC1, and Pop 3 involves

all genotypes placed in SC2 and C2. In contrary, as evidenced from Fig. 3B, the Bayesian analysis could not distinct 'GER' genotype from other genotypes and indicated a high relationship between *reticulatus* and *cantalupensis* landraces. Almost close results have been observed between Bayesian and UPGMA cluster analyses, hence both of them suggested for genetic population studies.

5. Conclusion

Judging from the notes, the classification of these genotypes is confusing and controversial. Consequently, according to our results, we propose to merging some groups which are genetically so close, namely, *ameri* group could be merged into *inodorus* group and *reticulatus* group merged into *cantalupensis* group, although further studies by more extant populations and molecular markers should be carry out to confirm this hypothesis. Given the fact that Iran is one of the main regions of melon diversity, there are still many other landraces that need to be identified and collected before their extinction. These landraces are the result of hundreds of years of selection by local farmers and are a legacy for the future, which can be applied to breeding programs and to release new elite cultivars.

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