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Analysis of Genetic Diversity among Watermelon [*Citrullus lunatus* Thunb (Matsum.) and Nakai] Accessions by Phenotypic and Molecular Markers

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ABSTRACT

In this study, genetic diversity among watermelon accessions was studied by analyzing morphological and physiological traits using Random Amplified Polymorphic DNA (RAPD). Thirty-seven morphological and physiological traits showed significant variation among the accessions. Some watermelon accessions showed typical attributes of seed characters and fruit flesh and skin color. Principle component analysis allocated the high variance percentage for fruit and seed characters. Cluster analysis of morphological and physiological characters separated *Citrullus colocynthis* in one independent cluster clearly. The 18 RAPD markers represented 126 polymorphic bands of 154 total bands. Cluster analysis using RAPD markers at similarity 0.54 also clarified colocynth genotypes in one separated group. Three main clusters distinguished for other accessions that were classified mainly by fruit shape and flesh color, then, by fruit skin color. The most similarity (1) was observed among three accessions in the same cluster, contrary to the different collecting areas, which may indicate that accessions were distributed in different areas from the same genetic sources. The Genetic Similarity coefficients (GS) among evaluated accessions ranged from 0.45 to 1.00, indicating that they had relatively high genetic diversity. Altogether, the high variation, especially for phenotypic traits, of watermelon accessions in Iran could be considered as a good resource for selection and breeding program.

Keywords: Genetic similarity, Principle component analysis, Random amplified polymorphic DNA.

INTRODUCTION

Genetic diversity is an evolutionary origin known-factor in biologic systems sustainability (Marques, 2001). Furthermore, genetic diversity assures population survival by generating consequent adaptations during the evaluation time. One of the important reasons for investigating genetic diversity is to indicate the relationship between species and sub-species and understand the evolutionary rate in plants (Engels and Visser, 2003). Geographic barriers, such as water availability and temperature, are important factors in diversification. Watermelon [*Citrullus lunatus* Thunb. (Matsum.) and Nakai] is an important worldwide species grown in temperate and warm climate (Wehner, 2008) and its genus belongs to the Cucurbitaceae family. During the last century, watermelon production has increased steadily and accounted for 2% of the world's area devoted to vegetable production (Levi *et al.*, 2001). Although many watermelon cultivars were developed worldwide during the last century, there is still an essential need for watermelon improvement, especially for biotic and abiotic stress resistance.

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According to the Food and Agriculture Organization (FAOSTAT, 2018), despite the semi-arid and arid climate, Iran is accounted for the production of 3,947,057 tons of watermelon, which puts it in second place internationally. Watermelon fruits have a major portion in the people's food basket, particularly in the warm and hot summer season. The nutritional value of fruits due to its lycopene content per cup, which is higher even than tomato (Chug-Ahuja *et al.*, 1993; Clinton, 1998; Holden *et al.*, 1999; Perkins-Veazie *et al.*, 2003). There are many types of watermelon in Iran that have been grown under upland farming system and some of them have been used just for their large seeds as appetizers. For effective conservation of watermelon germplasm, it is important to obtain information about genetic diversity within and between accessions. Morphological and molecular markers have been intensively used to determine genetic diversity of watermelon accessions (Hashizume *et al.*, 2003; Lee *et al.*, 1996; Che *et al.*, 2003; Levi *et al.*, 2013; Mujaju *et al.*, 2010; Park *et al.*, 2016).

Mashilo *et al.* (2017) analyzed the genetic diversity among citron watermelon landrace collections of South Africa by using Simple Sequence Repeat (SSR) markers. Yagcioglu *et al*. (2016) investigated Genome-Wide Association Analysis (GWAS) for some important watermelon traits such as yield, fruit weight, flesh color, total soluble solid, seed coat color, seed length, and flowering time by using 96 lines selected from 258 pure lines. Allocations of larger area culture of higher productive modern varieties, especially under high yielding environments, causes genetic erosion of valuable landraces and thus reducing genetic diversity. It should be emphasized that preservation and collection of watermelon landraces that are adapted to different environmental condition are the basis of food security and genetic pools for varieties improvement. Fortunately, accessions were collected and are now conserved mainly *ex situ* in national, regional gene banks in Iran. Thus, our study focused on the characterization of morphological, physiological, and molecular variations among different types of watermelon accessions that have been planted by local farmers in different parts of Iran and introducing the valuable accessions for cultivation and use in breeding programs.

MATERIALS AND METHODS

Plant Materials

A total of 30 accessions including 29 watermelons [*Citrullus lunatus* Thunb (Matsum.) and Nakai] and one accession of colocynth (*Citrullus colocynthis*) were collected from cultivation areas mainly in the western and southern parts of Iran. The seeds of these accessions were provided by the Gene Bank of Seed and Plant Improvement Institute, Karaj, Iran (Table 1).

Characterization of Morphological and Physiological Traits

All watermelon accessions were cultivated in the Research Station of Horticultural Science; the University of Tehran, in order to investigate morphological and physiological characteristics. The experiment was conducted in a completely randomized block design with three replications of each accession, and five plants in each replication. Plots were irrigated every week during the season and were weeded manually to maintain proper weeds control. Plants were fertilized with urea (46%) fertilizer up to pre-flowering stages and were exposed to foliar application of calcium nitrate once during fruit growth. To study leaf and seed characters, five leaves and five seeds were collected from mature fruits randomly. Most of the fruit characters were recorded after fruit harvesting. The total soluble solids was measured by a handheld refractometer. Lycopene content was determined using Sadler *et al.* (1990) method. Briefly, the lycopene was extracted from 2 g samples of

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Table 1. Accessions code and collection region for evaluation of morphological characters (TN-93-457 is colocynth).

Number	Accession code	Province	Number	Accession code	Province
1	TN.93.673	Kerman	16	TN-93-516	Hamedan
2	TN.93.766	Isfahan	17	TN.93.556	Fars
3	TN.93.774	Semnan	18	TN.93.767	Fars
4	TN.93.469	Markazi	19	TN.93.768	Isfahan
5	TN.93.756	Bushehr	20	TN.93.513	Hormozgan
6	TN.93.678	Kerman	21	TN.93.457	Sistan and Baluchstan
7	TN.93.677	Kerman	22	TN.93.758	Hormozgan
8	TN.93.765	Isfahan	23	TN.93.637	Isfahan
9	TN.93.514	Kerman	24	Birjand-2	Southern Khorasan
10	TN.93.591	Kerman	25	Birjand-3	Southern Khorasan
11	TN.93.485	Khorasan	26	Ajili	Khorasan Razavi
12	TN.93.540	Sistan & Baluchestan	27	TN-93-525	Ghazvin-Sharifabad
13	TN.93.512	Sistan and Baluchestan	28	TN-93-761	Fars
14	TN.93.470	Zanjan	29	Crimson Sweet	Common cultivar
15	TN-93-676	Kerman	30	TN-93-302	Kerman

fruit flesh with a 2:1:1 mixture of hexane:acetone:ethanol and 0.1% BHT followed by spectrophotometric measurement at 503 nm (Perkins-Veazie *et al.*, 2001). Also, total carotenoid content was determined using Ndolo and Beta (2013) method, and absorbance was measured at 450 nm using a spectrophotometer.

RAPD Analysis

Young leaves were ground in liquid nitrogen, and genomic DNA was extracted using the CTAB procedure (Murray and Thompson, 1980). The quality and quantity of each DNA sample were determined using a spectrophotometer and electrophoresis.

Each 10 µL PCR reaction constituted of 25 ng genomic DNA, 1 µL PCR buffer (Sigma, USA: 10 Mm Tris-HCl pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.1 mM dNTP), 0.5 µM RAPD primers and 0.25 U Taq polymerase (Sigma, USA). Amplification reactions were done by using an I-Cycler (Bio-Rad, USA). PCR amplification was initial denaturation at 95°C for 3 minutes, 40 PCR cycles at 94°C for 1 minute, 40°C for 2 minutes, and 72°C for 2 minutes, and then final extension at 72°C for 5 minutes. After amplification, the PCR products were run in 1.5% agarose gel at constant voltage (100V) using a horizontal gel electrophoresis system and were stained with ethidium bromide to obtain a scoring band.

Data Analysis

Factor analysis of morphological and physiological traits was done using a principal components analysis method based on a correlation matrix by SPSS software. RAPD bands were scored as 1 for present and 0 for absent. The estimate of similarity was based on the number of shared amplification products. Jaccard's coefficient was used as a genetic distance estimator. The original genetic distance matrix was compared with the corresponding cophenetic value matrix using the MXCOMP algorithm of the NTSYS, 2.2 software. The Polymorphic Information Content (PIC) was calculated using the formula $PIC= 1-\sum pi_2$, where, pi is the frequency of the ith allele (Smith *et al.*, 1997). The polymorphism percentage was calculated by polymorphisn band number ratio to total band number observed for each marker.

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RESULTS AND DISCUSSION

Diversity and Differentiation Based on Morphological and Physiological Traits

As illustrated in Figure 1, there were high variations among the evaluated accessions by phenotypic traits, which were more obvious in the case of fruit and seed characteristics. Principal component analysis was carried out to identify the most discriminative variables among the watermelon accessions. This analysis arranged all morphological and physiological traits in 11 major and independent factors with eigenvalues greater than 1, and accounted 85.939 % of the total variance (Table 2). The first principle component explained 14.627% of the total variance and had a high positive relationship with fruit weight, fruit length and width, mesocarp and pericarp thickness, fruit circumference, and fruit shape (Table 2). Seed attributes such as length, width, thickness, and weight loaded in the second factor and described 12.809% of the total variance. Some fruit physiological characters including total soluble solids, fruit luminescence (L), and a value, lycopene, and flesh color explained 11.895% in the third factor. The most important traits that contributed to variation among accessions were described in three factors. It could be concluded that fruit and seed characters played an effective role in population differences. Furthermore, leaf attributes with 7.780% of the total variance and skin color weighted a significant amount in other

loaded factors. Due to the fact that watermelon accession assessment and their relations depend on fruit characteristics (skin thickness and color, shape, sugar content, shape and color of the seed) these fruit traits are known as the main factor for assessment and evaluation of accessions (Levi *et al.*, 2000). The result of this study showed the significant contribution of fruit and seed attributes in the attained variance (Figure 1). As illustrated in this figure, whole and cut fruit shape represented the variation among accession within their seeds.

Cluster Analysis

Ward linkage cluster analysis of phenotypic traits divided the studied accessions into two distinct groups (Figure 2). At distances between 15 and 20, accessions were divided into 8 groups and 2 independent accessions. Group A, consisted of TN-93525, TN-93-512, TN-93-677 with dark green skin color and red flesh in the first sub-cluster, and TN.93.420 and TN.93.540 with dark green stripes on light green skin color and light reddish flesh color. In group B, two accessions with yellow flesh color, black seeds, and round fruit shape were placed in one sub-cluster and three accessions with light green skin color, pink flesh color, and the same seed characters were classified with each other, and TN.93.422 regarding different properties was placed far from these accessions in the same sub-cluster.

In group C, two sub-clusters were distinguished by accessions with the same fruit flesh color, skin color (light green), and seed characters grouped with each other, and accessions in the second sub-cluster had different skin color and texture but the same seed characters. One accession with dark green skin color, red flesh color, with large and light yellow seed color segregated as independent clad. Group D contained TN-93-513 and TN-93-514 with yellow flesh color and the same seed characters but different fruit shapes and was further than those two accessions as group E with dark green skin color and red flesh color with different seed characters were classified in the same group. Group F in the second main group included TN-93-556 and TN-93-758, which had elliptical fruit shape and the same seed characters and also one accession with green skin color and round shape.

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Table 2. Principal component analysis and variables contribution on each factor. [a]

Variables	PC.1	PC.2	PC.3	PC.4	PC.5	PC.6	PC.7	PC.8	PC.9	PC.10	PC.11
Male No	-.046	-.258	.022	.095	.138	-.024	**.879**	-.210	-.023	-.125	-.081
Female No	.055	-.123	.135	.011	.016	-.009	**.746**	.498	.202	-.155	.060
male/female	-.024	-.201	-.150	.015	-.089	.122	.052	**-.855**	-.144	.138	-.050
Shoot L	.130	**.544**	.220	.193	.334	.275	.136	.205	.234	.126	-.377
Internode L	.210	.342	.383	.048	.051	.363	.134	**.580**	.114	.198	-.270
Leaf L	.337	.142	.167	**.800**	-.018	.049	.155	.060	-.047	-.103	-.117
Leaf W	.324	-.083	.072	**.873**	-.041	-.037	.061	-.046	-.110	.073	-.097
Leaf Lobes	.066	.284	.135	**.562**	.228	.361	-.210	-.282	.086	.083	.309
Branch No	-.364	.023	-.168	**.554**	.144	.096	-.144	.045	.001	.412	-.009
Leaf angel	-.061	-.056	-.032	.103	-.248	.172	-.196	-.159	-.011	**.828**	.079
Leaf color	.435	.128	-.002	-.012	-.180	-.138	-.012	-.325	.384	-.108	**-.539**
Fruit weight	**.895**	-.154	.098	.169	-.076	-.006	-.021	-.017	.004	-.051	-.093
Longitudinal Diameter	**.739**	-.133	.241	.164	-.086	.355	-.001	-.287	.093	.189	.045
Transverse D	**.395**	.039	.345	.039	.134	-.248	.115	.108	.022	.532	-.267
Mesocarp D	**.860**	.031	.237	.187	-.105	-.005	.053	.223	.000	-.074	-.014
TSS	.470	-.144	**.670**	-.038	.072	-.290	.080	.032	.136	.150	.242
Rind thickness	**.874**	.244	.079	-.006	.146	-.083	-.124	-.098	.013	.122	.021
Skin color	.051	-.188	.008	-.042	.066	-.040	.134	.185	**.923**	.014	.009
Flesh color	.124	-.123	**.916**	.025	.004	.104	.034	-.033	.094	-.001	.179
perimeter	**.896**	.096	.230	.248	-.033	-.036	-.042	.169	-.014	-.081	.015
PH	.065	.038	.396	-.075	.228	-.099	.045	-.017	.104	-.007	**.722**
L	-.285	-.106	**-.574**	-.466	.230	.171	-.242	.014	.153	-.071	.090
a	.142	.076	**.861**	.015	-.101	-.053	.066	.161	-.226	.057	-.014
b	-.033	-.104	-.270	.003	**.889**	.156	.113	.075	-.003	-.159	.117
Mesocarp DW	-.205	-.236	.288	-.189	-.059	**-.627**	.105	.099	.450	.095	.083
Mesocarp FW	-.123	.325	-.045	.040	.125	**.789**	.032	-.050	-.001	.099	-.018
Seed L	-.089	**.942**	-.031	.048	-.040	.189	-.079	.075	-.065	.090	-.005
Seed wide	.007	**.933**	.110	.050	-.053	.214	-.031	.034	-.039	.017	.044
Seed thickness	.161	**.896**	-.170	.038	-.066	-.163	-.120	.085	-.071	-.160	-.088
seed weight	-.005	**.934**	.003	-.070	-.151	.084	-.074	.000	-.071	.018	-.005
Seed color	-.182	-.375	-.136	.057	.259	**.558**	.281	.409	-.024	.002	.191
Hue	.237	.220	.508	-.033	**-.619**	.010	.061	.126	-.314	-.079	-.059
croma	.001	-.137	.401	-.017	**.838**	.052	.125	.122	-.034	-.043	.093
Fruit shape	**.556**	.308	-.126	-.183	.013	-.191	.040	-.217	.364	.424	.065
Carotenoid	.092	-.193	-.321	**.565**	-.044	.252	.038	.131	.187	.001	.412
Lycopene	.238	.006	**.715**	.080	.096	-.085	-.198	.114	.343	-.225	-.027
Phenol	-.433	.167	-.010	.046	.199	.202	**.534**	.061	.292	.252	.246
Eigenvalue	5.412	4.739	4.401	2.879	2.544	2.265	2.102	2.068	1.962	1.765	1.66
%variance	14.627	12.809	11.895	7.780	6.876	6.123	5.680	5.590	5.303	4.77	4.49
Cumulative var%	14.627	27.436	39.331	47.111	53.987	60.110	65.790	71.379	76.682	81.45	85.93

[a] Values in bold indicate the most relevant characters (> 0.5) that contribute to the variation of the components.

In the other sub-cluster crimson sweet cultivar was at farther distance and classified with accessions TN-93-678 and TN-93-756 with the same fruit shape, flesh color, and seed characters. The other independent group including colocynth plant with silver-green leaves, the lowest leaf dimensions which had the shortest petiole, bush length, internodes length, and lowest fruit weight and the highest bitterness in its fruit. According to other studies on watermelon morphological characters, it was demonstrated that fruit traits among other characters had the main role for

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TN-93-766
Isfahan

TN-93-514
Kerman

TN-93-677
Kerman

TN-93-469
Markazi

Birjand 2(TN-93-420)
Southern Khorasan

TN-93-756
Bushehr

TN-93-774
Semnan

TN-93-673
Kerman

TN-93-591
Kerman

TN-93-485
Khorasan

Birjand -3(TN-93-422)

TN-93-540
Sistan and baluchestan

TN-93-302
Kerman

TN-93-512
Sistan and Baluchistan

TN-93-525
Sharifabad-Ghazvin

TN-93-470
Zanjan

TN-93-676
Kerman

Ajili(TN-93-725)
Sabzevar-Khorasan

TN-93-765
Isfahan

TN-93-556
Fars

TN-93-767
Fras

TN-93-761
Fars

TN-93-513
Hormozgan

TN-93-457
Sisatan and Baluchestan

TN-93-516
Hamedan

TN-93-758
Hormozgan

TN-93-768
Isfahan

TN-93-637
Isfahan

TN-93-678
Kerman

Crimson sweat, B32

Figure 1. Whole and half cut fruit shape of all studied accessions.

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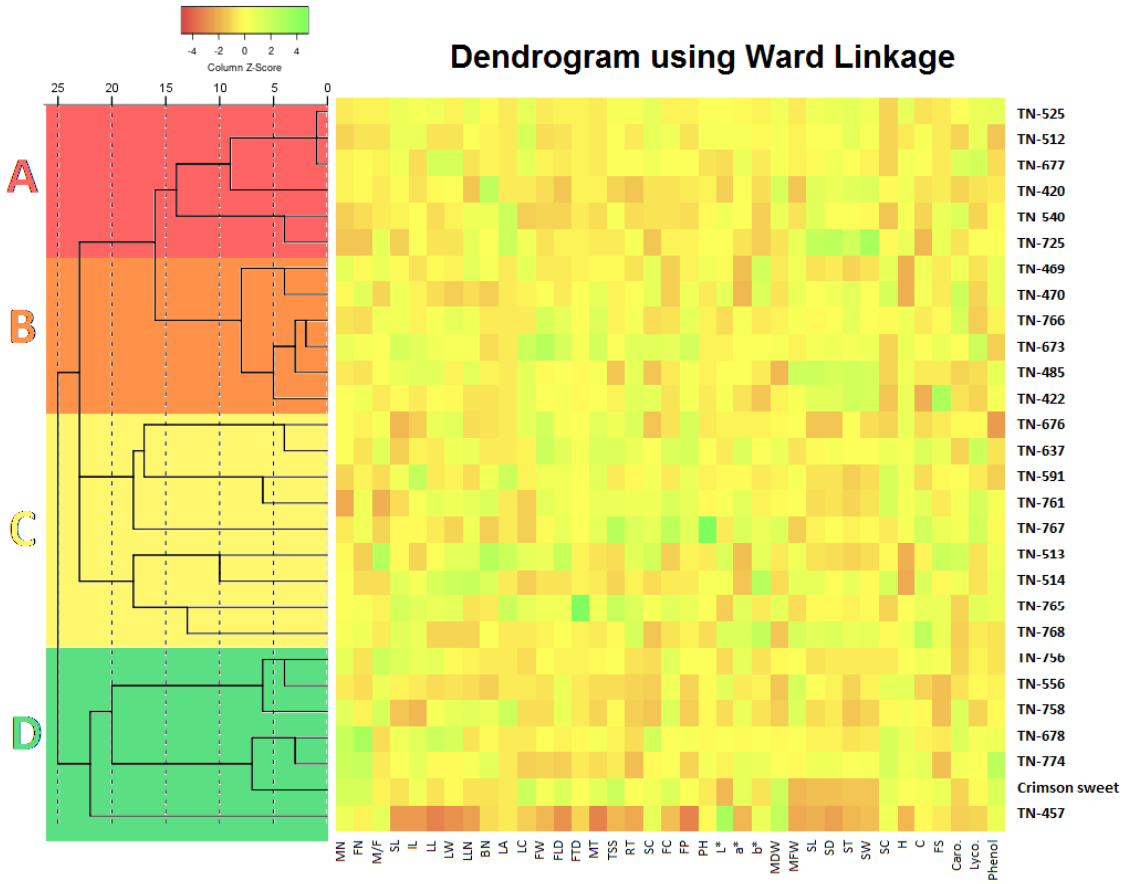

Figur 2. Heat map of the morphological traits measured on the studied accessions. Orange and yellow colors represent reduced and augmented representation levels of traits, respectively. MN: Male flowers Number; FN: Female flowers Number; M/F: Male/Female flowers ratio; SL: Shoot Length; IL: Internode Length; LL: Leaf Length; LW: Leaf Weigh; LLN: Leaf Lobes Number; BN: Branch Number; LA: Leaf Area; LC: Leaf Color; FW: Fruit Weight; FLD: Fruit Longitude Diameter ; FTD: Fruit Transvers Diameter; MT: Mesocarp Thickness; TSS: Total Soluble Solids; RT: Rind Thickness; SC: Skin Color; FC: Flesh Color; FP: Fruit Perimeter; PH: Ph; L*: L* Color component (lightness); a*: a* Color component; b*: b* Color component; MDW: Mesocarp Dry Weight; MFW: Mesocarp Fresh Weight 100 g^{-1} fruit; SL: Seed Length; SD: Seed Diameter; ST: Seed Thickness; SW: Seed Weight; SC: Seed Color; H: Hue; C: Chroma; FS: Fruit Shape index; Caro.: Carotenoids content; Lyco.: Lycopene content; Phenol: Phenols content.

classification of watermelon different landraces and also the wild type (Munisse *et al.*, 2011; Sheng *et al.*, 2012).

In this study, it became evident that geographic area could not explain the separation of accessions. This also has been reported in the literature from the work on some types of melons in various geographic areas (Yi *et al.*, 2009; Nhi *et al.*, 2010). Morphological dendrogram evaluation results from 30 watermelon accession that were collected from various separated geographical regions can convey a genetic connection among them. Also, in this study, accession did not segregate based on the geographic area in sub-clusters as reported by Mujaju *et al.* (2011).

Molecular Analysis Results

Eighteen primers were used to assess polymorphic DNA and 154 bands were produced. Among them, 28 bands were monomorphic among all genotypes, and 126 bands were polymorphic in at least two genotypes, which indicated a high percentage of polymorphic parts (80.59%) (Table3). In an

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Table 3. The RAPD primers name, total bunds number, polymorphic bands, percentage of polymorphism and Polymorphic Information Content (PIC) evaluated for watermelon accessions.

Primers	Total bands No (a)	Polymorphic bands No (b)	Polymorphism % (b/a)×100	PIC
TIBM BA02	10	9	90	0.404
TIBM BD09	9	8	88.89	0.640
TIBM BA20	12	12	100	0.851
TIBM BA06	9	7	77.78	0.230
TIBM BD10	9	8	88.89	0.630
TIBM BE02	9	7	77.78	0.540
TIBM BB14	7	5	71.43	0.317
TIBM BB08	6	3	50	0.182
TIBM BD12	11	9	81.82	0.504
TIBM BD01	8	5	62.50	0.240
TIBM BA04	11	9	81.82	0.437
TIBM BE01	11	9	81.82	0.373
TIBM BB20	6	3	50	0.213
TIBM BB19	5	5	100	0.449
TIBM BB13	7	6	85.71	0.396
TIBM BB18	9	8	88.89	0.71
M05	7	6	85.71	0.333
M07	8	7	87.50	0.363
Total	154	126	-	
Mean	8.55	7	80.59	0.434

investigation on African watermelon accessions using 138 RAPD molecular markers, 122 polymorph bands have been identified. Also, their polymorphism vicinity was 47% to 77% (Munjaju *et al.*, 2001). The largest number of multiplied parts were 12 bands (Primer: TIBM BA20) and the lowest of them were 3 bands (primer: TIBM BB08 and TIBM BB 20). The highest percentage of polymorphism (100%) was associated with TIBM BB19 and BM BA20 primers. The average mean for PIC value (0.434) showed the nearly informativeness of markers. The highest PIC value (0.851) was revealed by TIBM BA20.

Cluster Analysis

Based on the dendrogram that was obtained from the similarity matrix, the investigated watermelon accession with a similarity about 0.54 colocynth plant separated from all other accessions (Figure 3). For similarity of 0.78, two main groups could be identified. The first group included accessions with elliptical fruit shape and farther than those there were three accessions with the same fruit skin color, texture, and flesh color. In the second group, accession TN-93-678 with yellow fruit skin color was placed in an independent cluster and three accessions with higher similarity were classified with each other as one clad. Crimson sweet cultivar showed more affinity with TN-93- 761 and was placed in the same cluster. Accession with code numbers TN.93.725 and TN-93-422 from the same origin, have been cultivated for years and their seeds are red and larger than other types and are known in Iran as "Tokhmeh-Jabooni". They were classified in the same cluster.

Morphological attributes are affected by various factors, some of which are ineffective in DNA variation. These factors make those accessions differ from each other in morphological attributes in spite of the similarity in their DNA level. It should be noted that multiplied parts in RAPD are not necessarily identical regarding nucleotide sequence, and some parts with

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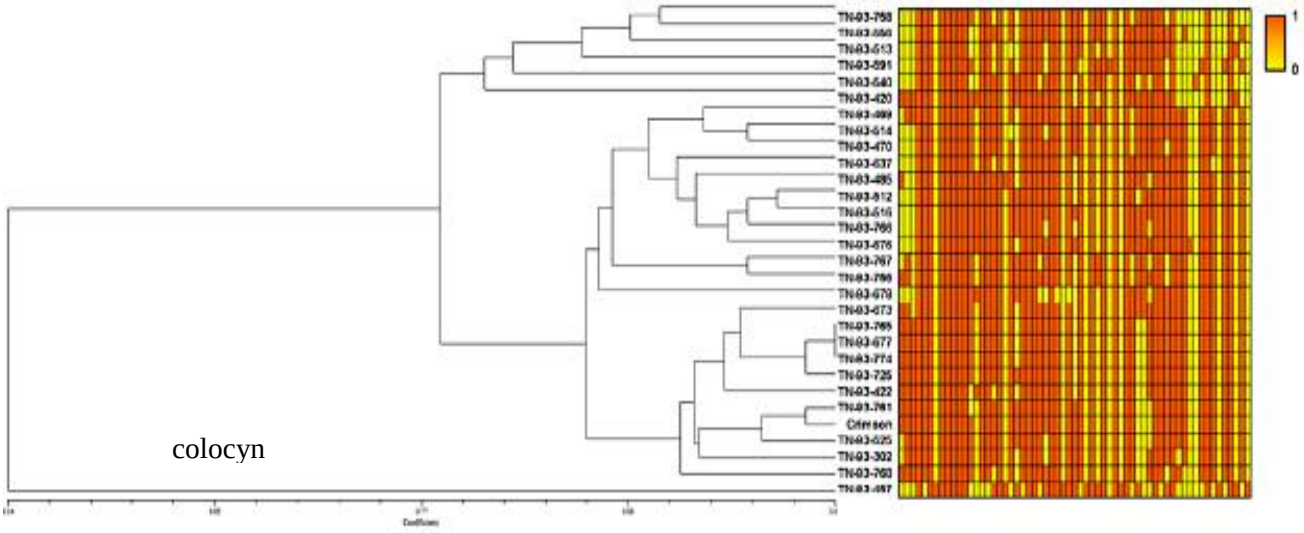

Figure 3. Genetic cluster analysis for the studied accessions according to RAPD results, Yellow and Orange represent the presence or absence of a band, respectively.

identical dimension, and might belong to various parts of the genome and different sequences. Therefore, it is possible that RAPD data does not fit to phenotypic characters. These results are consistent with those reported for watermelon accessions by Pandey *et al.* (2019).

Diversity, closeness and distance, and proximity of cultivars were studied in order to classify them, using morphological and molecular markers for different watermelon collections of different regions(Assefa *et al.*, 2020; Mandizvo *et al.*, 2021; Robert *et al.*,, 2018; Levi *et al.*, 2001). All researches reported that colocynth type separted from desert watermelon(*C. lunatus)*. Also, in our study, both morphological and RAPD markers distinguished the colocynth accession in one cluster farther than the other accessions.

Among the studied traits, fruit-related characteristics had the largest share in justifying the variations. In addition, most of the traits showed a high and positive correlation with each other.

In this evaluation, bitter apple watermelon (colocynth) contains high phenol and also the highest bitterness is a suitable genotype for the study of effective compounds and medicinal applications. Furthemore, the totoal soluble content as index of sweetness reported as affective attribute for classifying the different watermelon species. Selection force for fruit quality and flesh taste and sweetness made the distances between wild type and domesticte watermelon (Guo *et al.*, 2019). The results of the present study revealed significant phenotypic and molecular diversity of watermelon accessions that could be related to different plant responses to geographic areas, where the accessions were collected, are frequently subjected to drought, severe high temperature, and even low soil fertility. Given that most useful genes, such as genes for resistance to pests, diseases, and environmental stresses and genes for quality of the product, are commonly found in diversity centers, having accurate information on the genetic diversity of each plant to better utilize these resources for varieties improvement. Therefore, these promising watermelon accessions could be potentially utilized for the extreme environments and these data are useful for watermelon breeders.

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تحلیل تنوع ژنتیکی بین توده‌های هندوانه با استفاده از نشانگرهای فنوتیپی و مولکولی

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چکیده

در این تحقیق تنوع ژنتیکی بین توده‌های هندوانه با استفاده از نشانگرهای مورفولوژیکی، فیزیولوژیکی و مولکولی رپید مورد مطالعه قرار گرفته است. سی و هفت صفت مورفولوژیکی و فیزیولوژیکی تفاوت معنی داری بین توده‌ها نشان دادند. برخی از توده‌های هندوانه ویژگی‌های خاصی از صفات بذر، رنگ گوشت و رنگ پوست میوه نشان دادند. تجزیه به عامل‌های اصلی بیشترین واریانس بدست آمده را به صفات میوه و بذر اختصاص داد. تجزیه خوشه‌ای صفات مورفولوژیکی و فیزیولوژیکی به طور مشخص هندوانه ابوجهل را دریک خوشه مستقل قرار داد. 18 نشانگر رپید استفاده شده 126 باند پلی مورفیک از بین 154 باند تشکیل شده نشان داد. تجزیه خوشه‌ای حاصل از نشانگرهای رپید در حد تشابه 0/54 هندوانه ابوجهل را در یک گروه جداگانه از سایر توده‌ها طبقه بندی کرد. سایر توده‌های هندوانه در سه گروه اصلی قرار گرفتند که بیشتر بر اساس صفات شکل میوه، رنگ گوشت و رنگ پوست میوه دسته بندی شده بودند. بیشترین میزان شباهت (1) در سه توده‌ی هندوانه از مناطق جمع آوری متفاوت بدست آمد که به نظر می آید توده‌ها در مناطق مختلف از یک منبع ژنتیکی توزیع شده باشند. ضریب تشابه ژنتیکی بین 1-0/45 نشان داد که تنوع ژنتیکی نسبتا بالایی بین توده‌های ارزیابی شده، وجود دارد. به طور کلی تنوع بالا بویژه در صفات فنوتیپی در ایران، می‌تواند بعنوان منبع خوبی برای انتخاب و برنامه‌های اصلاحی پیشنهاد شود.

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