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Assessment of genetic diversity among local accessions of melon and identification of DNA markers linked with agro-morphological characteristics

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ABSTRACT

In this project, 14 local melon accessions were collected from five regions of Iran and examined in a randomized complete block design with three replications in the field over two consecutive years. The combined analysis of variance showed significant differences between accessions for the majority of characters, including days to flowering, flower petal width, fruit ripening time, peduncle diameter, fruit storage at room temperature, seed width, and seed length. The interaction effect of genotype×year was significant for variables including days to flowering, leaf tail length, number of seeds per fruit, thickness of fruit flesh, fruit fresh weight, 100-seed weight, and fruit width. Among the studied characters, fruit fresh weight and fruit length were selected through stepwise regression as remarkable variables that have direct and indirect effects, respectively, on total fruit yield. Regarding principal component analysis, the first two principal components (PCs) explained 54.5% of the data variability, and the studied accessions were distinguished into two groups based on their PC1 and PC2 scores. Using 12 RAPD primers, 146 loci were amplified across the studied melon accessions. Results showed that primer OPB13, with a polymorphism information content value of 0.38, has significant power in screening local melon germplasm. Classification of the studied melon panel using the Jaccard similarity coefficient and UPGMA algorithm produced three main groups. In this study, molecular classification did not coincide with agro-morphological classification. Here, co-localized genomic loci were identified that could potentially be utilized in local melon breeding programs through marker-assisted selection.

Key words: Genetic variability, Marker-trait association, RAPD marker, Stepwise regression.

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ABBREVIATIONS

DF (Days to flowering), FPL (Flower petal length), FPW (Flower petal width), 50% DF (Days to 50% flowering), LTL (Leaf tail length), 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 weight), FSRT (Fruit storage at room temperature), TFF (Thickness of fruit flesh), NSPF (Number of seeds per fruit), 100 SW (100-seed weight), SW (Seed width), SL (Seed length), FL (Fruit length), FW (Fruit width).

INTRODUCTION

The genus *Cucumis* from the family *Cucurbitaceae* includes important vegetable crops that can grow prominently in temperate and warm regions (Decker-Walters *et al.,* 2002). Among all species, *Cucumis melo* L. is the most polymorphic (Stepansky *et al.,* 1999; Szamosi *et al.,* 2010). Melon (*Cucumis melo* L.) is an annual, cross-pollinating, herbaceous plant that trails or creeps, with 2n = 2x = 24 (Napolitano *et al.,* 2020). Its economically important fruit contains carbohydrates, vitamin A, and other high nutritional content (Ermiş and Aras, 2017). The origin of *Cucumis melo* L. remains a subject of controversy. Some reports suggest an African origin due to its similar chromosome number to many African species of *C. silvestres* (Dhillon *et al.,* 2007), while others emphasize an Asian origin based on the indication that Australian *C. picrocarpus* and *C. melo* are sister species and likely wild progenitors of *C. trigonus* and *C. callosus*, both of which are Asian species (John, 2012).

The primary centers of diversification for melon are located in South Central Asia (Tzitzikas *et al.,* 2009), while the secondary centers comprise East Asian and Mediterranean regions (Blanca *et al.,* 2012). Melons exhibit a wide range of diversity within these primary and secondary diversification centers. Evaluating local germplasm resources is crucial for breeding and conservation efforts. Local varieties and landraces are especially valuable for their adaptation to local climates and soil conditions, and they often show better resistance to local pests and diseases and may possess other desirable attributes. In summary, assessing the genetic variability of local germplasm collections can significantly impact future breeding programs for species like melon (Solmaz *et al.,* 2016).

Genetic diversity in melon has been analyzed using various methods, including phenotypic (Szamosi *et al.,* 2010; Trimech *et al.,* 2013; Andrade *et al.,* 2019), isozymic (McCreight *et al.,* 2004), and molecular DNA markers (Guliyev *et al.,* 2018). Morphological characterization and the quantification of genetic variability are critical in pre- and post-improvement population studies to understand diversity and select appropriate plant groups. Multivariate analyses of agro-morphological traits are ideal for describing genetic diversity and can be performed using qualitative descriptors, quantitative descriptors, and binary data obtained through molecular information (Aragao *et al.,* 2013). According to the literature (Trimech *et al.,* 2013), melon germplasm from Tunisia was evaluated based on morphological traits, and PCA as a multivariate analysis identified and distinguished local melon accessions from others. Recently, Pandey *et al.* (2021) inspected 39 melon accessions from India, focusing on fruit morphology, floral characteristics, and nutritional attributes, demonstrating how floral diversity contributes to distinguishing melon accessions. Similarly, Andrade *et al.* (2019) examined 42 Brazilian melon accessions alongside four cultivars across three environments, using both quantitative and qualitative characteristics of fruit. They reported significant genotype×environment interactions for the majority of studied characters and classified the germplasm into four distinct groups. Overall, this indicates that melon morphological traits allow for significant differentiation among local and introduced varieties, likely a result of low levels of gene flow between them due to limited hybridization in melons (Trimech *et al.,* 2013).

In addition to agro-morphological traits, DNA molecular markers provide a straightforward, precise, and rapid method for examining divergence and grouping individuals (Hatami Maleki *et al.,* 2023). Several types of DNA markers have been introduced for plant germplasm evaluation, among which the RAPD (Random Amplified Polymorphic DNA) marker is frequently used due to its advantages: (i) suitability for working with anonymous genomes, (ii) applicability in situations with limited quantities of DNA, and (iii) low cost and high efficiency (Amiteye, 2021). The RAPD marker system has been implemented for genetic diversity analysis of several species and accessions within the *Cucurbitaceae* family (Tanaka *et al.,* 2007; Naznin *et al.,* 2023). Recently, Naznin *et al.* (2023) used 12 RAPD markers and seven SSR markers to assess genetic diversity among 62 accessions of Cambodian melons. In this regard, the evaluation of Iranian melon germplasm was conducted using ISSR (Ourang *et*

al., 2009), AFLP (Vafadar Shamasbi *et al.,* 2017), and RAPD (Feyzian *et al.,* 2007) markers, with all aforementioned research emphasizing the existence of genetic variability for melons in Iran. An additional advantage of DNA markers, besides genetic diversity analysis, is their ability to identify DNA markers tightly linked to a gene/locus of interest (Darvishzadeh *et al.,* 2014). Such DNA markers offer breeders the opportunity to apply marker-assisted selection (MAS) in their breeding programs. However, there are no reports on the identification of genomic loci associated with agro-morphological traits of endemic melon accessions.

In Iran, melon cultivation has a long historical background, and today, several bred cultivars alongside domesticated accessions are cultivated. In this context, greater attention to local accessions is vital to prevent the genetic erosion of melon materials. Despite several reports on melon germplasm from Iran, many accessions, landraces, and improved cultivars remain to be studied. In the present study, we evaluate the agro-morphological characteristics and genomic DNA fingerprinting of 14 collected melon accessions to assess germplasm variability and identify informative genomic loci related to the studied agro-morphological traits.

MATERIALS AND METHODS

Plant material and field experiment

Germplasm was collected in 2019 from five provinces of Iran (Table 1). Fourteen accessions of melon (Table 1) were acquired and evaluated over two consecutive years (2020 and 2021). The experiment was conducted in the research field of Islamic Azad University, Mianeh branch, Iran, using a randomized complete block design with three replicates. Seeds of each accession were directly sown in four rows (2 m apart) with 60 cm spacing. Irrigation (furrow system), fertilization, hand weeding, and other management practices were performed as needed throughout the growing period. Data were collected from five randomly selected plants from the middle row of each plot. Twenty-three agro-morphological characteristics were scored, including DF (Days to flowering), FPL (Flower petal length), FPW (Flower petal width), 50% DF (Days to 50% flowering), LTL (Leaf tail length), FRT (Fruit ripening time), LL (Leaf length), LW (Leaf width), ID (Internode distance), NAS (Number of arrows in the stem), PL (Peduncle length), PD (Peduncle diameter), PS (Plant size), FFW (Fruit fresh weight), TFW (Total fruit weight), FSRT (Fruit storage at room temperature), TFF (Thickness of fruit flesh), NSPF (Number of seeds per fruit), 100 SW (100-seed weight), SW (Seed width), SL (Seed length), FL (Fruit length), and FW (Fruit width) (Table 2).

Table 1. Names and origins of the collected melon accessions.

Code	Accession	Origin/province
G01	Ananasi	Gorgan
G02	Sefidkesh	Hamedan
G03	Balo	Urmia
G04	Keshavarz	Urmia
G05	Sabzevari	Khorasan
G06	Harati	Khorasan
G07	Khatooni	Khorasan
G08	Tashkandi	Khorasan
G09	Achachi	Miyaneh
G10	Nikabadchae	Miyaneh
G11	Bakermellon	Gorgan
G12	Mashhadi	Khorasan
G13	AtashiKolucheh	Miyaneh
G14	Atashimiyaneh	Miyaneh

Genomic DNA extraction and RAPD assay

DNA extraction from melon accessions was carried out using the CTAB method as detailed by Fulton *et al.* (1995). PCR amplifications were performed using 12 RAPD primers (Table 2). The amplification reactions were conducted in a total volume of 20 µl, containing 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, dATP, dCTP, dGTP, and dTTP each at 0.1 mM, 0.2 mM primer, 25-30 ng of genomic DNA, and 0.5 units of Taq DNA polymerase. Amplification was performed using a PCR instrument programmed for 40 cycles. After an initial denaturation step for two minutes at 94 °C, each cycle consisted of one minute at 94 °C, one minute at 36 °C, and two minutes at 72 °C. The 40 cycles were followed by a final extension step of seven minutes at 72 °C. PCR amplified products were subjected to electrophoresis on a 1.5% agarose gel in 1×TBE buffer at 120 V for three hours. The gels were then stained with ethidium bromide (1.0 µg ml^{-1}) and photographed under UV light using a Gel Documentation System (Bio-Rad, Canada).

Data analysis

Data obtained from the quantitative characteristics were subjected to analysis of variance (ANOVA). A combined analysis of the experiments was performed to assess significant differences among blocks, accessions, years, and the interaction between accessions and years. The homogeneity of residual variances was tested using Hartley's Fmax (1950) to validate the combined analysis of experiments. Stepwise multiple regression analysis was employed to identify agro-morphological characters that

Table 2. Code, sequence, and molecular characteristics of the applied RAPD primers.

Name	Sequence (5` to 3`)	Number of bands	Number polymorphic bands	PIC
OPA06	GGTCCCTGAC	13	13	0.37
OPB13	TTCCCCCGCT	13	13	0.38
OPD10	GGTCTACACC	11	9	0.30
OPD20	ACCCGGTCAC	14	12	0.31
OPE14	TGCGGCTGAG	11	10	0.25
OPF10	GGAAGCTTGG	11	6	0.18
OPG02	GGCACTGAGG	15	12	0.22
OPG10	AGGGCCGTCT	13	9	0.19
OPG12	CAGCTCACGA	12	9	0.25
OPJ16	CTGCTTAGGG	10	9	0.27
OPJ18	TGGTCGCAG A	13	12	0.31
OPM12	GGGACGTTGG	10	6	0.22

significantly contributed to total fruit weight, with total fruit weight as the dependent variable. Parameters with $P<0.01$ were subsequently included in the regression analysis as independent variables (Wada, 1986).

The path coefficient from an independent variable (X_i) to a dependent variable (Y) was calculated using the equation provided by Huo *et al.* (2010). Multivariate analysis through principal component analysis (PCA) was conducted using the mean values of the replicates for each character. The general divergence among accessions was estimated using a biplot produced from the first two components of the PCA analysis. All statistical analyses were conducted using IBM SPSS Statistics 23 software and Minitab 14.0.

For the RAPD data, polymorphic fragments were scored as present (1) or absent (0) for each of the 14 accessions. The number of loci, number of polymorphic bands, and polymorphism information content (PIC) were calculated for each RAPD primer. After calculating the Jaccard similarity matrix, the Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm was used for germplasm classification, conducted in SPSS 23.0 software. To identify informative markers linked with the studied agro-morphological characters, population structure was first analyzed using STRUCTURE software (Pritchard *et al.,* 2000), followed by an association analysis between markers and agro-morphological traits using TASSEL software (Bradbury *et al.,* 2007).

RESULTS AND DISCUSSION

The existence of genetic variability is a prerequisite for future breeding programs in melon, similar to other crops. The results showed significant differences among the studied melon germplasm for the majority of agro-morphological characters, including DF (Days to flowering), FPW (Flower petal width), FRT (Fruit ripening time), PD (Peduncle diameter), FSRT (Fruit storage at room temperature), SW (Seed width), and SL (Seed length) (Table 3). Additionally, some traits, such as 50% DF, LTL (Leaf tail length), NSPF (Number of seeds per fruit), TFF (Thickness of fruit flesh), FFW (Fruit fresh weight), 100 SW (100-seed weight), and FW (Fruit width), exhibited varied responses across the two years (Table 3). Previous studies (Feyzian *et al.,* 2007; Soltani *et al.,* 2022) have confirmed genetic diversity among Iranian local accessions of melon regarding morphological and fruit characteristics. Furthermore, Aragao *et al.* (2015) demonstrated the significance of year-to-year variability in melon plant performance. Consistent with our findings, literature reviews (Macedo *et al.,* 2017; Guliyev *et al.,* 2018) indicate remarkable morphological genetic diversity among melon accessions worldwide, suggesting that high genetic variability exists within *Cucumis melo*. The genetic variability observed among local melon accessions in this study could enhance the likelihood of successful selection in melon germplasm.

From a plant breeder's perspective, genetic variation for plant yield and agro-morphological traits is crucial, making it essential to examine the relationships between plant characteristics within a given germplasm (Hatami Maleki *et al.,* 2011). In this regard, path coefficient analysis is necessary for a better understanding of how the components of the dependent variable influence it. In this study, considering total fruit weight (TFW) as the dependent variable, stepwise regression analysis identified FFW, FPL, PD, PL, FSRT, and FL as effective traits influencing TFW of local melon germplasm (Table 4). The characters FFW and FL exhibited strong positive correlations of 0.95 and 0.81 with TFW,

Table 3. Combined analysis of variance for the anatomical characters of melon studied across the years 2019 and 2020.

Source of variation	DF	Mean of square										
		DF	FPL	FPW	50% DF	LTL	FRT	LL	LW	ID	NAS	PL	PD
Year	1	160.19**	3.69	1.31*	443.44**	287.00**	3936.01**	75.05**	307.05**	13.68	654.65**	1.24	1.44
Replication (year)	4	8.81	0.59	0.06	8.81	4.57	22.83	1.19	5.18	1.11	0.63	0.04	1.27
Accession	13	22.47*	0.15	0.15*	60.91	4.97	354.19**	4.04	7.53	3.46	20.13	0.11	2.14**
Year×accession	13	8.03*	0.11	0.05	35.7**	7.56*	39.11	3.47*	4.88	3.9	9.5	0.08	0.16
Error	52	3.84	0.14	0.06	3.76	3.71	22.71	1.5	3.39	2.62	10.86	0.10	1.34

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

*, ** and ^{ns} are significant in 1%, 5% and nonsigificant respectively.

Table 3 (Continued). Combined analysis of variance for the anatomical characters of melon studied across the years 2019 and 2020.

Source of variation	DF	Mean of square										
		PS	FFW	TFW	FSRT	TFF	NSPF	100 SW	SW	SL	FL	FW
Year	1	215369**	51.38**	75.68**	13.29	20.88**	1721884**	24.95**	0.67	48.76*	932.30**	3325.15**
Replication (year)	4	9278	0.05	1.18	73.30	0.52	9078	0.32	0.27	1.23	1.58	4.61
Accession	13	2353	0.77	1.13	2214.84**	0.27	27901	2.23	0.48	2.17**	64.19*	19.96
Year×accession	13	1068	0.77**	0.84	146.53	0.32*	30326**	1.07*	0.43	0.31	20.43	26.64**
Error	52	835	0.24	0.47	170.21	0.17	9085	0.57	0.29	0.85	13.00	4.28

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

*, ** and ^{ns} are significant in 1%, 5% and nonsigificant respectively.

Table 4. Path coefficient analysis based on two years of agro-morphological data for melon. Diagonal (underlined) values represent direct effects while off-diagonal values depict indirect effects.

Trait	FFW	FPL	PD	PL	FSRT	FL	Correlation
FFW	<u>0.88</u>	-0.11	-0.02	-0.05	0.04	0.2	0.95
FPL	-0.39	<u>0.25</u>	0.03	0.05	-0.04	-0.14	-0.24
PD	0.08	-0.05	<u>-0.17</u>	0.00	0.05	-0.01	-0.09
PL	-0.31	0.09	0.00	<u>0.14</u>	0.01	-0.1	-0.17
FSRT	-0.29	0.07	0.06	-0.01	<u>-0.13</u>	-0.04	-0.34
FL	0.78	-0.16	-0.01	-0.06	0.02	<u>0.23</u>	0.81

FFW: Friut fresh weight, FPL: Flower petal length, PD: Peduncle diameter, PL: Peduncle length, FSRT: Friut store at room temperature, FL: Friut length.

respectively (Table 4). The majority of the relationship between TFW and FFW can be attributed to the direct effect of FFW (0.88), while the relationship between TFW and FL is largely due to the indirect effect of FL (0.78) mediated by FFW (Table 4). Similarly, Zalapa *et al.* (2008) found that fruit length and weight had a strong association with yield, suggesting these traits as selection criteria. This research implies that direct selection based on higher mean values of these traits could improve yield.

To reduce data dimensionality and classify the studied melon germplasm, PCA (Principal Component Analysis) was performed (Table 5, Figure 1). Seven principal components (PCs) were identified, explaining 89.8% of the total data variation (Table 5). The loading coefficients of the studied characteristics, as criteria for character magnitude, were calculated for each PC (Table 5).

Table 5. Principal component analysis of 23 studied agro-morphological characters in 14 melon accessions.

Character	Components						
	PC1	PC2	PC3	PC4	PC5	PC6	PC7
DF	0.25	0.26	-0.15	0.06	0.16	0.11	0.10
FPL	-0.17	-0.03	0.46	-0.18	0.12	-0.04	-0.11
FPW	-0.24	-0.10	0.29	0.23	0.08	0.01	0.03
50% DF	0.26	0.23	-0.21	-0.05	0.18	0.03	-0.07
LTL	0.09	-0.33	0.29	0.23	-0.21	0.23	0.19
FRT	0.16	0.38	-0.09	-0.07	-0.21	-0.31	0.15
LL	0.22	-0.02	0.26	0.12	0.05	-0.21	0.43
LW	0.25	0.08	0.30	0.01	0.09	-0.06	0.28
ID	-0.19	-0.28	-0.14	0.27	-0.21	-0.09	0.10
NAS	-0.13	-0.24	-0.08	-0.36	0.02	-0.50	0.04
PL	-0.14	0.08	0.02	-0.03	0.64	-0.01	0.23
PD	-0.04	-0.26	-0.24	-0.31	-0.12	0.12	0.56
PS	0.21	0.03	0.05	-0.26	-0.33	0.35	0.12
FFW	0.28	-0.23	-0.07	-0.05	0.12	-0.07	-0.09
TFW	0.27	-0.22	0.03	-0.01	0.27	-0.08	-0.17
FSRT	0.01	0.42	0.31	-0.11	-0.19	0.01	0.09
TFF	0.04	-0.12	0.21	-0.56	0.14	0.35	-0.08
NSPF	0.27	-0.09	0.07	0.32	0.13	0.05	0.13
100 SW	0.25	-0.16	-0.15	-0.11	-0.14	-0.14	-0.12
SW	0.19	-0.01	0.26	0.03	-0.19	-0.05	-0.40
SL	0.19	-0.10	0.22	-0.13	-0.09	-0.47	0.01
FL	0.31	-0.09	-0.12	0.12	-0.02	0.12	-0.07
FW	0.25	-0.23	0.00	-0.03	0.14	0.08	-0.06
Eigen value	9.34	3.19	2.49	1.76	1.52	1.26	1.08
Variance (%)	40.6	13.9	10.8	7.7	6.6	5.5	4.7
Cumulative variance	40.6	54.5	65.3	73	79.6	85.1	89.8

DF: Day to flowering, FPL: Flower petal length, FPW: Flower petal width, 50% DF: Day to 50% flowering, LTL: Leaf tail length, FRT: Friut rippening 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: Friut fresh weight, TFW: Total friut weight, FSRT: Friut store at room temprature, TFF: Thickness of fruit flesh, NSPF: Number of seed per friut, 100 SW: 100-seed weight, SW: Seed width, SL: Seed length, FL: Friut length, FW: Friut width.

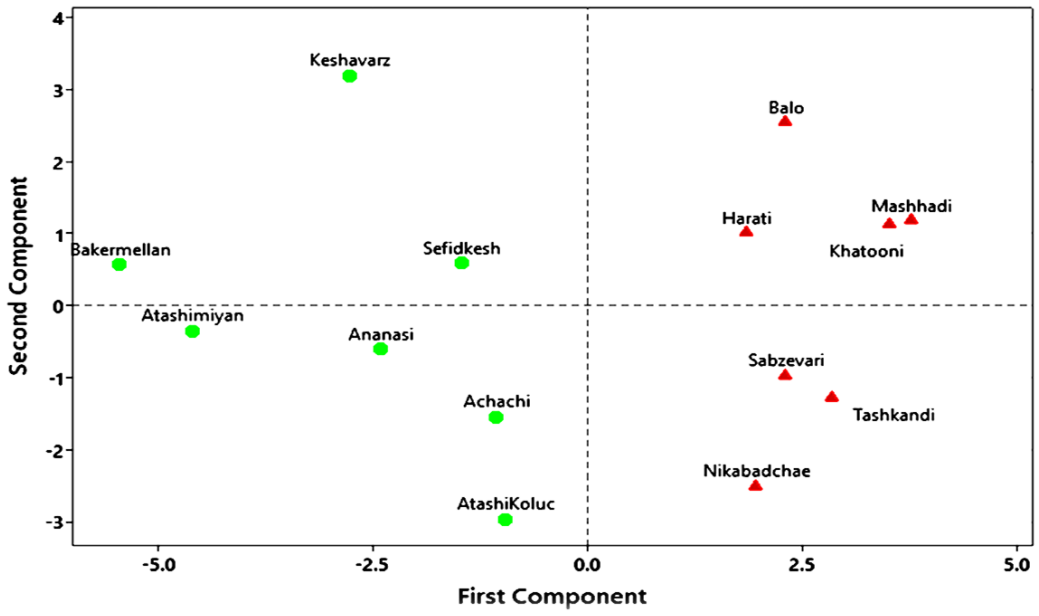

Figure 1. Scatter plot depicting the dispersion of studied melon accessions based on agro-morphological characters.

The first two PCs explained 54.5% of the data variability, with PC1 accounting for 40.6% and PC2 for 13.9%. Previous studies on melon (Trimech *et al.,* 2013) reported 49.68% of the explained variation for the first two components. According to Table 5, flower-related variables such as FPL, FPW, PL, and PD had negative coefficients in PC1, while fruit yield-related variables, including FFW, TFW, FL, FW, TFF, and flowering attributes, had positive loading coefficients. In PC2, most studied characteristics had negative relationships with this component, except for DF, 50% DF, FRT, LL, PL, PS, and FSRT. The loading coefficients were used to calculate the PC1 and PC2 scores for each accession, allowing for a two-dimensional arrangement of accessions (Figure 1). The scatter plot of melon accessions illustrates whether the agro-morphological dispersion corresponds with their geographical distribution. As shown in Figure 1, most accessions from the Khorasan region, except for "Balo" and "Nikabadchae," were clustered in the red group, while the remaining accessions from Gorgan, Hamedan, Miyaneh, and Urmia were placed in the green group. Although accessions from Khorasan and Miyaneh were distinguishable by PCA analysis, this method did not effectively separate other geographically distant melon accessions. In contrast to our findings, Trimech *et al.* (2013) emphasized PCA's ability to group melon accessions and reported a correlation between the geographical distribution of melon genotypes and their arrangement in the PCA plot.

In addition to agro-morphological traits, high molecular genetic variability was also observed among the studied melon accessions. In the RAPD assay, the number of loci detected for each RAPD primer ranged from 10 to 15 (Table 2). Most detected loci per primer were polymorphic (Table 2). This finding aligns with Naznin *et al.* (2023), who reported significant genetic variability in Cambodian melon landraces using RAPD markers. Similar to the present study, several investigations (Feyzian *et al.,* 2007) have highlighted the capability of RAPD markers to evaluate genetic diversity among Iranian melon accessions. The polymorphism information content (PIC), which indicates the primer's effectiveness in assessing genetic diversity, ranged from 0.18 (primer OPF10) to 0.38 (primer OPB13) (Table 2). Primers like OPB13, with a PIC value close to 0.5 (the maximum for any dominant marker), demonstrated promising potential for distinguishing melon germplasm in preliminary screenings. The classification of the studied melon germplasm using the UPGMA algorithm and Jaccard similarity matrix revealed three main groups at a distance of 22 (Figure 2).

As shown in Figure 2, two accessions from Mianeh, including "Atashi Kolucheh" and "Atashi Miyaneh," were located in the same group, while the accession named "Mashahdi" is located in a group alone. It is concluded that there is a minor coincidence between agro-morphological and molecular classification. Additionally, the complicated classification resulting from RAPD markers validates the existence of genomic mixture among the studied accessions over

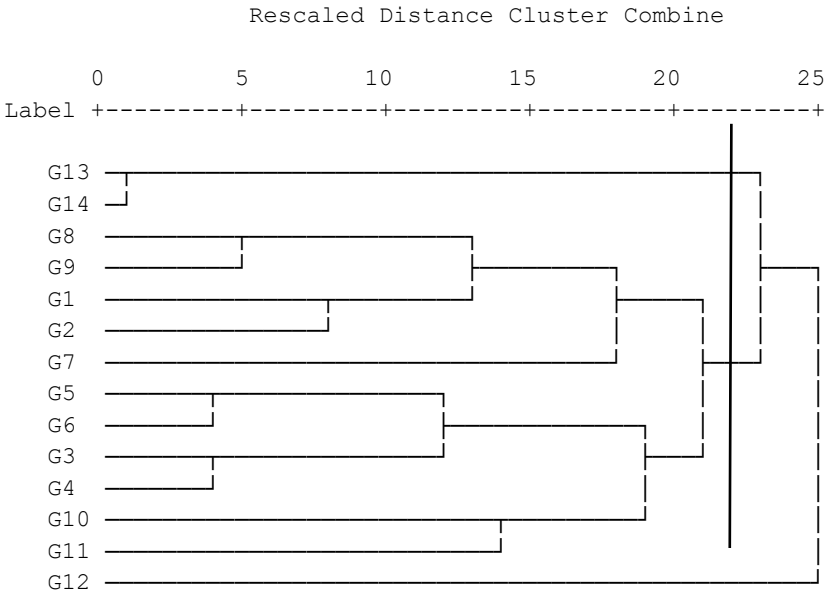

Figure 2. Classification of studied melon accessions based on RAPD data using the Jaccard similarity coefficient and the UPGMA algorithm.

Table 6. Results of the marker-trait association study using mixed linear model (MLM) analysis.

Trait	DNA marker	F_Marker	p_Marker	Trait	DNA marker	F_Marker	p_Marker
DF	OPD20-13	17.0175	0.00	FFW	OPA6-3	16.2469	0.00
	OPG10-13	17.0175	0.00		OPD20-1	8.7927	0.01
FPL	OPG10-2	14.1911	0.00		OPD20-4	11.8126	0.01
FPW	OPB13-13	8.9487	0.01	TFW	OPB13-3	9.4674	0.01
	OPJ6-3	9.8364	0.01		OPD10-1	11.7991	0.01
50% DF	OPJ6-3	10.2293	0.01		OPJ18-13	17.4763	0.00
LTL	OPJ18-9	8.8841	0.01		OPJ6-2	17.4763	0.00
	OPJ6-7	36.008	0.00		OPM12-5	17.4763	0.00
FRT	OPE14-10	14.8836	0.00	FSRT	OPF10-2	9.165	0.01
	OPE14-9	14.8836	0.00		OPG12-10	8.8857	0.01
LL	OPF10-9	9.1866	0.01	TFF	OPG10-5	13.353	0.00
LW	OPF10-9	10.398	0.01		OPJ18-10	9.8054	0.01
ID	OPD20-10	16.5018	0.00		OPJ6-1	11.5736	0.01
	OPD20-11	16.5018	0.00	NSPF	OPA6-4	19.4002	0.00
	OPG10-8	153.5444	0.00		OPD20-4	11.9457	0.01
	OPG10-9	153.5444	0.00	100 SW	OPJ6-3	9.6547	0.01
	OPG12-6	153.5444	0.00	SW	OPD20-14	9.9945	0.01
NAS	OPD20-10	9.8517	0.01	SL	OPF10-10	14.3753	0.00
	OPD20-11	9.8517	0.01		OPJ18-9	17.8028	0.00
	OPD20-13	9.1075	0.01	FL	OPG10-8	66.5277	0.00
	OPG10-13	9.1075	0.01		OPG10-9	66.5277	0.00
PL	OPG2-1	9.0413	0.01		OPG12-6	66.5277	0.00
PS	OPD10-7	10.0898	0.01	FW	OPD20-4	10.5483	0.01
					OPJ18-6	8.8755	0.01

DF: Day to flowering, FPL: Flower petal length, FPW: Flower petal width, 50% DF: Day to 50% flowering, LTL: Leaf tail length, FRT: Friut rippening 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: Friut fresh weight, TFW: Total friut weight, FSRT: Friut store at room temprature, TFF: Thickness of fruit flesh, NSPF: Number of seed per friut, 100 SW: 100-seed weight, SW: Seed width, SL: Seed length, FL: Friut length, FW: Friut width.

several decades. To identify markers associated with morphological traits in melon germplasm, an association analysis was performed using a mixed linear model (MLM) (Table 6). In this regard, a total of 36 different RAPD marker loci showed a significant relationship with the genes controlling the studied traits. Based on the MLM model, more than one genomic locus was identified for each of the traits, including DF, FPW, LT, FRT, ID, FFW, TFFW, FSRT, TFF, and NSPF (Table 6). The results of this research showed that there are 11 co-localized markers for different traits, and such markers can accelerate the breeding program (Darvishzadeh *et al*., 2014) through simultaneous selection for several traits. For traits ID with NAS, DF with NAS, FFW, and NSPF, as well as FW, LL with LW, ID with FL, FW and LT, and SL, FPW with 50% DF, and 100 SW, co-localized markers were identified (Table 6). The results of association mapping showed that, except for the PD trait, there is a significant positive marker for other traits, indicating the efficiency of RAPD markers in genome selection for melon plants.

CONCLUSION

The collected melon accessions from Iran exhibited high variability in both morphological and fruit-related characteristics, making them suitable for breeding programs aimed at developing more prolific cultivars, enhancing productivity, and improving adaptation to diverse production regions. Among the studied agro-morphological traits, fruit fresh weight and fruit length were identified as effective traits for selection in breeding programs focused on achieving high fruit yield.

This study concluded that RAPD markers can be effectively applied to evaluate the genetic variability of local melon accessions in Iran. Notably, the examined melon germplasm displayed distinct dispersion patterns in both molecular and agro-morphological data, suggesting that the classification of melon accessions does not strictly follow geographical distribution. However, the identified heterotic groups offer valuable potential for future melon breeding programs, leveraging the heterosis phenomenon and serving as parental lines for constructing mapping

populations.

Additionally, certain RAPD markers were identified as linked to multiple agro-morphological traits simultaneously. These co-localized loci could facilitate marker-assisted breeding, streamlining the selection process for desirable traits in melon development.

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