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Genetic Diversity in Iranian Melon Populations and Hybrids Assessed by IRAP and REMAP Markers

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ABSTRACT

Retrotransposons (RTNs) constitute informative molecular markers for plant species because of their ability to integrate into a multitude of loci throughout the genome and thereby generate insertional polymorphisms between individuals. In the present study, RTN-based molecular markers, IRAP (inter-retrotransposon amplified polymorphism) and REMAP (retrotransposon-microsatellite amplified polymorphism), were applied to study RTN integration events and genetic diversity in 100 melon genotypes (88 genotypes from 11 populations, three inbred lines, and 9 hybrids). A total of 94 and 262 loci were amplified using 5 IRAP and 15 REMAP primers, respectively. The percentage of polymorphic loci (PPL) in populations ranged from 39% (Zivari Shahrood) to 48% (Shadegani E). The Mantel test between IRAP and REMAP cophenetic matrices evidenced no significant correlation (r= 0.29). IRAP+REMAP-based cluster analysis using UPGMA algorithm and Dice similarity coefficient depicted 6 groups among 100 melon genotypes. AMOVA revealed the higher level of genetic variation within populations (67%) compared to among populations (33%). The mean *Fst* values of all groups, except for group VI, were more than 0.20, demonstrating differentiation among the populations and genetic structure of the studied melon collection.

Keywords: *Cucumis melo*, Genetic variability, *Reme1*, Retrotransposon.

INTRODUCTION

Melon (*Cucumis melo* L.) is an economically important crop of the *Cucurbitaceae* family grown in temperate and tropical climates worldwide. It is a diploid species (2n= 2x= 24), with a relatively small genome of 450 Mb, similar in size to that of rice (Garcia-Mas *et al*., 2012). It contains many landraces and numerous varieties, either consumed as a dessert fruit or a vegetable in Asia and Africa (Kirkbride, 1993).

Due to the abundance of retrotransposons (RTNs) in plant genomes and their ability to create new copies, they have been used as molecular markers (Kalendar *et al*., 1999; Mardi *et al*., 2011; Nasri *et al*., 2013). The melon genome contains approximately 20000 *Gypsy* and 6800 *Copia* RTN elements, comprising about 14.7% of its total size (Ramallo *et al*., 2008; Garcia-Mas *et al*., 2012). The haploid genome of melon (var. Piel de Sapo) contains about 120 copies of RTN *Reme1* (Ramallo *et al*., 2008). Several RTN-based molecular marker methods such as IRAP and REMAP have been developed, which rely on the principle that a joint is formed between the RTN and the genomic DNA during the integration process (Kalendar *et al*., 1999). The RTN-based markers have been applied successfully to the analysis of phylogenetic evolution and genetic diversity in genera and species as diverse as rice (Branco *et al*., 2007) alfalfa (Abdollahi Mandoulakani

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et al., 2012), apple (Kristiina *et al*., 2006), flax (Smykal *et al*., 2011), sunflower (Vukich *et al*., 2009) and grapevine (Onofrio *et al*., 2010).

To our knowledge, no IRAP and REMAP based study has been published in melon. Therefore, the aim of the present study was to use IRAP and REMAP markers for detecting integration events and activity of native (*Reme1* from melon) and non-native (RTNs originated from watermelon) RTN families in melon and to assess the genetic variability of a collection of melon populations and hybrids in order to evaluate the potential application of IRAP and REMAP techniques in melon breeding programs.

MATERIALS AND METHODS

Plant Material and DNA Isolation

A collection of 100 melon genotypes, including 88 genotypes from 11 melon populations (8 plants from each population), three inbred lines, and 9 hybrids (Table 1) were analyzed. Seeds of the genotypes were grown in small pots with 10 cm diameter containing mixture of garden soil and vermiculite in greenhouse with an ambient temperature 25°C. DNA was extracted from young leaves of 20-day seedlings, as described previously (Ausubel *et al*., 1995), with minor modifications intended to improve the quality of DNA: two consecutive extractions with Chloroform isoamyl alcohol (24:1) were performed followed by an additional wash with 5M NaCl for 20 minutes at -20°C, before final precipitation, to reduce the presence of polysaccharides. The quality and concentration of the DNA were measured using a spectrophotometer and electrophoresis in a 0.8% (w/v) agarose gel.

Table 1. Studied melon populations, hybrids, and inbred lines.

Name	Number	Abbreviation	Origin	Horticultural group
Khatooni Farimani	1-8	KhF	Iran-Khorasan-Fariman	Inodorus
Dargazi Tashkandi	9-16	DTa	Iran-Khorasan-Dargaz	Inodorus
Zivari Shahroud	17-24	ZSh	Iran-Semnan-Shahroud	Inodorus
Chahpaliz	25-32	Cha	Iran-Khorasan	Inodorus
Tashkandi M	33-40	TaM	Iran-Khorasan	Inodorus
Shadegani M	41-48	ShM	Iran-Khuzestan	Inodorus
Minoo	49-56	Min	Iran-Khorasan	Inodorus
Shadegani E	57-64	ShE	Iran-Khuzestan	Inodorus
Tal Shahroud	65-72	TSh	Iran-Semnan-Shahroud	Cantalupensis
Khagani M	73-80	KhM	Iran-Khorasan-Mashhad	Inodorus
Jalali	81-88	Jal	Iran-Semnan-Eyvanakey	Inodorus
637	89	Hyb1	Biotek-Turkey	Inodorus
Meanhno 9	90	Hyb2	Ergon-USA	-
Summit	91	Hyb3	Peto seed-USA	Cantalupensis
Durango	92	Hyb4	Peto seed-USA	Cantalupensis
Omega	93	Hyb5		Cantalupensis
Ananas	94	Hyb6	Enza Zaden-Netherlands	-
Super soykan	95	Hyb7	Biotek-Turkey	Inodrus
Behta N397	96	Hyb8	Behta-Iran	Inodrus
Dublone	97	Lin1	INRA, Avignon-France	Cantalupensis
PI414723	98	Lin2	INRA, Avignon-France	Momordica
Goldtropy	99	Hyb9	Canyon-USA	-
Charentais T	100	Lin3	INRA, Avignon-France	Cantalupensis

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IRAP and REMAP Reactions

The amplification reaction was performed as described by Abdollahi Mandoulakani *et al*. (2012). Five single and 10 IRAP primer combinations were used to study genetic diversity in 100 melon genotypes. RTN-based primers were designed based on *Reme1* (isolated from *C. melo*) (Ramallo *et al*., 2008) and RTNs characterized in watermelon (Table 2 and Supplementary Table 1). The amplification profile composed of an initial denaturation at 94°C for 4 minutes, followed by 35 cycles at 94°C for 45 seconds, 53 to 55°C (Table 2) for 40 seconds, 72°C for 2 minutes and a final extension of 5 minutes at 72°C. PCR products were separated by electrophoresis using 1.8% agarose gel in 0.5X TBE buffer with constant voltage of 65V for 3 to 4 hours. Gels were stained by ethidium bromide, then visualized under UV light and photographed using a gel documentation system.

For REMAP analysis, 5 IRAP primers combined with 15 3'-anchored ISSR primers, were tested on 8 melon genotypes to select the primer combinations producing scorable and discernible banding patterns (Table 2 and Supplementary Table 1). PCR reactions and temperature profile, electrophoresis and visualization of REMAP markers were the same as stated for IRAP. Annealing temperature of REMAP primer combinations depended on the primers (Table 2).

Table 2. Characteristics of the used IRAP and REMAP primer combinations in the current study.

Primer	Tm[a]	TL[b]	PL[c]	PPL[d]	He[e]	Ne[f]	I[g]	BS[h] (bp)
IRAP								
LTR4	54	20	20	100	0.40	1.72	0.58	400-3000
LTR2467	53	18	18	100	0.24	1.41	0.36	300-2000
LTR2476	54	14	12	85	0.33	1.56	0.50	200-3000
LTR4+LTR2453	53	24	23	95	0.38	1.66	0.55	75-2000
LTR2467+LTR2453	55	18	17	94	0.40	1.66	0.54	200-2000
Total		94	90					
Mean		18.8	18	95	0.35	1.60	0.51	
REMAP								
LTR4+A7	55	14	11	78	0.32	1.58	0.47	200-1500
LTR4+811	51	19	16	84	0.23	1.37	0.37	75-2000
LTR4+816	54	15	12	80	0.38	1.58	0.46	75-1500
LTR4+849	52	17	15	88	0.27	1.46	0.42	200-3000
LTR4+834	53	20	20	100	0.35	1.60	0.52	75-3000
LTR4+855	54	16	16	100	0.30	1.49	0.46	200-2000
LTR4+880	54	20	20	100	0.29	1.49	0.45	200-2000
LTR2467+855	53	16	14	87	0.31	1.52	0.47	75-2000
LTR2476+825	50	17	17	100	0.36	1.65	0.52	75-2000
LTR2467+826	52	17	17	100	0.34	1.59	0.51	200-2000
LTR2452+855	52	24	24	100	0.29	1.48	0.45	200-2000
LTR2452+825	50	19	17	89	0.29	1.51	0.44	300-2000
LTR2452+808	50	19	17	89	0.30	1.51	0.46	300-3000
LTR2452+840	50	16	16	100	0.39	1.71	0.57	400-2000
LTR2453+A7	50	13	12	92	0.311	1.51	0.47	200-1500
Total		262	244					
Mean		17.48	16.26	93	0.32	1.54	0.47	

[a] Annealing temperature; [b] Total Loci; [c] PL: Polymorphic Loci; [d] Percentage of Polymorphic Loci; [e] Mean of expected Heterozygosity; [f] Number of effective alleles, [g] Shannon's information index, [h] Band Size.

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Data Analysis

Each band row in the gels was considered as a locus, then amplification products were scored independently as 1 and 0 for presence and absence of the bands, respectively, and the attained binary data were used for analysis. Genetic similarity matrices between individual pairs of genotype were calculated for IRAP, REMAP and IRAP+REMAP data using Dice similarity coefficient in NTSYSpc 2.1 (Rohlf, 2000). These matrices were used to construct dendrograms using UPGMA algorithm. To verify the adjustment between genetic similarity matrices and respective dendrogram-derived matrices (cophenetic matrix), the cophenetic correlation coefficients (r) were estimated. Degree of correlation among the three cophenetic matrices for IRAP, REMAP and IRAP+REMAP was calculated using a Mantel's test of matrix comparison with 1,000 permutations in NTSYSpc 2.1 software. The statistical stability of the clusters was estimated by a bootstrap analysis with 1,000 replications with the Winboot software (Yap and Nelson, 1996). To evaluate the genetic structure of the melon collection and confirm the UPGMA clustering, Structure 2.3.1 (Falush *et al*., 2007) software was used with no admixture parameter, 50,000 generations of burn-in period, 100,000 MCMC iterations and different values of *K* for IRAP+REMAP data. The optimal *K* value (as true cluster number) was determined by the posterior probability [ln P(D)] and an ad hoc statistic ΔK based on the rate of change in [ln P(D)] between successive *K* (Evanno *et al*., 2005) using the software Structure Harvester. In order to partition the total genetic variation among and within populations, analysis of molecular variance (AMOVA) was carried out based on IRAP+REMAP data using GenAlEx 6.41 (Peakall and Smouse, 2006). Number of loci, percentage of polymorphic loci (PPL), number of loci with a frequency higher or equal to 5%, number of private loci, number of less common loci with frequency lower or equal to 25% and 50%, mean of expected heterozygosity (He), number of effective alleles (Ne) and Shannon's information index (I) were calculated for each population using IRAP, REMAP and IRAP+REMAP data. To measure the discriminating ability of each primer, *He, Ne*, and *I* were calculated for each primer as well.

RESULTS

IRAP Analysis

As expected, the IRAP analysis produced a high level of polymorphism (95%) (Table 2 and Figure 1-a). Out of 15 tested IRAP primers, 3 single and 2 primer combinations generated an average of 18.8 loci per primer; the highest and the smallest numbers of loci obtained with the primer LTR4-LTR2453 (24 loci) and LTR2476 (14 loci), respectively (Table 2). IRAP-based UPGMA dendrogram clustered 100 genotypes into 8 main groups (Supplementary Figure 1). No population-specific locus was obtained with IRAP markers. The percentage of IRAP polymorphic loci in population varied from 43% (ZSh) to 61% (Cha), averaging 52% (Supplementary Table 2).

REMAP Analysis

Out of 60 tested REMAP primer combinations, 15 primers generated a total of 262 loci, of which 92% were polymorphic (Table 2 and Figure 1-b). The primer combinations that amplified the highest and lowest number of loci were LTR2452-855 (24 loci) and LTR2453-A7 (13 loci), respectively (Table 2). Eight main groups were identified using REMAP-based UPGMA cluster (Supplementary Figure 2). The percentage of REMAP polymorphic loci in population varied from 37 (ZSh) to 46% (ShE), averaging 41%.

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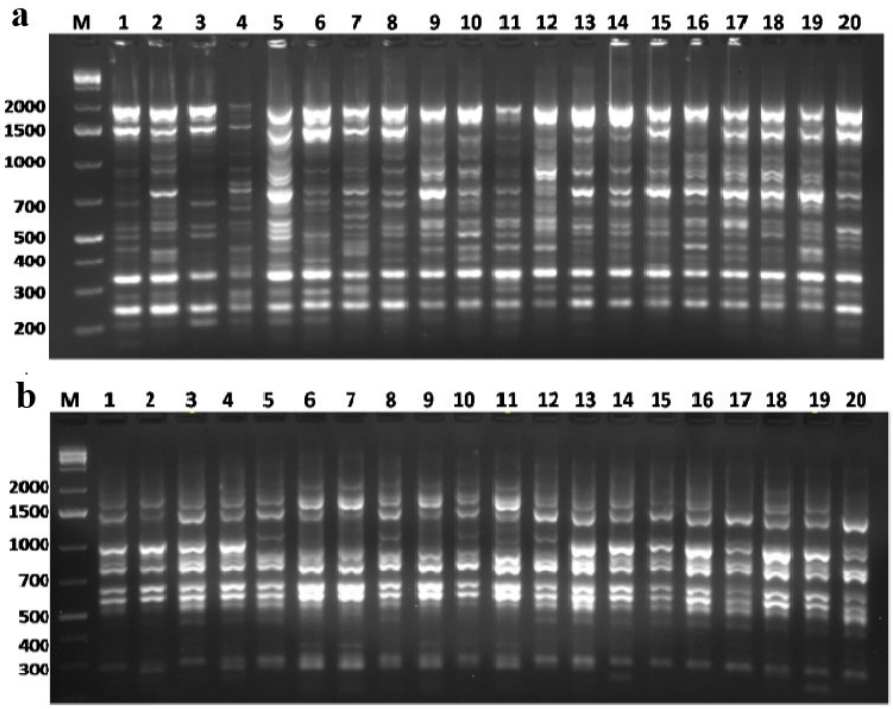

Figure 1. (**a**) Polymorphism detected by IRAP primer LTR2467. Lanes from left to right: (1 to 4) Individuals from population Minoo (Min); (5 to 12) Individuals from population Shadegani E (ShE), (13 to 20) Individuals from population Tal Shahroud (TSh), (**b**) Polymorphism detected by REMAP primer combination LTR2452+855. Lanes from left to right: (1 to 4) Individuals from population Tashkandi M (TaM); (5 to 12) individuals from population Zivari Shahroud (ZSh); (13 to 20) Individuals from population Dargazi Tashkandi (DTa), (M) 1kb O'GeneRuler™ DNA ladder (Fermentas) in base pair.

Five population-specific loci were detected in populations KhF, TaM, Min, ShE and Jal (Supplementary Table 3).

Combined Data Analysis

Mantel test between IRAP and REMAP cophenetic matrices revealed no significant correlation. Hence, combined data of IRAP and REMAP markers were used to build a dendrogram to accumulate the power of both techniques. IRAP+REMAP-based cluster analysis identified 6 groups among the studied genotypes (Figure 2). AMOVA revealed high level of genetic variation within-populations (67%) compared to among-populations (33%). The percentage of polymorphic loci in population varied from 39 (ZSh) to 48% (ShE), averaging 44%. The smallest values of *He, Ne*, and *I* were observed for population ZSh (Table 3).

DISCUSSION

RTN Insertional Polymorphism in Melon Genotypes

In this study, 5 IRAP and 15 REMAP primers amplified discernible banding pattern and were applied to study the used RTNs activity and genetic diversity among 100 melon genotypes. *Reme1* is the first complete *Copia* RTN, cloned, sequenced and characterized in melon (Ramallo *et al*., 2008). It is present in most of the various *Cucurbitaceae* species, indicating its presence before the species separation in this genus (Ramallo *et al*., 2008). In the current

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Table 3. Characteristics of the amplified IRAP+REMAP loci in studied melon populations.

Population[a]	KhF[a]	DTa[b]	ZSh[c]	Cha[d]	TaM[e]	ShM[f]	Min[g]	ShE[h]	TSh[i]	KhM[k]	Jal[l]	Mean
Number of loci	274	271	271	282	282	278	267	272	288	269	272	
Percentage of polymorphic loci	47	43	39	45	44	42	42	48	45	43	46	44
Number of private loci	1	0	0	0	1	0	1	1	0	0	1	
Number of loci with frequency ≥ 5%	274	271	271	282	282	278	267	272	288	269	272	
Number of less common loci (≤ 25%)	8	1	5	2	4	2	2	2	6	4	5	
Number of less common loci (≤ 50%)	17	12	23	13	18	19	14	17	23	20	19	
Mean of Heterozygosity (He)	0.17	0.16	0.15	0.16	0.17	0.16	0.16	0.18	0.17	0.16	0.17	0.16
Standard error of He	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Number of effective alleles (Ne)	1.29	1.27	1.26	1.28	1.30	1.29	1.28	1.31	1.30	1.29	1.30	1.29
Standard error of Ne	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Shannon's information index (I)	0.25	0.23	0.21	0.24	0.25	0.23	0.23	0.26	0.25	0.24	0.25	0.24
Standard error of I	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02

[a] Khatooni Farimani; [b] Dargazi Tashkandi; [c] Zivari Shahroud; [d] Chahpaliz; [e] Tashkandi M, [f] Shadegani M; [g] Minoo; [h] Shadegani E; [i] Tal Shahroud; [k]: Khagani M, [l] Jalali.

study, the transpositional activity and insertional polymorphism of *Reme1* and other long terminal repeat (LTR) RTNs, characterized in watermelon (LTR2452, LTR2453, LTR2467 and LTR2476), was demonstrated in melon genome. Several investigations have reported that primers designed based on LTR sequences of RTN families can be readily transferred among closely related genera (Lou and Chen, 2007; Kalendar *et al*., 2011).

Single IRAP primer LTR4 (designed based on *Reme1* RTN) amplified polymorphic banding pattern, showing its movement and transpositional activity in the studied melon genotypes. In addition, watermelon LTR-based primers produced polymorphic banding patterns, suggesting that these elements may have relatives in *C. melo* and probably come from the common ancestors before the divergence of melon and watermelon in evolutionary processes. Tam *et al*. (2005), in a study of comparative analyses of genetic diversity within tomato, stated that RTN sequences isolated from one species could be used in related *Solanaceae* genera. Primer LTR2453 generated much polymorphism in combination with LTR4 and LTR2467, indicating the insertion of this RTN near or into the other RTNs in melon genome. The insertion of the RTNs near or into each other has been reported in plant genomes (Branco *et al*., 2007; Vukich *et al*., 2009; Carvalho *et al*., 2010; Onofrio *et al*., 2010). The multiplicity of REMAP loci per each melon genotype reflected the insertion events between LTR sequences of the used RTNs and SSR motifs. The insertion of RTNs near different SSR motifs has been already documented in alfalfa (Abdollahi Mandoulakani *et al*., 2012) and wheat (Carvalho *et al*., 2012). *Reme1*-based REMAP patterns amplified bands with different SSR motifs, reflecting no preferential insertion of this RTN in SSR-rich region.

Genetic Relationship and Characterization of Melon Populations

The estimated correlations between the three generated cophenetic matrices from

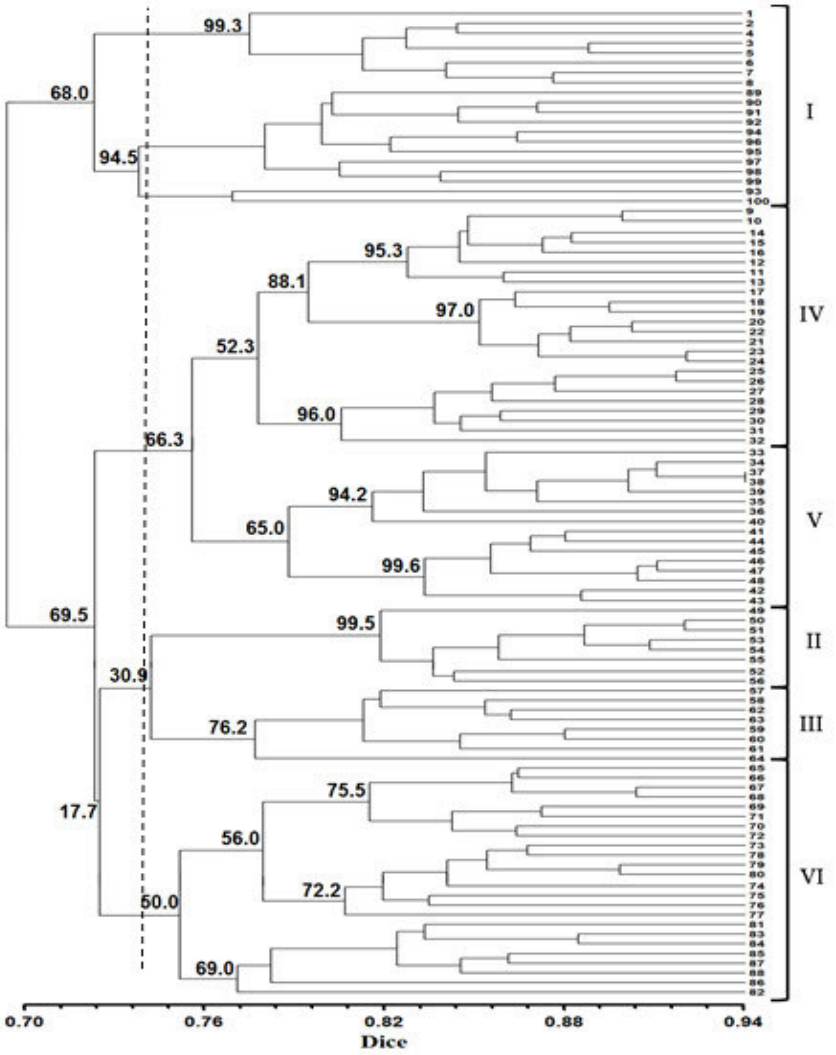

Figure 2. UPGMA dendrogram of 100 melon genotypes using IRAP+REMAP loci based on Dice similarity coefficient. The percentage values for groups represent 1,000 bootstrap cycles, Cophenetic correlation coefficient (r)= 0.74. Numbers: (1 to 8) Khatooni Farimani (KhF); (9 to 16) Dargazi Tashkandi (DTa); (17 to 24) Zivari Shahroud (ZSh); (25 to 32) Chahpaliz (Cha); (33 to 40) Tashkandi M (TaM); (41 to 48) Shadegani M (ShM); (49 to 56) Minoo (Min); (57 to 64) Shadegani E (ShE); (65 to 72) Tal Shahroud (TSh); (73 to 80) Khagani M (KhM); (81 to 88) Jalali (Jal); (89 to 96 and 99) Hyb 1 to Hyb 8 and Hyb 9, (97, 98 and 100) Lin1, Lin2 and Lin3.

IRAP, REMAP and IRAP+REMAP dendrogram evidenced a high and significant correlation of REMAP with IRAP+REMAP (r= 0.94), but the correlation of IRAP matrix with IRAP+REMAP was relatively low (0.41). However, the matrices estimated by the techniques individually also revealed a low and non-significant correlation (r= 0.29). These results suggest that, similarly to what is found in barley (Kalendar *et al*., 1999), rice (Branco *et al*., 2007) and wheat (Nasri *et al*., 2013), REMAP primers amplified DNA regions that could not be covered by IRAP. Therefore, to accumulate the power of both markers and get a confident classification of the studied melon

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collection, combined analysis of IRAP+REMAP data was used to reveal the association between individuals. UPGMA cluster using Dice similarity coefficient based on IRAP+RAMAP data identified 6 groups among 100 melon genotypes. The high cophenetic correlation coefficient (r= 0.74) suggest that this dendrogram is a good representation of our IRAP+REMAP data. Besides, percentage bootstrap values higher than 50% for most of the cluster forks corroborated the tree estimated based on IRAP+REMAP data. However, in order to determine whether this melon collection is differentiated in 6 groups, genetic structure analysis was performed using the software Structure 2.3.1. After performing several runs for different values of *K*, *K*= 6 was considered as the more suitable value to estimate the mean Fixation Index (Fst) for each group (Table 4). Except for group VI, all mean *Fst* values were higher than 0.20, indicating differentiation among the populations, genetic structure of the studied melon collection, and relatively low gene flow among them. This assumption was also corroborated by the allele frequency divergence estimated between pairs of populations (data not shown) and no mingles of the individuals from the different populations in the same cluster. Additionally, the Bayesian analysis was performed to assess the genetic relationships among the groups and occurrence of gene flow among them. This analysis revealed that the melon collection studied here was genetically structured in 6 groups (Figure 3). Hence, the genetic structure analysis performed using software Structure 2.3.1 corroborated the UPGMA clustering of the dendrogram presented in Figure 2. In this dendrogram, the individuals from each population usually were in the same cluster or sub-cluster. Hybrid cultivars and three inbred lines constituted two sub-clusters in group I. Populations DTa and Cha both originated from the same province (Khorasan) and horticultural group (Inodorus) clustered in group IV. Populations TSh, KhM, and Jal located in

Table 4. Mean *Fst* values, mean of expected heterozygosity and populations in each group obtained with *K*= 6 using Structure 2.3.1 software based on IRAP+REMAP data.

Group	Mean Fst for K=6	Mean of expected heterozygosity	Populations in each group
I	0.20	1.98	KhF, Hyb, Lin
II	0.34	2.06	Min
III	0.27	2.03	ShE
IV	0.20	2.01	DTa, Zsh, Cha
V	0.20	2.00	TaM, ShM
VI	0.17	2.01	TSh, KhM, Jal

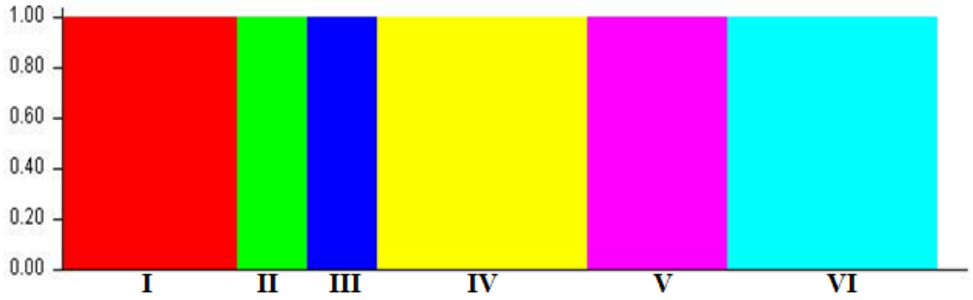

Figure 3. A Bayesian model-based clustering of the analyzed genotypes demonstrating the occurrence of 6 groups (I to VI) within the melon collection based on IRAP+REMAP data, bar colors and lengths represent inferred clusters and Q, respectively, identified by Structure for *K*= 6.

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group VI; KhM and Jal were from the same horticultural group (Inodorus). In addition, TSh and Jal originated from the same province (Semnan) although they were from different horticultural groups. In spite of the same origin of the populations Zsh and Tsh (both originated from Shahroud), they clustered in different groups. Population Zsh belonged to subspecies Inodorus of the C. *melo*, while Tsh was assigned to subspecies Cantalupensis (Fabriki Ourang *et al*., 2012). This might explain why these two populations from the same region occupied different clusters. However, none of the three IRAP, RAMAP, and IRAP+REMAP dendrograms was able to completely differentiate the genotypes and populations based on the origin or horticultural groups. Since melon is a cross pollinated plant and question of hybrid is of importance in melon, genotypes in different groups could be introduced as suitable parents in melon breeding programs. Higher levels of genetic variation detected among individual plants within each population (67%) compared to variation within populations (33%) might be related to the outcrossing nature of melon (Kirkbride, 1993), as well as the high activity and insertional polymorphism of the used RTNs in melon genome.

In conclusion, combined data of IRAP+REMAP markers proved to be reliable in the evaluation of genetic diversity in melon collections at the individual level, as it is for sunflower (Vukich *et al*., 2009), citrus (Biswas *et al*., 2010) and grapevine (Onofrio *et al*., 2010). The variable nature and activity of the LTR RTNs in melon genome should make them excellent sources of polymorphic markers and suggests that the use of these techniques in combination would allow melon breeders to obtain markers close to virtually any important agronomical trait. Besides, IRAP and REMAP markers based on *Reme1* and other LTR RTNs from watermelon proved to be useful for the determination of genetic relationships among melon genotypes and for the definition of the genetic structure of this melon collection. These data might be useful in the future for designing melon breeding programs and defining strategies for germplasm conservation.

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ارزیابی تنوع ژنتیکی در جمعیت ها و هیبریدهای ملون بر اساس نشانگرهای IRAP وREMAP

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چکیده

رتروترنسپوزون‌ها بخاطر توانایی ادغام در نواحی مختلف ژنوم و تولید چندشکلی در بین افراد به عنوان نشانگرهای ملکولی کارا در گیاهان محسوب می‌شوند. در این مطالعه از نشانگرهای ملکولی مبتنی بر رتروترنسپوزون‌ها (IRAP وREMAP) جهت مطالعه فعالیت ادغامی و تنوع ژنتیکی در ۱۰۰ ژنوتیپ ملون (۸۸ ژنوتیپ از ۱۱ جمعیت، ۳ لاین اینبرد و ۹ هیبرید) استفاده شد. در کل با استفاده از ۵ ترکیب آغازگری IRAP و ۱۵ ترکیب آغازگری REMAP به ترتیب ۹۴ و ۲۶۲ مکان تکثیر شد. درصد مکان‌های چندشکل بین جمعیت‌ها از ۳۹ (زیوری شاهرود) تا ۴۸ (شادگانی اصلاح شده) متغیر بود. آزمون مانتل بین ماتریس‌های کوفنتیک حاصل از نشانگرهای IRAP و REMAP معنی‌دار نبود (r=۰/۲۹) بنابراین تجزیه کلاستر بر اساس نشانگرهای IRAP+REMAP با استفاده از روش UPGMAو ضرایب تشابه دایس، افراد مورد مطالعه را در ۶ گروه قرار داد. تجزیهAMOVA، تنوع ژنتیکی زیادی را در درون جمعیت‌ها (۶۷ درصد) نسبت به بین جمعیت‌ها (۳۳ درصد) نشان داد. میانگین مقادیر Fst همه گروه‌ها بجز گروه ۶ بیشتر از ۰/۲ بود که بیانگر تمایز بین جمعیت‌های مورد مطالعه و وجود ساختار در ژرم پلاسم ملون مورد مطالعه می‌باشد.

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