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Genetic Diversity of Some Populations of Iranian Melon Using SSR Markers

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Abstract: To study the genetic diversity of the melon collection at the National Plant Genetic of Iran, 35 genotypes were planted at Bu Ali Sina University Hamedan in 2004 and were evaluated with 15 micro satellite SSR markers. Micro satellite markers in the upper levels showed polymorphisms and 87% of them are polymorphic and 63 alleles have been identified. The number of effective alleles in the polymorphic loci varied from 1.25-8.19 and the mean is 2.80. The percentage of genetic loci having polymorphism is 87.67%. Cluster analysis of genotypes was divided into 5 groups wherein genotype of foreign origin has smooth skin while different groups of Iran genotype are netted. Genetic distance between Iranian and foreign is 0.98. Results obtained from this study showed the significant difference between Iranian genotypes with other genotypes. These genotypes have high diversity which could be used in breeding programs.

Key words: Diversity, variability, Iranian melon, microsatellite, population, SSR marker

INTRODUCTION

Molecular studies using molecular markers in different melon populations to find out genetic characters have been done on diversity studies of different groups of melons in some collections (Garcia, 1998, 2000; Baudraeco-Arnas, 1996). Melons have a high variation in the fruit (Whitaker and Davis, 1962; Robinson and Dekker-Walkers (1997). For this reason Naudin (1859) and Pitrat *et al.* (2000) tried to classify them. Pitrat *et al.* (2000) determined the origin of the inodorus group as Turkey and Middle East. Melon diversity is very wide and Iran has diversity of the inodorus group. Kohpayegani (2004), studied the morphological characters of Iranian melons, but studies using molecular markers have not been done. Yet the first effort to study the characters of native cultivar was done using isozyme systems through Esquinas-Alcazar (1977). The development of micro satellites in melons was done for the first time by Katzir *et al.* (1996) and up to the present, diversity studies and gene mapping of the genus *Cucumis* and different groups of melons have used micro satellite markers (Ritschel *et al.*, 2004; Monforte *et al.*, 2003; Zhuang *et al.*, 2003; Staub *et al.*, 2000; Danin-Poleg *et al.*, 2000, 2001; Lopez-Sese *et al.*, 2002). To study the genetic diversity of melons and genetic distance, it is necessary to perform bulk sampling (Mliki *et al.*, 2001; Garcia *et al.*, 1998). The bulk sampling method gives little information on the diversity within the group and/or primarily gives no information in this regard. However, data on population structure can help in the management of germplasm

collection (Dje *et al.*, 2000). In this study, molecular diversity among 35 different cultivars of melon from different geographical areas and having different fruit shapes and foreign melons were investigated using molecular markers. This study is a preliminary evaluation of the Iranians structure collection and serves as a base data for the study of character relationship, duplicate ones, distinctiveness and general management of bigger collections.

MATERIALS AND METHODS

Plant materials and DNA extraction: Thirty one genotypes belonging to the inodorus group from the National Plant Gene bank of Iran and 4 ones from commercial imported cultivars obtained were used (Table 1).

For DNA extraction, the upper leaves of 5 plants were mixed and DNA extraction was done using a CTAB procedure (Lopez-Sese *et al.*, 2002). The amount of DNA was determined using spectrometer from Eppendorf Co. based on the company's directions and the final concentration was obtained from 0.1 M tris to 10 ng mL⁻¹.

Fifteen pairs of primers were used only one of them CSLHCPA was for cucumber (Katzir, 1996) and CMAT35 from Melon database. CMBR43, CMBR56 from enriched genomic libraries and the others from genomic library. (Ritschel, 2004) (Table 2). The primers were supplied by CMACCI 46 to CMACCI 68 (Danin-Poleg *et al.*, 2001) and Tib mol Bio Company. Optimization of PCR conditions for SSR markers was done by use the thermo gradient,

Table 1: Melon (*Cucumis melo* L.) examined in this study

No.	Gene bank No.	Local name	Origin	No.	Gene bank No.	Local name	Origin
1	357010	-	Gorgan	19	357534	-	Delijan
2	357013	-	Jiroft Kerman	20	357546	Hutak	Boshehr
3	357026	Gorgab	Arian Isfahan	21	357551	-	Nagade
4	357032	Tashkandi	Isfahan	22	357556	Aria Tokhm	Khoi
5	357052	-	Golpayegan	23	357564	-	Gonbad
6	357093	-	Ardecan Yazd	24	357566	Talnak	Sari
7	357100	To Sorkh Ibrahimi	Isfahan	25	357569	Torshak	Gorgan
8	357168	-	Damghan	26	357573	-	Tabriz
9	357212	-	-	27	357577	Shamama	Tabriz
10	357294	-	Naein Isfahan	28	357578	Hony dew	Tabriz
11	357303	-	Bakhtaran	29	357590	To Sorkh	Isfahan
12	357310	Salman Roshdi	Torbat Hydarie	30	357591	Haj Abadi	Isfahan
13	357314	Ghasri	Torbat Jam	31	357612	Bargardoni	Isfahan
14	357493	-	Bandar Hormoz	32	-	charante ¹	-
15	357522	Pashootori	Gorgan	33	-	Khaghani ¹	-
16	357524	Dashti	Boshehr	34	-	Pashootori ¹	-
17	357526	Gesh Sabo	Birjand	35	-	Ananasi ¹	-
18	357529	-	Dashtestan				

¹: Collect from market

Table 2: Description of single sequence repeat marker alleles (15 loci) detected among 46 melon (*Cucumis melo* L.) accession

SSR marker ^a	Core motif	Annealing temperature	No. of alleles among 35 accessions	Effective allele	Ho ^b	He ^c
CMACC146	(ACC)9	65	3.0	2.01	0.22	0.51
CMAT141	(AT)7(GT)6	65	4.0	3.08	0.85	0.68
CMCCA145	(CCA)5	65	2.0	2.00	1.00	0.50
CMCT44	(CT)10TGT(CT)3	63	4.0	2.11	1.00	0.53
CMCT134b	(TA)2(CT)8(AT)7	63	9.0	4.10	0.62	0.76
CMGA15	(GA)7	63	4.0	1.77	0.51	0.44
CMGA104	(GA)14AA(GA)3	65	7.0	4.12	0.79	0.76
CMGA172	(GA)9	63	1.0	1.00	0.00	0.00
CMGT108	(GT)9N65(CT)7	63	1.0	1.00	0.00	0.00
CMTC160a+b	(TC)2(TCC)2(CT)8N122(TC)8	58	2.0	1.25	0.00	0.20
CMTC168	(TC)14	63	7.0	4.92	0.88	0.80
CMBR43	(CT)20	63	9.0	8.19	0.65	0.89
CMBR56	(CT)3N2(CT)12(CCCT)2N8(CT)3(AT)3N3(TC)2	58	4.0	2.56	0.00	0.61
CMAT35	(TA)3AA(TA)2C(AT)7	65	2.0	1.99	0.94	0.50
CSLHCPA	(GA)8	58	4.0	1.89	0.00	0.48
Mean	-	-	4.2	2.799	0.497	0.51

^a: SSR markers described by Katzir *et al.* (1996), Danin-Poleg *et al.* (2001) and Ritschel *et al.* (2004); ^b: Observed heterosigosity; ^c: Expected heterosigosity

annealing temperature (Fazio *et al.*, 2002; Don *et al.*, 1991). The thermo-gradient needed was done using Eppendorf thermocycle and according to the directions of the manufacturers at 45-65°C. Tubes of 0.2 mL diameter, folded alternately in 6 different temperatures, were evaluated. PCR in volumes of 15 µL, according to Katzir *et al.* (1996), was done with a slight modification. PCR mixture containing 30 ng DNA, 3.7 µM from each primers, 2.5 mM MgCl₂ and 150 µM dNTP, 0.7U DNA polymerase Sinnagen and commercial 10x buffer, After adding PCR mixture, the content of each tube was covered with 15 µL mineral oil. Reaction cycles were done with initial temperature of 94°C for 3 min and 35 cycles, composed of 1 min at 94°C, 30 sec at thermo-gradient of 45-65°C, 1 min at 72°C and finally, 5 min at 72°C. After completion of cycles 5 µL loading buffer from each tube was added and denaturation was done for 5 min in 95°C.

Mixture of 5 µL was loaded on gel sequencing Sci plan 1600, with 6% acrylamide denaturing gel 1×TBE and 7 M urea were loaded and electrophoresed at 60 watt and 1500 watt. To be able to see the bands staining silver nitrate was done. Photographs of the gel were taken by flat scanner. Bands were scored from the lightest to the heaviest allele arranged in alphabetical order. Two alleles as heterozygous and one allele as homozygous were considered.

Statistical analysis: The band patterns of micro satellite were recorded in a matrix and to calculate the Nei distance coefficient (Nei, 1987) and from that the genetic distance was computed. Cluster analysis was calculated by mean genetic distance by UPGMA and to calculate the relation between genotypes, POPGENE software was used (Yeh *et al.*, 1997). The genetic diversity was studied by means of Shannon (1948) index.

RESULTS

Optimizing of the PCR conditions: In all of the loci the increase in temperature will reduce the formation of additional band (Fig. 1). The elimination of these bands will make the scoring of the band easier and more accurate. The appropriate temperature for the primer is shown in Table 2.

Diversity of microsatellites: In 15 gene loci, 63 alleles in 35 genotype were identified, two gene loci CMGA 172 and GMGT108 were monomorphic and 13 remaining loci were polymorphic which were composed of 3 (2 allele), 1 (3 allele), 5 (4 allele), 2 (7 allele) and 2 (9 allele). The number of effective alleles that were in the polymorphic loci varied from 1.25-8.19 and the percentage of genes loci that were polymorphic was 87.67% as well the number of observed alleles and effective alleles are shown in Table 2.

Polymorphic Information Content (PIC), taking in to consideration the number of alleles in every gene loci and relative frequency of them in the population was the criteria used for estimating the genetic distance (Rongwen *et al.*, 1995; Anderson *et al.*, 1993). All gene loci except CMCA145, CMCA172 and CMGT105 were able to identify at least one genotype (Table 2). The highest amount of PIC is 0.89 and belongs to CMCA43 and after CMCA168, CMCT134b and CMGA104 have the highest PIC, respectively. The gene loci with the high amount of PIC can be used to study other melon collections. The amount of PIC for neither of the loci is not zero and at least one of the loci under study is heterozygous. These amounts for the two genotypes 357212 and Bargardani Isfahan (357612) are 0.33 and more than the other genotypes (Table 3).

Mean Shannon similarity index in genotypes varied from 0.188 (Dashtestan) to 0.462 (357212, Bargardani Isfahan). And the mean index was 0.345. Mean expected heterozygosity between genotypes were 0.49 while the observed is 0.510 and Mean homozygosity in the observed and expected between genotypes were 0.51 and 0.49, respectively (Table 3). Observed and expected homozygosity between gene loci were 0.497 and 0.515, respectively (Table 2). The number of gene loci with polymorphism between genotypes varied from 2 (26.67%) to 9 (66.67%). The least polymorphism amount belonged to the genotype Torshak Gorgan (13%) and the highest belonged to genotype 357212 and Bargardani Isfahan (33%). The mean between genotypes is 7.45 (49.71%). The Hardy-Weinberg equilibrium is not significant for any of the gene loci of SSR which might be due to the artificial

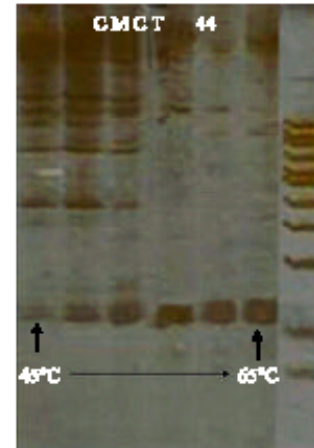


Fig. 1: Annealing temperature gradient (ATG) profile of CMCT44 SSR marker using DNA of melon lines Ananasi. Annealing temperature increases from 45°C (far left) to 65°C (far right) with a step of 3.3°C each wells and a 100 bp ladder

Table 3: Static of genetic variation for 35 Iranian melon accessions as measured by SSR loci

Accession	Ho ^a	He ^b	PIC ^c	I ^d
Gorgan	0.46	0.53	0.26	0.36
Jiroft Kerman	0.46	0.53	0.26	0.36
Gorgab Isfahan	0.60	0.40	0.20	0.27
Tahkandi Isfahan	0.40	0.60	0.30	0.41
Golpayegan	0.53	0.46	0.23	0.32
Ardekan Yazd	0.53	0.46	0.23	0.32
Tosorkh Isfahan	0.40	0.60	0.30	0.41
Damghan	0.66	0.33	0.16	0.23
212	0.33	0.66	0.33	0.46
Na'in Isfahan	0.53	0.46	0.23	0.32
Bakhtaran	0.53	0.46	0.23	0.32
Salman Roshdi	0.53	0.53	0.26	0.36
Gasri	0.46	0.53	0.26	0.36
Bandar Hormoz	0.57	0.42	0.21	0.29
Pashotori Gorgan	0.46	0.53	0.26	0.36
Dashti Boshahr	0.65	0.35	0.17	0.24
Kesh Sabo Birjand	0.46	0.53	0.26	0.36
Dashtestan	0.73	0.26	0.13	0.18
Delijan	0.46	0.53	0.26	0.36
Hutak Gorgan	0.46	0.53	0.26	0.36
Nagade	0.53	0.46	0.23	0.32
Aria Tokdun Khoi	0.46	0.53	0.26	0.36
Gonbad	0.46	0.53	0.26	0.36
Tahak Sari	0.66	0.33	0.16	0.23
Torshak Gorgan	0.46	0.53	0.26	0.36
Tahriz	0.40	0.60	0.30	0.41
Shamama Tabriz	0.46	0.53	0.26	0.36
Hony dew	0.66	0.33	0.16	0.23
To Sorkh Isfahan	0.40	0.60	0.30	0.41
Haj Abadi Isfahan	0.53	0.46	0.23	0.32
Bargardani Isfahan	0.33	0.66	0.33	0.46
charante	0.53	0.46	0.23	0.32
Khaghani	0.53	0.46	0.23	0.32
Pashotori	0.40	0.60	0.30	0.41
Ananasi	0.46	0.53	0.26	0.36
Mean	0.51	0.49	-	0.34

^a: Observed heterozygosity; ^b: Expected heterozygosity; ^c: Polymorphism information content; ^d: Shannon's Information index (Shannon, 1948)

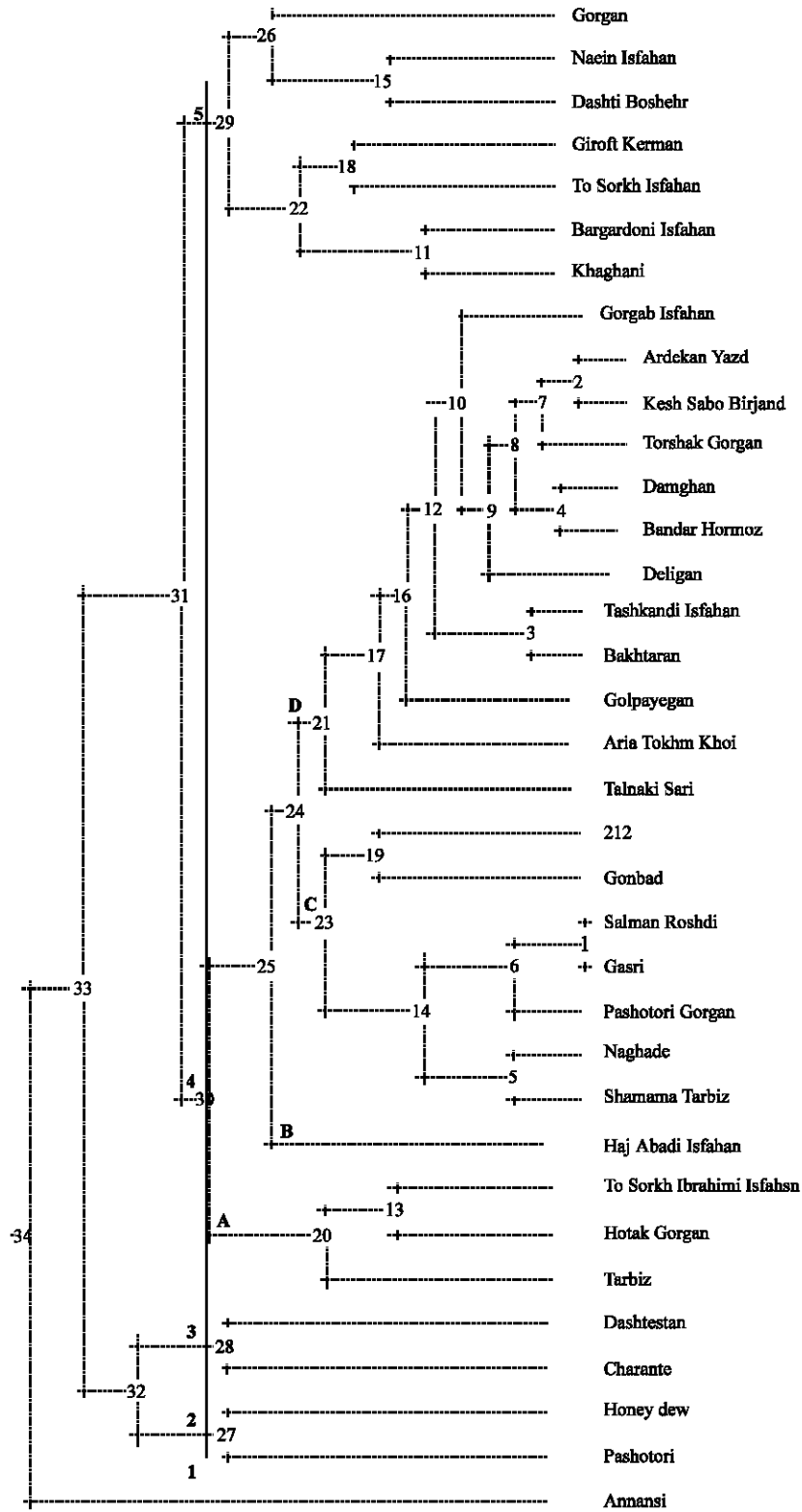


Fig. 2: Cluster analysis of 35 Iranian melon accessions by UPGMA grouped using genetic distance as estimated by 15 SSR loci (Nei's distance)

Table 4: Genetic similarity (upper diagonal) and genetic distance (lower diagonal) measures among the 35 accessions of Iranian melons as determined by 15 SSR loci using Nei' distance measure

Pop ID	010	013	026	032	052	093	100	168	212	294	303	310	314	493	522	524	526	529
010	***	0.704	0.65	0.744	0.733	0.689	0.558	0.703	0.595	0.666	0.733	0.568	0.568	0.727	0.545	0.711	0.704	0.543
013	0.350	***	0.739	0.790	0.644	0.755	0.581	0.724	0.619	0.778	0.755	0.590	0.590	0.772	0.659	0.733	0.772	0.543
026	0.426	0.301	***	0.801	0.787	0.851	0.645	0.857	0.707	0.638	0.787	0.783	0.783	0.805	0.696	0.702	0.870	0.520
032	0.295	0.234	0.220	***	0.796	0.864	0.642	0.785	0.780	0.728	0.932	0.697	0.699	0.812	0.799	0.658	0.884	0.516
052	0.309	0.439	0.239	0.227	***	0.782	0.568	0.813	0.652	0.673	0.826	0.755	0.755	0.755	0.689	0.608	0.800	0.572
093	0.372	0.280	0.161	0.145	0.245	***	0.659	0.917	0.699	0.739	0.847	0.755	0.757	0.889	0.755	0.673	0.933	0.490
100	0.582	0.542	0.437	0.441	0.564	0.415	***	0.654	0.731	0.523	0.637	0.604	0.608	0.604	0.604	0.500	0.697	0.385
168	0.351	0.321	0.153	0.241	0.206	0.086	0.426	***	0.693	0.667	0.792	0.767	0.767	0.916	0.682	0.667	0.852	0.509
212	0.517	0.478	0.346	0.247	0.426	0.357	0.312	0.366	***	0.559	0.722	0.738	0.739	0.738	0.762	0.536	0.762	0.526
294	0.405	0.251	0.448	0.317	0.394	0.302	0.647	0.404	0.580	***	0.673	0.644	0.646	0.644	0.644	0.804	0.755	0.572
303	0.309	0.280	0.239	0.069	0.191	0.165	0.451	0.232	0.324	0.394	***	0.689	0.681	0.778	0.778	0.630	0.866	0.531
310	0.565	0.526	0.244	0.359	0.280	0.280	0.502	0.264	0.302	0.439	0.372	***	1.000	0.704	0.909	0.577	0.818	0.543
314	0.565	0.526	0.244	0.359	0.280	0.280	0.502	0.264	0.302	0.439	0.372	0.000	***	0.704	0.909	0.577	0.818	0.543
493	0.318	0.257	0.216	0.205	0.280	0.117	0.502	0.086	0.302	0.439	0.251	0.350	0.350	***	0.681	0.644	0.818	0.501
522	0.606	0.416	0.362	0.234	0.372	0.280	0.502	0.382	0.270	0.439	0.251	0.095	0.095	0.383	***	0.533	0.818	0.501
524	0.340	0.309	0.353	0.415	0.496	0.394	0.692	0.404	0.623	0.217	0.461	0.548	0.548	0.439	0.628	***	0.689	0.654
526	0.350	0.257	0.138	0.123	0.222	0.068	0.359	0.159	0.270	0.280	0.142	0.200	0.200	0.200	0.200	0.372	***	0.501
529	0.609	0.609	0.653	0.666	0.557	0.711	0.954	0.673	0.642	0.557	0.631	0.609	0.609	0.689	0.689	0.424	0.689	***
534	0.257	0.318	0.190	0.205	0.222	0.195	0.326	0.134	0.302	0.372	0.222	0.257	0.258	0.207	0.352	0.340	0.120	0.776
546	0.565	0.739	0.426	0.465	0.405	0.439	0.205	0.414	0.335	0.761	0.405	0.526	0.521	0.488	0.648	0.761	0.416	0.648
551	0.715	0.761	0.415	0.564	0.394	0.461	0.487	0.404	0.324	0.650	0.496	0.142	0.142	0.510	0.251	0.737	0.372	0.557
556	0.488	0.416	0.244	0.205	0.280	0.222	0.465	0.321	0.404	0.405	0.222	0.257	0.257	0.350	0.257	0.405	0.200	0.776
564	0.565	0.452	0.331	0.326	0.405	0.340	0.582	0.351	0.270	0.628	0.340	0.416	0.416	0.318	0.416	0.670	0.318	0.648
566	0.552	0.382	0.365	0.298	0.373	0.286	0.566	0.328	0.542	0.404	0.344	0.446	0.446	0.382	0.446	0.538	0.264	0.935
569	0.287	0.318	0.138	0.177	0.168	0.142	0.465	0.134	0.270	0.309	0.195	0.173	0.173	0.146	0.257	0.372	0.095	0.609
573	0.625	0.502	0.338	0.405	0.487	0.415	0.241	0.423	0.455	0.692	0.382	0.326	0.326	0.465	0.359	0.647	0.393	0.848
577	0.606	0.526	0.331	0.465	0.405	0.372	0.359	0.351	0.369	0.587	0.372	0.228	0.228	0.452	0.287	0.670	0.350	0.609
578	0.670	0.712	0.425	0.606	0.612	0.503	0.566	0.415	0.468	0.980	0.574	0.414	0.414	0.480	0.480	0.612	0.552	0.465
590	0.502	0.264	0.403	0.479	0.564	0.487	0.441	0.390	0.279	0.451	0.564	0.502	0.502	0.428	0.625	0.382	0.428	0.548
591	0.587	0.510	0.266	0.382	0.245	0.362	0.525	0.344	0.391	0.496	0.461	0.405	0.405	0.405	0.548	0.570	0.340	0.711
612	0.478	0.302	0.346	0.455	0.539	0.426	0.577	0.433	0.393	0.357	0.539	0.517	0.517	0.440	0.645	0.391	0.403	0.522
700	0.628	0.510	0.517	0.451	0.532	0.570	0.787	0.538	0.391	0.496	0.532	0.548	0.548	0.548	0.548	0.570	0.474	0.424
701	0.670	0.439	0.415	0.647	0.784	0.609	0.564	0.612	0.500	0.532	0.737	0.439	0.439	0.628	0.510	0.461	0.587	0.631
702	0.502	0.625	0.713	0.559	0.738	0.692	0.602	0.829	0.494	0.647	0.564	0.465	0.465	0.816	0.326	0.692	0.625	0.512
703	0.739	0.526	0.692	0.542	0.761	0.670	0.625	0.757	0.558	0.548	0.628	0.893	0.893	0.693	0.788	0.628	0.648	0.609
Pop ID	534	546	551	556	564	566	569	573	577	578	590	591	612	700	701	702	703	
010	0.772	0.568	0.489	0.613	0.568	0.575	0.750	0.535	0.545	0.511	0.604	0.555	0.619	0.533	0.511	0.604	0.477	
013	0.727	0.477	0.466	0.659	0.636	0.682	0.727	0.604	0.590	0.490	0.767	0.600	0.738	0.600	0.644	0.535	0.590	
026	0.826	0.652	0.659	0.783	0.718	0.694	0.870	0.712	0.718	0.653	0.668	0.766	0.707	0.595	0.659	0.490	0.500	
032	0.812	0.621	0.568	0.814	0.721	0.741	0.837	0.666	0.621	0.545	0.619	0.685	0.634	0.637	0.523	0.571	0.581	
052	0.800	0.668	0.673	0.755	0.666	0.688	0.844	0.614	0.666	0.542	0.568	0.782	0.582	0.587	0.456	0.477	0.466	
093	0.822	0.644	0.630	0.800	0.711	0.750	0.866	0.658	0.689	0.604	0.614	0.697	0.658	0.565	0.545	0.500	0.511	
100	0.721	0.814	0.614	0.628	0.558	0.567	0.628	0.785	0.697	0.567	0.642	0.591	0.561	0.455	0.568	0.547	0.535	
168	0.874	0.660	0.667	0.724	0.703	0.720	0.874	0.654	0.703	0.660	0.676	0.708	0.648	0.583	0.542	0.436	0.469	
212	0.738	0.715	0.722	0.667	0.762	0.581	0.762	0.634	0.691	0.626	0.756	0.676	0.675	0.676	0.606	0.609	0.572	
294	0.689	0.466	0.521	0.666	0.533	0.667	0.733	0.500	0.555	0.375	0.637	0.608	0.699	0.608	0.587	0.523	0.577	
303	0.800	0.666	0.608	0.800	0.711	0.708	0.822	0.682	0.689	0.563	0.568	0.630	0.582	0.587	0.478	0.568	0.533	
310	0.772	0.599	0.866	0.777	0.659	0.639	0.840	0.721	0.795	0.669	0.608	0.666	0.595	0.577	0.644	0.628	0.409	
314	0.772	0.599	0.866	0.777	0.659	0.639	0.840	0.721	0.795	0.660	0.604	0.666	0.595	0.577	0.644	0.628	0.409	
493	0.818	0.613	0.600	0.704	0.727	0.682	0.863	0.628	0.636	0.618	0.651	0.666	0.643	0.577	0.533	0.442	0.500	
522	0.704	0.522	0.778	0.772	0.659	0.639	0.772	0.697	0.750	0.618	0.535	0.577	0.524	0.577	0.600	0.721	0.454	
524	0.711	0.466	0.478	0.666	0.511	0.583	0.689	0.523	0.511	0.542	0.682	0.565	0.676	0.565	0.630	0.500	0.533	
526	0.886	0.659	0.689	0.818	0.727	0.767	0.909	0.674	0.704	0.575	0.651	0.711	0.667	0.622	0.555	0.535	0.522	
529	0.459	0.522	0.572	0.459	0.522	0.392	0.543	0.428	0.543	0.627	0.577	0.490	0.592	0.654	0.531	0.599	0.543	
534	***	0.636	0.668	0.750	0.659	0.724	0.863	0.676	0.681	0.597	0.697	0.711	0.643	0.622	0.577	0.511	0.500	
546	0.452	***	0.624	0.545	0.659	0.511	0.681	0.697	0.636	0.511	0.628	0.577	0.595	0.444	0.466	0.395	0.568	
551	0.405	0.474	***	0.644	0.733	0.542	0.733	0.659	0.911	0.646	0.568	0.717	0.676	0.543	0.695	0.637	0.377	
556	0.287	0.606	0.439	***	0.659	0.724	0.795	0.721	0.704	0.554	0.581	0.711	0.595	0.533	0.622	0.558	0.500	
564	0.416	0.416	0.309	0.416	***	0.554	0.750	0.581	0.750	0.575	0.651	0.733	0.762	0.600	0.622	0.488	0.613	
566	0.321	0.670	0.612	0.321	0.590	***	0.724	0.611	0.618	0.420	0.545	0.667	0.581	0.500	0.458	0.436	0.426	
569	0.146	0.383	0.309	0.228	0.287	0.321	***	0.651	0.727	0.597	0.674	0.733	0.691	0.622	0.600	0.488	0.522	
573	0.393	0.359	0.415	0.326	0.542	0.492	0.428	***	0.744	0.589	0.547	0.568	0.561	0.409	0.591	0.547	0.511	
577	0.383	0.452	0.092	0.350	0.287	0.480	0.318	0.295	***	0.703	0.604	0.755	0.738	0.489	0.778	0.651	0.386	
578	0.515	0.670	0.436	0.590	0.552	0.867	0.515	0.529	0.351	***	0.545	0.583	0.514	0.500	0.583	0.654	0.405	
590	0.359	0.465	0.564	0.542	0.428	0.606	0.393	0.602										

Table 4: Continue

Pop ID	534	546	551	556	564	566	569	573	577	578	590	591	612	700	701	702	703
591	0.340	0.548	0.332	0.347	0.309	0.406	0.309	0.564	0.281	0.538	0.451	***	0.763	0.607	0.652	0.477	0.489
612	0.447	0.517	0.391	0.517	0.270	0.542	0.369	0.577	0.302	0.665	0.216	0.262	***	0.582	0.839	0.512	0.619
700	0.472	0.817	0.609	0.628	0.510	0.692	0.474	0.892	0.715	0.692	0.487	0.496	0.539	***	0.478	0.614	0.622
701	0.543	0.761	0.362	0.474	0.474	0.779	0.510	0.525	0.251	0.538	0.382	0.427	0.175	0.737	***	0.614	0.533
702	0.669	0.921	0.451	0.582	0.716	0.829	0.716	0.602	0.428	0.423	0.741	0.738	0.668	0.487	0.487	***	0.511
703	0.693	0.563	0.973	0.693	0.488	0.852	0.648	0.669	0.951	0.903	0.582	0.715	0.478	0.474	0.628	0.669	***

700: Sharante 701: Khaghani 702: Pashotori 703: Ananasi

selection which benefit one allele and loss to the other alleles through human intervention that prevents the movement of genotypes toward reaching equilibrium.

Relationship of different genotypes of melon:

Dendrogram shows that the genotypes from genetic distance of 31 are divided into 5 groups (Fig. 2 and Table 4). In the first group is only one genotype, the Annanasi cv. The second group is composed of genotypes Pashotori and Honeydew, while the third group is made up of Charante and Dashtestan. These three groups are composed of imported cultivars and are characterized by smooth skin. The majority of the genotypes are found in the fourth group. For cluster analysis, this group is subdivided to four subgroups. In subgroup A, the genotypes are Tabriz, Hotak Gorgan and To Sorkhe Isfahan. In subgroup C, the genotypes have the least distance between each other such that two genotypes, Salman Roshdie, Torbat Heidarieh, Ghasri Torbatjam did not show any difference and were same morphologically the same genotypes found in subgroup D were collected from the central region of Iran. The fifth group composed of 7 genotypes which are mostly from Semnan province. Most of these genotypes, are the same as those used in a study by Kohpayegani (2004) which are near each other based on fruit characters. This group have medium fruits with net area, fruit long, number fruit per plant and 100 seed weight, but sub cluster 1, 2 fruits are without net, oblong and low 100 seed weight. The Iranian ones usually are netted, long. The A group in subcluster 4 is very netted and 100 seed weight but 2 fruit per plant, so it is separate in sub cluster 4 in B group lonely. The others C and D groups have many fruits per plant and 100 seed weight with short length and medium net area.

The genetic distance of the cultivars were measured based on Nei (1972) method. This distance varied from 0.0000-0.98. The least genetic distance belonged to Salman Roshdie (No. 357310) and Ghasri (No. 357314), while the largest genetic distance belonged to Honeydew (No. 357578) and Naein Isfahan (No. 357294). In the study by Lopez-Sese *et al.* (2002) the mean genetic distance between Spanish melons is 0.85 and the maximum genetic distance between genotypes is 0.491, whereas in the present study, despite the genotypes being within the

group 37.65% of those genotypes have genetic distance more than 0.500. This wide distance is due to the large diversity of melons in Iran and in which the local genotypes are produced from open-pollination under different climatic conditions.

DISCUSSION

The mean number of allele in one gene loci is 4.2. Studies by Danin-Poleg *et al.* (2001) using 30 SSR primers on 13 genotypes, found 3.5 alleles while Lopez-Sese *et al.* (2002) found 2.4 alleles on Spanish melons and the mean of effective alleles was 1.5, while Monforte found 6.3 alleles, this high number is due to between the group genotypes. The percentage of gene loci that Katzir *et al.* (1996) obtained using 7 SSR primers on 8 genotypes is 0.71%. Studies by Monforte *et al.* (2003), found 100% polymorphism. The difference between the number of alleles in each locus and number of effective loci shows the existence of rare alleles.

Rare alleles are those which have low frequency and on the other hand occur on one or more genotypes. From these alleles, we could use to identify the genotypes by means of combination of some genetic loci. For example, using the table, CMAT15 can be used as a primer to identify genotypes where the gene loci are different from each other. Mean observed heterozygosity compared with the Lopez-Sese *et al.* (2002) is much higher, which is probably due to the wide diversity of genotypes used in the study and their being native varieties. Significance of the Hardy-Weinberg equilibrium is due to artificial selection by human which will benefit one allele but will be a loss to other alleles, which prevents the movement of genotypes toward reaching equilibrium.

Melons from different geographical region which were evaluated in this study were very diverse which showed the wide genetic base of Iranian melons and their high heterogeneity. Some observations in cluster analysis are in accordance with the study of Kohpayegani (2004), who evaluated 100 Iranian melons for morphological characters.

Lopez-Sese *et al.* (2002) did not observe a relation between geographical location and diversity brought about by RAPD markers among 125 Spanish melons.

However, in the present study some of the genotypes belonging to the same geographical area are found located near each other. Notwithstanding similar accessions with different names belonging to different origins may be located at the same cluster due to transferring seed by farmers, therefore, cluster analysis can be helpful in identification and management of same accessions.

In this study, there exists a wide genetic distance between Honeydew and Naein Isfahan. Other genotypes from foreign sources have wide genetic distance with Iranian genotypes. The mean genetic distance that Lopez-Sese *et al.* (2002) obtained on Spanish genotypes is 0.285 and the maximum genetic distance between genotypes was 0.491. Whereas the present study the mean was 37.65%. This genetic distance shows the important of Iranian population that was created in area zone of Iran and is important for conservation and use in improving cultivars.

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