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Genetic diversity among melon accessions (*Cucumis melo*) from Turkey based on SSR markers

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ABSTRACT. Melon (*Cucumis melo*) is an important vegetable crop in Turkey, where it is grown in many regions; the most widely planted lines are local winter types belonging to the var. *inodorous*. We examined 81 melon genotypes collected from different provinces of Turkey, compared with 15 reference melon genotypes obtained from INRA/France, to determine genetic diversity among Turkish melons. Twenty polymorphic primers were used to generate the SSR markers. PCR amplification was performed and electrophoresis was conducted. SSR data were used to generate a binary matrix. For cluster analysis, UPGMA was employed to construct a clustering dendrogram based on the genetic distance matrix. The cophenetic correlation was compared with the similarity matrix using the Mantel matrix correspondence test to evaluate the representativeness of the dendrogram. A total of 123 alleles were amplified using the 20 SSR primer sets. The number of alleles detected by a single primer set ranged from 2 to 12, with an average of 6.15. The similarity ranged from 0.22 to 1.00 in the dendrogram developed from microsatellite analysis. Based on this molecular data, we concluded that genetic diversity among these Turkish accessions is relatively high.

Key words: *Cucumis melo*; Genetic resources; Microsatellite analysis; Genetic diversity

INTRODUCTION

Melon (*Cucumis melo* L.) is a temperate and warm season crop belonging to the genus *Cucumis* in the family Cucurbitaceae. It is considered to be the most diverse species, and several intraspecific classifications have been reported (Robinson and Decker-Walters, 1997; Nuñez-Palenius et al., 2008; Szamosi et al., 2010). Recently, Pitrat et al. (2000) classified melons into 16 groups, 5 of which (*conomon*, *makuwa*, *chinensis*, *momordica*, and *acidilus*) can be assigned to the subsp *agrestis* and 11 (*cantalupensis*, *reticulatus*, *adana*, *chandalak*, *ameri*, *inodorus*, *flexuosus*, *chate*, *tibish*, *dudaim*, and *chito*) to the subsp *melo*. As stated by McCreight et al. (1993), var. *cantalupensis* and var. *inodorus* are the most economically significant melons grown in the US and in European, Mediterranean and Asian countries.

Turkey is the second leading country after China in melon production, with 1749 million tons of melon grown (FAO, 2009). The country is also located in a secondary genetic diversity center (Pitrat et al., 1999). Turkey has been reported to be rich in melon genetic resources, as Turkish melons are morphologically diverse, especially in regard to fruit traits (Sari and Solmaz, 2007; Sensoy et al., 2007; Solmaz et al., 2010). However, these valuable genetic resources are being compromised by the cultivation of commercial varieties that have a higher yield and are of better quality. Due to environmental and economic factors, the local landraces are likely to become extinct in the near future. Nevertheless, Turkish melon strains potentially have useful genes that can be utilized in breeding programs.

As stated by Escribano and Lázaro (2009), morphological analyses are an absolute requirement for the initial evaluation of genetic resources and the accurate identification of local landraces. Several studies have morphologically characterized local germplasms and found them to represent different genetic sources (Decker-Walters et al., 2002; Liu et al., 2004; Staub et al., 2004; Laghetti et al., 2008; Escribano and Lázaro, 2009; Szamosi et al., 2010). Molecular markers have been shown to be useful to assess genetic diversity in a number of plant species (Bretting and Widrlechner, 1995; Staub et al., 2000). These molecular markers provide complete morphological and phonological data because they are plentiful, free of tissue and environmental factors and allow for cultivar identification in the early stages of development. Esquinas-Alcazar (1977) used isozymes to perform the preliminary study of melon to determine genetic relationships. In 1996, Katzir et al. (1996) developed the first simple sequence repeats (SSR) markers in melons. Molecular characterization of melons was performed using techniques including cleaved amplified polymorphic sequences (Zheng et al., 1999), amplified fragment length polymorphism (Garcia-Mas et al., 2000), random amplified polymorphic DNA (RAPD) (Stepansky et al., 1999; Mliki et al., 2001; Staub et al., 2004; Nakata et al., 2005; Sensoy et al., 2007; Tanaka et al., 2007) and SSR (Danin-Poleg et al., 2001; Szabo et al., 2008; Tzitzikas et al., 2009). Several additional studies compared different types of molecular markers to determine the genetic diversity of melons. Silberstein et al. (1999) revealed molecular variation by restriction fragment length polymorphism and RAPD, Stepansky et al. (1999) used RAPD and inter-simple sequence repeat for intraspecific classification; López-Sesé et al. (2002) assessed between and within accession variation in Spanish melon germplasm by RAPD and SSR; Staub et al. (2000) used both RAPD and SSR markers to characterize melon groups and reported that lower coefficients of variation can be attained when using RAPDs compared to SSRs, and Nakata et al. (2005) assessed the genetic diversity of Japanese melon cultivars by RAPD and SSR markers. Additionally,

morphological and molecular (SSR, ITS) characterization of 47 melon genotypes was carried out to determine an extinct medieval type (Szabo et al., 2008).

SSR markers, represented by the repeats of 1-6 nucleotide-long DNA motifs arranged in tandem, have been considered one of the most powerful Mendelian markers (Jarne and Lagoda, 1996) because of their high reproducibility, co-dominance inheritance, multi-allelic character, and extensive genome coverage (Powell et al., 1996). The polymorphism of SSRs, primarily resulting from the variation of repeat numbers, can be easily detected by a simple PCR technique. Together, these features are advantageous for genetic mapping, quantitative trait loci association, population genetics, and evolutionary studies (Hu et al., 2010).

The aim of this study was to determine the genetic diversity of melon genotypes collected from different provinces of Turkey compared with reference genotypes representing different melon varieties using SSR markers.

MATERIAL AND METHODS

This study was carried out in the Department of Horticulture, Faculty of Agriculture, University of Cukurova, Turkey.

Plant material

A total of 96 melon genotypes were used as plant material. Eighty-one melon genotypes were collected from different provinces of Turkey and 15 reference genotypes were obtained from INRA/France (Table 1).

DNA isolation

Young leaves were collected from each melon genotype and immediately frozen in liquid nitrogen and stored at -80°C. High molecular weight genomic DNA was extracted from the leaf samples following the CTAB miniprep protocol (Edwards et al., 1991). DNA concentration was measured with a NanoDrop ND 100 spectrophotometer (NanoDrop Technologies, Inc.) and gel electrophoresis. DNA was diluted in water to a final concentration of 50 ng/µL and stored at -20°C.

SSR analysis

Twenty primers (CMCT44, CMGA104, CMACC146, CMCTT144, CMTC47, CMAT141, CMCCA145, CMTC168, CMGA172, CMTC123, CMTG108, CMTAA166, CMTA134a, CMTC160a+b, CSCTTT15a, CMGAN92, CMGAN10, CMGAN59, CMGAN68, and TJ24) were used to generate the SSR markers. Amplification reactions were performed in 10-µL volumes containing 2X PCR Mastermix (Fermentas K0171), 1 U Taq DNA polymerase (Fermentas EP0402), 25 mM $MgCl_2$, 1 µM forward and reverse primers and 25 ng melon DNA. The mixtures were prepared at 0°C and transferred to the thermal cycler. The amplification was performed in a model Master Gradient thermal cycler (Eppendorf) using a program consisting of an initial denaturation step of 2 min at 94°C followed by 35 cycles of 2 min at 94°C, 1 min at 55°C and 2 min at 72°C; the program ended

Table 1. Melon germplasms used in this study.

No.	Genotype	Local name	Origin	Horticultural variety (group)
1	KAV 1	Asma kavunu	Diyarbakır	var. *inodorus*
2	KAV 3	Unnamed	Mardin	var. *inodorus*
3	KAV 5	Unnamed	Şanlıurfa	var. *inodorus*
4	KAV 8	Unnamed	Şanlıurfa	var. *inodorus*
5	KAV 19	Unnamed	Gaziantep	var. *inodorus*
6	KAV 26	Kışlık kavun	İzmir	var. *inodorus*
7	KAV 27	Gönen kavunu	Bursa	var. *inodorus*
8	KAV 28	Melemen kavunu	Manisa	var. *inodorus*
9	KAV 29	Çengel Tatar kavunu	Manisa	var. *inodorus*
10	KAV 36	Unnamed	Uşak	var. *inodorus*
11	KAV 37	Unnamed	İstanbul	var. *inodorus*
12	KAV 39	Lambada kavunu	Manisa	var. *inodorus*
13	KAV 40	Dilim kavunu	Manisa	var. *inodorus*
14	KAV 42	Unnamed	Tekirdağ	var. *inodorus*
15	KAV 43	Unnamed	Tekirdağ	var. *inodorus*
16	KAV 45	Sülüklü kışlık kavun	Tekirdağ	var. *inodorus*
17	KAV 46	Unnamed	Uşak	var. *inodorus*
18	KAV 48	Unnamed	Uşak	var. *inodorus*
19	KAV 50	Cavdan kavunu	Manisa	var. *inodorus*
20	KAV 51	Unnamed	Balıkesir	var. *inodorus*
21	KAV 52	Acur kavunu	Balıkesir	var. *inodorus*
22	KAV 54	Siyah kavun	Manisa	var. *inodorus*
23	KAV 56	Unnamed	Çanakkale	var. *inodorus*
24	KAV 59	Girnogi	Adıyaman	var. *inodorus*
25	KAV 61	Bal kavunu	Adıyaman	var. *inodorus*
26	KAV 62	Kelek bal kavun	Adıyaman	var. *inodorus*
27	KAV 64	Şemamok	Mardin	var. *dudaim*
28	KAV 65	Şelengo	Şanlıurfa	var. *cantalupensis*
29	KAV 66	Yabani kavun	Şanlıurfa	var. *agrestis*
30	KAV 67	Unnamed	Ankara	var. *inodorus*
31	KAV 70	Kuşçular	Unknown	var. *inodorus*
32	KAV 71	Yabani	Unknown	var. *agrestis*
33	KAV 72	Unnamed	Sakarya	var. *inodorus*
34	KAV 73	Unnamed	Şanlıurfa	var. *inodorus*
35	KAV 74	Unnamed	Unknown	var. *inodorus*
36	KAV 79	Unnamed	Ankara	var. *inodorus*
37	KAV 82	Unnamed	Uşak	var. *inodorus*
38	KAV 84	Unnamed	Manisa	var. *inodorus*
39	KAV 85	Unnamed	Nevşehi	var. *inodorus*
40	KAV 87	Unnamed	Niğde	var. *inodorus*
41	KAV 90	Unnamed	Elazığ	var. *inodorus*
42	KAV 170	Unnamed	Antalya	var. *inodorus*
43	KAV 171	90625/C9C2	INRA, France	var. *acidulous*
44	KAV 173	Carosello	INRA, France	var. *chate*
45	KAV 174	Chandaljak	INRA, France	var. *chandalak*
46	KAV-175	ME 0705 = Cuba 1	INRA, France	var.*chito*
47	KAV-176	ME 0733 = Fazaizabadi	INRA, France	var. *flexuosus*
48	KAV 177	Honeydew Green Flesh	INRA, France	var. *inodorus*
49	KAV 179	Khatoni	INRA, France	var. *ameri*
50	KAV 180	MR 1	INRA, France	var. *momordica*
51	KAV 183	PI 414723/TG	INRA, France	var. *momordica*
52	KAV 186	Queen's Pocket Melon	INRA, France	var. *dudaim*
53	KAV-187	ME 0241 = Shiro Uri Okayama	INRA, France	var. *conomon*
54	KAV 188	Snakemelon	INRA, France	var. *flexuosus*
55	KAV 189	Tibish 93-2	INRA, France	var. *tibish*
56	KAV 190	Vedrantais	INRA, France	var. *cantalupensis*
57	KAV 191	Unnamed	Kahramanmaraş	var. *inodorus*
58	KAV 213	Tırtıllı kara kavun	Ankara	var. *inodorus*
59	KAV 214	Altınbaş	Ankara	var. *inodorus*
60	KAV 221	Kışlık sarı kavun	Ankara	var. *inodorus*
61	KAV 226	Altın	Ankara	var. *inodorus*

Continued on next page

Table 1. Continued.

No.	Genotype	Local name	Origin	Horticultural variety (group)
62	KAV 228	Uzun yuva	Ankara	var. *inodorus*
63	KAV 232	Çikolata	Ankara	var. *inodorus*
64	KAV 234	İpsala	Ankara	var. *inodorus*
65	KAV 235	Mühürlü siyah kavun	Ankara	var. *inodorus*
66	KAV 237	Portakal kavun	Ankara	var. *inodorus*
67	KAV 239	Dilimli	Ankara	var. *inodorus*
68	KAV 242	Yerli kavun (muz kavunu)	Ankara	var. *inodorus*
69	KAV 249	Kırkağaç	Ankara	var. *inodorus*
70	KAV 250	Şememe	Ankara	var. *dudaim*
71	KAV 252	Tepeköy kavunu	Ankara	var. *inodorus*
72	KAV 255	Kışlık kavun	Konya	var. *inodorus*
73	KAV 265	Unnamed	Erzurum	var. *inodorus*
74	KAV 266	Unnamed	Erzurum	var. *inodorus*
75	KAV 267	Unnamed	Erzurum	var. *inodorus*
76	KAV 269	Unnamed	Erzurum	var. *inodorus*
77	KAV 274	Unnamed	Erzurum	var. *inodorus*
78	KAV 277	Unnamed	Erzurum	var. *inodorus*
79	KAV 278	Unnamed	Erzurum	var. *inodorus*
80	KAV 279	Unnamed	Erzurum	var. *inodorus*
81	KAV 280	Unnamed	Erzurum	var. *inodorus*
82	KAV 282	Kış kavunu	Elazığ	var. *inodorus*
83	KAV 284	Malbora kavunu	Elazığ	var. *inodorus*
84	KAV 287	Kışlık beyaz kavun	Elazığ	var. *inodorus*
85	KAV 291	VA435	INRA, France	var. *cantalupensis*
86	KAV 292	Yabani	Mersin	var. *agrestis*
87	AC 01	Beyaz Acur Kısa Meyve	Ankar	var. *flexuosus*
88	AC 05	Hıtta	Şanlıurfa	var. *flexuosus*
89	AC 07	Kızılören 1	Kayseri	var. *flexuosus*
90	AC 16	Adana	Adana	var. *flexuosus*
91	AC 33	TR 47808	İzmir	var. *flexuosus*
92	AC 47	TR 51559	İzmir	var. *flexuosus*
93	AC 51	Hıtta	Şanlıurfa	var. *flexuosus*
94	AC 54	Unnamed	Siirt	var. *flexuosus*
95	AC 56	Unnamed	Nevşehir	var. *flexuosus*
96	AC 60	Tüylü Acur	Şanlıurfa	var. *flexuosus*

with a 10-min elongation step at 72°C. PCR products were stored at 4°C prior to analysis. After amplification, 1-25 µL of loading buffer containing 95% formamide, 10 mM EDTA, pH 8.0, 0.025% xylene cyanol, and 0.025% bromophenol blue were added to each reaction tube. The samples were heat-denatured for 5 min at 95°C and quickly transferred to ice. After loading 1.0 µL of each sample, PCR products were separated on 6% denaturing polyacrylamide gel that had been preheated for 25 min. Electrophoresis was conducted at 1500 V, 50 W, 35 mA, and 48°C using a Li-Cor DNA Analyzer 4300. A 50-350 bp DNA ladder (MWG Biotech AG, Ebersberg, Germany) was run alongside the amplified PCR products to determine DNA sizes.

SSR data were coded with a 1 to indicate the presence of a band or 0 to indicate its absence for the generation of a binary matrix. For cluster analysis, the clustering procedure unweighted pair-group method using the arithmetic average (UPGMA) was employed to construct the clustering dendrogram based on the genetic distance matrix using the NTSYS-PC version 2.02i program (Rohlf, 1998). The Mantel matrix correspondence test (Mantel, 1967) was used to evaluate the representativeness of the dendrogram by estimating the cophenetic correlation for the dendrogram compared with the similarity matrix. The result of this test is a cophenetic correlation coefficient, r, indicating how well the dendrogram represents similarity data.

RESULTS AND DISCUSSION

Genetic diversity among Turkish melon genotypes was evaluated by SSR markers. Amplification was successful with 20 markers assayed. A total of 123 alleles were generated using the 20 SSR primer sets listed in Table 2. The polymorphism rate was 97.5% among 96 genotypes. The number of alleles detected by a single primer set ranged from 2 to 12, with an average of 6.15 (Table 2). This average was higher than those of many previous reports. Tzitzikas et al. (2009) used SSR markers to investigate the genetic diversity and population structure of traditional Greek and Cypriot melon cultigens. They reported that all SSR markers were polymorphic with a total number of 81 alleles, averaging 4.7 alleles per locus. In another study, a total of 232 SSR alleles and an average of 10.3 alleles per SSR were obtained for Indian snap melons (*C. melo* var. *momordica*) (Dhillon et al., 2007). Kong et al. (2007) used EST-SSR markers in *C. melo* and found that the number of alleles ranged from 2 to 5 with an average of 2.9 alleles per locus.

Table 2. Simple sequence repeat marker bands used to assess the genetic diversity of melons.

No.	Primer	Size (bp)	Polymorphic band No.	Polymorphism (%)
1	CMCT44	130, 140, 143, 145, 148, 150, 152, 154, 156, 158, 175	11	100
2	CMGA104	161, 164, 170, 173, 176, 180, 182, 184	8	100
3	CMACC146	155, 160, 170	3	100
4	CMCTT144	185, 198, 200, 207, 210, 212, 215, 218	8	100
5	CMTC47	172, 175, 185, 190	4	100
6	CMAT141	185, 187, 190, 195, 198	5	100
7	CMCCA145	145, 148, 150, 173	4	100
8	CMTC168	200, 208, 210, 215, 218, 220	6	100
9	CMGA172	125, 130, 132, 138, 142, 145	6	100
10	CMTC123	122, 125	1	50
11	CMTG108	204, 206, 208	3	100
12	CMTAA166	170, 180, 185, 200, 204, 206	6	100
13	CMTA134a	165, 166, 170, 173, 180, 190, 200, 202, 210, 225, 250, 255	12	100
14	CMTC160a+b	232, 235, 240, 245, 250	5	100
15	CSCTTT15a	204, 206	2	100
16	CMGAN92	160, 170, 176	3	100
17	CMGAN10	183, 184, 190, 195, 204, 208, 218, 220	8	100
18	CMGAN59	71, 74, 76, 81, 85, 86, 87, 88, 91, 94	10	100
19	CMGAN68	123, 125, 128, 131, 136, 140, 144	7	100
20	TJ24	150, 156, 157, 160, 174, 180, 182, 185, 189, 197	10	100
Total			122	97.5

The results showed that the SSR polymorphism rate (97.5%) was higher than that reported in much of the literature [71%, Katzir et al. (1996); 86%, Danin-Poleg et al. (2001); and 66.7%, López-Sesé et al. (2002)]. The reason for obtaining high SSR polymorphism values may be due to the large number of genotypes used and the detection of a higher number of alleles, as explained by Monforte et al. (2003).

The matrix correlation coefficient was calculated to be $r = 0.94$ for the dendrogram obtained based on the SSR analysis. This value indicates that the similarity index is well represented in the dendrogram (Rohlf, 1998). Interpretation of the correlation coefficient matrix was as follows: $r \geq 0.9$, very good; $0.8 \leq r < 0.9$, good; $0.7 \leq r < 0.8$, poor; $r < 0.7$, very poor.

The similarity coefficient ranged from 0.22 to 1.00 as a result of microsatellite analysis. UPGMA employing SSR data resulted in a dendrogram with two main branches as shown in Figure 1. The genotypes were determined to be highly variable, up to 80%.

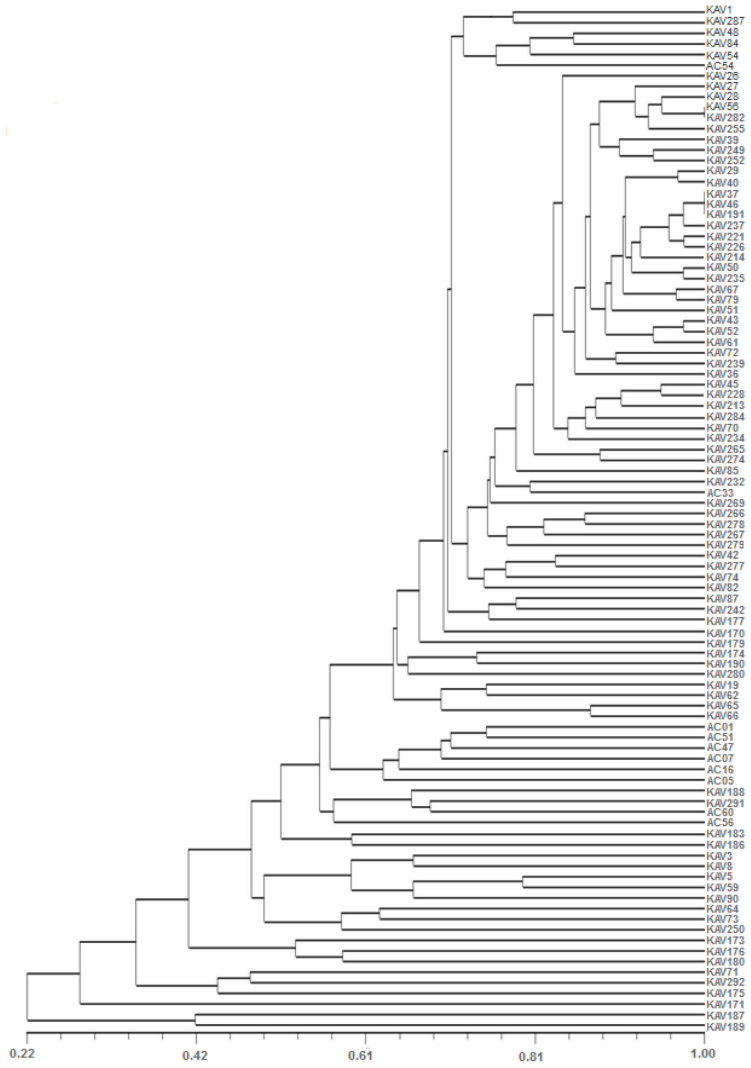

Figure 1. Dendrogram based on simple sequence repeat analysis of 96 melon genotypes.

One of the primary branches of the dendrogram consisted of two reference genotypes (Kav 189 var. *tibish* and Kav 187 var. *conomon*) provided by INRA France. However, the similarity coefficient was relatively low (42%) because they belong to different botanical groups. The second main branch contained all the other 94 genotypes. SSR analysis supports the separation of reference accessions and Turkish landraces with some exceptions. Most of the snakemelon genotypes, belonging to var. *flexuosus*, were clustered with the reference accessions as expected.

Based on the results, wild genotypes (Kav 66, Kav 71 and Kav 292), collected from different provinces of Turkey and belonging to var. *agrestis*, were dispersed among the reference accessions.

Cluster analysis also indicated that there was no correlation between grouping and geographical origin of the genotypes.

It was obvious that genotypes belonging to the *cantalupensis* and *inodorus* varieties were clustered together in different subgroups with varying (70-100%) similarity rates. These subgroups contained Kirkagac, Yuva, Kuscular, and different local melons, which are morphologically different. In these subgroups, Kav 56 and Kav 282 were identical and classified in var. *inodorus*. These genotypes are known as the Kirkagac type, which is characterized by an orange rind with green spots. Kav 37, Kav 46 and Kav 191 could not be distinguished from each other as well. This result is consistent with the study of Tzitzikas et al. (2009), in which Greek and Cypriot traditional cultigens were classified within the subspecies *melo* and found to be different from the *flexuosus* accessions. Another study, by Staub et al. (2004), used RAPD markers and did not show a distinction between Greek *flexuosus* landraces and Greek *inodorus* landraces. The authors speculated that their results were attributed to using a different germplasm or to the discrimination power of the marker type. Genetic differences depending on the marker system were also reported by Staub et al. (2000), Nakata et al. (2005) and Aierken et al. (2011), indicating that the ability of SSR markers to discriminate was better than that of RAPD markers.

The genetic diversity of Turkish melons based on phenotypic characters and RAPD markers was investigated by Sensoy et al. (2007). These researchers reported that the genetic variation among Turkish melon genotypes was very high. This finding is in concordance with our finding of high genetic diversity using SSR markers.

CONCLUSIONS

Turkey is a secondary center of origin of melons, and it has important melon genetic resources. The present study used SSR markers to detect high genetic variation among the melon genotypes collected from different Turkish provinces. Genetic resources and their conservation and utilization are very important for breeding new cultivars. The genetic resources provided by the diverse melon genotypes in Turkey indicate that Turkey can play an important role in future breeding strategies with these genotypes as candidates for breeding lines.

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