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Molecular Genetic Diversity in the Turkish National Melon Collection and Selection of a Preliminary Core Set

Anne Frary[1]
Department of Molecular Biology and Genetics, Izmir Institute of Technology, Urla, 35430 Izmir, Turkey

Hasan Özgür Şığva
Biotechnology Program, Izmir Institute of Technology, Urla, 35430 Izmir, Turkey

Ayfer Tan, Tuncer Taşkın, Abdullah İnal, Sevgi Mutlu, and Mehmet Haytaoğlu
Department of Plant Genetic Resources, Aegean Agricultural Research Institute, Menemen 35660 Izmir, Turkey

Sami Doğanlar
Department of Molecular Biology and Genetics, Izmir Institute of Technology, Urla, 35430 Izmir, Turkey

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Abstract. Turkey is a secondary center of diversity for melon (*Cucumis melo*) and is home to a variety of regional morphotypes. This diversity is housed in a national germplasm repository with more than 500 accessions. Molecular genetic variability of 209 melon genotypes from 115 accessions of this collection was characterized using amplified fragment length polymorphisms (AFLPs). Ten AFLP primer combinations yielded 279 reproducible fragments, which were used for dendrogram and principal coordinate analyses. These analyses showed two major clusters of Turkish melons: one group contained highly similar genotypes (maximum Dice dissimilarity coefficient of 0.18), whereas the other group was genetically more diverse (maximum dissimilarity 0.41). Although average dissimilarity was low (0.13), a broad range of genetic diversity was observed in the collection. A marker allele richness strategy was used to select a core set of 20 genotypes representing the allelic diversity of the AFLP data. The core set had double the average diversity (0.26) of the entire set and represented the major morphotypes present in the collection. Molecular genetic diversity of the core set was further validated using simple sequence repeat marker data (116 polymorphic fragments), which confirmed that the selected core set retained high levels of molecular genetic diversity.

Melon is a diploid (2n = 2x = 24), morphologically diverse crop of commercial importance as a dessert fruit. Melon was first used as food in ancient Egypt and Iran during the second and third centuries B.C.E. (Dhillon et al., 2011; Janick et al., 2007; Zeven and de Wet, 1982) and melon cultivation then spread to nearby areas. In 2010, 25 million tonnes of melon were produced on 1 million hectares [Food and Agricultural Organization of the United Nations (FAO), 2012]. According to some authors, wild-type melon originated from south and east Africa (Mallick and Masui, 1986). However, recent work suggests that melon originated in Asia (Sebastian et al., 2010). This idea is supported by the fact that the primary center of diversity of many commercially important melons is the Near East and central Asia (Jeffrey, 1980; Luan et al., 2008). Turkey is an important secondary center of diversity for melon and other cucurbits (Sari et al., 2008) and was considered by Harlan (1951) to be a microcenter for melon landraces. Turkey consistently ranks second behind China in worldwide melon production with 1.6 million tonnes produced in 2010 (FAO, 2012).

The Turkish national melon germplasm collection is housed at the National Seed Genebank at the Aegean Agricultural Research Institute (AARI), Menemen, Izmir, Turkey. The seed bank contains 571 accessions of *Cucumis melo,* many of which have been collected and submitted by farmers from throughout the country (Sari et al., 2008). Ex situ conservation of plant germplasm is expensive and labor-intensive. Although a collection may contain hundreds or thousands of accessions, these accessions may be redundant or genetically similar. In addition, depending on collection method, accessions may be mixtures of individuals with different morphologies. Such populations are difficult to fully characterize and may not be favored by breeders who prefer to work with homogeneous material. Therefore, at least preliminary morphological and molecular characterization is essential for efficient management and use of germplasm collections. Molecular genetic characterization of plant accessions has become routine and several molecular marker methods have been used for determination of genetic variability within melon accessions including isozymes (Akashi et al., 2002), restriction fragment length polymorphisms (Neuhausen, 1992), random amplified polymorphic DNA [RAPDs (Garcia et al., 1998; Luan et al., 2008; Nhi et al., 2010; Sensoy et al., 2007; Staub et al., 2004; Yildiz et al., 2011)], simple sequence repeats [SSRs (Danin-Poleg et al., 2001; Monforte et al., 2003)], intersimple sequence repeats [ISSRs (Perl-Treves et al.,

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[1]Corresponding author. E-mail: annefrary@iyte.edu.tr.

1998; Yildiz et al., 2011)], AFLPs (Nimmakayala et al., 2009), and single nucleotide polymorphisms (Deleu et al., 2009; Szabo et al., 2005).

In this study, we characterized the molecular genetic diversity of a portion of the Turkish national melon germplasm collection (209 genotypes from 115 accessions) using AFLP markers. The AFLP technique was selected because it provides a high number of reproducible polymorphic fragments distributed throughout the genome. The genotypes represented eight morphotypes including both widely grown (Ananas, Casaba, Charentais, Winter) and regional (Altınbaş, Yuva-Hasanbey, Mollaköy, Topatan) types. The regional melons are Turkish in origin or are variants of more widely grown melons, which have been selected according to local preferences. Altınbaş is a Kırkağaç type of melon. This type is of Turkish origin and has yellow skin with dark green spots. Yuva-Hasanbey melons are Casaba types with dark green or gray–green skin. Mollaköy is a Charentais type with yellow skin and green sutures. Topatan is an Ananas type with yellow skin and less netting than Ananas. The AFLP data indicated the level of diversity present in the national collection and were used to select a core set of genotypes. This core set was then analyzed with SSR markers to confirm that it represented the molecular diversity present in the entire set of genotypes.

Fig. 1. Map of Turkey showing the origins of melon genotypes used in diversity analysis. Numbers in parentheses indicate the numbers of genotypes from each area.

Materials and Methods

Plant material. A total of 115 Turkish melon accessions were obtained from AARI (Supplementary Table 1). These accessions were randomly selected from the national collection. During Summer 2006, 10 seeds of each accession were planted and grown in a greenhouse at AARI. Morphologically distinct plants (genotypes) within each accession were selected and self-pollinated to produce seed for the molecular analysis. Thus, a total of 209 genotypes were sampled from the 115 original accessions. These genotypes represented eight morphotypes. Four were widely grown types [Ananas (43 genotypes), Casaba (16), Charentais (seven), Winter (two)] and four were regional types [Altınbaş (65 genotypes), Yuva-Hasanbey (50), Mollaköy (three), Topatan (16)]. Five genotypes had intermediate morphotype, one had long cylindrical fruit, and one had unknown morphotype because it did not produce fruit. The origins of the material encompassed all of the major melon-growing regions of the country: the Aegean (44 genotypes), East Anatolian (32), Southeast Anatolian (64), Central Anatolian (17), Marmara (31), and Black Sea regions (18) (Fig. 1). Three genotypes were from unrecorded locations in Turkey. Additional members of the Cucurbitaceae family were used as outgroups: *Luffa cylindrica*, *Luffa sicercia*, *Cucurbita maxima* (two accessions), *Momordica charantia*, *Cucurbita pepo*, *C. pepo* var. *turbaniformis, Cucurbita moschata* (two accessions), and *C. melo* var. *flexuosus* (Supplementary Table 2). These outgroup accessions were obtained from N. Sari (Cukurova University, Adana, Turkey).

DNA extraction. Ten seeds of each genotype were planted and germinated in a greenhouse (20 to 24 °C, 16-h photoperiod, ≈300 μmol·m^{-2}·s^{-1}) at Urla, Izmir, in Mar. to May 2007. Total genomic DNA was extracted from fresh leaf tissue of each seedling at the two- to four-leaf stage with a cetyltrimethyl ammonium bromide extraction protocol modified according to Fulton et al. (1995) and with a Wizard Genomic DNA purification kit (Promega, Madison, WI). The DNA was quantified with a spectrophotometer (Nanodrop ND-1000; Thermo Fisher Scientific, Waltham, MA) following the manufacturer's protocol. After quantification, the 10 samples from each genotype were bulked in equal concentrations. All genomic DNAs were stored at –20 °C in Tris-EDTA buffer.

Amplified fragment length polymorphism analysis. For AFLP analysis, bulked genomic DNAs were double-digested with the restriction enzymes *Eco*RI and *Mse*I (Vos et al., 1995). Preselective amplification was carried out using DNA fragments ligated to restriction half-site specific adapters for the *Eco*RI and *Mse*I sites. Selective amplification was carried out using 10 AFLP primer combinations from the AFLP Core Reagent Kit and AFLP Starter Primer Kit (Invitrogen, Carlsbad, CA) (*Mse*I-CTC/*Eco*RI-AAC, *Mse*I-CTC/*Eco*RI-AAG, *Mse*I-CTC/*Eco*RI-ACA, *Mse*I-CTA/*Eco*RI-ACG, *Mse*I-CTA/*Eco*RI-ACC, *Mse*I-CTA/*Eco*RI-ACT, *Mse*I-CAT/*Eco*RI-AAG, *Mse*I-CAT/*Eco*RI-ACA, *Mse*I-CAC/*Eco*RI-AAC, and *Mse*I-CAC/*Eco*RI-AAG). Primers were fluorescently labeled with blue dye and amplification products were diluted in sample loading solution (SLS) with 0.5 μL size standard 600 and analyzed using a CEQ 8800 Sequencer (Beckman-Coulter, Fullerton, CA). The default Frag 4 separation method was used: capillary temperature 50 °C, denaturation temperature 90 °C for 120 s, injection voltage 2.0 kV for 30 s, and separation voltage of 4.8 kV for 60.0 min.

Data analysis. AFLP primer combination data were scored as present (1) or absent (0). Polymorphism information content (PIC) values were calculated according to Roldan-Ruiz et al. (2000). Distance matrices were generated with the Dice coefficient (Dice, 1945) and used to draw a dendrogram with the unweighted neighbor joining method using the Darwin computer program (Perrier and Jacquemoud-Collet, 2006). To evaluate the efficiency of clustering, the cophenetic correlation coefficient was calculated with the Mantel method (Mantel,

1967). Principal coordinate analysis (PCoA) was also performed and multidimensional plots were produced with the NTSYS-pc Version 2.2 (Exeter Software, Setauket, NY) software program. PIC value means comparisons for morphotypes represented by more than 10 accessions were done with Tukey's honestly significant difference test as used by JMP software (SAS Institute, Cary NC). The AFLP data were also used to select a core set of genotypes using the M strategy and modified heuristic algorithm of PowerCore 1.0 software (Kim et al., 2007).

Validation of the core set. The genetic diversity of the core set was checked by comparing SSR marker data for the core set with all genotypes. For this, polymerase chain reaction (PCR) amplifications were carried out using 12 SSR markers. Four expressed sequence tag (EST)–SSR markers (MU118, FR14G19, SSH6I23, and PH8C1) were generated from the Cucurbita Genomics Database (International Cucurbit Genomics Initiative, 2012) melon EST library using the default parameters of the PBC Public SSR Discovery Input web-based freeware program. The other eight SSR markers included three EST-SSRs (CMCTN86, TJ10, TJ27) and five genomic SSRs (CMCTN5, CMGAN25, CMCTN35, CMAGN68, CMGAN80). These markers were chosen from the melon SSR markers mapped by Gonzalo et al. (2005). All 12 of the SSR markers were labeled with M13(-21) tail (5-TGT AAA ACG ACG GCC AGT-3) (Schuelke, 2000) for more cost-effective band detection using the Beckman-Coulter CEQ 8800 Sequencer. The PCR mixture contained 0.75 µL (3.2 pmol) of each reverse and FAM-labeled M13(-21) primer and 0.75 µL (0.8 pmol) forward primer in a 20-µL reaction volume with 2 µL 10× PCR buffer, 0.4 µL (0.2 mM) dNTPs, 1 U AmpliTaq DNA polymerase, 13.95 µL sterile distilled H_2O, and 50 to 100 ng template DNA. The PCR amplification protocol consisted of 5 min initial denaturation at 94 °C, then 30 cycles of 30 s of denaturing at 94 °C, 45 s at 56 °C for annealing, and extension at 72 °C for 45 s followed by eight cycles: 30 s of denaturation at 94 °C, 45 s for annealing of M13 fluorescent-labeled primer at 53 °C, 45 s for extension at 72 °C, and final extension at 72 °C for 10 min. After amplification, 27-µL SLS and 0.5 µL size standard 400 were added to 3 µL PCR product and one drop mineral oil was added for separation in the sequencer using the Frag 4 method. All analyses were similar to those described for AFLP.

Results

Morphological examination of 115 randomly selected accessions from the Turkish national melon collection indicated that some were mixtures of individuals. Heterogeneity within accessions was especially evident after fruit set because plants of such accessions often produced fruit with different morphotypes (Fig. 2). This was not unexpected because many samples were collected and submitted by farmers. Plants representing the most prevalent morphologies were selected and self-pollinated to produce the 209 genotypes used in the molecular study (Supplementary Table 1).

Diversity of melon in the national collection. Ten AFLP primer combinations were used to assess genetic diversity among the 209 melon genotypes and 10 outgroups. All of the AFLP primer combinations were polymorphic and provided a total of 345 polymorphic bands, thus averaging 34.5 polymorphic bands per combination. The combinations *Mse*I-CTC/*Eco*RI-ACA and *Mse*I-CTA/*Eco*RI-ACT gave the most polymorphic bands with 42 each, whereas the combination *Mse*ICAC/*Eco*RI-AAC gave the fewest polymorphic bands with 21. Some of the AFLP fragments had poor sample-to-sample reproducibility. These fragments were excluded and 279 fragments were selected for further analyses. PIC values were calculated for each of these markers and then averaged across morphotypes (Table 1). PIC values varied from 0.09 (Mollaköy) to 0.20 (Casaba and Topatan) for the different morphotypes indicating fairly low polymorphism. Means comparison for morphotypes represented by more than 10 accessions indicated that PIC values were significantly higher for Casaba and Topatan genotypes (0.20) than for Yuva-Hasanbey genotypes (0.15). In general, PIC values for regional melon types were not significantly different from those for more widely grown types such as Casaba and Ananas.

Principal coordinate analysis was performed for the Turkish melons using the AFLP data. The first, second, and third axes for PCoA explained 61%, 9%, and 4% of the total variance, respectively. The bivariate plot showed tight clustering of many Turkish melon genotypes indicating very high genetic similarity (Cluster A in Fig. 3). The remaining genotypes were more dispersed indicating greater genetic variability. No relationship was observed between grouping and origin or morphotype of the melon genotypes.

An unweighted neighbor joining dendrogram of the 209 national melon genotypes and 10 outgroups was drawn based on the Dice coefficient results (Supplementary Fig. 1A). According to a Mantel test, the correlation between the Dice distance matrix and the dendrogram was very high (0.99). The dendrogram scale varied from 0 to 0.69 with an average dissimilarity of 0.34. When only Turkish melon genotypes were considered, the scale varied from 0 to 0.52 with an average genetic dissimilarity of 0.13. Five pairs of genotypes were genetically identical (84-3 and 93-2; 41-3 and 67-7; 43-8 and 145-4; 47-9 and 48-1; 27-9 and 126-1). Interestingly, although these genotypes were genetically identical according to the AFLP analysis, none of the pairs had the same morphotype. The Turkish melons fell into two clusters, whereas the outgroups clustered separately as expected. Cluster A of the AFLP dendrogram contained the most genetically similar genotypes (Supplementary Fig. 1B). Genetic dissimilarity ranged from 0 to 0.18 for the 146 genotypes in this cluster. The tightly grouped genotypes found in Cluster A of the PCoA (Fig. 3) were also found in Cluster A of the dendrogram. Cluster B of the dendrogram was smaller (63 genotypes) but more diverse (Supplementary Fig. 1C). Dissimilarity in this cluster varied from 0.03 to 0.41. Clustering was not related to origin or morphotype. The outgroups had a minimum dissimilarity of 0; the two *C. moschata* accessions were genetically identical according to AFLP analysis (Supplementary Fig. 2D). As expected, the two *C. pepo* and two *Luffa* accessions were also closely related to each other. However, the *Cucurbita* species accessions did not cluster, an unexpected result based on their classification. Because AFLP is a nonspecific marker system, it is possible that some amplified fragments were nonallelic across different species. In any event, the dendrogram is not intended to show the true phylogenetic relationships among Cucurbitaceae species, but instead to illustrate the appropriateness of the chosen accessions as outgroups for this study.

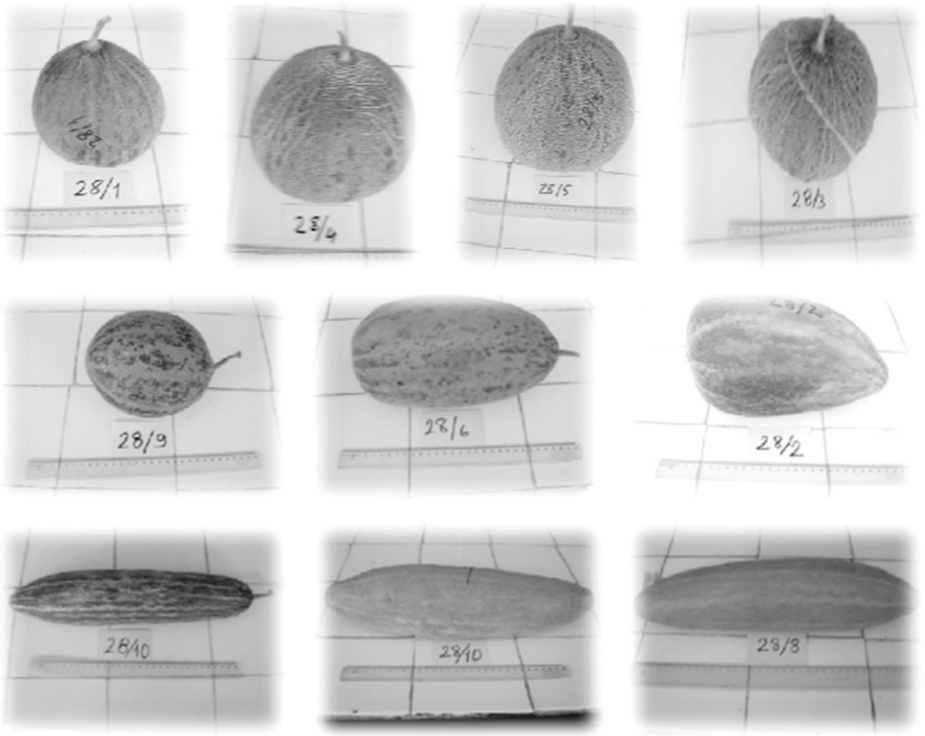

Fig. 2. Fruit from the 10 individual plants grown for melon accession TR47874 showing heterogeneous fruit morphology.

Table 1. Average polymorphism information content (PIC) values for amplified fragment length polymorphism (AFLP) and simple sequence (SSR) markers calculated for each melon morphotype.[z]

Morphotype	Genotypes (no.)	AFLP [mean ±SE (PIC)][y]	SSR [mean ±SE (PIC)]
Casaba	16	0.20 ± 0.01 a[x]	0.28 ± 0.02 a
Topatan	16	0.20 ± 0.01 a	0.30 ± 0.02 a
Altınbaş	65	0.19 ± 0.01 ab	0.33 ± 0.01 a
Ananas	43	0.16 ± 0.01 ab	0.34 ± 0.01 a
Yuva-Hasanbey	50	0.15 ± 0.01 b	0.31 ± 0.01 a
Charentais	7	0.10 ± 0.01	0.29 ± 0.02
Mollaköy	3	0.09 ± 0.01	0.18 ± 0.02
Winter	2	0.14 ± 0.01	0.19 ± 0.02
Other[w]	7	0.19 ± 0.01	0.20 ± 0.02

[z]Means comparison analysis was not performed on morphotypes represented by fewer than 10 genotypes.
[y]PIC values as calculated according to Roldan-Ruiz et al. (2000) range from 0 to 0.50.
[x]Within columns, values followed by different letters are significantly different at $P \leq 0.05$ as determined by Tukey's honestly significant difference test.
[w]Other category includes unknown and intermediate types.

Core set selection. The AFLP data were used to select a core set of melon genotypes to represent the molecular genetic diversity present in the entire data set. The PowerCore program selected 20 (10%) genotypes from the collection (Table 2). Not all morphotypes were represented in the core set, which contained Ananas, Altınbaş, Yuva-Hasanbey, Topatan, and Casaba types. The 279 distinct AFLP fragments identified in all genotypes were retained in the core set. Both the Shannon-Weaver and Nei's diversity indices were higher for the core set than the entire data set (0.50 vs. 0.34 and 0.40 vs. 0.28, respectively). Diversity analysis of the core set with the same methods used for the entire set indicated an average dissimilarity of 0.26 with genetic dissimilarity ranging from 0.04 to 0.52. Like with the entire data set, the core set genotypes fell into two main clusters and cluster identity (A or B) matched that seen in the entire set dendrogram (Fig. 4). Cluster A contained the more similar genotypes, whereas Cluster B had more dissimilar genotypes. Very little clustering by origin or morphotype was observed with the exception that none of the Ananas genotypes were found in Cluster B.

Validation of core set with simple sequence repeat analysis. Both the entire and core genotype sets were analyzed with SSR markers to confirm that the core set was representative of the molecular genetic diversity of the original germplasm. For this analysis, both new and previously published SSR markers were assayed. To develop new EST-SSR primers, the Cucurbit Genomics Database melon EST database containing 3522 melon unigenes was examined for SSRs. More than 400 SSRs were detected and flanking primers were designed for these SSRs. Thirty primer pairs were tested for their polymorphism on a subset of melon genotypes. In this way, four reproducible and highly polymorphic markers (MU118, FR14G19, SSH6I23, PH8C1) were selected for use in the diversity analysis (Supplementary Table 3). Eight frequently used and previously mapped melon SSR markers were also included in the analysis (CMCTN5, CMCTN86, CMGAN25, CMCTN35, CMAGN68, CMGAN80, TJ10, TJ27). Five of the markers were genomic SSRs, whereas seven were EST-SSRs. A mixture of both types of SSRs was used to ensure inclusion of both coding and noncoding regions. All 12 of the SSR primers were polymorphic and provided 116 polymorphic bands on the 209 melon genotypes. Thus, the number of polymorphic bands per primer was 9.7. Average PIC value was 0.34 for all genotypes. PIC values for the SSR markers as averaged across morphotypes were moderate with a minimum of 0.18 for Mollaköy and a maximum of 0.34 for Ananas types. PIC values did not show significant differences among types, which were represented by more than 10 genotypes (Table 1). Like with AFLP, the SSR data yielded a dendrogram that divided the Turkish melon genotypes into two groups (data not shown). However, the grouping did not exactly match that seen in the AFLP dendrogram and

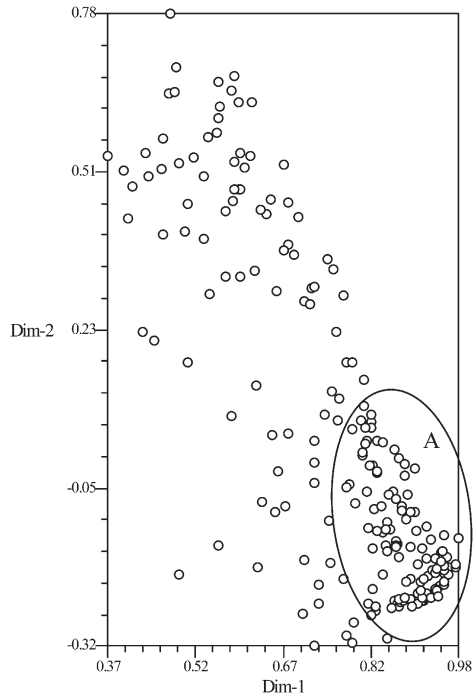

Fig. 3. Two-dimensional principal coordinate analysis of the 209 melon genotypes calculated from 279 amplified fragment length polymorphism marker fragments. The first and second axes accounted for 61% and 9% of the total variance, respectively. The most similar genotypes are labeled Cluster A.

a Mantel test of the data matrices for the AFLP and SSR data gave a low correlation ($r = 0.17$). These disparities may reflect that the two marker systems were sampling different portions of the melon genome. PCoA analysis of the core set with the SSR data also showed clustering that was similar to that observed with the AFLP data (Supplementary Fig. 2). When only the core set was examined, 101 polymorphic SSR fragments were detected indicating that 87% of the SSR alleles were preserved in the core set. Average PIC value for the core set was 0.37.

Discussion

Diversity of Turkish melons. In this work, genetic diversity of Turkish melon genotypes was examined using both AFLP and SSR markers. The AFLP results confirmed previous research indicating the efficiency of this marker type for diversity studies in melon with 34.5 polymorphic fragments detected per combination. SSR markers also provided multiple polymorphic fragments per primer combinations with nearly 10 polymorphic bands identified per marker. Both of these values are higher than those obtained for other studies of melon diversity using AFLP and SSR markers (López-Sesé et al., 2002; Monforte et al., 2003; Nakata et al., 2005; Nimmakayala et al., 2009; Szabo et al., 2005). However, none of the other studies used as many melon genotypes. For example, AFLP was used to analyze 38 Ukrainian melon genotypes and an average of 21.2 polymorphic fragments were identified (Nimmakayala et al., 2009). AFLP markers were fourfold more polymorphic than SSRs when compared on the basis of polymorphic fragments per primer combination. Similar results were observed by Nimmakayala et al. (2009) who found a sixfold difference in polymorphism of these two marker systems in 38 Ukrainian melon accessions.

Table 2. Turkish melon genotypes in the core set.[z]

Genotype no.	Accession no.	Origin	Morphotype
1-8	None	Denizli	Yuva-Hasanbey
7-1	TR 31586	Diyarbakır	Ananas
7-9	TR 31586	Diyarbakır	Casaba
18-9	TR 46438	Turkey	Intermediate
19-5	TR 46489	Sivas	Altınbaş
25-5	TR 47812	Adıyaman	Altınbaş
26-7	TR 47813	Adıyaman	Altınbaş
37-1	TR 33380	Tekirdağ	Topatan
38-1	TR 40280	Gaziantep	Ananas
51-2	TR 47885	Urfa	Ananas
55-6	TR 50747	Erzurum	Ananas
85-3	TR 37394	Çorum	Ananas
87-7	TR 43024	Çanakkale	Yuva-Hasanbey
88-1	TR 43041	Çanakkale	Topatan
101-1	TR 2092	Turkey	Altınbaş
113-2	TR 61659	Mugla	Yuva-Hasanbey
115-3	TR 61812	Denizli	Topatan
122-3	TR 68913	Konya	Yuva-Hasanbey
123-5	TR 68934	Manisa	Casaba
126-8	TR 69425	Ankara	Altınbaş

[z]Core set was selected based on analysis of the amplified fragment length polymorphism marker data (279 fragments) using PowerCore 1.0 software (Kim et al., 2007).

AFLP markers revealed a low average genetic dissimilarity of the Turkish melon genotypes of only 0.13. However, maximum genetic dissimilarity was 0.52 showing the presence of genetically distinct types within the collection. This maximum value indicated that genotypes within the Turkish germplasm have very good genetic diversity as was suggested in a previous report, which examined 56 genotypes (not from the national melon collection) with RAPD markers (Sensoy et al., 2007). More recently, Yildiz et al. (2011) used ISSR, RAPD, and sequence-related amplified polymorphism markers to characterize 63 Turkish genotypes and found very high levels of polymorphism. The maximum dissimilarity values obtained in the present study are comparable to those observed in Chinese melons (Luan et al., 2008). Both China and Turkey are secondary centers of melon diversity; therefore, it is expected that these regions will be rich in genetic diversity. Spanish (López-Sesé et al., 2002) and Greek (Staub et al., 2004) melons also had similar levels of molecular genetic diversity. It is probable that melon spread to European countries like Greece and Spain from central Asia through Turkey (Paris et al., 2012); therefore, similar levels of genetic diversity may suggest that similarities in climate and cultivation conditions allowed maintenance of a wide variety of germplasm. In contrast, melons from Vietnam (Nhi et al., 2010), Ukraine (Nimmakayala et al., 2009), and Myanmar (Yi et al., 2009) were much less diverse.

Selection of a core set. Although the Turkish melon germplasm contained molecular genetic diversity, many of the genotypes were very similar. Thus, the molecular diversity within the collection can be represented with far fewer lines. Maintenance of a core set of accessions is logistically and economically easier than preservation of all genotypes. A marker allele richness (M) strategy for core selection was used using the AFLP data and the PowerCore software program, which

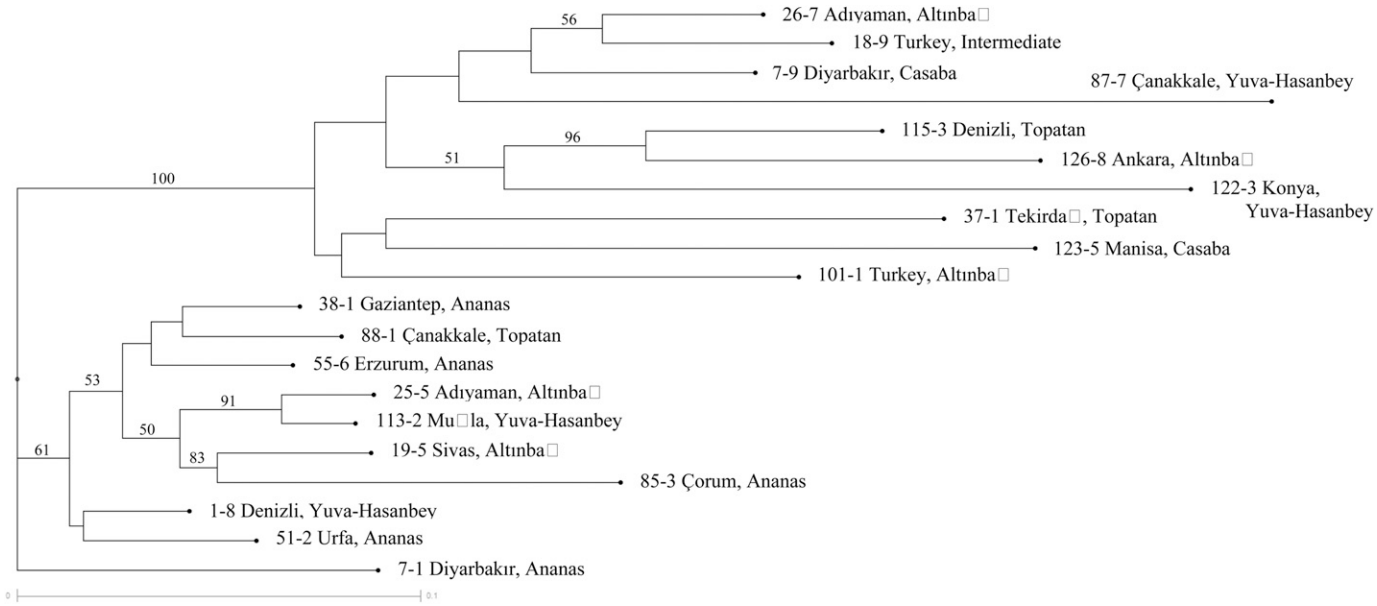

Fig. 4. Neighbor joining dendrogram based on Dice matrix calculated from the amplified fragment length polymorphism data (279 fragments) for the 20 genotypes of the Turkish melon core set. Origin and morphotype are given after genotype codes. Bootstrap values (1000 iterations) greater than 50 are shown on branches.

does a heuristic search for the core set (Kim et al., 2007). This analysis resulted in a core set of 20 genotypes with higher average diversity than the entire genotype set. These genotypes encompassed all of the AFLP diversity and 87% of the SSR diversity present in the entire set. The core set included representatives of five of the eight morphotypes present in the entire set. Mollaköy, Charentais, and Winter melons were not included in the core set. These melon types were rare in the collection, each representing 1% to 3% of the 209 genotypes tested in the study. Our results point out a serious limitation of using only molecular data for selection of a core set. Rarer morphotypes may be lost. Mollaköy is a regional Charentais-type melon; therefore, the exclusion of both Mollaköy and Charentais in the core set would be a significant loss. Another illustration of the limitations of selecting a core set based solely on molecular data is our finding that in four instances, different morphotypes showed the same AFLP profiles. This is not an unexpected result given that morphological variation in crop plants is often controlled by a few major loci (Gross and Olsen, 2010). Because molecular marker analysis may not capture information related to important phenotypic traits, final core set selection must be based on a combination of morphological and molecular analyses. However, selection of a preliminary core set using molecular markers can help to prioritize genotypes for additional characterization of traits such as fruit quality, yield, and disease and pest resistances.

In conclusion, our results stress the importance of conservation of Turkish melon germplasm to maintain diversity in the crop and to provide material for future genetic improvement. High levels of molecular genetic similarity among some genotypes suggested that the Turkish national germplasm would benefit from the establishment of a core collection and that AFLP marker data are suitable for preliminary selection of this core set based on molecular markers. The final core set should contain a subset of morphologically and molecularly distinct genotypes, which could be preserved and maintained with less expense and labor than the entire collection of over 500 accessions. Detailed phenotypic characterization of the core set would be feasible, thus providing information of use to breeders who would like to take advantage of this resource for melon improvement.

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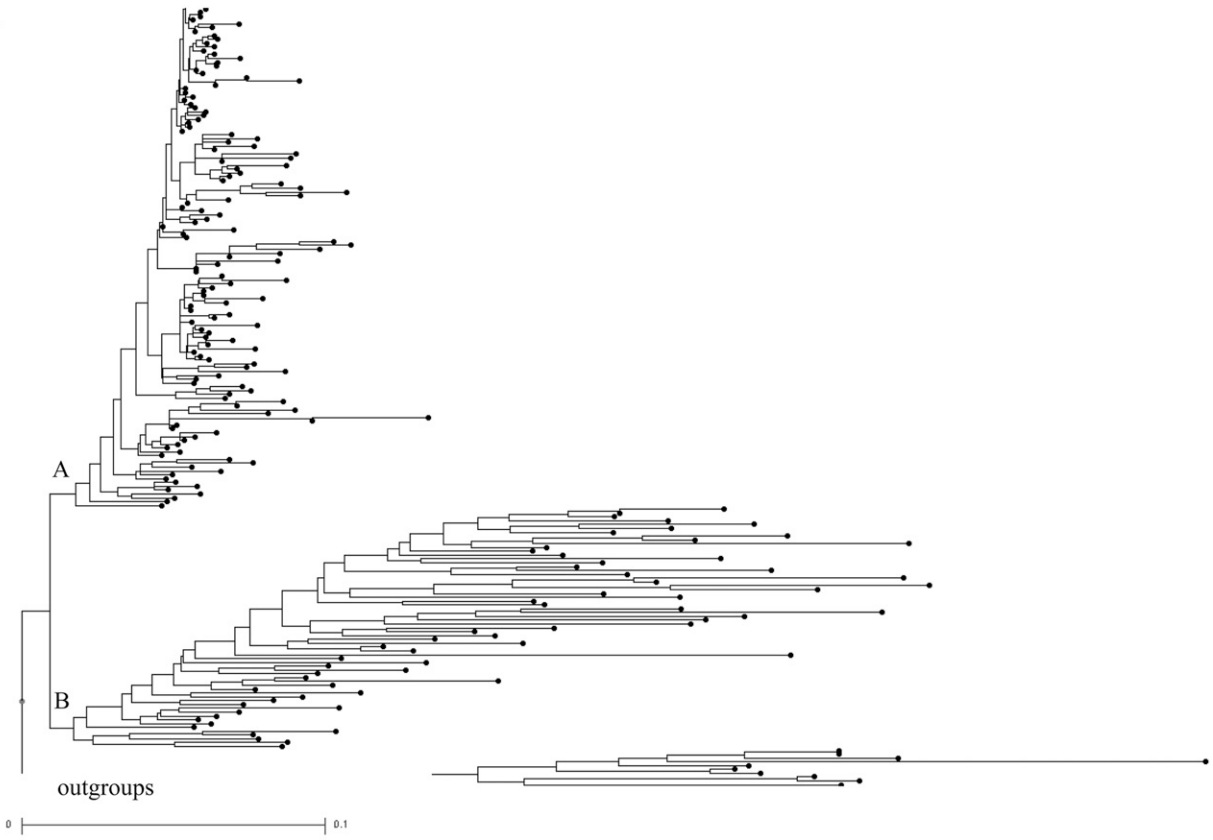
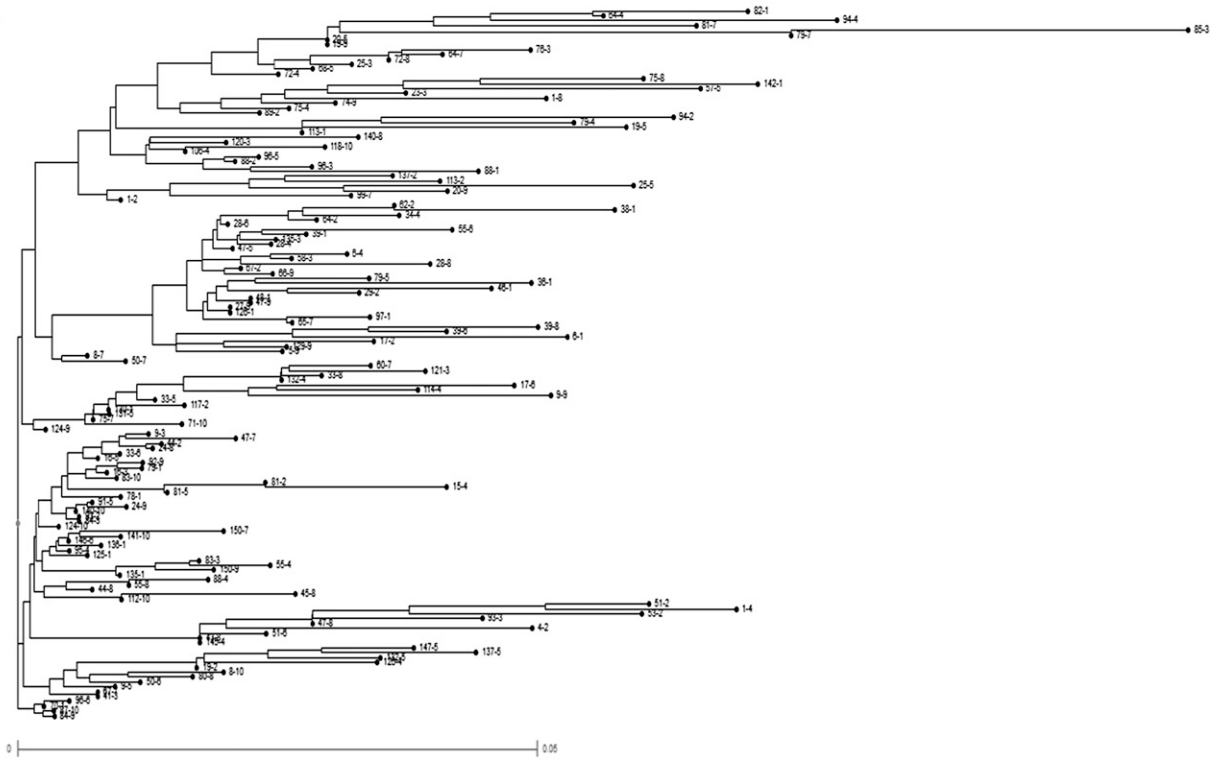
1A



1B



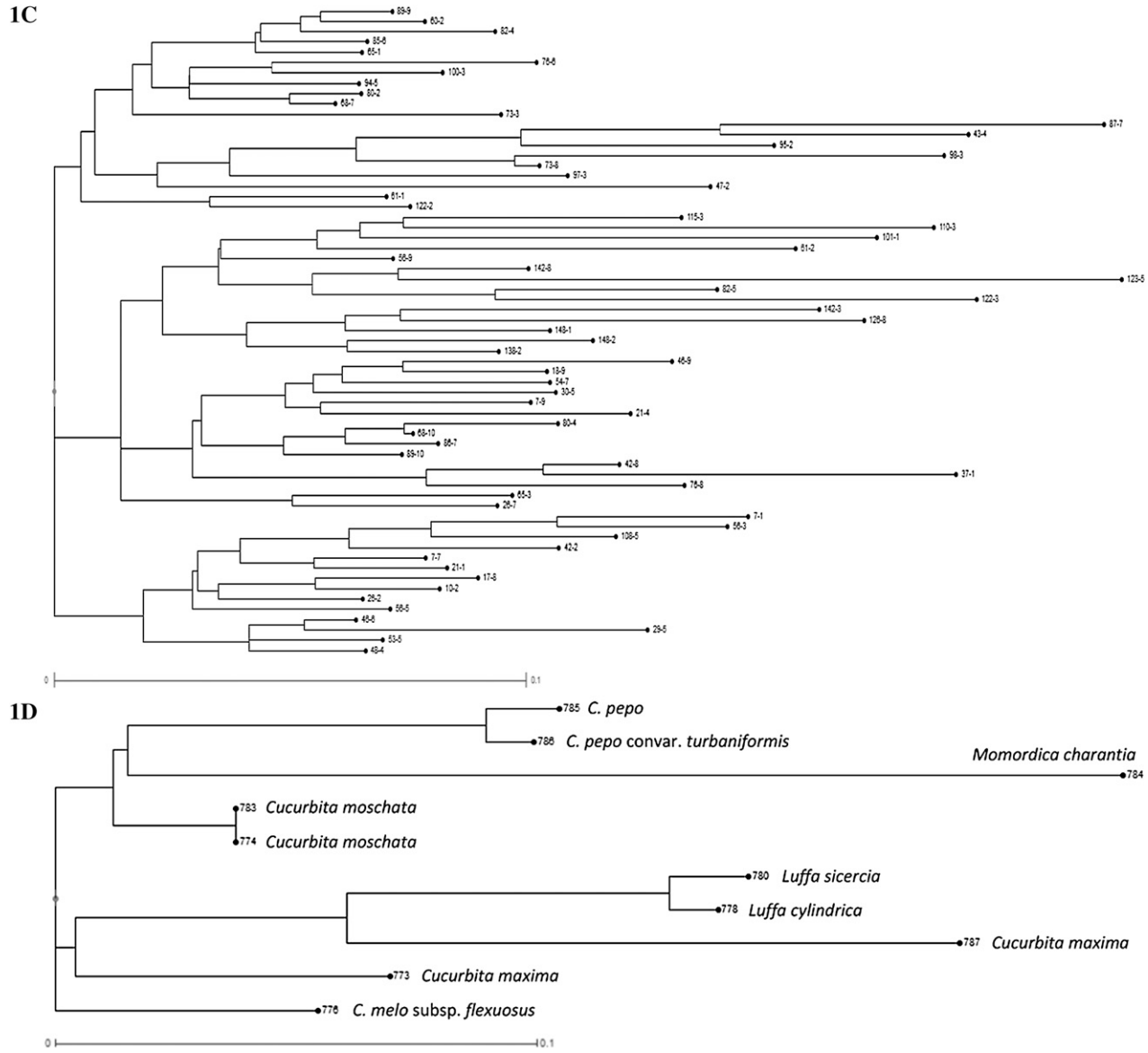


Fig. S1. Neighbor joining dendrograms of 209 Turkish melon and ten outgroup genotypes based on 279 amplified fragment length polymorphism (AFLP) fragments and the Dice dissimilarity matrix. **A**. Entire dendrogram showing topology of three main clusters: A, B and outgroup. **B**. Detail of neighbor joining dendrogram showing Cluster A from Fig. 1A. Dendrogram contains 146 Turkish melon genotypes and was redrawn for legibility using the same data as in Fig. 1A. **C.** Detail of neighbor joining dendrogram showing Cluster B from Fig. 1A. Dendrogram contains 63 Turkish melon genotypes and was redrawn for legibility using the same data as in Fig. 1A. **D.** Detail of neighbor joining dendrogram showing outgroup from Fig. 1A. Dendrogram contains ten accessions of Cucumis, Cucurbita, Luffa and Momordica species and was redrawn for legibility using the same data as in Fig. 1A.

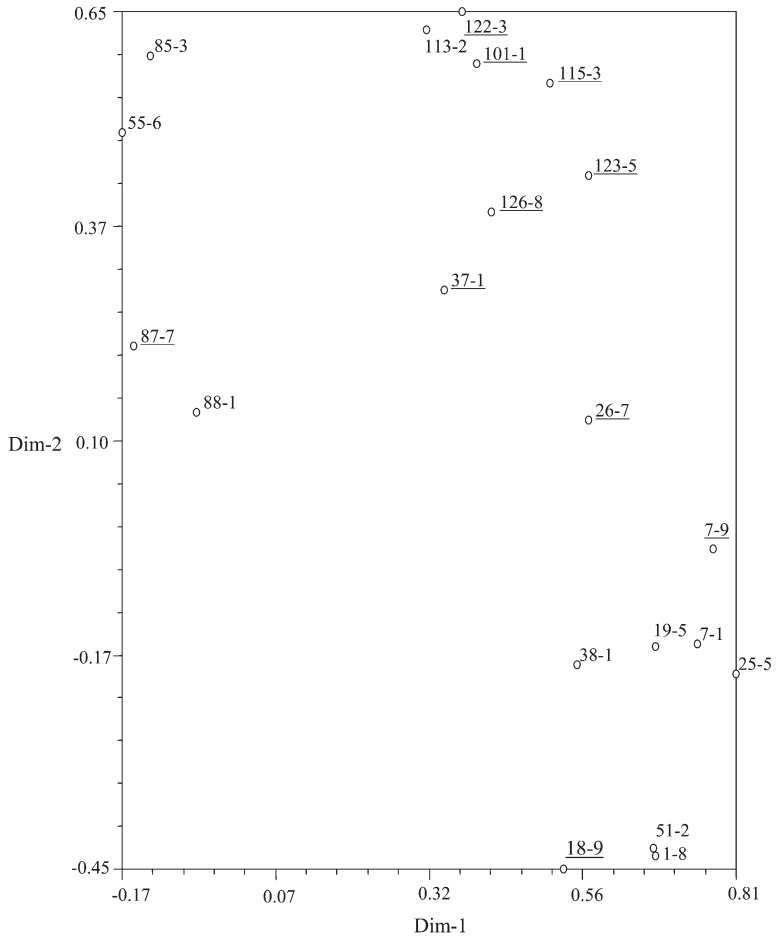


Fig. S2. Two-dimensional principal coordinate analysis (PCoA) of the 20 core set genotypes based on simple sequence repeat (SSR) marker data. The first and second axes accounted for 27% and 16% of the total variance, respectively. Underlined genotypes are those which grouped in Cluster B of the AFLP (amplified fragment length polymorphism) core set neighbor joining dendrogram (Fig. 4).

Supplementary Table 1. Identities of Turkish melons used in the study including origin and morphotype. A total of 209 genotypes were used representing 115 different accessions from the national melon collection.

Genotype no.	Accession no.	Origin	Morphotype	Genotype no.	Accession no.	Origin	Morphotype
1-2	None	Denizli	Long cylinder	72-4	None	Çanakkale	Altınbaş
1-4	None	Denizli	Altınbaş	72-8	None	Çanakkale	Charentais
1-8	None	Denizli	Yuva-Hasan	73-3	TR 40503	Van	Casaba
4-2	TR 38116	Balıkesir	Altınbaş	73-8	TR 40503	Van	Altınbaş
5-9	TR 38125	Balıkesir	Casaba	74-9	TR 43015	Çanakkale	Ananas
6-1	TR 38479	Tekirdağ	Casaba	75-4	TR 43023	Çanakkale	Ananas
6-4	TR 38479	Tekirdağ	Altınbaş	75-7	TR 43023	Çanakkale	Ananas
7-1	TR 31586	Diyarbakır	Ananas	75-8	TR 43023	Çanakkale	Ananas
7-7	TR 31586	Diyarbakır	Ananas	76-3	TR 43105	Çanakkale	Yuva-Hasan
7-9	TR 31586	Diyarbakır	Casaba	76-6	TR 43105	Çanakkale	Yuva-Hasan
8-7	TR 31588	Diyarbakır	Yuva-Hasan	76-8	TR 43105	Çanakkale	Yuva-Hasan
8-10	TR 31588	Diyarbakır	Altınbaş	78-1	TR 43749	Balıkesir	Altınbaş
9-3	TR 31589	Diyarbakır	Yuva-Hasan	79-1	TR 45896	Kars	Topatan
9-5	TR 31589	Denizli	Casaba	79-4	TR 45896	Kars	Yuva-Hasan
9-9	TR 31589	Denizli	Casaba	79-5	TR 45896	Kars	Yuva-Hasan
10-2	TR 26762	Bilecik	Yuva-Hasan	79-7	TR 45896	Kars	Topatan
15-4	TR 40534	Elazığ	Ananas	80-2	TR 46437	Erzincan	Yuva-Hasan
16-3	TR 45791	Erzurum	Ananas	80-4	TR 46437	Erzincan	Topatan
16-5	TR 45791	Erzurum	Yuva-Hasan	80-8	TR 46437	Erzincan	Topatan
17-2	TR 45883	Kars	Ananas	81-2	TR 47845	Adıyaman	Altınbaş
17-6	TR 45883	Kars	Yuva-Hasan	81-5	TR 47845	Adıyaman	Ananas
17-8	TR 45883	Kars	Altınbaş	81-7	TR 47845	Adıyaman	Ananas
18-9	TR 46438	Turkey	Intermediate	82-1	TR 47867	Urfa	Altınbaş
19-2	TR 46489	Sivas	Altınbaş	82-4	TR 47867	Urfa	Altınbaş
19-5	TR 46489	Sivas	Altınbaş	82-5	TR 47867	Urfa	Altınbaş
19-9	TR 46489	Sivas	Yuva-Hasan	83-10	TR 49591	İzmir	Topatan
20-5	TR 47776	Gaziantep	Ananas	83-3	TR 49591	İzmir	Altınbaş
20-9	TR 47776	Gaziantep	Casaba	84-3	TR 35299	Mardin	Yuva-Hasan
21-1	TR 47783	Gaziantep	Altınbaş	84-9	TR 35299	Mardin	Casaba
21-4	TR 47783	Gaziantep	Altınbaş	85-3	TR 37394	Çorum	Ananas
23-3	TR 47804	Adıyaman	Ananas	85-6	TR 37394	Çorum	Yuva-Hasan
24-8	TR 47805	Adıyaman	Ananas	86-7	TR 40379	Diyarbakır	Ananas
24-9	TR 47805	Adıyaman	Altınbaş	87-10	TR 43024	Çanakkale	Yuva-Hasan
25-3	TR 47812	Adıyaman	Altınbaş	87-7	TR 43024	Çanakkale	Yuva-Hasan
25-5	TR 47812	Adıyaman	Altınbaş	88-1	TR 43041	Çanakkale	Topatan
26-2	TR 47813	Adıyaman	Intermediate	88-2	TR 43041	Çanakkale	Yuva-Hasan
26-7	TR 47813	Adıyaman	Altınbaş	88-4	TR 43041	Çanakkale	Yuva-Hasan
27-9	TR 47833	Adıyaman	Yuva-Hasan	89-10	TR 43135	Çanakkale	Altınbaş
28-4	TR 47874	Urfa	Topatan	89-2	TR 43135	Çanakkale	Yuva-Hasan
28-6	TR 47874	Urfa	Ananas	89-9	TR 43135	Çanakkale	Yuva-Hasan
28-8	TR 47874	Urfa	Topatan	91-5	TR 43265	Tekirdağ	Altınbaş
29-2	TR 48527	Amasya	Altınbaş	92-9	TR 43746	Balıkesir	Altınbaş
29-5	TR 48527	Amasya	Altınbaş	93-2	TR 43835	Balıkesir	Altınbaş
30-5	TR 48566	Tokat	Yuva-Hasan	93-3	TR 43835	Balıkesir	Yuva-Hasan
33-5	TR 48671	Adıyaman	Altınbaş	94-2	TR 46503	Tokat	Intermediate
33-6	TR 48671	Adıyaman	Yuva-Hasan	94-4	TR 46503	Tokat	Altınbaş
33-8	TR 48671	Adıyaman	Yuva-Hasan	94-5	TR 46503	Tokat	Ananas
34-4	TR 26195	Konya	Altınbaş	95-2	TR 47811	Adıyaman	Altınbaş
36-1	TR 31534	Amasya	Yuva-Hasan	95-4	TR 47811	Adıyaman	Yuva-Hasan
37-1	TR 33380	Tekirdağ	Topatan	96-3	TR 47822	Adıyaman	Altınbaş
38-1	TR 40280	Gaziantep	Ananas	96-5	TR 47822	Adıyaman	Ananas
39-1	TR 40284	Gaziantep	Altınbaş	96-6	TR 47822	Adıyaman	Yuva-Hasan
39-6	TR 40284	Gaziantep	Ananas	97-1	TR 48541	Tokat	Intermediate
39-8	TR 40284	Gaziantep	Yuva-Hasan	97-3	TR 48541	Tokat	Intermediate
41-3	TR 40514	Bingöl	Yuva-Hasan	98-3	TR 48611	Gaziantep	Ananas
42-2	TR 40530	Elazığ	Altınbaş	99-7	TR 48650	Urfa	Mollaköy
42-8	TR 40530	Elazığ	Altınbaş	100-3	TR 49583	İzmir	Altınbaş

continued next page

Supplementary Table 1. Continued.

Genotype no.	Accession no.	Origin	Morphotype	Genotype no.	Accession no.	Origin	Morphotype
43-4	TR 40559	Malatya	Ananas	101-1	TR 2092	Turkey	Altınbaş
43-8	TR 40559	Malatya	Ananas	106-4	TR 57782	İzmir	Altınbaş
44-2	TR 40563	Malatya	Ananas	108-5	None	Turkey	unknown
44-8	TR 40563	Malatya	Ananas	110-3	TR 61573	Aydın	Altınbaş
45-8	TR 43744	Bursa	Altınbaş	112-10	TR 61627	Muğla	Altınbaş
46-1	TR 47797	Adıyaman	Altınbaş	113-1	TR 61659	Muğla	Charentais
46-6	TR 47797	Adıyaman	Altınbaş	113-2	TR 61659	Muğla	Yuva-Hasan
46-9	TR 47797	Adıyaman	Altınbaş	114-4	TR 61714	Muğla	Charentais
47-2	TR 47825	Adıyaman	Yuva-Hasan	115-3	TR 61812	Denizli	Topatan
47-5	TR 47825	Adıyaman	Casaba	117-2	TR 62023	İzmir	Altınbaş
47-7	TR 47825	Adıyaman	Yuva-Hasan	118-10	TR 62060	İzmir	Ananas
47-8	TR 47825	Adıyaman	Altınbaş	120-3	TR 62474	Çanakkale	Charentais
47-9	TR 47825	Adıyaman	Yuva-Hasan	121-3	TR 66755	Sakarya	Topatan
48-1	TR 47846	Adıyaman	Altınbaş	122-2	TR 68913	Konya	Yuva-Hasan
48-4	TR 47846	Adıyaman	Ananas	122-3	TR 68913	Konya	Yuva-Hasan
50-6	TR 47884	Urfa	Altınbaş	123-5	TR 68934	Manisa	Casaba
50-7	TR 47884	Urfa	Altınbaş	124-10	TR 68940	Kütahya	Yuva-Hasan
51-2	TR 47885	Urfa	Ananas	124-9	TR 68940	Kütahya	Altınbaş
51-6	TR 47885	Urfa	Ananas	125-1	TR 69022	Eskişehir	Altınbaş
53-2	TR 50719	Kars	Ananas	126-1	TR 69425	Ankara	Winter
53-5	TR 50719	Kars	Ananas	126-4	TR 69425	Ankara	Altınbaş
54-7	TR 50728	Kars	Topatan	126-8	TR 69425	Ankara	Altınbaş
55-4	TR 50747	Erzurum	Ananas	129-9	TR 69689	Balıkesir	Yuva-Hasan
55-6	TR 50747	Erzurum	Ananas	130-1	TR 69895	Balıkesir	Yuva-Hasan
55-8	TR 50747	Erzurum	Ananas	131-5	TR 71500	Tunceli	Yuva-Hasan
56-3	TR 51531	Kastamonu	Ananas	132-4	TR 71540	Kütahya	Altınbaş
56-5	TR 51531	Kastamonu	Mollaköy	132-5	TR 71540	Kütahya	Casaba
56-9	TR 51531	Kastamonu	Ananas	135-1	TR 71571	Kütahya	Yuva-Hasan
57-5	TR 51550	Kastamonu	Mollaköy	135-3	TR 71571	Kütahya	Casaba
58-3	TR 51561	Kastamonu	Charentais	136-1	TR 71616	Bilecik	Ananas
60-2	TR 51676	Tokat	Yuva-Hasan	137-2	TR 64142	Kütahya	Altınbaş
60-7	TR 51676	Tokat	Yuva-Hasan	137-5	TR 64142	Kütahya	Yuva-Hasan
61-1	TR 51763	Sivas	Ananas	138-2	TR 64154	Bilecik	Casaba
61-2	TR 51763	Sivas	Altınbaş	140-10	TR 66004	Kütahya	Altınbaş
62-2	TR 49882	Ankara	Yuva-Hasan	140-8	TR 66004	Kütahya	Ananas
64-2	TR 38484	Çanakkale	Yuva-Hasan	141-10	TR 66008	Kütahya	Altınbaş
64-4	TR 38484	Çanakkale	Yuva-Hasan	142-1	TR 66005	Kütahya	Casaba
64-7	TR 38484	Çanakkale	Yuva-Hasan	142-3	TR 66005	Kütahya	Casaba
65-1	TR 39655	Kars	Altınbaş	142-8	TR 66005	Kütahya	Altınbaş
65-3	TR 39655	Kars	Altınbaş	145-4	TR 66002	Bursa	Charentais
65-7	TR 39655	Kars	Ananas	146-6	TR 61543	Aydın	Altınbaş
66-9	TR 39683	Siirt	Topatan	147-5	TR 63230	Muğla	Casaba
67-2	TR 15776	Ankara	Yuva-Hasan	148-1	TR 66366	Uşak	Winter
67-7	TR 15776	Ankara	Topatan	148-2	TR 66366	Uşak	Charentais
68-5	TR 40345	Urfa	Topatan	150-7	TR 69873	Kırşehir	Altınbaş
68-7	TR 40345	Urfa	Altınbaş	150-9	TR 69873	Kırşehir	Altınbaş
68-10	TR 40345	Urfa	Topatan				
70-1	TR 40380	Diyarbakır	Ananas				
71-10	TR 40382	Diyarbakır	Ananas				

Supplementary Table 2. Cucurbit species used as outgroups in the study.

Genotype no.	Species
773	*Cucurbita maxima*
774	*Cucurbita moschata*
776	*Cucumis melo* var. *flexuosus*
778	*Luffa cylindrica*
780	*Luffa sicercia*
783	*Cucurbita moschata*
784	*Momordica charantis*
785	*Cucurbita pepo*
786	*Cucurbita pepo turbaniformis*
787	*Cucurbita maxima*

Supplementary Table 3. Primer sequences for the four simple sequence repeat (SSR) markers developed and used to measure molecular diversity of melon in the study.

Marker	Forward primer	Reverse primer
MU118	TGTGTGCTGTACTCCTGAAA	CGGTTCTTTCTTCTCTTCCT
FR14G19	TCTTTGTCTACCACCAAACC	GTTTGAGAGGAGGAAGAGGT
SSH6I23	CCGCTTCTTCTTCTTCTTCT	CTAGGACCGGAATCGTAATG
PH8C1	CGTGAATTTCCTCGTTTTTCA	TTGGCTCTGGAAATCAGTTG