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RESEARCH ARTICLE

Evaluation of genetic diversity in Turkish melons (*Cucumis melo* L.) based on phenotypic characters and RAPD markers

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Abstract The genetic relationships among 56 melon (*Cucumis melo* L.) genotypes collected from various parts of Turkey were determined by comparing their phenotypic and molecular traits with those of 23 local and foreign melon genotypes to investigate the taxonomic relationships and genetic variation of Turkish melon germplasm. Sixty-one phenotypic characters and 109 polymorphic RAPD markers obtained from 33 primers were used to define the genetic similarity among the melon genotypes by dendrograms or two and three dimensional scaling. There were high correlations ($r \geq 0.97$) among the four resulting matrices used in molecular characterization. The correlations between phenotypic (Euclidean) and molecular Euclidean, Jaccard, Simple matching, and Nei analyses were $r = 0.41$, $r = -0.40$, $r = -0.43$ and $r = -0.40$, respectively. Related genotypes or genotypes collected from similar regions were partitioned to similar clusters. Both analyses (phenotypic and molecular) indicated that non-sweet melon types were dissimilar from sweet types and diversity of Turkish melon genotypes was higher than that of sweet foreign cultivars examined, but similar to that of the reference accessions employed. It was also observed that sweet Turkish melon genotypes belonging to groups inodorus and group cantalupensis were highly variable and could have intermated or have crossed with other non-sweet types.

Keywords *Cucumis melo* L. · Genetic variation · Marker · Melon · Molecular · Phenotypic

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Introduction

Melon (*Cucumis melo* L.; $2n = 2x = 24$) is an important vegetable both worldwide and in Turkey with a 26.7 million tons and 1.7 million tons of production on 1.2 millions and 115,000 ha area, respectively (Anonymous 2003). Moreover, Turkey is located in the secondary genetic diversity center, from Minor Asia to Japan (Pitrat et al. 1999; Jeffrey 2001). A large diversity has been observed among melon genotypes and different researchers have classified them into various groupings (Pitrat et al. 2000; Jeffrey 2001). Robinson and Decker-Walters (1997) classified melons into two major groups as *Cucumis melo* L. subsp. *agrestis* (Naud.) Pangola and *Cucumis melo* subsp. *melo* and the latter was then divided into six informal subgroups,

cantalupensis (*Cucumis melo* L. subsp. *melo* var. *cantalupensis* Naudin), inodorus (*Cucumis melo* L. subsp. *melo* var. *inodorus* H. Jacq.), flexuosus (*Cucumis melo* L. subsp. *melo* var. *flexuosus* (L.) Naudin), conomon (*Cucumis melo* L. subsp. *melo* var. *conomon* (Thunb.) Makino), dudaim-chito (*Cucumis melo* L. *dudaim* (L.) Naudin-(*Cucumis melo* L. subsp. *melo* var. *chito* (C. Morren) Naudin)) and momordica (*Cucumis melo* L. subsp. *melo* var. *momordica* (Roxb.) Duthie et J.B. Fuller).

It has been observed that local melon genotypes in Turkey are rich in diversity and group cantalupensis type melons spread to Europe from the Eastern part of Turkey (Zhukovsky 1951; Günay 1993). Thus, Turkish local melon genotypes have been collected for use in breeding programs where reasonable collection of germplasm exist at Aegean Agricultural Research Institute-Izmir, Turkey and Cukurova University Faculty of Agriculture-Adana, Turkey (Küçük et al. 2002).

Germplasm managements and breeders have attempted to evaluate these collections to discard identical accessions. A comparison of the plant phenotype is the simplest approach for the detection of mislabeled genotypes and the assessment of genetic diversity. However, phenotypic evaluation is influenced by environment and might not distinguish between closely related genotypes. Molecular DNA marker analyses, which are not affected by environment have been suggested for the determination of genetic similarity among genotypes (Gilbert et al. 1999).

Although there has been high phenotypic variation among modern commercial melon cultivars, the genotypic variation among them found to be lower than expected (Stepansky et al. 1999; Silberstein et al. 1999). Therefore, it is wise to search for greater variation among local melon genotypes or wild relatives.

Morphological markers have been known for very long time and these visually observed markers are small in number and might have epistatic effects (Meglic and Staub 1996). Markers aided by the polymorphisms in proteins and DNA structures have become popular in order to compensate for the disadvantages of morphological markers. There are also a few isozyme markers that may also be affected by environment and post-translational modification, and the use of them is very restricted (Meglic and Staub 1996; Akashi et al. 2002). However, DNA markers such as RFLP, RAPD, AFLP, and microsatellites have been beneficial by being large in number and not affected by the environment, especially in fingerprinting, marker assisted selection and genome mapping (Waugh and Powel 1992; Rafalski and Tingey 1993; Lee 1995; Winter and Kahl 1995; Park et al. 2000; Yıldırım and Kandemir 2001).

Genetic distance values among genotypes are measured by fingerprinting with molecular markers and this helps the evaluation and classification of genotypes in germplasm management and aids the protection of breeders' rights (Badenes and Parfitt 1998; Cansian and Echeverrigaray 2000; Li and Quiros 2000). Molecular DNA marker methods have been increasingly employed in melon genetic studies (Shattuck-Eidens et al. 1990; Neuhausen 1992; Baudracco-Arnas and Pitrat 1996; Katzir et al. 1996; Staub et al. 1997, 2000, 2004; Wang et al. 1997; Garcia et al. 1998; Silberstein et al. 1999, 2003; Stepansky et al. 1999; Garcia-Mas et al. 2000; Danin-Poleg et al. 2001; Mliki et al. 2001; Decker-Walters et al. 2002; Lopez-Sese et al. 2002; Monforte et al. 2003; Zhuang et al. 2004) as well many other plant species (Al-Zahim et al. 1997; Horejsi and Staub 1999; Cansian and Echeverrigaray 2000; Brown-Guedira et al. 2000; Mc Gregor et al. 2000). So far, melon genotypes from Spain, Greece, Africa, East and South Asia have been studied and compared with sweet and non-sweet reference accessions by using molecular or phenotypic markers. In most of the studies mentioned above, RAPD markers proved generally to be effective in the determination of genetic similarity among melon genotypes and were in agreement with the other molecular DNA markers (Garcia et al. 1998; Silberstein et al. 1999; Garcia-Mas et al. 2000; Mliki et al. 2001; Staub et al. 2004).

In the present study, we employed molecular RAPD and phenotypic character data; (1) to define genetic similarity among melon genotypes collected in Turkey; (2) to compare them with foreign sweet and non-sweet melon accessions; and (3) to determine the concordance among molecular and phenotypic data.

Materials and methods

Plant material

The majority of plant material employed in the study was chosen from the melon germplasm of the Cukurova University Department of Horticulture which contains about 400 melon accessions collected mostly from different regions of Turkey. Initial selection for the study was made based on their fruit characteristics, regional origins and other germplasm data (Table 1, Figs. 1 and 2).

Phenotypic evaluation

Seeds were sown in pots in a greenhouse on April 19th in 2002 and then six plants of each genotype transplanted into plastic high tunnels on May 25th at the Experimental Area of the Horticulture Department of Yuzuncu Yil University, Van, Turkey. The plastic of the tunnels was removed at the beginning of the July. Plants were furrow irrigated and fertilized with 100 kg N and 50 kg P_2O_5 ha^{-1}. Phenotypic descriptions of genotypes were determined at three stages: cotyledon, flowering and fruit maturation. At harvesting, mature fruits were described and photographed. In all 67 phenotypic traits were scored, but six traits (cotyledon length, ovary width, ovary length, fruit diameter, fruit length/diameter, groove depth) were discarded and not considered for the phenotypic evaluation due to very high correlations with some other traits (see at http://www.suatsensoy.net/). Quantitative traits were converted into 3–5 discrete classes (as in Stepansky et al. 1999) and traits used in the phenotypic evaluation are given in a website (http://www.suatsensoy.net). Modified descriptions of the UPOV (The International Union for the Protection of New Varieties of Plants) criteria were followed in the present study. At least three mature fruits from each genotype were harvested, measured, and analyzed. The length measurements were performed by a ruler or a caliper compass; total soluble solids (TSS) was analyzed by a hand refractometer (Atago N1); pH was analyzed by a pH-meter (Hach 50050).

DNA extraction

About 20 seeds of each genotype were sown in a greenhouse at the University of Yuzuncu Yil, Van for molecular work. Genomic DNA was extracted from young leaf tissues (from at least 15 plants) sampled at the two-to-three leaf stages employing the CTAB procedure (Doyle and Doyle 1987). DNA was quantified by Biotech UV 1101 photometer.

RAPD amplification

Thirty-three 10-mer primers either from Operon Technologies or the University of British Columbia were chosen according to previous melon diversity analyses (Silberstein et al. 1999; Stepansky et al. 1999; Garcia-Mas et al. 2000; Staub et al. 2000; Mliki et al. 2001; Lopez-Sese et al. 2002). The optimized reaction contained 30 ng DNA, 0.2 μM primer, 100 μM dNTPs, 1 U Taq DNA Polymerase (Fermentas), 100 mM Tris–HCl, 1.5 mM $MgCl_2$, and 50 mM KCl, pH 8.8, in a 15-μl final volume. DNA reactions were performed in a Model 212-1CE thermal cycler (Lab-Line Instruments Inc.). After 5 min of heating at 94°C, amplifications were performed under the following regime: 40 cycles of 60 s at 94°C, 63 s 36°C, 59 s ramps, 120 s 72°C, a final extension reaction of 10 min at 72°C. Reactions were replicated at least twice to control reproducibility of patterns. After amplification, PCR products were analyzed in 1.5% agarose gels in 1× TAE at 90 V using a Model 192 horizontal gel electrophoresis system (BIO-RAD) for 3 h and stained with ethidium bromide and photographed by the gel documentation analysis system (Syngene, UK).

Data analysis

Fifty-six Turkish melon and 23 local and foreign melon genotypes were investigated for their taxonomic relationships; their phenotypic and molecular data are presented in tables available on a website (http://www.suatsensoy.net/). Sixty-one of scored phenotypic traits and polymorphisms detected at 109 loci by using 33 RAPD

Table 1 Origins, donors, and names of melon accessions used for examination of genetic relationships

Genotype	Origin	Original donor	Donor ID	Seed donor
CU-1	Van	YYU[a]	A01	CU
CU-2	Banaz (Usak)	YYU	A02	CU
CU-4		CU[b]	Ananas selection	CU
CU-15	Midyat (Mardin)	YYU	A22	CU
CU-20	Yalova	YYU	A32	CU
CU-23	Yalova	YYU	A35	CU
CU-33	Bilecik	YYU	B13	CU
CU-37		Ankara Univ.		CU
CU-38		Ankara Univ.		CU
CU-45	Turkmenistan	Turkmenistan	Turkmen 2	CU
CU-56[a]	Kirklareli	YYU	B42	CU
CU-57	Elazig	YYU	B43	CU
CU-63	Mardin	YYU	C05	CU
CU-65	Erzincan	YYU	C07	CU
CU-69	Adiyaman	YYU	C12	CU
CU-73	Yalova	YYU	C16	CU
CU-78	Van	YYU	D01	CU
CU-101	Turkmenistan	Turkmenistan		CU
CU-104		Agromar	Kis kavunu	CU
CU-106		Agromar	Hasanbey	CU
CU-116		Adiyaman	Saf turuncu	CU
CU-120	Polatli (Ankara)	YYU	A14	CU
CU-129[a]	Azerbaijan	Azerbaijan	Nahcivan 3	CU
CU-131	Ankara	Kazan	Yuva	CU
CU-135		EARI[c]	Cinikiz	CU
CU-139		EARI	Cesme 1/8	CU
CU-151	Van	Suat Sensoy	2000–3 (Mezmeze)	CU
CU-175	Viransehir (Sanliurfa)	CU	Salengo 5	CU
CU-192	Viransehir (Sanliurfa)	CU	Sekerpare	CU
CU-196	Midyat (Mardin)	CU		CU
CU-198	Midyat (Mardin)	CU		CU
CU-203	Silvan (Diyarbakir)	CU	Haci Haso	CU
CU-206	Batman	CU	Azizo 2	CU
CU-208	Diyarbakir	CU		CU
CU-229	Balikesir	CU	Hirsizcalmaz	CU
CU-234[a]	Susurluk (Balikesir)	CU	Acur Kavunu	CU
CU-236	Gonen (Balikesir)	CU	Gonen Beyazi	CU
CU-237	Balıkesir	CU	Gonen Beyazi	CU
CU-238	Gonen (Balikesir)	CU	Gonen Sarisi	CU
CU-240	Biga-Erdek (Canakkale)	CU		CU
CU-252	Kayadibi (Canakkale)	CU	Kirkagaç	CU
CU-258	Van	YYU	65ER05	YYU
CU-269	Van	YYU	65ERC02-Semame	YYU
CU-272	Van	YYU	65ERC05	YYU
CU-280	Van	YYU	65ERC13	YYU
CU-286	Van	YYU	65ERC19	YYU
CU-305	Adana	CU	Yabani kavun	CU
CU-307	Tekirdag	CU	Topatan	CU
CU-308	Tekirdag	CU	Suluklu	CU
CU-309	Tekirdag	CU	Ziraat-Beyaz	CU
CU-310	Kirklareli	CU	Bagributun	CU
CU-311	Kirklareli	CU	Sari Kislik	CU
CU-315	Edirne	CU	Cobanaldatan	CU
CU-323	Ankara	CU	Karakavun	CU
CU-326	Ankara	CU		CU
CU-327	Ankara	CU		CU

Table 1 continued

Genotype	Origin	Original donor	Donor ID	Seed donor
T1	Turkey	Pinaper Seed	Kirkagaç 637 (Altınbas) (group inodorus)	Pinaper Seed (Reference accession)
T5	Turkey	Istanbul Toh.	Ananas (group cantalupensis)	Istanbul Toh. (Reference accession)
T6	Turkey	Bursa Toh.	Acur (group flexuosus)	Bursa Toh. (Reference accession)
T8	Turkey	Anadolu Toh.	Galia (group cantalupensis)	Anadolu Toh. (Reference accession)
Y1	USA	Pitrat, France	Topmark (group cantalupensis)	Pitrat, France (Reference accession)
Y2	USA	Pitrat, France	Hale's Best (group cantalupensis)	Pitrat, France (Reference accession)
Y3	France	Pitrat, France	Vedrantais TG-94 (group cantalupensis)	Pitrat, France (Reference accession)
Y4	Israel	Pitrat, France	Ogen 2 (group cantalupensis)	Pitrat, France (Reference accession)
Y5	USA	Pitrat, France	Honeydew Green Flesh (group inodorus)	Pitrat, France (Reference accession)
Y6	Spain	Pitrat, France	Piel de Sapo 134 (group inodorus)	Pitrat, France (Reference accession)
Y7	Spain	Pitrat, France	Rochet 2 (group inodorus)	Pitrat, France (Reference accession)
Y8	Spain	Pitrat, France	Amarillo 140 (group inodorus)	Pitrat, France (Reference accession)
Y9	Japan	Pitrat, France	Shiro Uri Okoyama (group conomon)	Pitrat, France (Reference accession)
Y10	Cuba	Pitrat, France	Cuba 3 (group chito)	Pitrat, France (Reference accession)
Y11	Turkey	Pitrat, France	PI 177362 (group dudaim)	Pitrat, France (Reference accession)
Y12	India	Pitrat, France	PI 414723 TG95 (group momordica)	Pitrat, France (Reference accession)
Y13	Sudan	Pitrat, France	Humaid 95–1 (group agrestis)	Pitrat, France (Reference accession)
Y14	Sudan	Pitrat, France	HSD 192 (group flexuosus)	Pitrat, France (Reference accession)
Y15	France	Pitrat, France	Isabelle (group cantalupensis)	Pitrat, France (Reference accession)
Y17	Leen de Mos	Staub, USA	Ref no: 23849 (group cantalupensis)	Staub, USA (Reference accession)
Y31	Israel	Perl-Treves, Israil	Arava F #165 (group cantalupensis)	Perl-Treves, Israel (Reference accession)
Y62	India	Pitrat, France	PI124112 (group momordica)	Pitrat, France (Reference accession)
Y63	Korea	Pitrat, France	PI161375 (group conomon)	Pitrat, France (Reference accession)

[a] YYU: Yuzuncu Yil University, [b] CU: Cukurova University, [c] EARI: Aegean Agricultural Research Institute

primers were used in the genetic evaluation of 79 melon genotypes (Table 2).

The phenotypic genetic diversity among melon genotypes was determined by using Euclidean distance matrix. A presence(1)/absence(0) binary data matrix obtained from scoring polymorphic RAPD bands was used to calculate Euclidean, Jaccard (Jaccard 1908), Simple matching (Sokal and Sneath 1963), and Nei (Nei 1972) dis/similarity coefficients to estimate the molecular genetic diversity among melon genotypes. The unweighted pair-group method using arithmetic average (UPGMA) cluster analysis, the resulting dendrograms and multidimensional scalings (MDS) were performed on the genetic distance matrices using the computer program NTSYpc version 2.02 k (Rohlf 1997). MDS produces a statistic called *Stress* indicating a goodness of fit of the distances in the configuration space to the monotone transformation function of the original distances. *Stress* values vary between 0 and 1 (0.40: poor; 0.20: fair; 0.10: good; 0.00: perfect goodness of fit) (Kruska 1964; Rohlf 1997).

In all dendrograms and MDS scalings (Figs. 2 and 3), foreign non-sweet genotypes (Y9, Y10, Y11, Y12, Y13, Y14, Y62 and Y63), foreign group cantalupensis genotypes (Y1, Y2, Y3, Y4, Y15, Y17, and Y31), foreign group inodorus genotypes (Y5, Y6, Y7, and Y8) and distinct genotypes of Turkey are depicted in different colors. CU4, CU15, CU37, CU38, CU237, CU258, CU272, T5, and T8 genotypes were considered as group cantalupensis of Turkish germplasm group inodorus genotypes of Turkish germplasm were classified in even more detail, as *Kirkagac* genotypes, which have generally light green skin color with dark green spots at first, yellow at maturity (CU2, CU135, CU139, CU252 and T1), *Yuva-Hasanbey* genotypes, which have dark green or greenish grey skin color (CU20, CU73, CU106, and

Fig. 1 Mature fruit from the examined melon (*Cucumis melo* L.) genotypes

CU131), *Kislik Sari* genotypes, which have bright yellow skin color (CU104 and CU311) and *Hirsizcalmaz* genotypes, which have cream to greenish yellow skin color (CU229, CU315 and CU323).

Genetic variation among genotypes observed in the dendrograms and MDS charts was determined in molecular data by genetic variation measurements such as Shannon's information index, Nei's gene diversity and percentage of polymorphic loci. Genotypes were divided into main three groups as Turkish genotypes (total 58 genotypes, # from 1 to 61 except genotypes from Turkmenistan (CU45 and

Fig. 2 Map of Turkey

CU101) and Azerbaijan (CU129) (# 10, 18 and 24)), foreign sweet genotypes (genotypes # 61, 62, 63, 64, 65, 66, 67, 68, 75, 76 and 77 besides 10, 18 and 24) and foreign non-sweet genotypes (genotypes # 69, 70, 71, 72, 73, 74, 78 and 79). Foreign sweet ones were also divided into group cantalupensis (genotypes # 61, 62, 63, 64, 75, 76 and 77) and group inodorus (genotypes # 10, 18, 24, 65, 66, 67 and 68).

The computer program POPGENE (Yeh et al. 1997) was used to calculate the statistical measures of genetic variation (i.e., Nei's gene diversity (Nei, 1943), Shannon's information index (Shannon and Weaver 1949) and percentage of polymorphic loci) as measured by RAPD markers for Turkish and foreign melon genotypes.

Results

Genetic dis/similarities among melon genotypes

Correlations among the four different dis/similarity estimators (Euclidean distance (*E*), Jaccard's coefficient (*J*), Simple matching coefficient (*S*), and Nei's genetic distance (*N*)) employed in molecular evaluation were found to be very high ($r \geq 0.97$; E–$J = -0.97$; E–$S = -0.99$; E–$N = -0.97$; J–$S = 0.97$; J–$N = 0.99$; S–$N = 0.97$) (data matrices available on a web site; http://www.suatsensoy.net/). Correlations between Euclidean distance matrix used in phenotypic evaluation and the four different dis/similarity matrices (Euclidean distance, Jaccard's coefficient, Simple matching coefficient, and Nei's genetic distance) used in molecular evaluation were found to be $r = 0.41$, $r = -0.40$, $r = -0.43$ and $r = -0.40$, respectively. Relationships among genotypes were best visualized by comparing their clustering and MDS charts. Therefore, dendrograms, 2D and 3D scalings based on phenotypic, molecular (RAPD), and combined phenotypic and molecular Euclidean distance values were formed (Figs. 3 and 4, and http://www.suatsensoy.net) and evaluated. The *stress* values for all evaluated GD matrices in MDS scalings ranged from 0.11 to 0.14, indicating good fit of the data.

Based on the phenotypic Euclidean distance matrix, the most similar genotypes were Y1 (Topmark) and Y2 (Hale's Best) (4.00E + 14) followed by Y7 (Rochet 2) and CU120 (4.35E + 14) and by Y31 (Arava) and CU15 (4.47E + 14); the most dissimilar ones were T6

Table 2 Polymorphisms detected at 109 loci by using 33 RAPD primers

Primer name	Number and positions of polymorphic bands	Primer name	Number and positions of polymorphic bands	Primer name	Number and positions of polymorphic bands
OP-A04[a]	3 (550, 650, 750 bp)	OP-D20	1 (1200 bp)	OP-AE06	1 (900 bp)
OP-A07	5 (600, 750, 850, 1400, 1800 bp)	OP-E14	2 (500, 600 bp)	OP-AG15	4 (850, 900, 1000, 1400 bp)
OP-A10	2 (1500, 1550 bp)	OP-F04	3 (800, 900, 1400 bp)	OP-AJ18	6 (600, 750, 850, 1200, 1600, 1750 bp)
OP-A18	3 (1400, 1500, 2000 bp)	OP-G17	1 (800 bp)	OP-AK16	6 (550, 600, 650, 700, 1100, 1150 bp)
OP-B06	5 (500, 900, 1100, 1400, 1800 bp)	OP-H05	1 (1900 bp)	OP-AL05	2 (500, 700 bp)
OP-C01	3 (600, 800, 900 bp)	OP-L07	2 (1600, 1700 bp)	OP-AW14	4 (900, 1500, 1550, 1600 bp)
OP-C08	2 (1400, 3000 bp)	OP-R01	5 (350, 400, 1400, 1500, 1600 bp)	OP-AX16	6 (700, 800, 1200, 1250, 1300, 1500 bp)
OP-D02	4 (950, 1000, 1300, 3000 bp)	OP-R02	1 (1500 bp)	BC-231[b]	3 (750, 800, 850 bp)
OP-D07	3 (1100, 1200, 1300 bp)	OP-R10	1 (900 bp)	BC-388	9 (750, 800, 1200, 1300, 1400, 1500, 2400, 2500, 2600 bp)
OP-D08	2 (1500, 1600 bp)	OP-T20	5 (650, 750, 1000, 1100, 1200 bp)	BC-526	2 (950, 1000 bp)
OP-D13	4 (600, 650, 700, 2300 bp)	OP-AD14	1 (400 bp)	BC-551	7 (600, 650, 700, 1000, 1200, 1600, 2200 bp)

[a] OP: Operon, [b] BC: Univ. British Columbia

(Turkish group flexuosus) and Y4 (Ogen 2) (1.35E + 15) followed by Y13 (group agrestis) and T8 (Galia) (1.31E + 15) and by CU258-T6, CU258-Y62 (group conomon), CU280-Y13, T8-Y6, Y4-Y62 and Y13-CU65 genotype pairs (1.30E + 15). Of all evaluated genotypes, the most distinct ones were T6, Y13 and Y62 while the least distinct ones were CU252, CU120 and CU135. The genotypes CU305, CU65 and CU286 were the most distinct Turkish genotypes beside T6.

According to the phenotypic dendrograms, 2D and 3D scalings, Y10 (group chito), Y13 (group agrestis) and CU305 (group agrestis-like genotype of Turkey) were the most distant genotypes. Group flexuosus (Y14 and T6), group conomon (Y9 and Y63), group momordica (Y12 and Y62), group dudaim (Y11), and genotypes CU192, CU196, CU198 and CU 269 had also very distinctive positions. Moreover, genotypes CU101, CU129, CU175, CU203 and CU208 had position different from other genotypes. There was a relatively definite clustering among group inodorus and group cantalupensis genotypes, and there was wide variation among them. On the other hand, some related genotypes (e.g., Y1 and Y2 (US genotypes), Y6, Y7 and Y8 (Spanish genotypes), *Kirkagac* genotypes or some genotypes collected from the same regions of Turkey (e.g., CU236 and CU237 or CU326 and CU327) tended to be grouped.

Based on the molecular Euclidean distance matrix, the most similar genotypes were Y6 (Piel de Sapo 134) and Y7 (Rochet 2) (2.24E + 14) followed by CU236 and CU237 (2.83E + 14) and by CU104-CU310, CU104-CU311 (*Kislik Sari* genotypes) and CU309-310 genotype pairs (3.00E + 14); the most dissimilar ones were Y62 (group conomon) and CU101 (7.68E + 14) followed by and by Y9-CU56, and Y13 (group agrestis)-CU45 genotype couples (7.62E + 14). Of all evaluated genotypes, the most distinct ones were Y13 (group agrestis), Y10 (group chito) and Y63 (group momordica) while the least distinct ones were CU326, CU315 and Y8 (Amarillo 140). The genotypes CU175 and CU116 were the most distinct genotypes in the germplasm.

According to the molecular dendrograms, 2D and 3D scalings, Y9 (group conomon), Y10

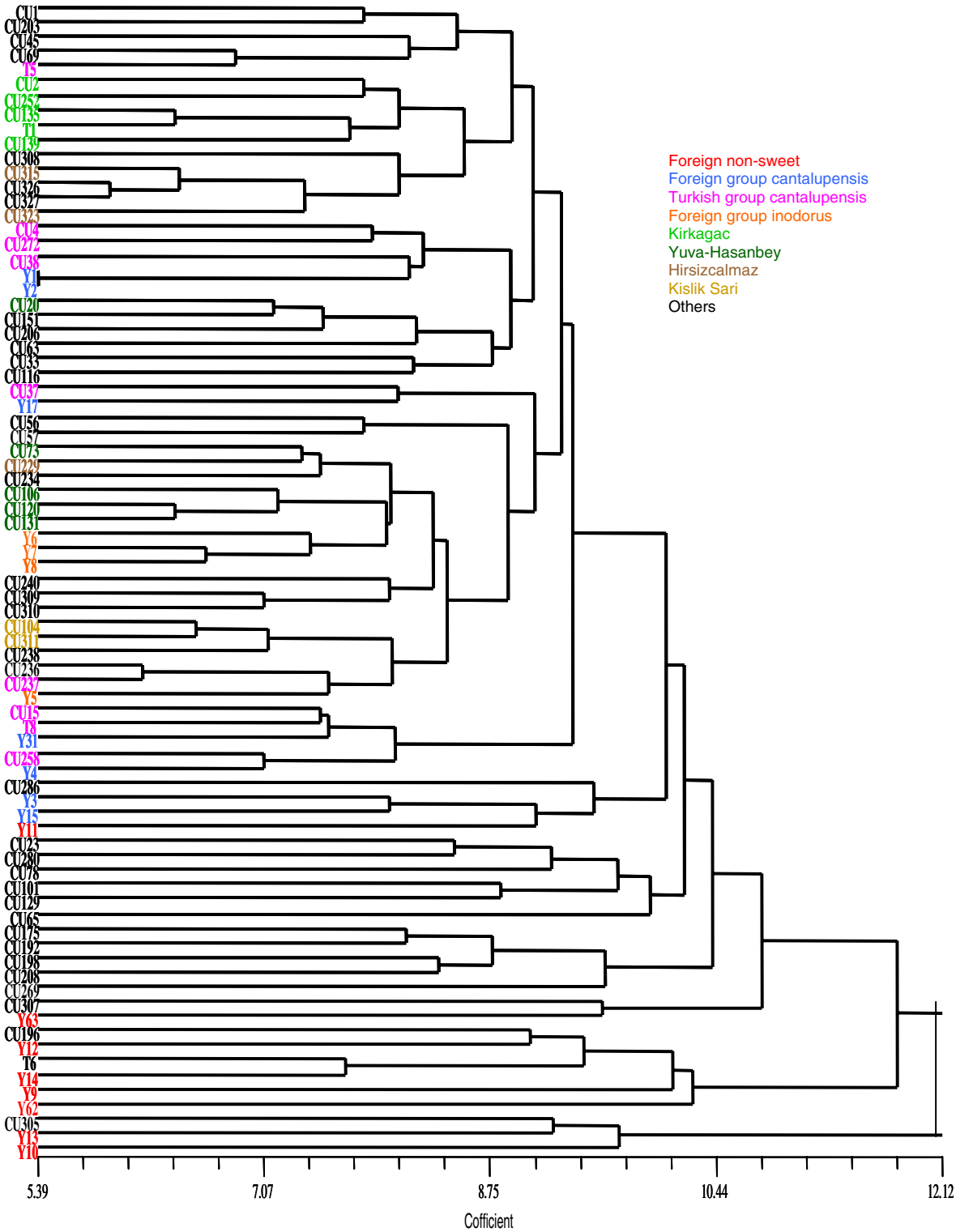

Fig. 3 Associations among Turkish melon genotypes revealed by UPGMA clustering analysis on the basis of the combined phenotypic and molecular Euclidean distance values

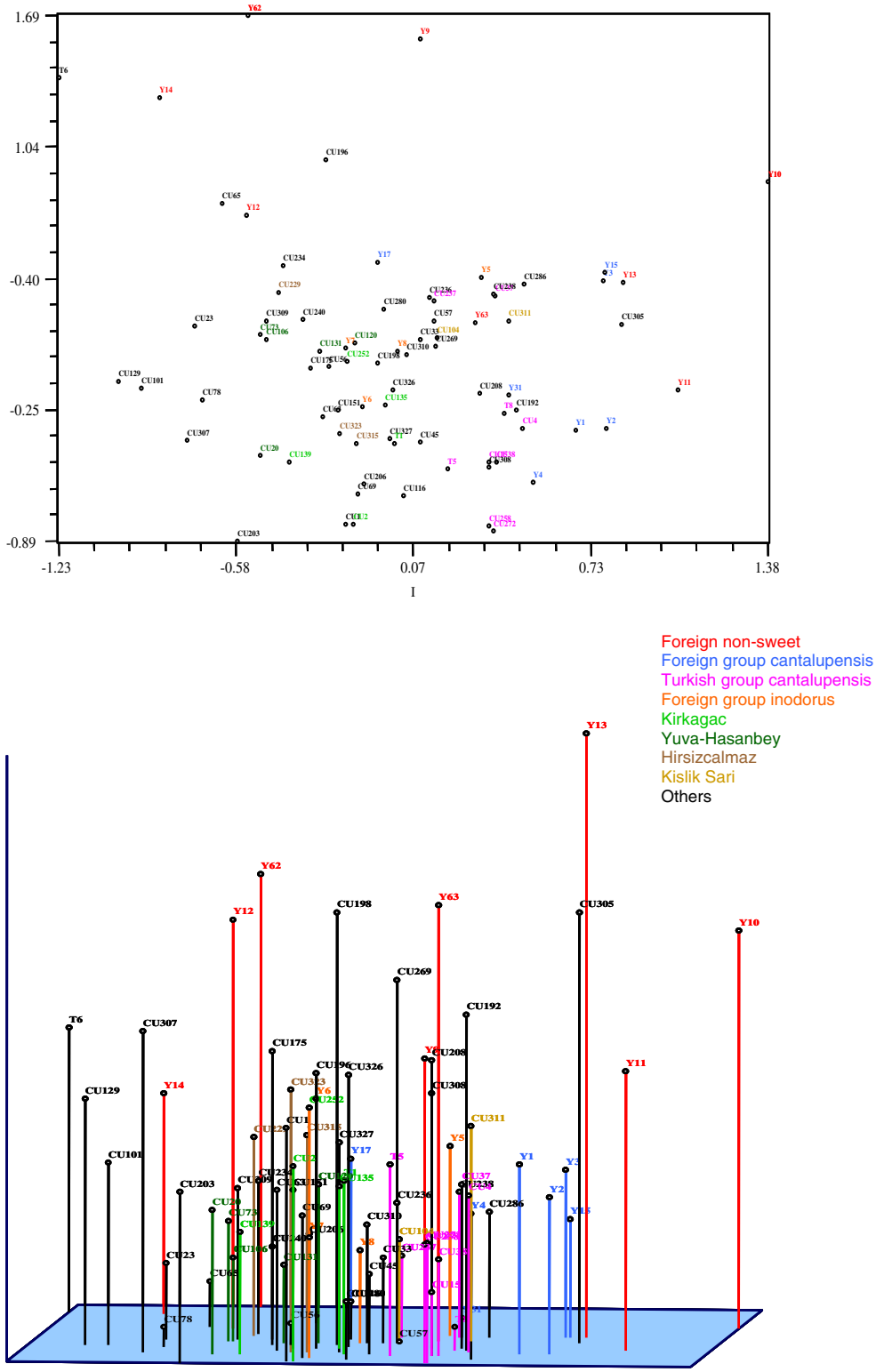

Fig. 4 Associations among Turkish melon genotypes revealed by 2D and 3D scaling analysis on the basis of the combined phenotypic and molecular 3D Euclidean distance values

(group chito), Y13 (group agrestis), Y62 (group momordica), and Y63 (group conomon) were the most distant genotypes. Y11 (group dudaim), Y12 (group momordica), CU101, CU175, CU192, CU198, CU208, and CU305 genotypes had also very distinctive positions. However, especially group flexuosus genotypes (Y14 and T6) were not distinctly separated from the other sweet genotypes. There was also a relatively distinct clustering among group inodorus and group cantalupensis genotypes. Variation among Turkish genotypes was definitely greater than that of foreign group inodorus and group cantalupensis genotypes. Moreover, most related genotypes (e.g., Y5, Y6, Y7, and Y8 (foreign group inodorus genotypes)) or most genotypes collected from the same regions (e.g., CU236 and CU237 or CU326 and CU327) tended to be grouped.

Based on the combined phenotypic-molecular Euclidean distance matrix, the most similar genotypes were Y1 and Y2 (US genotypes) (5.39E + 14) followed by CU326 and CU327 (5.92E + 14) and by CU236 and CU237 (6.16E + 14); the most dissimilar ones were Y13 (group agrestis) and T8 (Galia) (1.49E + 15) followed by Y13 and CU65 and by Y13 and CU280 (1.48E + 15). Of all evaluated genotypes, the most distinct ones were Y13, Y62 (group conomon) and T6 (Turkish group flexuosus) while the least distinct ones were CU135, CU236 and Y7 (Rochet 2). The genotypes CU305, CU198 and CU196 were the most distinct genotypes in the germplasm beside T6.

According to the combined phenotypic-molecular dendrograms, 2D and 3D scalings, Y9 (group conomon), Y10 (group chito), Y12 (group momordica), Y13 (group agrestis), Y14 (group flexuosus), Y62 (group momordica), Y63 (group conomon), T6 (group flexuosus), and CU305 were the most distant genotypes. CU101, CU129, CU175, CU192, CU196, CU198, CU208, CU269, CU307 and Y11 (group dudaim) genotypes had also very distinctive positions. There was a more distinct clustering among group inodorus and group cantalupensis genotypes. Variation among Turkish genotypes was definitely larger than that of foreign group inodorus and group cantalupensis genotypes. Moreover, most related genotypes (e.g., Y1 and Y2 (US genotypes) or Y6, Y7 and Y8 (Spanish genotypes)) or most genotypes collected from the same regions (e.g., CU236 and CU237 or CU326 and CU327) tended to be grouped.

Genetic variation among melon genotypes

The statistical variation measures showed that the genetic diversities among these populations were significantly different (Table 3). The genetic diversity among Turkish melon genotypes was equal to that of all foreign melon genotypes, and especially higher than that of sweet ones. Moreover, Turkish melon genotypes found to be very polymorphic.

Discussion

In the present study, RAPD method was used to assess the genetic relationship among Turkish melon genotypes because of its simplicity and cost efficiency (Mc Gregor et al. 2000; Yıldırım and Kandemir 2001). Moreover, Garcia-Mas et al. (2000) compared the AFLP, RAPD and RFLP techniques in their melon genetic study comparing six genotypes, belonging to groups inodorus, agrestis, conomon and momordica and found that all methods gave similar results and the correlations among them were very high. Especially the correlations of RAPD method with other

Table 3 Statistical measures of genetic variation as measured by RAPD markers for Turkish and foreign melon genotypes

Genotypes	N^a	H^b	I^c	% Polymorphism[d]
Turkish genotypes	58	0.29	0.43	89.9
Foreign genotypes	21	0.31	0.47	94.5
Non-sweet foreign genotypes	7	0.26	0.39	74.3
Sweet foreign genotypes	14	0.25	0.38	72.5
Foreign group cantalupensis	7	0.19	0.28	55.1
Foreign group inodorus	7	0.20	0.30	56.0

[a] N = Number of genotypes in each population

[b] H = Nei's gene diversity

[c] I = Shannon's information index

[d] Percentage of polymorphic loci

methods were above $r = 0.90$. This shows the efficiency of RAPD method in melon genetic studies. The correlations among the four genetic distance estimators used were found to be very high ($r \geq 0.97$) in the molecular evaluation. This is in agreement with the findings of Staub et al. (2000). These researchers studied 46 melon genotypes (33 group cantalupensis, 11 group inodorus, 1 group conomon and 1 group flexuosus) with 135 RAPD markers and 54 SSR markers and found that there were very high correlations ($r \geq 0.98$) among the genetic estimators (Jaccard's coefficient, Simple matching coefficient and Nei's genetic distance).

Correlations between the Euclidean distance matrix used in phenotypic evaluation and the four different dis/similarity matrices used in molecular evaluation were found to be about $r = 0.42$ in the present study. Garcia et al. (1998) studied 32 melon breeding lines belonging to group cantalupensis and group inodorus (most of them were Piel de Sapo or Galia types) with 115 RAPD markers and 24 qualitative agronomic traits and found that the correlation between phenotypic and molecular Jaccard's coefficients was $r = 0.79$. These researchers studied lower numbers of genotypes with presence(1)/absence(0) binary data matrix used both in molecular and agronomic data than the present study. The phenotypic traits of the present study contained quantitative data, which most probably were affected by environment. Moreover, when compared to more objective molecular DNA markers, these quantitative phenotypic traits may co-vary or even be co-inherited (Stepansky et al. 1999). In another study, Lopez-Sese et al. (2002) examined 16 melon genotypes from Spain (15 group inodorus and 1 group flexuosus) with 100 RAPD markers and 12 SSR markers and discovered that the correlation between RAPD and SSR matrices was $r = 0.34$ likely because of an insufficient number of SSR markers. The low number of phenotypic markers compared to RAPD markers in the present study could be another explanation of the lower correlation between the mentioned markers. Staub et al. (1997) reported that 35 should be the minimum number of markers in melon and Staub et al. (2000) reported that 80 should be an adequate number of markers in melon.

The relationships among Turkish melon genotypes were determined in an acceptable manner; however, we encountered some deficiencies in sole application of either phenotypic or molecular evaluation. Phenotype is influenced by environment and molecular bands might be in positions not much related to important agricultural traits. Therefore, combined evaluation of the phenotypic and molecular data was also employed. Molecular evaluation was more favorable than phenotypic evaluation because it had more markers and represented neutral traits of simple inheritance.

Most non-sweet genotypes (groups agrestis (Y13), conomon (Y9 and Y63), chito (Y10), dudaim (Y11) and momordica (Y62)) were separated from sweet genotypes; however, group flexuosus (Y14 and T6) and Y12 (group momordica) was placed in sweet melon genotypes in molecular evaluation of the present study. Although many melon studies using RAPD markers (Silberstein et al. 1999; Stepansky et al. 1999; Garcia-Mas et al. 2000; Staub et al. 2000, 2004; Mliki et al. 2001; Zhuang et al. 2004) separated non-sweet genotypes from sweet genotypes, most reported that some group flexuosus and group momordica genotypes had been clustered with sweet genotypes. Group flexuosus and group momordica genotypes might have been selected among sweet genotypes or vice versa at different domestication centers. For example Turkish group flexuosus genotype (T6) was found to be closely related to CU234 (Acur kavunu = snakemelon melon). Distinction among group inodorus and group cantalupensis (sweet) genotypes was also not very significantly different in the evaluations. This is in line with the findings of other mentioned researchers, indicating some close relationships between group inodorus and group cantalupensis despite the significant agricultural differences between them. Relatively small number of genes might be responsible for the difference among sweet genotypes and between sweet genotypes and some non-sweet genotypes (e.g., group flexuosus).

The statistical measures of genetic variation as measured by RAPD markers revealed the high genetic diversity among Turkish melon genotypes. Calculated genetic diversity indices were in

agreement with the literature (Lopez-Sese et al. 2002; Staub et al. 2004). While Staub et al. (2004) studied the genetic diversity among melon populations from Greece, Japan, Africa and Spain, Lopez-Sese et al. (2002) studied the genetic diversity among melon population only of Spain. The genetic variation estimates for Turkish melon genotypes ($H = 0.29$, $I = 0.43$ and 90% polym.) were higher than those of Spanish genotypes (the highest estimates were $H = 0.17$, $I = 0.25$ and 44% polym.) (Lopez-Sese et al. 2002). The genetic variation estimates for sweet melon genotypes in the present study ($H = 0.25$, $I = 0.37$ and 72% polym.) were similar to the sweet genotype results of Staub et al. (2004) ($H = 0.23$, $I = 0.35$ and 55% polym.). The genetic diversity among Turkish genotypes was only a little less than that of African landraces ($H = 0.34$ and $I = 0.50$). On the other hand, the percentage of polymorphic loci among Turkish melon genotypes (90%) was even higher than that of African landraces (85%).

The relationships of Turkish melon genotypes with other foreign melon cultivars and non-sweet wild genotypes were clearly defined. The Turkish melon germplasm has some unique genotypes. Presence of group dudaim genotypes (such as Y11 and CU269) in Turkey has long been known (Silberstein et al. 1999; Stepansky et al. 1999; Jeffrey 2001). The genotype CU305 collected from the province of Adana was very similar to non-sweet wild genotypes, especially to group agrestis. This reinforced the position of Turkey in the secondary genetic diversity center of melon.

Conclusions

The genetic variation among foreign sweet melon cultivars was found to be narrower than that of Turkish genotypes. The genetic variation among foreign sweet melon cultivars has decreased most probably due to monoculture practices. Nevertheless, melon genotypes similar to foreign sweet melon genotypes were also observed in Turkish germplasm, but the genetic variation among Turkish melon genotypes was very high. For example, *Kirkagac* melon cultivars (group inodorus, e.g., CU252 and T1) had more distinct positions than foreign group inodorus genotypes. One of the reasons of this large variation was the inevitable out-crossing among melon genotypes in Turkey. Intermediate forms might have been formed among group inodorus and group cantalupensis due the old farming practices employed by some local small-scale melon producers for centuries. Several melon genotypes grow together in several regions of Turkey and introgression of genotypes occurs naturally. Presence of group dudaim genotypes in Turkey has long been known. Moreover, some melon genotypes collected from South Eastern Part of Turkey (CU175, CU192, CU196 and CU198) were found to be related with especially group conomon and group momordica. Intra-specific hybrids among melon genotypes are rather common in Turkey. Therefore, it is no surprise to observe such a broad genetic diversity among Turkish melon genotypes. There might be hidden treasures to be revealed among them such as resistance to a/biological factors. Taken as a whole, the results clearly show that Turkish melon germplasm with this broad genetic diversity could play an important role in the preservation of melon genetic diversity and the enhancement of modern melon cultivars.

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