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Morphological and Molecular Characterization of Turkish Landraces of *Cucumis melo* L.

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Abstract

Twenty-four accessions covering different groups of *Cucumis melo* L. from Eastern and South-eastern Anatolian regions of Turkey were characterized by using 43 morphological traits and 207 markers obtained from 31 ISSR and 16 SSR primers. The genetic relatedness was studied by examining the Euclidian/UPGMA dendrogram obtained from the combined phenotypic-molecular data. In the combined morphological-molecular dendrogram, there were two main clusters. Sweet and non-sweet melon groups were separated and the *flexuosus* group accessions were discriminated from the sweet ones, but the *momordica* group accession was clustered with the sweet ones. Unclear South-eastern Anatolian accessions were sub-clustered separately among the sweet ones. Principle component analysis (PCA) of morphological characters was used in detail to discriminate melon accessions. The cumulative proportion of variation reached 44% by first three PCA axes. The first component was mainly based on sex expression, ovary index, ovary shape, flesh thickness, seed cavity length, seed cavity width, soluble solids content, fruit shape, aroma, netting, and taste. The PCA plot based on all measured traits allowed distinction between *flexuosus* group, subsp. *agrestis* and *reticulatus* group. A high variation among groups was observed for the fruits characters. Netting, aroma and abscission of peduncle represent *reticulatus* group; a small fruit size, strong typical aroma and secondary colour distribution characterize *dudaim* group. Monoecy, very long fruit shape and mature fruit rind colour discriminate *flexuous* group; Ovary index, fruit size and flesh width distinguish subsp. *agrestis* group. These findings indicated wide range of variations for investigated characteristics in Turkish gene pool that provides a good source of diversity to use in melon improvement program for better yield and other traits of interest.

Keywords: diversity, ISSR, morphological characters, PCA, SSR

Introduction

Cucurbitaceae family contains several economically important cultivated cucurbit species such as watermelon (*Citrullus lanatus* L.), squash (*Cucurbita pepo* L.), cucumber (*Cucumis sativus* L.) and melon (*Cucumis melo* L.). Allogamous fertilization habit helps gen flow among the genotypes (Robinson and Decker-Walters, 1997). A large diversity has been observed among melon genotypes; therefore, there are several classification for melon; in one of them Pitrat *et al*. (2000) have classified them into various groupings: *C. melo* ssp. *agrestis* (groups of *conomon* Thunb., *makuwa* Makino, *chinensis* Pangalo, *momordica* Roxb., and *acidulous* Naud.) and *C. melo* ssp. *melo* (groups of *cantalupensis* Naud., *reticulatus* Ser., *adana* Pangalo, *chandalak* (Pangalo) Filov, *ameri* Pangalo, *inodorus* Naud., *flexuosus* L., *chate* (Hasselquist) Filov, *tibish* Mohamed, *dudaim* L. and *chito* Morren).

Cantalupensis and *inodorus* market types are the most economically important ones in USA, Europe, Mediterranean and Asian countries (McCreight *et al*., 1993). Fruit shape morphology is one of the most diverse trait, and depends on geographic origin (i.e., adaptation to environmental factors), cultural traditions, culinary attributes, and market characteristics and requirements (Staub *et al*., 2000).

The 85% of Turkish melon genetic resources belongs to *inodorus* group (Kirkagac, Hasanbey, Yuva and Kislik Sari) and the rest belongs to *cantalupensis* group (Abak, 2001). Turkey is the secondary genetic diversity centre, from Minor Asia to Japan (Pitrat *et al*., 1999; Jeffrey, 2001). Although there are disputed theories about the origins and domestication of melons, it is agreed that initial domestication probably occurred in the Middle East (Robinson and Decker-Walters, 1997; Jeffrey, 2001; Luan *et al*., 2008). Moreover, Pitrat *et al*. (2000) reported that Turkey and Central Asia could be origins of *inodorus* group. Turkey is the second biggest producer for melon production after China with 1.7 million tons of production and 102, 000 ha harvested area (FAO, 2014). Turkey melon production is realized in mainly five regions (20% in Aegean region, 19% in Central Anatolia, 17% in Marmara region, 16% in Mediterranean region, and 19% in Eastern and South-eastern Anatolian regions). In Eastern and South-eastern Anatolian regions, *inodorus* and *reticulatus* groups are mainly cultivated and *flexuosus* group

is also produced in some areas. Still some farmers grow local melon landraces; therefore, there is large genetic diversity in melon (Abak, 2001).

It has been reported by many earlier researchers that local melon genotypes in Turkey are rich in diversity and *cantalupensis* group type melons spread to Europe from the Eastern part of Turkey (Zhukovsky, 1951; Sensoy *et al*., 2007). Thus, Turkish local melon genotypes have been collected for use in breeding programs, and reasonable collection of germplasm exist at Aegean Agricultural Research Institute-Izmir, Turkey and Faculty of Agriculture, University of Cukurova, Adana, Turkey (Küçük *et al*., 2002; Sensoy *et al*., 2007). Yildiz *et al*. (2011) also stated that there is a large genetic diversity among melon genotypes in Turkey and most of the melon genotypes in Eastern and South-eastern Anatolian region have distinct groupings in the obtained dendograms.

Escribano and Lazaro (2009) stated that morphological analysis is an important requirement for the initial evaluation of genetic resources and the accurate identification of local landraces. Initially, morphological assessment led to the taxonomic and horticultural classification of Turkish melon germplasm (Sari and Solmaz, 2007; Sensoy *et al*., 2007). Sensoy *et al*. (2007) used random amplified polymorphic DNA markers (RAPD) to differentiate Turkish melon germplasm. Yildiz *et al*. (2011) used ISSR and SRAP to assess genetic diversity of Turkish melon germplasm. More recently, microsatellite analyses (Kacar *et al*., 2012) have also been used to estimate genetic diversity among Turkish melon accession.

SSR are PCR-based markers with a relatively low cost, with the advantage of being stable across laboratory. They are abundant in the genome and specific to species and genera, or to a lesser extent, can be preserved among plant family (Katzir *et al*., 1996; Gong *et al*., 2012). Genomic resources present in economically important species might be exploited in marginal crops with lower molecular resources. For example, successful SSRs transferability between species within a genus has been reported in *Prunus* (Wuensch and Hormaza, 2002), *Cucumis* (Danin-Poleg *et al*., 2001), *Apiacea* (Cavagnaro *et al*., 2011) and *Cucurbita* (Gong *et al*., 2012). The availability of SSR markers among the related species has been evaluated in many laboratories.

The preset study aimed to investigate the morphological and molecular diversity and to employ morphological and molecular (31 ISSR primers and 16 SSR primers derived from *Cucurbita* spp) traits together in the characterization of melon accessions mainly from Eastern and South-eastern Anatolian regions of Turkey.

Materials and methods

Plant Materials

Eastern and South-eastern Anatolian melon landraces and reference cultivars were chosen based on the study of Yildiz *et al*. (2011) from collections of the University of Yuzuncu Yil and the University of Cukurova. Total 19 local landraces (6 from Eastern Anatolia, 10 from South-eastern Anatolia, 2 from Mediterranean, 1 from Marmara regions) and 5 foreign reference cultivars were used as plant material in the study. Among them, there were 2 accessions in *inodorus* group, 1 accession in *cantalupensis* group, 4 accessions in *reticulatus* group, 3 accessions in *conomon* group, 4 accessions in *flexuosus* group, 2 accessions in ssp. *agrestis*, 1 accession in *dudaim* group, 1 accession in *momordica* group, and 6 *unknown* accessions (Tab. 1).

Tab. 1. List of melon accessions used for examination of genetic relationships

Accession No/Cultivar	Hort. Group	Province/Country	Original donor
Turkey landraces			
CU-152	*cantalupensis*	Van - East Anatolia	YYU*
CU-161	*reticulatus*	Van - East Anatolia	YYU
CU-171	*unclear*	Sanliurfa - South East Anatolia	CU**
CU-175	*unclear*	Sanliurfa - South East Anatolia	CU
CU-177	*conomon*	Mardin/Midyat - South East Anatolia	CU
CU-179	*unclear*	Mardin/Sashane - South East Anatolia	CU
CU-181	*inodorus*	Mardin/Harbuni - South East Anatolia	CU
CU-196	*conomon*	Mardin/Midyat - South East Anatolia	CU
CU-199	*unclear*	Sanliurfa - South East Anatolia	CU
CU-203	*reticulatus*	Diyarbakir - South East Anatolia	CU
CU-205	*unclear*	Batman - South East Anatolia	CU
CU-234	*flexuosus*	Balikesir - Marmara	CU
CU-279	*inodorus*	Van - East Anatolia	YYU
CU-280	*reticulatus*	Van - East Anatolia	YYU
CU-305	*agrestis*	Adana - Mediterranean	CU
CU-341	*dudaim*	Hakkari - East Anatolia	M. Pitrat
CU-343	*flexuosus*	Gaziantep - South East Anatolia	M. Pitrat
CU-347	*unclear*	Tunceli - East Anatolia	CU
CU-349	*flexuosus*	Kahramanmaras - Mediterranean	CU
CU-360	*reticulatus*	USA	M. Pitrat
CU-365	*conomon*	Japan	M. Pitrat
CU-375	*agrestis*	-	P-Treves
CU-385	*flexuosus*	Sudan	M. Pitrat
CU-386	*momordica*	India	M. Pitrat

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Morphological evaluation

An evaluation of the morphology and productivity of 24 melon accessions was carried out at the research and implementation area of Faculty of Agriculture, University of Cukurova, Adana in 2008. Ten seeds of each genotype were initially sown in a compost media. Seedlings at the two-leaf stage were transplanted to greenhouse. Plant evaluation was based on standard vegetative descriptors (Sensoy *et al*., 2007). Phenotypic descriptions of genotypes were determined at three stages: cotyledon, flowering and fruit maturation. Total forty-three phenotypic traits were evaluated (Tab. 2). At least three mature fruits from each genotype were harvested, measured, and analyzed. The length measurements were performed by a ruler or a calliper compass; soluble solids content (SSC) was analyzed by a hand refractometer (Atago N1).

Tab. 2. Principal component (PC) analysis of characters associated with 24 melon accessions

Traits	PC axis								
	1	2	3	4	5	6	7	8	9
Eigen-values	8.57	6.30	3.70	3.36	2.77	2.35	2.28	1.77	1.63
Explained Proportion of Variation (%)	20	15	9	7.7	6.5	6	5	4	4
Cumulative Proportion of Variation (%)	20	35	44	51.7	58.2	64.2	69.2	73.2	77.2
Character	*Eigen vectors*								
Hypocotyl length	-0.02	0.16	-0.24	-0.23	0.15	-0.15	-0.19	-0.13	0.11
Cotyledon width	0.05	0.27	-0.16	-0.04	-0.14	-0.07	0.24	0.16	-0.11
Cotyledon length	0.00	0.28	-0.26	0.11	0.13	0.14	-0.08	0.02	-0.11
Stem pubescence	0.09	0.04	0.12	-0.35	0.04	0.27	-0.08	-0.09	0.19
Leaf colour	0.10	0.05	0.29	0.17	0.03	0.06	0.00	0.17	-0.30
Lobes in a leaf blade	0.01	-0.04	0.28	0.10	0.09	0.03	0.35	-0.10	-0.13
Dentations of margin in a leaf blade	-0.08	-0.04	-0.02	-0.20	0.08	-0.38	-0.12	0.29	-0.02
Undulation of margin in a leaf blade	-0.09	0.03	0.17	0.14	-0.11	-0.18	0.25	0.29	0.34
Blistering in a leaf blade	0.03	-0.08	0.06	-0.15	-0.08	0.39	-0.16	-0.26	-0.04
Sex expression	-0.26	0.14	-0.04	0.13	0.04	-0.03	-0.13	-0.11	-0.06
Ovary index	-0.28	0.12	0.07	0.14	-0.02	0.01	0.03	-0.11	-0.05
Ovary shape	-0.29	0.06	0.06	0.16	-0.08	-0.05	-0.01	-0.07	-0.02
Fruit length	-0.17	0.29	-0.02	0.04	-0.06	0.10	0.08	0.05	-0.05
Fruit width	0.18	0.27	-0.11	-0.08	0.08	-0.03	0.17	0.03	-0.02
Flesh thickness	0.20	0.25	-0.01	-0.02	-0.14	0.20	-0.09	0.10	0.01
Skin thickness	-0.07	0.03	-0.04	-0.04	0.29	-0.29	0.12	-0.10	-0.30
Seed cavity width	-0.21	0.17	-0.02	0.05	-0.18	0.08	-0.06	0.14	0.00
Seed cavity length	0.21	0.10	-0.13	0.05	0.24	-0.09	-0.02	0.05	0.02
Soluble solids content	0.23	0.13	0.14	0.22	-0.04	-0.11	0.04	-0.14	-0.07
Immature fruit skin colour	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Immature fruit skin colour intensity	0.16	-0.07	-0.05	-0.23	-0.20	0.22	0.11	0.12	0.05
Fruit shape	-0.29	0.11	-0.02	0.11	-0.07	0.04	0.09	-0.06	0.08
Aroma	0.24	0.02	0.01	0.13	0.18	-0.08	-0.24	-0.06	-0.04
Mature fruit skin colour	0.07	-0.01	0.26	-0.13	0.17	0.14	0.04	0.31	-0.19
Mature fruit skin colour intensity	0.14	0.07	-0.03	-0.01	-0.32	-0.17	-0.07	-0.07	-0.01
Secondary skin colour	0.11	-0.16	-0.26	-0.06	0.07	0.16	0.18	0.22	-0.15
Distribution of secondary skin colour	0.13	-0.12	-0.24	0.04	0.01	0.12	0.19	0.15	0.09
Easy separation peduncle	0.16	-0.08	-0.04	0.38	0.13	0.11	-0.06	-0.08	0.13
Fruit shape of base	0.16	-0.08	-0.12	-0.04	0.07	-0.18	0.19	-0.34	0.08
Fruit shape of apex	0.18	-0.16	-0.02	0.24	-0.07	-0.05	0.14	0.02	0.26
Fruit ribs	-0.01	-0.03	-0.22	0.15	0.34	0.07	0.16	0.05	0.29
Netting	0.21	0.15	0.20	0.12	0.01	-0.03	-0.25	-0.07	0.15
Fruit skin wrinkle	-0.05	0.13	0.02	-0.25	0.07	-0.04	0.15	-0.27	0.09
Flesh colour	0.03	0.15	-0.02	0.20	0.11	0.19	0.02	-0.04	0.09
Flesh colour intensity	0.12	0.07	0.08	0.06	-0.17	-0.15	-0.27	0.22	0.23
Colour between rind and flesh	-0.08	0.07	0.30	-0.15	0.26	0.13	0.03	0.15	0.17
Taste	0.24	0.16	0.13	0.04	0.11	-0.09	-0.14	0.04	-0.23
Seeds weight	0.00	0.27	-0.08	-0.10	0.00	-0.11	-0.12	0.06	0.26
Seeds hilum end shape	-0.14	0.08	-0.13	0.21	0.22	0.26	-0.13	0.13	0.06
Shape of cross section of seed	0.10	0.22	0.17	-0.07	0.01	-0.07	0.26	0.03	0.16
Seeds colour	0.08	0.24	0.24	-0.03	0.04	0.05	0.20	-0.25	0.15
Seeds number	-0.15	-0.04	0.16	-0.17	0.38	-0.03	-0.10	0.12	0.13
Fruit weight	0.08	0.33	-0.15	-0.07	-0.03	0.08	0.14	0.05	-0.16

Genetic Characterization

DNA isolation

The twenty-four accessions were sown in the greenhouse and minimum of six seedlings (three weeks old) per genotypes were sampled in bulk, and lyophilized for DNA extraction. Total genomic DNA was isolated following the protocol of Doyle and Doyle (1990), with the minor modifications incorporated by Boiteux *et al*. (1999). DNA was normalized to 20 ng/ µl for PCR analysis.

SSR markers

A total of 40 SSR markers, 16 derived from *C. pepo* and 24 from *Cucurbita moschata*, were selected based on their *C. pepo* genome location (2 SSRs for each linkage group; Gong *et al*., 2008) and evaluated as described by Cavagnaro *et al*. (2011). Briefly, PCR reactions were performed in 15 µl volume reaction, employing 7.15 µl water, 1.5 µl 10 x DNA polymerase buffer, 1.2 µl dNTPs (2.5 mM each), 1 µl of each primer at 5 µM, 0.15 µl Taq Polymerase at 10 u/ µl (Fermentas) and 20 ng DNA. The amplification protocol consisted of one initial denature time of 3 min. at 94 °C, followed by 40 cycles with a denature time of 20 s at 94 °C, a primer specific annealing temperature for 1 min., an extension time of 1 min. at 72 °C, and an additional final extension of 5 min. at 72 °C. The amplicons were size fractionate during five hours using 4% (w/v) high resolution agarose gel (Gene- Pure Hi-Res Agarose, ISC Bioexpress, Kaysville, UT), 0.5X TAE buffer and visualized with Ethidium bromide.

ISSR markers

Thirty-one ISSR primers were used. Amplification reactions were performed according to Yildiz *et al*. (2011). The ISSR PCR reactions were performed as follow: 3 min. at 94 °C, 30 s at 94 °C, 45 s at 55 °C, 2 min. at 72 °C for 45 cycles and a final extension of 5 min. at 72 °C. The amplicons were size fractionate during three hours using 2% (w/v) agarose gel, 0.5X TAE buffer and visualized with Ethidium bromide.

Data analysis

The morphological and combined morphological-molecular genetic diversity among melon accessions were determined by using SM and Euclidean distance matrix. A presence (1) / absence (0) binary data matrix obtained from scoring polymorphic ISSR, SSR bands was used to calculate Euclidean similarity coefficients to estimate the genetic diversity among melon genotypes. Quantitative traits were converted into 3-5 discrete classes (as in Garcia *et al*., 1998; Stepansky *et al.,* 1999; Sensoy *et al*., 2007). The UPGMA cluster analysis and the resulting dendrogram were performed on the Euclidean genetic distance matrix using the computer program NTSYpc version 2.02k (Rohlf, 1997).

All the original data were transformed into standardized data to eliminate the difference in the variance of each character. The principal component analysis (PCA) based on morphological traits was used to identify the patterns of variation within set melon landraces and reference cultivars. The PCA analyses were carried out using PRINCOM procedure implemented in SAS (SAS Institute 2005) using 43 characteristics and 24 landraces.

Results

Transferability of SSR markers

There were amplifications at twenty-four out of forty cucurbit SSRs in melon. There were no amplification at nine *C. moschata* SSRs out of 24 and also five of them were monomorphic. There were no amplification at six *C. pepo* SSRs out of 16 and also four of them were monomorphic. Among the polymorphic SSRs, ten of them belong to *C. moschata* (41.7%) and six of them belong to *C. pepo* (37.5%).

A total of 74 bands were identified by the SSR analysis of the 24 accessions. The number of bands per marker ranged from 2 (CMTm14, CMTm224, CMTp86, CMTp132, and CMTp210) to 11 (CMTm48) with average 4.9 bands. Fifty-five of the obtained seventy-four bands (74.3%) were polymorphic (Tab. 3).

Cluster analysis

As seen from the dendogram having 43 morphological traits and two main clustering (Fig. 1), *flexuosus* group having distinct ovary length, fruit shape, mature fruit colour, fruit flesh colour, and sex expression were grouped together; *reticulatus* group having distinct rind netting, fruit colour, abscission of peduncle, and external aroma were grouped together; *conomon* group having fruit shape, fruit colour, and taste were grouped together; small fruited *agrestis* group and *dudaim* group having distinct cotyledon ratio, fruit weight, fruit shape, taste, and soluble solid content were grouped together. In the first main cluster, CU365 (*conomon* group) was outlier and there were two sub-clusters; 4 accessions of *flexuosus* group (CU385, CU343, CU349, and CU234) were in one sub-cluster and 2 accessions of *conomon* group (CU177, and CU196) were in the other. In the second main cluster, there were also two sub-clusters; 2 accessions of *agrestis* group (CU305 and CU375) were in one sub-cluster and two sub-sub-clusters in the other: one having *dudaim* group, *unclear*, and *momordica* group genotypes (CU341, CU347, and CU386, respectively), the other having *reticulatus* group, *cantalupensis* group, *inodorus* group and *unclear* genotypes.

In morphological dendrogram, sweet and non-sweet genotypes were generally discriminated from each other. Unclear genotypes CU205 having orange flesh colour was grouped with four *reticulatus* group genotypes (CU161, CU280, CU203, and CU360). Three unclear genotypes (CU199, CU179 and CU171) were grouped with *cantalupensis* group (CU152) and *inodorus* group (CU181) due to their fruit shape and flesh colour. Unclear genotypes CU347 having yellow-orange fruit colour and white flesh colour was grouped with *dudaim* group (CU341) and

momordica group (CU386) genotypes.

Polymorphisms detected at 152 loci by using thirty-one ISSR primers, 55 loci by using 16 SSR primer pairs (Tab. 3), and 43 morphological traits (Tab. 2) were used also used together in the genetic evaluation of 24 melon genotypes. In the combined morphological-molecular dendrogram, there were also two main clusters (Fig. 2). The first main cluster contained two accessions of *agrestis* group (CU305 and CU375) and one accession of *dudaim* group (CU342). The second main clusters divided into two sub-clusters (2a and 2b). The sub-cluster 2a contained *conomon* group and *flexuosus* group genotypes, while sweet melons appeared in the sub-cluster 2b. Unclear genotypes (CU171, CU175, CU179, CU199 and CU347) were grouped together with an *inodorus* group accession (CU181). US originated *reticulatus* group genotype (CU360) and India originated *momordica* group genotype (CU386) were outliers in the sub-cluster 2b. Turkey originated all *reticulatus* group genotypes were all grouped together. Moreover, all *inodorus* group and *reticulatus* group genotypes originated from Van province were also grouped together.

Tab. 3. Polymorphisms detected at 207 loci by using ISSR, and SSR methods

Locus name	No. of bands	Polym. bands	Polym. (%)	Locus name	No. of bands	Polym. bands	Polym. (%)
ISSR (total)	239	152	63.6				
B2	9	7	77.8	813	6	6	100
B4	6	4	66.7	816	4	2	50
B5	5	3	60	825	8	5	62.5
B10	5	1	20	826	11	4	36.4
cbt5	5	3	60	829	5	2	40
issr3	7	3	42.9	834	11	8	72.7
issr4	6	3	50	Average	7.7	4.9	
issr5	6	3	50	SSR (total)	74	55	74.3
issr6	9	3	33.3	CMTm48	11	9	81.8
issr7	12	12	100	CMTm68	8	6	75
phv7	7	2	28.6	CMTm84	5	4	80
Sola1	14	12	85.7	CMTm111	5	2	40
Sola2	10	5	50	CMTm120	9	8	88.9
Sola3	7	4	57.1	CMTm130	4	2	50
Sola4	3	3	100	CMTmC14	2	1	50
Sola5	11	7	63.6	CMTm206	7	6	85.7
Sola6	8	6	75	CMTmC67	3	1	33.3
Sola7	6	6	100	CMTm224	2	1	50
Sola10	10	5	50	CMTp86	2	1	50
Sola11	10	6	60	CMTp132	2	1	50
125	4	1	25	CMTp138	4	3	75
131	5	4	80	CMTp174	8	7	87.5
808	12	12	100	CMTp210	2	2	100
809	10	4	40	CMTp68	2	1	50
812	7	6	85.7	Average	4.9	3.4	

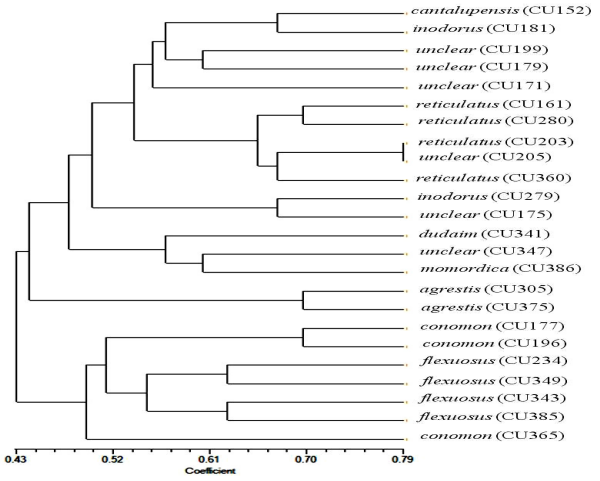

Fig. 1. Associations among melon accessions revealed by UPGMA clustering analysis on the basis of phenotypic SM distance values

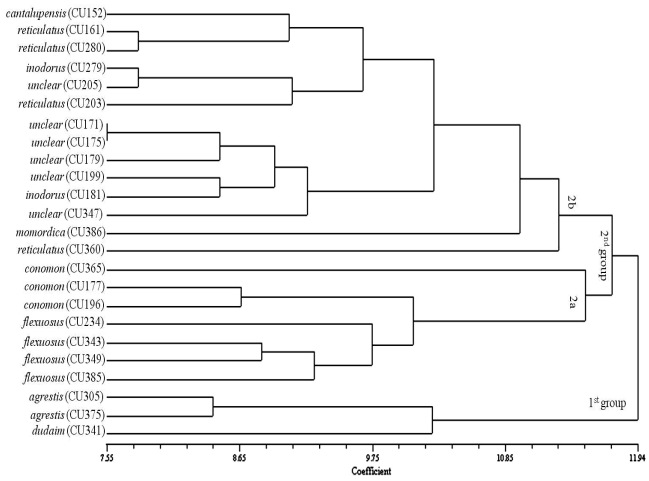

Fig. 2. Associations among melon accessions revealed by UPGMA clustering analysis on the basis of combined phenotypic and Molecular Euclidian distance values

Principal component analysis (PCA)

Principal component analysis was used to evaluate and describe the variation in melon accessions. The first nine factor scores (PC axes) contained 77.2% the total multivariate variation (Tab. 2). The first PC axes account for 20% of the total multivariate variation, the second for 15% and the third for 9%, and the principal characters with higher eigenvectors that delineated the accessions into separate groups in the nine components are present in Tab. 2. The cumulative proportion of variation reached 44% in the first three axes. Twenty-four melon genotypes were scattered by their component scores of the first and the second components (Fig. 3a) and of the first and the third (Fig. 3b).

The distribution along the first component is mainly based on sex expression, ovary index, ovary shape, flesh thickness, seed cavity length, seed cavity width, soluble solids content, fruit shape, aroma, netting, and taste. In this component, accessions of *flexuosus* group and *conomon* group were discriminated based on sex expression, ovary shape and index, seed cavity length and fruit shape, and were on the left upper side of Fig. 3a. The accessions of *inodorus* group, *reticulatus* group, *cantalupensis* group and all unclear accessions were located closely to each other in right upper side of Fig. 3a based on aroma, netting and taste component.

The second component is mainly related to variation of fruit weight, fruit length and width, flesh thickness, cotyledon width and length, and seeds weight. In this component, subsp. *agrestis* accessions (CU305 and CU375) and *dudaim* group (CU341) were discriminated from all accessions based on fruit size and small cotyledon length and width; subsp. *agrestis* accessions were distributed in left lower side Fig. 3a and *dudaim* group accession was located in right lower side of Fig. 3a.

With respect to third PC axis, it reflected the variation of colour between rind and flesh, fruit ribs, secondary skin colour, mature fruit skin colour, secondary skin colour, development of lobes in a leaf blade, leaf colour, and cotyledon length, which located sweet and non-sweet accessions into left upper and right upper side of Fig. 3b, respectively.

Abscission of peduncle, flesh colour, fruit skin wrinkle, stem pubescence had higher coefficients in the fourth PC axis, while seeds number, colour between rind and flesh, fruit ribs, mature fruit skin colour intensity, and skin thickness had higher coefficients in the fifth PC axis. The sixth PC axis separated dentations of margin in a leaf blade, undulation of margin in a leaf blade skin thickness, and seed hilum end shape, while seventh separated development of lobes in a leaf blade, dentations of margin in a leaf blade, flesh colour intensity, and shape of cross section of seed. Dentations of margin in a leaf blade, undulation of margin in a leaf blade, blistering in a leaf blade, mature fruit skin colour, fruit shape of base, fruit skin wrinkle, and seeds colour had high coefficients in the eighth PC axis while seeds weight, fruit ribs, fruit shape of apex, skin thickness, undulation of margin in a leaf blade, and leaf colour had high coefficients in the ninth PC axis.

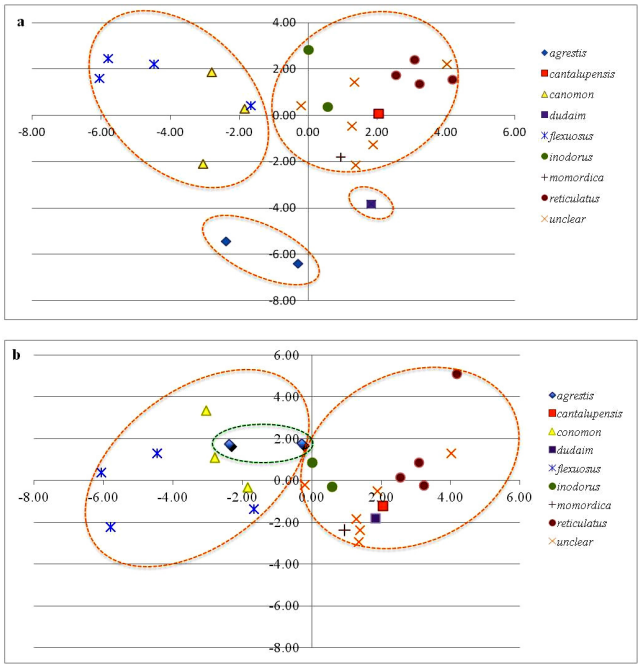

Fig. 3. Scatter diagram of 24 melon accession on the plants defined by the PC 1-2 (A) and 1-3 (B)

Discussion

Same traits presented in different botanical groups could be an outcome of parallel evolution and also intercrossing between groups later by selection for preferred alleles (Pitrat, 2013). In the dendrogram generated by morphological characters, similar genotypes in terms of fruit characteristics have generally positioned in similar clusters. The groups' *flexuosus* and *conomon* were separated from the other groups based on fruit shape. Beside this trait, different sex expression and creamy rind colour at the maturity helped to discriminate *flexuosus* group from the others. The netting, the most discriminative traits among the morphological ones, clustered *reticulatus* group accessions together. Similarly, small-fruited subsp. *agrestis* accessions were clustered together based on fruit weight and rind colour at the maturity. Unclear South-eastern Anatolian accessions were clustered with sweet melons (*inodorus* group, *cantalupensis* group, and *reticulatus* group). This might be because of close cultivation of these accessions with each other and with the cultivars of above mentioned sweet melons.

Pitrat (2013) have summarized that genetic control of a majority of the diversification traits in melon such as sex expression, fruit shape, sutures, number of placentas, gelatinous sheath around the seeds, white flesh colour etc. with recessive genetic control and other phenotypic traits such as orange flesh colour, netting, and colour of mature fruit are controlled dominant genes. We believe dominant traits such as orange flesh colour and netting were transmitted to South-eastern Anatolian melon accessions from *cantalupensis* group and *reticulatus* group and fixed by farmer selections.

Total discrimination of sweet and non-sweet accessions in the combined molecular and morphological dendrogram

was also observed in the other studies (Stepansky *et al*., 1999; Garcia-Mas *et al*., 2000; Staub *et al*., 2000; Mliki *et al*., 2001); however, some studies have placed *flexuosus* group and *momordica* group accessions among the sweet ones (Stepansky *et al*., 1999, Staub *et al*., 2004, Sensoy *et al*., 2007; Yildiz *et al*., 2011). The researches assumed that different outcomes could be results of the different marker types or the germplasm. In the present study, the accessions of *flexuosus* group were discriminated from the sweet ones, but the accession of *momordica* group was clustered with the sweet ones. It has been stated that genetic diversity could vary based on the marker methods (Staub *et al.,* 2000; Aierken *et al*., 2011). By virtue of their uniqueness, SSRs are most valuable for studying intra- and inter-specific relationships. In the present study, SSRs from two *Cucurbita* species were employed; therefore, we think the use of *Cucumis* specific SSRs could increase the power in discrimination.

It was clearly visible that genotypes belonging to *reticulatus* group, *cantalupensis* group and *inodorus* groups were clustered together in different subgroups. Similar results have been determined by Sensoy *et al*. (2007) that distinction amon *inodorus* group and *cantalupensis* group (sweet) genotypes was also not very significantly different in their evaluations. Also, Kacar *et al*. (2012) have been documented that *cantalupensis* group and *inodorus* group were clustered together with varying (70-100%) similarity rates.

Diversification is a second step after domestication, having the cultivars, cultigroups and botanical groups (Pitrat, 2013). All regions and countries have special cultivar requirements. Commercial melon cultivars are employed at all regions in Turkey, but local groups are still requested by some consumers. Despite being less compared to the past, these landraces are still produced. However, as in other members of *Cucurbita* family, there is high rate of out crossing in melon landraces and cultivars, which causes gene flow and lose of characteristic traits among them. In the present study, South Eastern Anatolian melon accessions were in a separate cluster although they had some common traits from *cantalupensis* group, *reticulatus* group, and *inodorus* group. They were different than *inodorus* group based on aroma, abscission of peduncle and flesh colour and different than *cantalupensis* group and *reticulatus* group based on netting, soluble solids content and taste.

A total of forty-three morphological characters related to three stages: cotyledon, flowering and fruit maturation were used to assess the variation of different melon groups. The PCA plot based on all measured traits allowed distinction between *flexuosus* group, subsp. *agrestis* and *reticulatus* group. A high variation among groups was observed for the fruits characters. Netting, aroma and abscission of peduncle represent *reticulatus* group; a small fruit size, strong typical aroma and secondary colour distribution characterize *dudaim* group. Monoecy, very long fruit shape and mature fruit rind colour discriminate *flexuous* group; ovary index, fruit size and flesh width distinguish subsp. *agrestis*. Pitrat (2013) stated that domestication traits are present in almost all cultivars and absent in wild accession. On the opposite, diversification traits are present in only some cultivars or cultigroups. For example, orange flesh colour, and andromonoecy are found in cultivated melons, while wild ones have green flesh colour and monoecy.

Conclusions

Turkey is among the secondary diversity centre for melon and melon landraces are still produced at the request of the consumers in some regions in Turkey. In the present study, some melon groups that are still produced in the Eastern and South-eastern Anatolian regions of Turkey were characterized by morphologically and molecularly, and determined that unclear accessions were sub-clustered separately among the sweet melon groups. The findings have great importance in conservation of germplasm and in the development of new groups by breeding efforts. These cultured local genotypes still require protection against genetic erosion. This characterization study will be useful to germplasm conservation attempts in other regions. Information presented herein is also valuable to characterize and preserve other melon germplasm.

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