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Chinese melon (*Cucumis melo* L.) diversity analyses provide strategies for germplasm curation, genetic improvement, and evidentiary support of domestication patterns

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Abstract The genetic diversity of melon market types (*Cucumis melo* L., 2n = 2x = 24) in China, an important secondary center of diversity, has not been examined. Therefore, reference accessions from India and Africa, Crete/Greece, Japan, Europe, U.S.A., Spain, and 68 Chinese cultigens (fresh-market non-netted thin-skinned; non-netted thick-skinned; netted thick-skinned; and non-netted thin-skinned, and vegetable) were evaluated by using 17 10-mer RAPD primers (32 mapped loci), days to flower, sex expression, lateral-branch number, and fruit number and weight per plant. While Chinese thin-skinned melons differed from vegetable melon types only in sex expression, the U.S. Western Shipping market type reference accession "Top Mark" and Chinese thick-skinned melons were similar for all of the morphological traits examined. The average similarity (Jaccard Coefficient) between any two pairs of accessions examined as estimated by RAPD variation was 0.47 ± 0.14. Within-group genetic similarities ranged between 0.94 (thin-skinned type) and 0.08 (non-netted thick-skinned type). The average/standard deviation, maximum, and minimum similarity between any two Chinese reference accessions was 0.41 ± 0.13, 0.75, and 0.12, respectively. Cluster analysis partitioned accessions into two main branches consisting of Group Cantalupensis and Inodorus reference accessions (clade 1) and Chinese accessions (clade 2). A second cluster analysis partitioned China, India, and Africa accessions into one major group, and accessions from Japan, Europe, and U.S.A. into another. Results indicate that Chinese accessions are a rich source of genetic diversity for plant improvement, and that molecular assessments support previously described theoretical melon domestication patterns constructed from historical and archeological evidence.

Keywords Genetic similarity · Morphological traits · Multivariate analysis · RAPD

Abbreviations

THIN	Fresh-market, non-netted, thin-skinned melon
THICK	Non-netted, thick-skinned melon
NET	Netted, thick-skinned melon
VM	Non-netted, thin-skinned, vegetable melon
DF	Days to 50% flower
LBN	Lateral-branch number on main stem
FN	Fruit number per plant
FW	Fruit weight per plant

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JC	Jaccard's similarity coefficient
SSR	Simple sequence repeat
RAPD	Random amplified polymorphic DNA
PCR	Polymerase chain reaction
LSD	Least significant difference
PCA	Principal component analyses
UPGMA	Unweighted pair-group method using arithmetic average
RMA	Reference marker array
SRA	Standard reference accessions

Introduction

Cucumis species of the family Cucurbitaceae (115 genera) having varying chromosome numbers (i.e., 2n = 14, 24, and 48) are indigenous to the native flora of Africa, India, and various regions of the Middle East. There are 30 2n = 24 *Cucumis* African species of various ploidy levels comprising six taxonomic groups distributed across Africa, the Middle East, Pakistan, and Asia (Kroon et al. 1979; Kirkbride 1993). Two *Cucumis* species, cucumber (*C. sativus* L.; 2n = 2x = 14) and melon (*Cucumis melo* L.; 2n = 2x = 24), are important economically worldwide but do not share close genetic affinities as once thought (Chung et al. 2006; Renner et al. 2007). Cucumber originated in Asia, likely on the Indian subcontinent. In contrast, melon, a morphologically diverse outcrossing species, is thought to have originated in Africa (Kirkbride 1993; Robinson and Decker-Walters 1997) where many wild, free-living var. *agrestis* Naud. morphotypes exist near regions of agricultural cultivation (Rubatzky and Yamaguchi 1997; Whitaker and Bemis 1976). However, domestication patterns of melon have not been clearly elucidated.

Edible melons are divided into six botanical groups including Flexuosus (snake melon; Middle East), Conomon (Asia), Cantalupensis (Middle East), Inodorus (Middle East, Southern Europe), Chito (mango melon; Asia) Dudaim (Queen's pocket melon; Asia), and Momordica (Phoot or snap melon; Asia) (Robinson and Decker-Walters 1997). Several of these groups are economically important in developed countries based on their culinary attributes (Staub et al. 2000). These include Group Cantalupensis (e.g., "Earl's", "House", "Galia", "Charentais", and "Ogen" market types), Group Inodorus (e.g., "Honeydew" and "Casaba" market types) and Group Conomon (e.g., "Oriental" market types) which differ markedly in fruit characteristics such as netting, shape, interior texture, flavor, aroma, and shelf life to form specific commercial market classes.

Simple sequence repeat (SSR) and random amplified polymorphic DNA (RAPD) markers have been used broadly to define genetic relationships among botanical groups and commercial market classes (e.g., Charentais versus Ogen) (García et al. 1998; Katzir et al. 1996; Monforte et al. 2003; Silberstein et al. 1999; Staub et al. 1997; Stepansky et al. 1999). Standard marker arrays and reference accessions have also been employed to assess the genetic diversity of melon landraces and cultivars from Europe and the U.S.A. (Staub et al. 2000), Africa (Akashi et al. 2006; Mliki et al. 2001), Spain (López-Sesé et al. 2002), Crete/Greece (Staub et al. 2004), India (Dhillon et al. 2007), Japan (Nakata et al. 2005), and Turkey (Senory et al. 2007). These genetic analyses have provided diversity assessments of all major primary and secondary centers of melon diversity, except China. The genetic analysis of Chinese melon, along with ancient agricultural trade and archeological information, could facilitate the development of genetic enhancement strategies to increase genetic diversity of major market classes and to rigorously appraise centers of diversity to provide insights into the species domestication. Therefore, we used a previously defined standard RAPD marker array (Staub et al. 2000), and several morphological traits to: (1) assess the genetic diversity of Chinese melons from diverse geographical origins; (2) determine their relationship to a previously defined set of African, Japanese, Greek, Spanish, Turkish, U.S., and European reference accessions, and; (3) use this information in conjunction with historical and archeological evidence to provide a more comprehensive understanding of melon domestication patterns.

Materials and methods

Plant materials

Chinese market class melons are defined by fruit epidermal (skin) characteristics (i.e., netting and thickness), and culinary uses. Seeds of 68 phenotypically diverse Chinese fresh-market non-netted thin-skinned

(32), non-netted thick-skinned (18), netted thick-skinned (10), and non-netted thin-skinned vegetable (8) cultigens (landraces, inbred lines, and hybrids) were obtained from various seed companies, academic and cultural institutes, and local growers (Table 1). These melon accessions [landraces and open-pollinated (OP) or OP selfed local varieties] are regularly grown in 13 provinces and five city–states, and represent the phenotypic variation typically seen in virtually all Chinese melon growing regions (Fig. 1). Hybrid melon cultivars are presently not widely used in Chinese agricultural production.

Fourteen previously fingerprinted, genetically diverse melon accessions [two Chinese accessions; #1 (Q3-2-2) and #35 (Yuan H3), nine Indian accessions, and three U.S. accessions; Table 1] were used as reference lines for initial comparative analysis of Chinese accessions (data not presented). These accessions are representative of the genetic diversity present in Group Cantalupensis and Group Inodorus market classes and important primary (India) and secondary centers of diversity (McCrieght et al. 2004; Staub et al. 1997). The Indian accessions used (Table 1; accessions 72–80) were the result of a relatively recent collection expedition, and typify current Indian landrace diversity (McCrieght et al. 2004). The 80 [66 (Chinese) +14 (reference)] accessions were analyzed using a standardized set of 17 RAPD primers (32 mapped loci; Staub et al. 2000; Zalapa et al. 2007) based on their discriminatory power (Mliki et al. 2001; Staub et al. 2000) (Table 2).

Subsequently, the genotypic variation in the 66 Chinese accessions (as defined by these 32 RAPD loci) was compared to a set of 97 accessions drawn from the analysis of 22 commercial U.S. and European accessions (Staub et al. 2000), 15 African accessions (Mliki et al. 2001), 15 Spanish accessions (López-Sesé et al. 2002), 17 Crete/Greece accessions (Staub et al. 2004), 19 Japanese accessions (Nakata et al. 2005), and nine Indian accessions (McCreight et al. 2004). These accessions generally circumscribe the genetic variation (i.e., as assessed by the standard marker array) in the geographic regions examined, and are thus designated herein as standard reference accessions.

Morphological evaluation

An evaluation of morphology and comparative productivity based on yield components as defined by Zalapa et al. (2007), of Chinese melon accessions was

Table 1 Melon (*Cucumis melo* L.) accessions used in diversity analysis

ID no.	Origin	Accession name or no.	Region[a]	Market class[b]	Population[c]	Seed source[d]
1	China	Q 3-2-2	1	THIN	L	Farmer
2	China	Taitian2-4-5	1	THIN	L	Farmer
3	China	Yucui	1	THIN	L	Farmer
4	China	Tianshuai	1	THIN	L	Farmer
5	China	Chaotianbaishami	1	THIN	OPS	HAALC
6	China	Gailiangpopihong	1	THIN	OPS	HAALC
7	China	Tedahongchengcui	1	THIN	OPS	HAALC
8	China	Qitian N 1	1	THIN	OPS	HAALC
9	China	Qitian N 2	1	THIN	OPS	HAALC
10	China	Jindaowang	1	THIN	OPS	HAALC
11	China	Qitian N 8	1	THIN	OP	QAI
12	China	Chaojimibaowang	1	THIN	OPS	HAALC
13	China	Tedalvmagua	1	THIN	OPS	HAALC
14	China	Tedalongtian N 1	1	THIN	OPS	HAALC
15	China	Tedalongtian N 3	1	THIN	OPS	HAALC
16	China	Tedabalixiang	2	THIN	OPS	HAALC
17	China	Tiancuiwang	2	THIN	OPS	HAALC
18	China	Shengkaihua	2	THIN	OPS	CKSC
19	China	Gaotangyucui	2	THIN	OP	JSCJ

Table 1 continued

ID no.	Origin	Accession name or no.	Region[a]	Market class[b]	Population[c]	Seed source[d]
20	China	Jizaoshulaiwang	2	THIN	OP	JSCJ
21	China	Jingpinxuemeiren	2	THIN	OP	JSCJ
22	China	Tiancuihuapi	2	THIN	OP	JSCJ
23	China	Gailianghuangjindao	3	THIN	OPS	HAALC
24	China	Yunmi N 1	3	THIN	OPS	YSCL
25	China	Balengcui	3	VM	OP	YSCL
26	China	Tianba N 1	3	THIN	OPS	YSCL
27	China	Mengtianbaibao	3	THICK	OP	YSCL
28	China	Tedahuishuzi	4	THIN	OPS	HAALC
29	China	Shidaogou	5	THIN	OPS	BCB
30	China	Baiyu N 1	5	THICK	OPS	BCB
31	China	Chaojitiandiaoya	6	THIN	OPS	HAALC
32	China	Yilishabai 1	6	NET	L	SSLC
33	China	Yilishabai 2	6	NET	L	SSLC
34	China	Fengtian N1	6	THICK	L	SSLC
35	China	Yuan H3	6	THICK	L	SSLC
36	China	Sucuibaicaigua	6	VM	OP	SSSCH
37	China	Hongxiangsu	7	THICK	OP	JDSCT
38	China	Cuitianbaibao	7	THICK	OP	JDSCT
39	China	Jinmitianshuai	7	THICK	OP	JDSCT
40	China	Huangjinmi	8	THIN	OPS	LSCS
41	China	Qingpisugua	9	VM	OPS	LSCH
42	China	Gexinghuatai	10	THIN	OPS	YSC
43	China	Saixue N 2	10	THIN	OPS	YSC
44	China	Huapimian	10	THICK	OP	KDSC
45	China	Heipimiangua	10	THICK	OP	KSC
46	China	Heipicaigua	10	VM	OP	ZSC
47	China	M-012	11	THICK	L	JHI
48	China	M-008	11	THICK	L	JHI
49	China	M-021	11	NET	L	JHI
50	China	Yinxiangyu N1	11	THICK	H	JHI
51	China	Yinmi	11	NET	H	JHI
52	China	Annong N3	11	NET	L	JHI
53	China	Qingpicaigua2	11	VM	OP	HFSC
54	China	Huapicaigua	11	VM	OP	HFSC
55	China	Qingpicaigua 1	12	VM	OP	LSC
56	China	Qinglongcaigua	13	VM	OP	WWC
57	China	M-130	14	NET	L	JHI
58	China	CX 178	15	NET	OP	NCRP
59	China	M-135	15	THICK	L	JHI
60	China	CG 188	16	THICK	OP	NCRP
61	China	Kang 2	17	NET	L	SSLCS
62	China	BF	18	THICK	L	SSLCS
63	China	BT3	18	NET	L	SSLCS
64	China	S3	18	THICK	L	SSLCS

Table 1 continued

ID no.	Origin	Accession name or no.	Region[a]	Market class[b]	Population[c]	Seed source[d]
65	China	M-074	18	NET	L	JHI
66	China	TN	18	THICK	L	SSLCS
67	China	Chang S3	18	THICK	L	SSLCS
68	China	T6	18	THICK	L	SSLCS
69	U.S.A.	GFHD	19	Honey dew	OP	HSC
70	U.S.A.	WI998	19	U.S.W.	L	USDA
71	U.S.A.	TM	19	U.S.W.	OP	USDA
72	India	PI 614433	20	VM	OP	NCRP
73	India	PI 614201	20	VM	OP	NCRP
74	India	PI 614222	20	VM	OP	NCRP
75	India	PI 614281	20	VM	OP	NCRP
76	India	PI 614572	20	VM	OP	NCRP
77	India	PI 614580	20	VM	OP	NCRP
78	India	PI 614526	20	VM	OP	NCRP
79	India	PI 614355	20	VM	OP	NCRP
80	India	PI 614540	20	VM	OP	NCRP

[a] Geographic region sampled; where 1 = Heilongjiang Province, 2 = Jilin Province, 3 = Liaoning Province, 4 = Neimenggu Municipality, 5 = Beijing City, 6 = Hebei Province, 7 = Tianjing City, 8 = Shanxi Province, 9 = Shandong Province, 10 = Henan Province, 11 = Hefei City, 12 = Nanjing City, 13 = Wuhan City, 14 = Gansu Province, 15 = Xinjiang Province, 16 = Guangxi Province, 17 = Hongkong Municipality, 18 = Taiwan, 19 = U.S.A., and 20 = India (see Fig. 1)

[b] Market class; where THIN = non-netted thin skin, THICK = non-netted thick skin, NET = netted thick skin, VM = non-netted thin vegetable melon, and U.S.W. = U.S. Western shipping (Reference Array)

[c] OP = open-pollinated variety, OPS = open-pollinated which had been selfed, L = inbred line, and H = commercial or experimental F_1 hybrid

[d] Commercial seed company, academic institute, or farmer; where BCB = Baohongyun Company in Beijing, Beijing City, CKSC = Changchun Kefeng Seed Company, Changchun, Jilin Province, HAALC = HLJ Aolong Agriculture Limited Company, Harbin, Heilongjiang Province, HFSC = Hefeng Seed Company, Hefei, Anhui Province, HSC = Hollar Seeds Company, Rocky Ford, Colorado, JHI = Jianghuai Horticultural Institute, Hefei, Anhui Province, JSCJ = Jixiangdi Seed Company, Changchun, Jilin Province, KDSC = Kedadi Seed Company, Henan Province, KSC = Keda Seed Company, Xinxiang, Henan Province, LSC = Lixiang Seed Company, Nanjing, Jiangsu Province, LSCH = Longfeng Seed Company, Hezhe, Shandong Province, LSCS = Lvbao Seed Company, Shanxi, Shanxi Province, NCRP = North Central Regional Plant Introduction Station, Ames, Iowa, QAI = Qiqihar Agricultural Institute, Qiqihar, Heilongjiang Province, SSSCH = Shijiazhuang Shuangxing Seed Company, Shijiazhuang, Hebei Province, JDSCT = Jinnan Dist Seed Company, Tianjin City, SSLCS = Shuangxing Seed Limited Company, Shijiazhuang, Hebei Province, WWC = Wangwang Company, Wuhan, Hubei Province, YSC = Yimin Seed Company, Liaoning Province, YSCL = Yifeng Seed Company, Yi County, Liaoning Province, and ZSC = Zhumadian Seed Company, Zhumadian City, Henan Province

carried out in 2005. Seeds of 80 melon accessions (68 Chinese, nine Indian, and three U.S.; Table 1) melon accessions were sown on May 16, and seedlings at the two-leaf stage were "hardened-off" outdoors for three days, and then transplanted to rows covered with 1 mm black plastic at the University of Wisconsin experimental farm in Hancock, Wis. Plants were spaced 0.3 m within rows on 2 m centers (~14,300 plants/ha) in Planefield loamy sand (Typic Udipsamment) soil. Seedlings were arranged in a randomized complete block design consisting of three replications with five plants per plot.

Plant evaluation was based on standard vegetative descriptors of difference (Zalapa et al. 2007). Plants were assessed for days to flower (DF), sex expression on the main stem (SE), lateral branch number on main stem (LBN), and fruit number (FN) and fruit weight (FW) per plant. Days to anthesis was recorded as the number of days from transplanting to the time where ~50% of the plants were flowering within a plot. Sex expression was determined by calculating the percentage of pistillate flowers between nodes 10 and 20. The number of primary branches for each plant was counted 30 days after transplant to include all

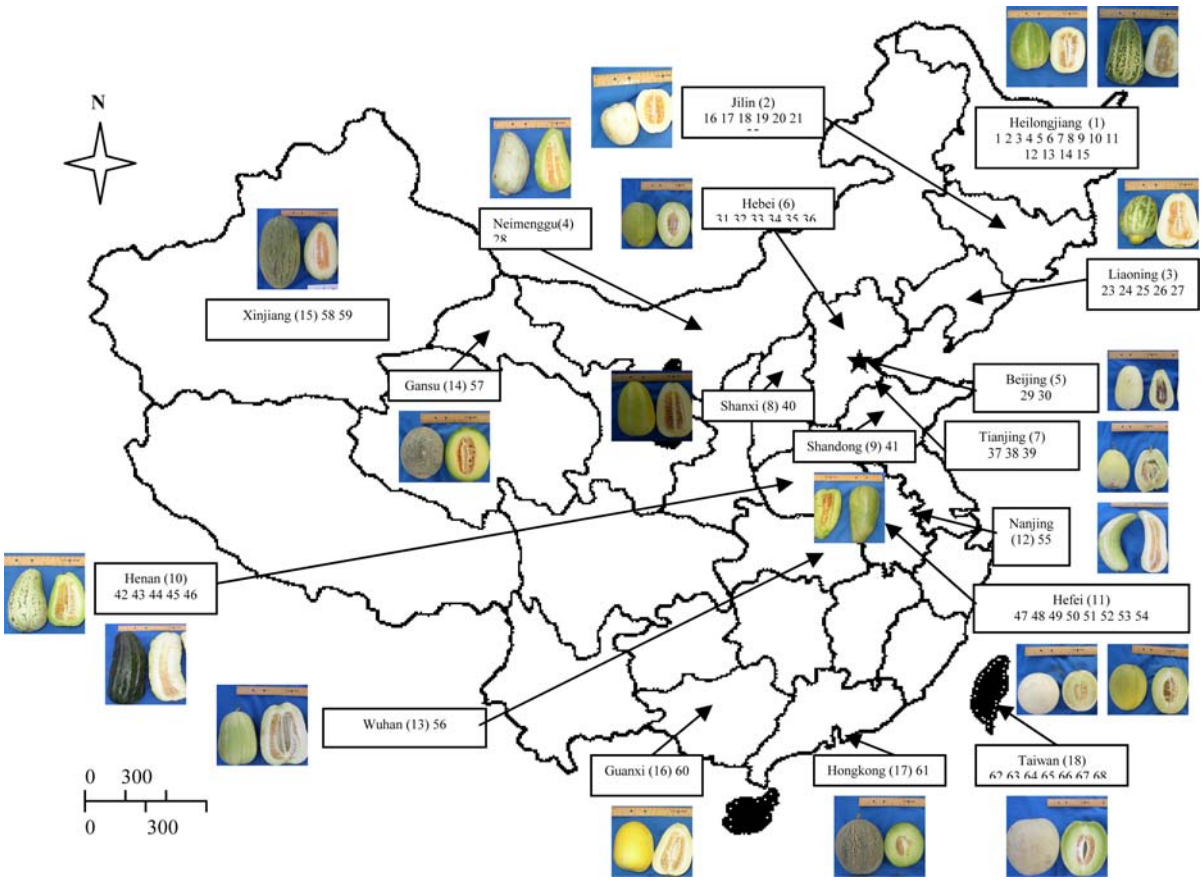

Fig. 1 Origin of Chinese melon (*Cucumis melo* L.) accessions examined given by geographic regions (autonomous community) and numbered according to Table 1. Region numbers are in parentheses

branches more than 12.5 cm in length below the fourth node. Fruit number and fruit weight (kg) data were collected per plant when fruit were mature (at the full-slip maturity) over a 25-day harvest period. The average weight per fruit was calculated for each plant by dividing the total number of fruit per plant by the total weight of the fruit per plant.

DNA extraction and RAPD amplification

Fifteen to 20 seeds of each accession were germinated in a greenhouse at the University of Wisconsin, Madison. Genomic DNA was extracted from leaf tissue sampled at the 2- to 3-leaf stage employing a CTAB procedure (Maniatis et al. 1982) modified according to Staub et al. (1996) by using 2-β-mercaptoethanol.

Seventeen 10-mer primers either from Operon Technologies (OP; Alameda, CA) or the University of British Columbia (BC; Vancouver, BC, Canada), were chosen based on their repeatability, reaction product intensity, and level of polymorphism observed in previous melon diversity analyses (López-Sesé et al. 2002; Mliki et al. 2001; Nakata et al. 2005; Staub et al. 2000, 2004). All polymerase chain reaction (PCR) solutions were purchased from Promega (Madison, Wisconsin), and PCR was performed according to Staub et al. (1996), where reaction conditions followed López-Sesé et al (2002). After amplification, PCR products were electrophoresed following Horejsi and Staub (1999) in 1.6% agarose gels at 120 V with a Model H4 horizontal gel electrophoresis system (BRL, Life Technologies, Gaithersburg, Maryland) for 3 h. *Hind*III + *Eco*RI digested lambda-phage DNA was used as a standard size marker. Each heritable polymorphic band considered as a marker was given a unique identifier by its RAPD primer denomination with base pair size given as a subscript (e.g., $OPB12_{500}$) (Zalapa et al. 2007).

Table 2 Relative frequency of random amplified polymorphic DNA (RAPD) marker bands in Chinese melons (*Cucumis melo* L.) of four market classes

No.	Primer designation[a]	RAPD frequency (%)[b]				
		Non-netted thin skin[c]	Non-netted thick skin[c]	Net thick skin[c]	Non-netted vegetable[d]	Average[e]
1	$C1_{920}$	2	42	100	0	36
2	$C1_{600}$	100	38	18	100	64
3	$C1_{300}$	0	0	0	0	0
4	$D7_{1350}$	0	0	0	0	0
5	$D7_{1250}$	16	27	25	35	26
6	$D7_{1050}$	16	13	12	47	22
7	$I4_{900}$	28	6	18	0	13
8	$I16_{1600}$	54	13	0	50	29
9	$I16_{950}$	62	42	100	29	58
10	$W7_{800}$	3	13	42	13	18
11	$AD14_{400}$	27	50	53	100	58
12	$AF14_{750}$	75	76	65	35	63
13	$AF14_{400}$	3	16	13	0	8
14	$AG15_{950}$	24	9	7	13	13
15	$AK16_{1200}$	100	59	65	100	81
16	$AT1_{1100}$	58	47	12	13	33
17	$AT1_{650}$	31	25	50	0	27
18	$AT5_{800}$	3	18	6	6	8
19	$AT5_{500}$	64	100	67	39	68
20	$AT15_{850}$	21	50	53	39	41
21	$AT15_{300}$	31	57	33	65	47
22	$AU2_{850}$	11	15	0	6	8
23	$AU2_{650}$	55	38	25	100	55
24	$AU2_{620}$	59	38	53	29	45
25	$AX16_{2000}$	52	13	12	47	31
26	$AX16_{1600}$	0	0	0	0	0
27	$AX16_{1200}$	100	41	18	65	56
28	$BC299_{750}$	7	3	6	0	4
29	$BC526_{800}$	7	15	18	0	10
30	$BC526_{700}$	81	42	25	65	53
31	$BC551_{700}$	31	13	12	65	30
32	$BC551_{550}$	74	31	18	100	56
Average[f]		37	30	29	36	33

[a] Primers C through AX obtained through Operon Technologies Inc., Alameda, CA, U.S.A., and BC primers are from British Columbia University, Vancouver, Canada

[b] Bands identified by the RAPD primer and PCR product fragment size which is given as a subscript after the primer designation (e.g., $B12_{500}$ designates a 500-base pair band produced by primer B12)

[c] Exocarp of thin- and thick-skinned fresh market is (mean $\pm$ standard deviation) 2.03 ± 0.31 and 7.30 ± 0.92, repectively

[d] Fruit are cooked before consumption

[e] Average band presence for each primer across market classes examined (see Table 1)

[f] Average band precence across all markers for a given market class

Data analysis

Morphological data were subjected to analyses of variance (ANOVA) followed by least significant difference (LSD) mean comparisons with the statistical program SAS (SAS Institute 1992). Morphological data were used in principal component analyses (PCA) performed in SAS (SAS Institute 1992) to define relationships among accessions (Harris 1975).

A binary data matrix obtained from scoring polymorphic RAPD bands was used to calculate Jaccard similarity coefficients (JC; Jaccard 1908), and then to estimate the genetic diversity among the Chinese melon accessions, and between these and the reference accessions. This genetic similarity estimator was based on its utility in previous melon diversity analyses (Staub et al. 2000) and its concordance with other distance estimators (García et al. 1998; Mliki et al. 2001). Genetic similarity estimates were calculated as the complement of each coefficient ($1 - J_{ij}$) as described by Spooner et al. (1996).

Unweighted pair-group method using arithmetic average (UPGMA) cluster analyses of JCs were conducted to visualize relationships among accessions using the "Tools for Population Genetic Analyses" (TFPGA) computer application (Miller 1997). In addition to the analysis of Chinese accessions, a comparative analysis was performed by including data from reference accessions from Africa (Mliki et al. 2001), Crete (Staub et al. 2004), Europe and U.S.A. (Staub et al. 2000), Japan (Nakata et al. 2005), and Spain (López-Sesé et al. 2003). This was possible because all accession genotyping employed the same standard marker array. All cluster analyses were also subjected to "bootstrap analyses" (Miller 1997) (bootstrap values after 1,000 re-samplings) in order to estimate the reliability of the clustering pattern. These accessions are assumed to represent a collection of populations which have experienced relatively consistent rates of evolution over time.

Statistical measures of genetic variation (i.e., Nei's genetic diversity, Shannon's information index, heterozygosity) were calculated by using the computer program POPGENE (Yeh et al. 1997), and applied for comparative analyses as described by López-Sesé et al. (2002) based on accession origin and horticultural/market type grouping. Results from Sensoy et al. (2007) could be included for comparison since that study was performed using an equivalent number of RAPD markers and the analyses were performed using the algorithms employed herein. Distinctive accessions were identified by their unique RAPD profiles and their relationship to other accessions after PCA. Data matrices, and JC (similarity coefficient) are available at the website: http://vcru.wisc.edu/staublab/.

The relative frequencies of RAPD marker bands observed for each of the 32 primers (reference marker array; RMA; López-Sesé et al. 2003) employed herein were calculated for the Chinese accessions. Frequency differences were used for comparative analyses among market types, to define the most discriminatory primers, and for formulating potential strategies for subsequent diversity analyses. Similarly, RAPD frequencies of this RMA were used for comparative analysis between Chinese accessions and standard reference accessions (SRA) from primary and secondary centers of diversity (López-Sesé et al. 2003; Mliki et al. 2001; Nakata et al. 2005; Staub et al. 2000, 2004).

Results

Morphological comparisons

Chinese melon market types differ dramatically in exterior and interior fruit morphology (Fig. 1). Thick-skinned (netted and non-netted) melon fruit typically have a longer post-harvest storage life (weeks–months) than their thin-skinned counterparts (vegetable and non-vegetable; Table 1). Thin-skinned melon fruit is consumed either as fresh (non-vegetable) or cooked (vegetable) fruit, and the relative sweetness of fruit of thick- or thin-skinned melons is often greater than that of vegetable melon.

Among and between accession variations were detected in the Chinese melon germplasm examined based on descriptive vegetative characteristics (Table 1; Figs. 1, 2). Because developmental competition among fruits on a given plant gives rise to several cycles of fruiting in indeterminate melon types (Rosa 1924), fruit yield and weight data were partitioned into two groups based on mature fruit harvest (i.e., crown and distal fruit set). These groupings reflected two distinct fruiting periods each of about 10 days in duration with an intervening five-day quiescent Period.

When morphological data were collectively analyzed with PCA, 66% of the observed variation was explained (Fig. 2; 44 and 22% in principal components

Market class	V1	V2	V3	Harvest 1 V4	Harvest 1 V5	Harvest 2 V6	Harvest 2 V7	Total V8	Total V9	V10
Thin skinned	19.5	2.2%	4.2	2.2	0.8	1.2	0.4	3.4	1.1	0.3
Thick skinned	21.0	2.0%	3.9	0.8	0.4	2.2	1.4	3.0	1.8	0.7
Net thick skinned	21.3	0.3%	4.1	0.0	0.0	2.3	2.0	2.3	2.0	1.0
Vegetable	21.0	27.0%	4.1	2.3	1.2	2.1	0.9	4.4	2.1	0.5
Green flesh honeydew	20.7	0.0%	4.1	0.0	0.0	1.7	2.3	1.7	2.3	1.4
W1998	20.7	40.0%	3.8	0.0	0.0	1.6	0.9	1.6	0.9	0.7
Top mark	21.7	0.0%	3.7	0.0	0.0	2.4	2.1	2.4	1.1	0.8
Grand mean	20.4	5.1%	4.1	1.4	0.6	1.8	1.0	3.2	1.6	0.6
LSD (0.05)	3.6	0.20	0.7	1.5	0.5	1.6	1.3	2.0	1.1	0.5
CV (%)	9.2	201.25	9.0	53.5	42.4	48.0	66.9	32.0	7.3	43.3

V1: Days to flower
V2: Sex expression (%)
V3: Lateral branch number
V4: Fruit number of harvest 1
V5: Fruit weight of harvest 1
V6: Fruit number of harvest 2
V7: Fruit weight of harvest 2
V8: Total average number of fruit per harvest
V9: Total average weight of fruit per plot
V10: Total average weight per fruit

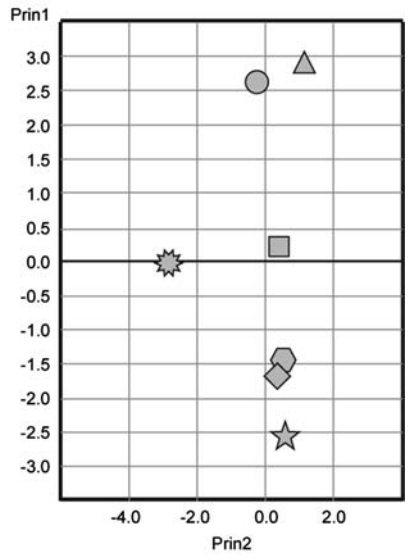

Fig. 2 Principal component analysis of Chinese and reference melon (*Cucumis melo* L.) accessions (see Table 1) using mean values of five morphological traits

1 and 2, respectively). Harvest 2 fruit weight and total fruit weight were primary contributors to principal components 1 and 2, respectively. Chinese thin-skinned and vegetable melons were similar to each other, but different from other melon types examined. Nevertheless, Chinese thick-skinned and WI 998 were more similar to each other than to Chinese netted thick-skinned melons. In contrast, "Top Mark" and "Green Flesh Honeydew" (GFHD) were morphologically similar for the traits examined. While Chinese thin-skinned melons differed from vegetable melon types only in sex expression, "Top Mark" and Chinese thick-skinned melon were similar for all of the traits examined.

Genetic relationships among melon germplasm from China

The 17 RAPD primers used allowed for the genetic assessment of 68 Chinese melon varieties at 32 loci (Table 2; McCrieght et al. 2004). The amplicon sizes ranged from approximately 300–1,200 bp, and the mean number of loci examined per primer was 1.9. Inspection of marker frequency distributions among Chinese market classes indicated that while polymorphic bands were most frequently visualized at $AK16_{1200}$, nine loci ($C1_{600}$, $I16_{950}$, $AD14_{400}$, $AF14_{750}$, $AT5_{500}$, $AU2_{650}$, $AX16_{1200}$, $BC526_{700}$, and $BC551_{550}$) provided moderate (>50% but <80% band frequency) utility for discrimination among market classes during genotyping (Table 2).

The average JC between any two pairs of accessions examined as estimated by RAPD variation was 0.47 ± 0.14 (raw data not presented). Genetic similarities ranged between 0.94 in the most closely related lines [varieties 2-Taitian 2-4-5 (thin-skinned type) and 27-Mengtianbaib (non-netted thick-skinned type)] to 0.08 in distantly related lines 55-Qingpicaigua (vegetable type) and 66-TN (non-netted thick-skinned type). Genetic similarity estimation allowed for the identification of a set of 16 reference accessions (7-Tedahongchengcui, 8-QitianN1, 21-Jingpinxuemeiren, 24-YunmiN1, 27-Mengtianbaibao, 29-Shidaogou, 30-BaiyuN1, 31-Chaojitiandiaoya, 39-Jinmitianshuai, 50-YinxiangxuN1, 54-Huapicaigua, 55-Qingpicaigua1, 57-M-130, 59-M-135, 61-Kang2, and 68-T6) that circumscribed the variation of the Chinese accessions examined. The mean ± standard deviation and maximum and minimum JC between any two of these Chinese reference accessions were 0.41 ± 0.13, 0.75 (for 8-QitianN1 and 24-YunmiN1) and 0.12 (for 55-Qingpicaigua and 57-M-130), respectively.

Cluster analysis based on RAPD data resulted in a dendrogram with two main branches (Fig. 3, Node 1) clearly separating the previously defined reference

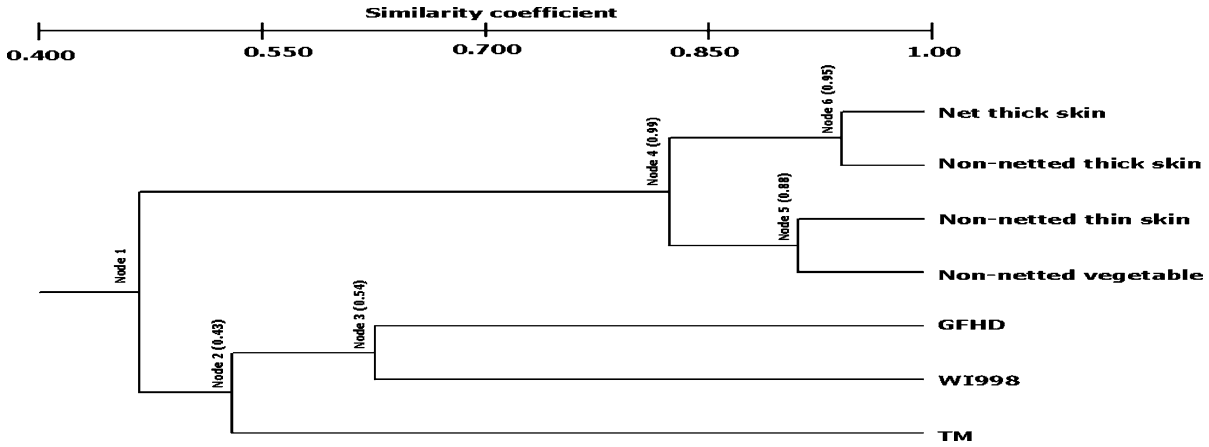

Fig. 3 Cluster analysis (by UPGMA) of Chinese and reference melon (*Cucumis melo* L.) accessions (see Table 1) grouped using genetic similarities (Jaccard's coefficient). Groupings estimated by 32 RAPD loci as framing criteria according to market class designations according to Table 1. Bootstrap values are in parentheses

accessions ("Top Mark", WI 998, and GFHD; Staub et al. 1997) from Chinese accessions. "Top Mark" (U.S. Western Shipping type) and Chinese vegetable melon were most distant from each other (JC = 0.28), and Chinese netted, thick-skinned and Chinese non-netted, thick-skinned melons were most similar (JC = 0.94). While WI 998 and GFHD were similar (JC = 0.69), they were predictably different from "Top Mark" (JC = 0.62 for both) (Nodes 2 and 3). Chinese non-netted thin-skinned and vegetable types were genetically similar (JC = 0.91), but differed from netted and non-netted thick-skinned market types (Node 4).

Genetic affinities and variation within and among market classes

There were remarkable differences detected among a broad array of melon market types from diverse origins (Fig. 4; Table 4). Cluster analysis employing a standard marker array resulted in the division of accessions into two major groups (Fig. 4, Node 1), where one group contained accessions from Spain, Europe, U.S.A., Japan, and Crete, and the other contained accessions from Africa, and India, and all of the Chinese accessions examined. Accessions from Spain were dissimilar (JC = 0.84) from the rest of the accessions in the first major group (Node 2). The remaining accessions in the first group were also genetically distinct from each other [Nodes 3 and 4; Europe/U.S.A. versus Japan and Crete (JC = 0.87) and Japan versus Crete (JC = 0.93)]. While African accessions differed genetically from Indian and Chinese accessions (Node 5; JC = 0.79), Indian and Chinese accessions showed some genetic affinities (Node 6; JC = 0.84), with Indian accessions resembling Chinese vegetable and thin-skinned market types (JC = 0.85) slightly more strongly than Chinese netted and non-netted thick-skinned types (JC = 0.83).

Statistical measures of variation indicate that the population structure of the germplasm examined differed with regard to their origin and market class orientation (Table 4). The genetic variation among Chinese non-netted vegetable melons (56.3% polymorphisms) was dramatically less than the other Chinese market types (mean polymorphism level = 82.3%) examined. The variation of the Chinese accessions examined taken collectively (90.6% polymorphisms) was higher than that of reference accessions from diverse origins, except for Turkish accessions (89.9%). Moreover, with the exception of non-netted vegetable melon, Chinese market types possessed more variation than did accessions from Crete, India, and Spain. The comparatively high level of polymorphisms in China accessions collectively is due to the calculated polymorphism percentage at particular loci (i.e., various market class components possess different polymorphic loci; Table 2). For example, loci $C1_{600}$ illustrated the genetic disparity between thin-skinned and vegetable melon when compared to thick-skinned and netted thick-skinned melon. Band presence of loci composing the SMR in thin-skinned and vegetable melon accessions was 100%, while presences in thick-skinned and netted thick-skinned were 38 and 18%, respectively.

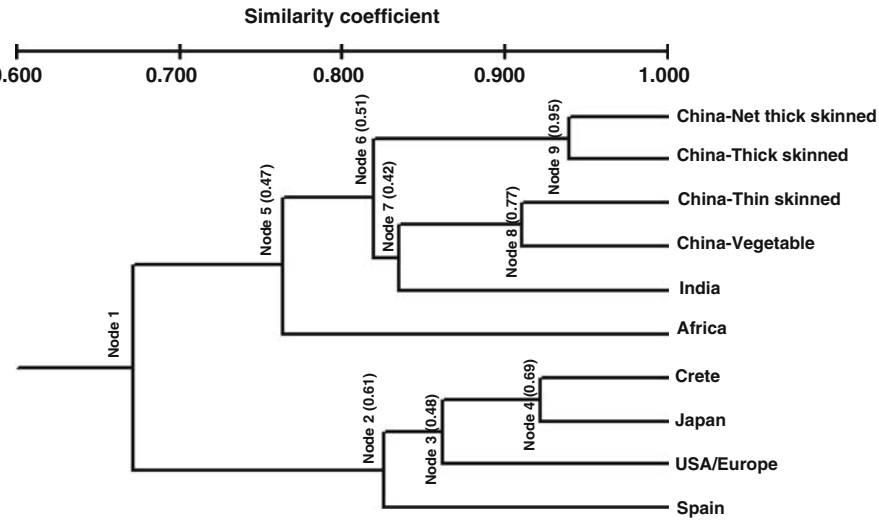

Fig. 4 Cluster analysis of Chinese and standard reference (SRA) melon (*Cucumis melo* L.) accessions (see Table 1; SRA according to Staub et al. 2004) grouped by UPGMA using genetic similarities (Jaccard's coefficient) using 32 RAPD loci as framing criteria. Bootstrap values are in parentheses

Discussion

Critical assessments of the genetic diversity of melon have been made using a broad array of informative genetic markers (Akashi et al. 2002; Dhillon et al. 2007; García et al. 1998; Mliki et al. 2001; Monforte et al. 2003; Sensoy et al. 2007; Silberstein et al. 1999; Staub et al. 2000; Stepansky et al. 1999). These studies have provided descriptive appraisals of major primary and secondary germplasm pools and defined melon market class relationships. Each study has provided a representative sample of the diversity (genotypic and phenotypic) available within the germplasm pool (SRA). This, along with the use of the same molecular array (SMR), allowed us to make direct comparative analyses. Our study expands these genetic appraisals by defining genetic variation among a diverse group of Chinese melon accessions, the last undocumented major germplasm pool. The number of Chinese accessions analyzed is relatively small. Nevertheless, it is clear that the genetic variation of the Chinese melon accessions examined herein warrants their distinction as market classes based both on phenotypic (vegetative) and genotypic (RAPD) differences (Table 2; Fig. 2; supplemental website Fig. 1), and as a unique center of melon diversity worldwide (Tables 3, 4; Fig. 4; supplemental website Fig. 2). Several RAPD marker loci (i.e., $C1_{920}$, $C1_{600}$, $D7_{1250}$, $D7_{1050}$, $I16_{950}$, $AF14_{750}$, $AK16_{1200}$, $AT1_{1100}$, $AT5_{500}$, $AT15_{850}$, $AT15_{300}$, $AU2_{620}$, and $BC526_{700}$) were found to be particularly useful for detecting polymorphisms in accessions of diverse origin, and, therefore, are likely to be useful in describing other Chinese melon collections (Table 3).

Domestication and the development of distinct gene pools

Although the domestication origins of melons are disputed (Lebeda et al. 2006; Robinson and Decker-Walters 1997; Yashiro et al. 2005), most authorities agree that initial domestication events probably occurred in the Middle East (e.g., Iran; ~3,000 BC) well after the beginnings of plant domestication (Fertile Crescent ca. 7,000 BC) (Robinson and Decker-Walters 1997). These events lead to instances where seeds of wild *C. melo* subsp. *agrestis* and possibly selected forms more closely resembling currently cultivated *C. melo* (Dhillon et al. 2007) were likely introduced from Africa into the Middle East (e.g., Turkey, Iraq and Iran) and Asia (e.g., India, China, and Japan) along land and sea commerce routes most certainly by 1,500–2,000 BC (Fujishita 1992; Kajale 1979; Walters 1989). The free-living, wild "chate" or "orange melon", *C. melo* var. *chate* Forsk., in fact, inhabits tropical Africa, especially the upper Nile valley and what was previously considered Nubia and eastern Sudan (Andrews 1956). Melon seeds, likely *C. melo* subsp. *agrestis*, dating to 2,000 BC have also been found in the Indus River region (i.e., Harappan excavation) (Vats 1974).

The center of diversity and perhaps the origin of some principal melons of world commerce (i.e., the *C. melo* Inodorus and Cantalupensis Groups; sweet

Table 3 Random amplified polymorphic DNA (RAPD) marker bands used in a genetic diversity assessment of melon (*Cucumis melo* L.)

No.	Primer designation[a]	RAPD frequency % (band presence)[b]									
		China	China RA[c]	Africa[d]	Crete[e]	Europe & U.S.A.[f]	India[g]	Japan[h]	Spain[i]	Average[j]	Total (%)[k]
1	$C1_{920}$	20	24	47	100	79	100	100	32	63	54
2	$C1_{600}$	53	42	100	65	63	100	68	100	74	68
3	$C1_{300}$	0	0	0	3	0	0	5	3	1	1
4	$D7_{1350}$	0	0	40	36	40	0	14	100	29	23
5	$D7_{1250}$	21	25	57	100	70	53	60	63	56	49
6	$D7_{1050}$	17	29	100	100	44	53	77	63	60	49
7	$I4_{900}$	18	10	32	76	44	100	44	6	41	31
8	$I16_{1600}$	30	24	7	67	51	6	31	53	34	36
9	$I16_{950}$	52	35	37	100	56	53	68	100	63	62
10	$W7_{800}$	11	13	55	100	100	33	77	74	58	44
11	$AD14_{400}$	41	40	14	34	18	33	31	37	31	35
12	$AF14_{750}$	67	55	63	100	52	12	100	100	69	70
13	$AF14_{400}$	7	18	32	51	7	53	35	48	31	24
14	$AG15_{950}$	16	11	48	51	18	0	100	100	43	41
15	$AK16_{1200}$	76	100	14	57	79	100	100	74	75	73
16	$AT1_{1100}$	40	32	63	100	79	13	100	100	66	65
17	$AT1_{650}$	27	18	48	36	30	6	54	63	35	36
18	$AT5_{800}$	8	3	0	100	44	0	77	73	38	35
19	$AT5_{500}$	65	65	22	100	40	21	49	19	48	52
20	$AT15_{850}$	35	37	27	100	57	21	77	100	57	55
21	$AT15_{300}$	42	42	23	65	79	50	60	100	58	56
22	$AU2_{850}$	9	13	23	7	10	33	5	11	14	11
23	$AU2_{650}$	48	39	48	48	33	53	39	55	45	46
24	$AU2_{620}$	48	39	32	100	100	42	100	100	70	69
25	$AX16_{2000}$	33	29	27	76	70	33	49	7	41	41
26	$AX16_{1600}$	0	0	74	36	15	0	44	4	22	18
27	$AX16_{1200}$	54	50	63	16	17	18	24	14	32	37
28	$BC299_{750}$	6	4	55	6	35	21	14	7	19	16
29	$BC526_{800}$	10	13	37	20	42	35	49	63	34	27
30	$BC526_{700}$	54	50	100	16	100	100	17	3	55	47
31	$BC551_{700}$	19	27	32	23	36	67	54	74	42	34
32	$BC551_{550}$	49	37	32	6	2	100	5	37	34	33
Average[l]		31	29	42	59	47	41	54	56	45	

[a] Primers C through AX obtained through Operon Technologies Inc., Alameda, CA, U.S.A., and BC primers are from British Columbia University, Vancouver, Canada

[b] Bands are identified by the RAPD primer and the PCR product fragment size which is given ia as a subscript after the primers (e.g., $B12_{500}$ designates a 500-base pair band from the B12 primer)

[c] Chinese reference array was developed by selecting the most diverse individuals of each market class (thin skin, thick skin, net thick skin, and vegetable) using multidimensional analyses

[d] Reference array according to Mliki et al. (2001)

[e] Reference array according to Staub et al. (2004)

[f] Reference array according to Staub et al. (2000)

[g] Reference array according to McCreight (2004)

[h] Reference array according to Nakata et al. (2005)

[i] Reference array according to Lopez-Sesé et al. (2003)

[j] The average band presence for each primer across all populations

[k] Percent polymorphisms across populations

[l] Average band presence across all markers for a given population

Table 4 Statistical measures of genetic variation as measured by RAPD markers for melon accessions (*Cucumis melo* L.) grouped by origin

Origin	N[b]	H[c]	I[d]	Percentage of Polym.[e]	Reference
China[a]	66	0.33	0.49	90.6	
Non-netted thin skinned	31	0.26	0.40	81.3	
Non-netted thick skinned	18	0.31	0.46	87.5	
Netted thick skinned	9	0.26	0.40	78.1	
Non-netted thin skin vegetable	8	0.21	0.30	56.3	
Reference Accessions					
Africa	15	0.35	0.51	84.4	Mliki et al. (2001)
Crete	17	0.24	0.35	65.6	Staub et al. (2004)
Europe and U.S.A.	22	0.34	0.50	87.5	Staub et al. (2000)
India	9	0.25	0.37	65.6	McCreight et al. (2004)
Japan	19	0.31	0.46	81.3	Nakata et al. (2005)
Spain	15	0.24	0.36	71.9	López-Sesé et al. (2003)
Turkey	58	0.29	0.43	89.9	Sensoy et al. (2007)

[a] Exocarp width (mean ± standard deviation) of thin- and thick-skinned fresh market class is 2.03 ± 0.31 and 7.30 ± 0.92, respectively. Genetic estimates of individual Chinese market types do not include reference accessions

[b] N = number of accessions in each population

[c] H = Nei's gene diversity (Nei 1973)

[d] I = Shannon's information index

[e] Percentage of polymorphic loci

melons) is located in the Near East and adjacent central Asia (Jeffrey 1980). Ancient historical and archaeological records indicate early melon cultivation in Egypt [second millennia BC; as appearing in paintings (Pagalo 1929) and the Bible (Numbers 11:5 as "quishu'im"; probably a non-sweet *C. melo* type)] and Iran (third millennia BC) (Karchi 2000; Robinson and Decker-Walters 1997; Stepansky et al. 1999). It is likely that Indian subsp. *melo* types (Group Momordica) were developed independently from those in Europe and the Middle East (Dhillon et al. 2007; Staub et al. 2004). The comparative analysis of allelic frequencies among European, Mediterranean and Indian accessions examined herein (Table 3) and those of previous studies (McCreight et al. 2004; Sensoy et al. 2007; Staub et al. 2000, 2004) supports the hypothesis of well-differentiated gene pools in these regions.

From the Middle East (perhaps Iran), melon most certainly spread to Turkey, China, and Afghanistan (secondary centers of diversity), and subsequently to Europe (Roman and Greek periods) (Andrews 1956; Jeffrey 2001; Szabo et al. 2005). Linguistic implications regarding Arabic and Turkish names for melon led Pitrat et al. (1999) to hypothesize that there were three independent introductions of melon in Europe from the east (Russia, Bulgaria, and Hungary), southeast (Greece, Albania, and Romania) and the south (Italy). Ensuing selection after these events led to market class gene pools. Melon was subsequently introduced to Central America in 1516, and rapidly expanded with colonization in the New World leading to more recent economically important tertiary centers of diversity (e.g., Virgnia 1609 and New York 1629) (Ware and McCollum 1980). The RAPD-based analysis of several secondary centers of diversity supports the basic tenets of these domestication patterns (Europe, U.S.A., Staub et al. 2000; Middle East, Sensory et al. 2007).

Melon domestication events may have occurred independently in Africa and Asia (Bates and Robinson 1995; Esquinas-Alcazar and Gulik 1983; Jeffrey 1980; Stepansky et al. 1999). The comparatively higher frequency of edible, sweet wild or feral melons found in Asia supports the hypothesis that more extensive domestication occurred in Asia (secondary domestication pools) after their introduction from Africa (Jeffrey 1980; Kirkbride 1993). Northern and southern African germplasm are genetically different

(Mliki et al. 2001), and it is likely that major Indian introductions events originated from southern Africa (Mliki et al. 2001), whereas the Middle East was the beneficiary of North African introductions (Senory et al. 2007; Staub et al. 2004). Polyphyletic relationships inferred by melon chloroplast genome analyses suggest that primitive melon types originated from central and southern Africa, and that large- and small-seeded types found in India (Akashi et al. 2002, 2006; Yashiro et al. 2005) were derived from northern and southern Africa, respectively (Tanaka et al. 2006). The data presented herein lends support to these hypotheses and the development of distinct secondary and tertiary centers of crop diversity.

Recent archaeological evidence dates melon to China ca. 2,000 (Hou-ma in Shaanxi) to 3,000 (Ch'ien Shan Yang in Zhejiang) BC, and to western Japan as early as 100 BC (Fujishita 1992; Yamazaki 2007; Walters 1989; Watson 1969). Isozyme analyses and seed sizes allowed Akashi et al. (2002) to hypothesize that at least some Chinese melon types [i.e., Group Cantalupensis (Kua) and Conomon (Yueh Kua)] may have been derived from early melon introductions from central India (at least by 100 BC via Laos and eastern China). Certainly, melon types were introduced to western China via the Silk Road (from Baghdad to Iran to Kashmir and then to China; ca. 700–1,000 AD; Kitamura 1951). In contrast, Oriental Asian melon types (Group Conomon) are from a distinct germplasm pool, which form two discrete botanical varieties, vars. *makuwa* Makino (cultivated in northern China) and *conomon* Thunberg (cultivated in southern China), which may have been introduced into China from India (Akashi et al. 2002; Bates and Robinson 1995; Nakata et al. 2005; Pitrat et al. 2000). Based on genetic affinities presented herein (Table 4; Figs. 3, 4) and historical records (Walters 1989), it is likely that Chinese thick-skinned melon (netted and non-netted), non-netted thin-skinned and vegetable melon were introduced into China from the Middle East and India, respectively, resulting in unique gene pools.

Plant improvement

The genetic characterization (both morphological and DNA-based) of melon populations representing all significant centers of diversity is essential for the deployment of effective and efficient breeding strategies seeking to broaden market class diversity. Relatively moderate polymorphism levels (i.e., allelic frequency) were detected in accessions from Africa, Europe, U.S.A., and the Mediterranean (Crete/Greece and Spain) (Tables 3, 4). This was due to a fixation (e.g., $C1_{300}$, $D7_{1350}$, $AG15_{950}$, $AT5_{800}$,) or near-allelic fixation (e.g., $I16_{1600}$, $AU2_{850}$, $BC299_{750}$, $BC551_{550}$) at particular loci and to moderate polymorphism levels at other loci (e.g., $AD14_{400}$, $AX16_{1200}$). Thus, the genetic diversity in primary (Africa, Middle East, and India) and secondary (China, and Japan) regions of diversity (i.e., gene pools) show allelic fixation which supports recent observations of Dhillon et al. (2007) indicating a need for implementation of aggressive germplasm enrichment (collection) and enhancement (trait introgression for increased diversity) strategies.

The reference accessions "Top Mark" (Group Cantalupensis) and "Green flesh honeydew" (Group Inodorus) used herein represent two horticultural groups that typify the diversity among major, economically important market classes (Staub et al. 1997, 2000). Although a relatively small array of germplasm was examined, it typified Chinese melon diversity, and thus the genetic differences among the Chinese market classes and these reference accessions characterize their comparative uniqueness and potential as a germplasm source for melon improvement (Figs. 2–4). Data suggest that the genetic diversity in the Group Cantalupensis and Inodorus market types (e.g., the Mediterranean, Spain, Europe, and U.S.A.) could be enhanced by the introgression (e.g., backcross or pedigree breeding) of genes from Chinese market types, especially netted, thick-skinned forms. Given the physiological and genetic differences between sweet and non-sweet melons and the RAPD-based distinctions between primary and secondary centers of diversity (see also Dhillon et al. 2007; Senory et al. 2007; data presented herein), strategic, broad-based population development (e.g., mass selection using Middle Eastern, Chinese, Indian, and African accessions) might be effective for increasing the genetic diversity in Group Inodorus and Cantalupensis market types (i.e., Japanese, European, and U.S. netted thick-skinned market and shipping types).

The comparatively narrow genetic diversity detected in Asian wild or feral populations is likely due to intensive selection of horticultural traits resulting in population bottlenecks (Kerje and Grum 2000; Mliki et al. 2001; Staub et al. 2004). The Chinese and

standard reference accessions used herein where chosen because they circumscribe the known morphological and/or genetic (DNA-based) diversity in melon. The stark differences in DNA-based genetic variation between India and China (Table 4) lend support to the contention of the occurrence of possible bottlenecks and/or geographic or political isolation during more recent domestication after permanent trade routes were established (>700 AD) (Akashi et al. 2006; Lebeda et al. 2006; Robinson and Decker-Walters 1997). Such isolating events result in distinct gene pools that are sources of variation for melon improvement. Chinese melon market types are truly a rich source of genetic diversity. Knowledge of genetic affinities, along with historical and archeological data, allows for a more strategic deployment of this gene pool for plant improvement.

Management of large collections

Molecular-marker analysis of large germplasm collections may also foster curatorial efficiency and effectiveness through the creation of "core collections" (Frankel 1984). Based on isozyme analyses, historical and geographical information, and disease-evaluation data, a functional core collection was, in fact, constructed for *C. sativus* within the U.S. National Plant Germplasm System (US NPGS) (Staub et al. 2002). The RAPD marker array (SMR) used herein provided critical initial information for the establishment of a core collection of Spanish landrace melons (López-Sesé et al. 2002). Similarly, reference accessions (SRA) that circumscribe the genetic diversity of melon germplasm from primary, secondary, and tertiary centers of diversity have also been identified with the same standard RAPD array (McCreight et al. 2004; Mliki et al. 2001; Nakata et al. 2005; Staub et al. 2000; and accessions in Staub et al. 2004 used herein). Additional melon reference accessions (10–15) from Turkey, an important secondary center of diversity, can be drawn from a recent analysis [i.e., 56 accessions examined by 109 RAPD markers (33 primers)] reported by Sensoy et al. (2007). These reference accessions along with those identified herein could be used to designate a core collection for melon (~120 accessions). Many of the ~4,000 genetically diverse accessions in the US NPGS have accompanying passport and evaluation data, but have not been marker-genotyped. If these accessions were genotyped with the standard RAPD marker array, then an initial working core collection of perhaps 250 accessions [120 (previous studies and those identified herein) + 130 US NPGS] could be established based on marker profiles and phenotypic variation.

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