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Genetic diversity of Japanese melon cultivars (*Cucumis melo* L.) as assessed by random amplified polymorphic DNA and simple sequence repeat markers*

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Abstract

The genetic diversity among 67 melon (*C. melo* L.) cultivars from five Japanese seed companies was assessed using 25 10-mer RAPD primers (56 bands) and nine SSR (36 alleles) markers. These cultivars belong to three horticultural varieties (synom. Groups) spanning eight melon market classes: Group Cantalupensis (market classes Earl's, House, Galia, Charentais, and Ogen), Group Inodorus [Honeydew and Casaba melons (market classes Amarillo, Piel de Sapo, Rochet, Negro, Crenshaw, and Tendral)], and Group Conomon (market class Oriental). Genetic variation among these cultivars was compared to variation in a reference array (RA) consisting of 34 selected melon accessions from previous studies. Cluster analysis resulted in 11 of 15 Japanese Oriental accessions forming a group with South African RA accessions. The remaining Group Conomon Japanese accessions grouped either with Casaba or with Honeydew cultivars. Japanese Group Conomon accessions and South African RA accessions formed a genetic group that was distinct from all other accessions studied, and suggests either an Asiatic origin for the South African melon germplasm examined or an independent domestication involving similar ancestors. The majority of Japanese House and Earl market class accessions shared genetic affinities, and were genetically different from the Japanese Group Inodorus accessions examined. These Japanese accessions were most similar to Casaba RA accessions. Japanese Galia accessions were similar to either House and Earl's market classes or to Galia, Ogen, Casaba, and Honeydew RA accessions. Genetic differences exist between melon types that were domesticated from wild, 'free-living' subspecies *agrestis* and from *melo*.

Introduction

Melon (*Cucumis melo* L.; $2n = 2x = 24$, Cucurbitaceae) is a morphologically diverse, outcrossing horticultural crop of broad economic importance (Kirkbride 1993). Although the origin of melon is in dispute, most authorities consider that it originated in Africa (Robinson and Decker-Walters 1997). *C. melo* varies widely in leaf, vine, plant habit, and fruit characters, and this morphological diversity allowed Naudin (1859) to subdivided the species into 10 groups denominated as botanical varieties or tribes. Attempts have been made to

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simplify Naudin's classification (Grebenščikov 1953; Pangalo 1929). Munger and Robinson's (1991) most recent revision includes the following groups where trinomial names exist for each: (1) *C. melo agrestis* Naud. (wild melon); (2) *C. melo flexuosus* Naud. (snake melon); (3) *C. melo conomon* Mak. (pickling melon, Chinese white cucumber); (4) *C. melo cantalupensis* Naud. (cantaloupe or muskmelon); (5) *C. melo inodorus* Naud. (winter melons, honeydew, Casaba); (6) *C. melo chito* (mango melon) and *dudaim* Naud. (Queen's pocket melon); and (7) *C. melo momordica* (Phoot or snap melon). More recently, Pitrat et al. (2000) proposed a synthesis of the infraspecific classification of melons based on the identification of the different synonymous epithets used in the literature. They identified 16 groups, where five were assigned to subsp. *agrestis* Jeffrey and 11 to the subsp. *melo* Jeffrey. Those groups are denominated *varietas* or *variety* following the International Code of Botanical Nomenclature (ICBN) rules for the lower-ranking taxa (Greuter et al. 2000; Pitrat et al. 2000). Subspecies *agrestis* include, among others, the varieties *conomon* Thunberg and *momordica* Roxburgh, and subspecies *melo* include the varieties *cantalupensis* Naud., *inodorus* Jacquin, *flexuosus* L., *dudaim* L., and *chito* Morren (Pitrat et al. 2000). However, because this taxonomic classification is not universally accepted, and in order to simplify terminology according to groupings in previous works (Mliki et al. 2001; Staub et al. 2000) and based on Munger and Robinson (1991), we designate these varieties herein as horticultural groups (i.e., Group Cantalupensis, Inodorus, etc.)

Wild forms of *C. melo* subsp. *agrestis* are found south of the Sahara, in eastern tropical Africa (Whitaker and Bemis 1976) and throughout the Old World tropics. Wild forms of *C. melo* subsp. *melo* can be found in the Middle East and Asia (Staub et al. 1987; McCreight et al. 1993; McCreight and Staub 1993; Rubatzky and Yamaguchi 1997). Wild *C. melo* subsp. *agrestis* forms are morphologically distinct from the *C. melo* landraces consumed by endemic African cultures which are themselves genetically distant from European and US commercial melon (Mliki et al. 2001).

Groups Flexuosus, Conomon, and Dudaim are mostly likely indigenous to Middle East (e.g., Armenia), Asia, and Africa, respectively (Nayar and Singh 1998). Based on ancient Asian trade routes, it is possible that melon germplasm could have been exchanged from Southest Asia to North China and Japan as early as 200 BC (Curtin 1984). Melon of Chinese and Japanese origin was then later imported to South Asia in the 17th century AD.

Groups Cantalupensis and Inodorus are of commercial importance in the United States and Europe as well as in Mediterranean and Asian countries (McCreight et al. 1993; Table 1). Their diverse fruit morphology allowed for further partition into important market classes (Staub et al. 2000) which might be considered as putative cultivar-groups in future classification based on one or more distinctive criteria (Spooner et al. 2003). For example, within the Group Cantalupensis, the popular market classes Charentais, Shipper (US Western and European), Ogen, Galia, and Japanese Earl's and House might be designated as cultivar-groups. While there is a substantial introgression of British melon germaplasm in the pedigree of Earl type melons (Sakata and Sugiyama 2002), House types likely originated in isolation on the Japanese mainland in the 9th century. Likewise, Group Inodorus includes Honeydew and a broad array of Casaba melons such as Rochet, Piel de Sapo, and Amarillo, might also be designated as cultivar-groups.

The genetic diversity of several commercially important melon groups (principally Groups Cantalupensis and Inodorus) has been characterized using molecular analyses (Staub et al. 1997; Silberstein et al. 1999; Stepansky et al. 1999). Simple sequence repeat (SSR) and random amplified polymorphic DNA (RAPD) markers have been used to differentiate elite melon germplasm (Katzir et al. 1996; García et al. 1998), and Spanish melon landraces (López-Sesé et al. 2002). Likewise, Mliki et al. (2001) used these marker types to define the diversity among African melon accessions and genetic differences between African and US/European melon market classes.

Japanese Group Cantalupensis, Inodorus and Conomon melons differ morphologically from African accessions and commercial US/European melon germplasm (RA), and the genetic diversity among Japanese melon accessions has not been rigorously assessed. In order to facilitate the

Table 1. Plant habit, flowering and fruit characteristics of Japanese melon (*Cucumis melo* L.) accessions.

Horticultural variety (Group)[a]	Market class (type)[b]	Fruit traits						Relative days to anthesis	Relative canopy size[f]	Sex expression
		Diameter (cm)	Weight (kg)	Shape	Flesh color	Netting or Striping[e]	Relative days to maturity			
Cantaloupensis	House	10–12	1.0–1.5	Globular	Green	P	100–120	50–57	Medium	A[g]
Cantaloupensis	Earl's	11–13	1.3–1.6	Globular	Yellow	P	110–120	55–58	Medium	A
Cantaloupensis	Charentais	10–12	0.8–1.2	Globular	Orange	A or P	85–95	30–40	Large	A
Cantaloupensis	Galia	10–12	0.8–1.2	Globular	Green	A	80–90	30–40	Large	SA
Cantaloupensis	Ogen	10–12	0.7–1.2	Globular	Green	P	85–95	25–40	Medium	SA
Cantaloupensis	US Western Shipper	10–14	1.0–1.5	Globular	Orange	A	85–95	25–35	Large	A
Cantaloupensis	European Shipper[c]	10–12	0.8–1.2	Globular	Orange	A or P	85–95	30–40	Large	SA
Cantaloupensis	US Eastern Market	12–15	1.2–2.0	Globular	Orange	A/P	80–90	25–35	Large	A
Conomon	Oriental	8–10	0.6–2.0	Globular/Ovoid	White/Orange	A, N	90–110	35–45	Medium	A
Conomon	Oriental	5–6	0.8–1.5	Elongate	White	A, N	80–90	25–35	Medium	SA
Flexuosus		2–4	0.5–0.8	Elongate	White	A, N, C	80–90	25–35	Large	A
Inodorus	Rochet (Casaba)	13–16	1.5–2.5	Elongate	White	A or P, N, C	85–95	30–40	Large	SA
Inodorus	Negro (Casaba)	13–16	1.5–2.5	Elongate	White	A, N, C	80–90	30–40	Large	A
Inodorus	Amarillo (Casaba)	14–17	1.5–2.0	Ovoid	White	A, N	80–90	30–40	Medium	A
Inodorus	Piel de Sapo (Casaba)	13–16	1.5–2.5	Elongate	White	A, N, C	80–90	30–40	Large	A
Inodorus	Crenshaw (Casaba)	13–16	1.5–2.5	Tear drop[d]	White	A, N, C	80–90	30–40	Large	A
Inodorus	Tendral (Casaba)	13–16	1.5–2.5	Elongate/Ovoid	White	A, N, C	80–90	30–40	Medium	A
Inodorus	Honeydew type	14–16	1.5–2.5	Globular	White	A, N	85–95	30–40	Large	A

[a] According to Staub et al. (2000) based on Munger and Robinson (1991).
[b] Epidermal fruit coloration at maturity for Casaba market classes are: Rochet is green with yellow speckles/spots; Negro is black; Amarillo is yellow; Piel de Sapo is green with dark green blotches; Crenshaw is cream/tan to yellowish green; and Tendral is dark green.
[c] Although unique in shelf-life characteristics, fruit morphology can be either like Galia or Charentais market classes depending on market.
[d] Fruit larger in diameter near the blossom-end.
[e] A – absent; P – present; A/P – mostly absent, but some stripping can be detected; A or P – variable presence or absence; N – netting apparent; and C – corrugated.
[f] Relative size, where medium denotes canopies <1.0 m, and large >1.0 m in diameter.
[g] A – andromonoecious (male and hermaphroditic flowers); and SA – strongly andromonoecious (a greater proportion of hermaphroditic flowers).

effective use of melon diversity for plant improvement it is critical to determine the genetic relationships among these economically important melon germplasm pools (i.e., US, European, African, and Japanese). Therefore, we designed an experiment employing RAPD and SSR markers to: (1) assess the genetic variation in Japanese melon cultivars, and; (2) compare that variation to variation previously defined in a set of African (Mliki et al. 2001), and US and European reference accessions (Staub et al. 2000).

Materials and methods

Germplasm

Genetic variation in 101 *C. melo* accessions was examined (Table 2). Seeds of 67 cultivars from five Japanese seed companies [Sakata (59), Yokohama Ueki (2), Nihon Engei Kenkyuukai (1), Kobayashi (4), and Tohoku (1)] were obtained for molecular analyses (nos. 1–67). These cultivars belonged to the Groups Inodorus (13), Cantalupensis (39), and Conomon (15), and spanned eight market classes: Earl's, House, Galia, Charentais, and Ogen as Group Cantalupensis types, the Group Inodorus types Honeydew and Casaba (Amarillo, Piel de Sapo, Rochet, Negro, Crenshaw, and Tendral market classes), and Oriental market class in the Group Conomon (Table 1).

A reference array (RA) of 34 accessions (nos. 68–101) drawn from previous analyses of African (Mliki et al. 2001; nos. 68–82) and commercial US and European germplasm (Staub et al. 2000; nos. 83–101) was used for comparative analysis (Table 2). Seeds of African accessions were received from the US Department of Agriculture, North Central Regional Plant Introduction Station, Ames, Iowa. Seed of commercial germplasm used in a previous study (Staub et al. 2000) was obtained from five seed companies: Rijk Zwaan Seeds (De Lier, The Netherlands), Leen de Mos BV [Granvendzade, The Netherlands (now Numhems Seeds)], Zaadunie BV [Enkuizen, The Netherlands (now Syngenta)], Peto Seed Company [Woodland, CA (now Seminis)], and Harris Moran Seed (Modesto, CA). This germplasm represented accessions from four subsp. *melo* Groups (Cantalupensis, Conomon, Inodorus, and Flexuosus), and consisted of a diversity array of US and European Cantalupensis and Inodorus market classes: Galia, Ogen, Charentais, US and European Shipper, Honeydew, and Casaba. Fruit of accessions of African origin are of diverse shapes, sizes, and epidermis and mesocarp colors (Mliki et al. 2001), and do not fit into either typical US or European market classes (Table 1).

DNA extraction

Fifteen to 20 seeds of each accession were germinated in vermiculite under identical conditions (20–24 °C, 300 μmol m^2 s^{-1}; 16-h photoperiod) in a greenhouse at the University of Wisconsin, Madison, WI. Genomic DNA was extracted from tissue sampled at the two- to three-leaf stage employing a CTAB procedure (Maniatis et al. 1982) modified according to Staub et al. (1996) by using 2-β-mercaptoethanol. The bulked DNA from 15 plants of each accession was quantified on a Hoefer TKO 100 mini-fluorometer (Hoefer Scientific Instruments, San Francisco, CA) following the manufacturer's protocol, and the final DNA concentration was adjusted to 3 ng/μL with 0.1 M Tris buffer.

RAPD amplification

Twenty-five 10-mer primers were purchased either from Operon Technologies (OP; Alameda, CA) or the University of British Columbia (BC; Vancouver, BC, Canada). These primers were chosen based on the level of polymorphism observed in previous melon diversity analyses (Staub et al. 2000) and their predictable genetic basis (Staub 2001). All polymerase chain reaction (PCR) solutions were purchased from Promega (Madison, WI), and PCR was performed according to Staub et al. (1996). The optimized reaction contained 15 ng DNA, 0.3 mM primer, 0.3 mM dNTPs, 4.0 mM $MgCl_2$, commercial Taq DNA polymerase buffer, and one unit of Taq DNA polymerase in a 15-μL final volume. Amplification reactions were performed in a Perkin-Elmer Gene AmpPCR System 9600 thermocycler (Norwalk, CT). After 1 min of heating at 94 °C, amplifications were performed under the following regime: 50 cycles of 93 °C, 15 s for denaturing, 35 °C, 45 s annealing, and 72 °C, 60 s for extension. After amplification, 7 μL of loading dye (0.25% bromophenol blue,

Table 2. Melon (*Cucumis melo* L.) germplasm used for genetic diversity comparisons.

Origin[a]	No.	Seed source[b]	Accession name	Horticultural variety (Group)	Genetic Type[c]	ID[d]	Market class	Cluster analysis[e]	
								A	B
Japan (Jpn)	1	Sakata	Earl's knight sohshunbanshu	Cantaloupensis	C	E	Earl's	10	14
	2	Sakata	Earl's knight shyunzyu	Cantaloupensis	C	E	Earl's	10	14
	3	Yokohama ueki	Earl's miyabi	Cantaloupensis	C	E	Earl's	10	14
	4	Sakata	Sk6-175	Cantaloupensis	C	H	House	11	14
	5	Sakata	Florence	Cantaloupensis	C	H	House	11	15
	6	Sakata	Sk5-167	Cantaloupensis	C	H	House	9	15
	7	Yokohama ueki	Quincy	Cantaloupensis	C	H	House	11	15
	8	Sakata	Sk5-326	Cantaloupensis	C	G	Galia	3	7
	9	Sakata	Gordes	Cantaloupensis	C	Ch	Charentais	7	12
	10	Sakata	Casals	Cantaloupensis	C	Ch	Charentais	7	12
	11	Nihon engei kenkyuukai	Takami	Cantaloupensis	C	H	House	9	15
	12	Sakata	Honeydew PF	Inodorus	C	CH	Honey dew	5	10
	13	Sakata	Marco polo	Inodorus	C	CH	Honey dew	5	10
	14	Kobayashi	Homerun star	Inodorus	C	CH	Honey dew	5	10
	15	Sakata	Utopia	Inodorus	C	CA	Casaba (Amarillo)	5	10
	16	Sakata	Resort	Conomon	C	OR	Oriental	5	10
	17	Sakata	Kinsyou2	Conomon	C	OR	Oriental	5	10
	18	Sakata	Kinsyou	Conomon	C	OR	Oriental	2	4
	19	Sakata	Andes	Cantaloupensis	C	H	House	9	15
	20	Sakata	Earl's knight natsu no.2	Cantaloupensis	C	E	Earl's	10	14
	21	Sakata	Prince	Conomon	C	OR	Oriental	2	4
	22	Sakata	Columbus	Conomon	C	OR	Oriental	2	4
	23	Sakata	Midori no Yousei	Cantaloupensis	C	H	House	9	15
	24	Sakata	Amur	Cantaloupensis	C	H	House	3	7
	25	Sakata	Cygnus	Cantaloupensis	C	H	House	9	15
	26	Sakata	Nile	Cantaloupensis	C	H	House	9	15
	27	Sakata	Volga	Cantaloupensis	C	H	House	11	15
	28	Sakata	A-one	Cantaloupensis	C	O	Ogen	3	7
	29	Sakata	Earl's cruise syunzyu	Cantaloupensis	C	E	Earl's	10	14
	30	Sakata	Earl's knight syunzyu no.2	Cantaloupensis	C	E	Earl's	10	14
	31	Sakata	Earl's knight natsu no.1	Cantaloupensis	C	E	Earl's	10	14
	32	Sakata	Earl's knight seika	Cantaloupensis	C	E	Earl's	10	14
	33	Sakata	Prince PF	Conomon	C	OR	Oriental	2	4
	34	Sakata	Prince PF6	Conomon	C	OR	Oriental	2	4
	35	Sakata	Prince PF17	Conomon	C	OR	Oriental	2	4
	36	Sakata	Prince PF19	Conomon	C	OR	Oriental	2	4
	37	Sakata	Sweet heart	Cantaloupensis	C	Ch	Charentais	7	11
	38	Sakata	Golden sweet 9	Conomon	C	OR	Oriental	2	4
	39	Sakata	New melon	Conomon	C	OR	Oriental	2	4
	40	Sakata	Vernet melon	Cantaloupensis	C	E	Earl's	8	13
	41	Sakata	Kenkyaku	Cantaloupensis	C	H	House	7	11
	42	Sakata	Kyouei	Cantaloupensis	C	H	House	9	15
	43	Sakata	Paradise	Cantaloupensis	C	USC	US Cantaloupe	11	15
	44	Sakata	Sweet surprise	Cantaloupensis	C	USC	US Cantaloupe	5	10
	45	Sakata	Rugger	Cantaloupensis	C	USC	US Cantaloupe	7	12

Table 2. Continued.

Origin[a]	No.	Seed source[b]	Accession name	Horticultural variety (Group)	Genetic Type[c]	ID[d]	Market class	Cluster analysis[e]	
								A	B
	46	Sakata	Judo	Cantaloupensis	C	G	Galia	3	7
	47	Sakata	Aikido	Cantaloupensis	C	G	Galia	3	7
	48	Sakata	Karate	Cantaloupensis	C	G	Galia	3	7
	49	Sakata	White rosa	Inodorus	C	CH	Honeydew	5	10
	50	Sakata	Temptation	Inodorus	C	CH	Honeydew	5	10
	51	Sakata	Sunrise	Cantaloupensis	C	G	Galia	11	15
	52	Sakata	Harvest king	Cantaloupensis	C	G	Galia	11	15
	53	Sakata	Concert	Cantaloupensis	C	G	Galia	11	15
	54	Sakata	Zilba	Cantaloupensis	C	G	Galia	3	10
	55	Sakata	Pegasus	Inodorus	C	CPS	Casaba (Piel de Sapo)	5	10
	56	Sakata	Sonic	Inodorus	C	CR	Casaba (Rochet)	5	10
	57	Sakata	Pelayo	Inodorus	C	CN	Casaba (Negro)	5	10
	58	Sakata	Carnival	Inodorus	C	CC	Casaba (Crenshaw)	5	10
	59	Sakata	Tucan	Inodorus	C	CA	Casaba (Amarillo)	5	10
	60	Sakata	Kinka	Inodorus	C	CT	Casaba (Tendral)	5	10
	61	Sakata	Eagle	Inodorus	C	CA	Casaba (Amarillo)	5	10
	62	Sakata	Parisien	Cantaloupensis	C	Ch	Charentais	7	11
	63	Sakata	Artix	Cantaloupensis	C	Ch	Charentais	7	11
	64	Tohoku	Ougon makuwa	Conomon	C	OR	Oriental	2	4
	65	Kobayashi	Inami pecchin uri	Conomon	C	OR	Oriental	2	4
	66	Kobayashi	Akapuruko	Conomon	C	OR	Oriental	5	10
	67	Kobayashi	Sankyuu	Conomon	C	OR	Oriental	5	10
Africa (Afr)	68	Senegal	PI 436534	NA	LR (RA)	NA	G22843		4
	69	Zimbabwe	PI 482411	NA	LR (RA)	NA	TGR554		1
	70	Zimbabwe	PI 482422	NA	LR (RA)	NA	TGR1687		3
	71	Mali	PI 490388	NA	LR (RA)	NA	Kankani		5
	72	Zambia	PI 505602	NA	LR (RA)	NA	ZM/A5384		3
	73	Morocco	PI 102077	NA	LR (RA)	NA	NA		5
	74	Egypt	PI 288233	NA	LR (RA)	NA	NA		5
	75	Egypt	PI 525103	NA	LR (RA)	NA	NO.22		5
	76	Libya	NA	NA	LR (RA)	NA	Adzhur		5
	77	Tunisia	NA	NA	LR (RA)	NA	Chaman/Machmoum		5
	78	Senegal	PI 274954	NA	LR (RA)	NA	NA		4
	79	Kenya	PI 385966	NA	LR (RA)	NA	Ein Dor		5
	80	Senegal	PI 436532	NA	LR (RA)	NA	G22841		4
	81	Zimbabwe	PI 482400	NA	LR (RA)	NA	TGR234		1
	82	Tunisia	NA	NA	LR (RA)	NA	Galaoui		5
US/EU (RA)	83	RZ	1	Cantalupesis	IL (RA)	G	Galia		7
	84	RZ	3	Cantalupesis	IL (RA)	G	Galia		7
	85	RZ	4	Cantalupesis	IL (RA)	O	Ogen		7
	86	RZ	6	Cantalupesis	IL (RA)	O	Ogen		7
	87	RZ	10	Cantalupesis	IL (RA)	G	Galia		7
	88	LM	'Printadoux', 23840	Cantalupesis	OP (RA)	Ch	Charentais		11
	89	LM	'Mango', 23848	Cantalupesis	IL (RA)	ES	European shipper		10
	90	LM	23851	Inodorus	IL (RA)	CH	Honeydew		7
	91	LM	24138	Inodorus	OP (RA)	CH	Honeydew		10
	92	Zu	M-473	Cantalupesis	IL (RA)	G	Galia		7
	93	Zu	M-442	Inodorus	IL (RA)	CR	Casaba (Rochet)		10
	94	Zu	M-126	Inodorus	IL (RA)	CPS	Casaba (Piel de Sapo)		10
	95	Zu	MY-124	Inodorus	IL (RA)	CA	Casaba (Yellow Canari)		10
	96	Peto	Dorado	Inodorus	F1 (RA)	CA	Casaba (Yellow Canari)		7
	97	Peto	Snake melon	Flexuosus	NA (RA)	Flex			10

Table 2. Continued.

Origin[a]	No.	Seed source[b]	Accession name	Horticultural variety (Group)	Genetic Type[c]	ID[d]	Market class	Cluster analysis[e]	
								A	B
	98	Peto	Freeman cucumber	Conomon	NA (RA)	Con	Oriental		4
	99	HM	Hmx 2608	Cantalupesis	IL (RA)	USE	US eastern market		11
	100	USDA	Top mark	Cantalupesis	OP (RA)	USW	US western market		15
	101	ARO	Galia (original)	Cantalupesis	F1 (RA)	G	Galia		7

[a] Accessions selected from previous and current studies: Japan (Jpn) – Japanese accessions; US/EU (RA) – US and European market reference array (Staub et al. 1997, 2000); Africa (Afr) – African landraces (Mliki et al. 2001).
[b] RZ – Rijk Zwaan Seeds, De Lier, The Netherlands; LM – Leen de Mos BV, Granvendzade, The Netherlands (now Nunhems BV); Zu – Zaadunie BV, Enkuizen (now Syngenta); Peto – Peto Seed Company (now Seminis), Woodland, CA; HM – Harris Moran Seed, Modesto, CA; USDA – United States Department of Agriculture, Agricultural Research Service, Salinas, CA; ARO – Agricultural Research Organization, Israel.
[c] C – cultivar; IL – inbred line; LR – landrace; OP – open pollinated variety; F1 – single cross hybrid; NA – not applicable; RA – reference accession used in all analyses abstracted from either Staub et al. (2000) or Mliki et al. (2001).
[d] ID – identification by cultivar-group as depicted in Figure 1 to include Earl's (E), House (H), US cantaloupe (USC), Galia (G), Charentais (Ch), Casaba-Honeydew (CH), Casaba-Piel de sapo (CPS), Casaba-Rochet (CR), Casaba-Negro (CN), Casaba-Crenshaw (CC), Casaba-Amarillo (CA), Oriental (OR) Cononom (Con), Flexuosus (Flex), Ogen (O), European Shipper (ES), US western shipper (USW), and US eastern market (USE).
[e] Cluster node of RAPD analyses as depicted in Figure 1 (Panels A and B).

0.25% xylene cyanol FF, 15% Ficol) was added to each reaction tube. PCR products were electrophoresed according to Horejsi and Staub (1999) in 1.6% agarose gels with 0.5 μg/mL of ethidium bromide in 0.5×TBE buffer (4.84% Tris, 2.28% boric acid, 0.30% EDTA) at 120 V using a Model H4 horizontal gel electrophoresis system (BRL, Life Technologies, Gaithersburg, MD) for 4.5 h. Gels were then photographed using GelExpert Software and its associated video system (NucleoTech Corporation, 1996, San Mateo, CA). *Hin*dIII+
*Eco*RI-digested λ DNA was used as a standard marker for estimating the size of PCR products by migration distance comparison.

Each polymorphic band considered as a marker was identified by its RAPD primer denomination and base-pair size given as a subscript (e.g., $OPB12_{500}$). Only consistent, reproducible, and Mendelian-inherited bands produced by these primers were scored (Staub 2001; Staub et al. 2000). Each polymorphic RAPD band was scored as either present (1) or absent (0).

SSR amplification

The 12 SSR markers used were: CMTC13, CMGA15, CMCT44, CMGA104, CMACC146, CMCTT144, CMTC47, CMAT141, CMCCA145, CMGT108, CMTC160a+b, and CMAT35. Their characteristics and primers information have been previously described by Katzir et al. (1996) and Danin-Poleg et al. (2001).

Amplification reactions of SSR loci were carried out in Madison WI as follows: 1 min of heating at 94 °C, followed by 15 s of denaturing at 93 °C, 65 °C, for annealing 45 s, and then extension at 72 °C for 60 s. After amplification, 7 μL of loading dye (0.25% bromophenol blue, 0.25% xylene cyanol FF, 15% Ficol) was added to each reaction tube. PCR products were electrophoresed in 2% agarose gels with 0.5 μg/mL of ethidium bromide in 0.5×TBE buffer (4.84% Tris, 2.28% boric acid, 0.30% EDTA) at 200 V. In order to separate adequately some PCR products, electrophoresis was performed on a DNA sequencing gel containing 6% polyacrylamide and 1×TBE at 1000v for 1.5 h, and stained with ethidium bromide. Gels were photographed using the same software and imaging system used for RAPD analysis. A 100-bp DNA ladder was used as a standard marker for estimating the size of PCR products by migration distance comparison. The presence and number of bands obtained (alleles) from amplifications at SSR loci were scored based on their relative mobility as present (1), absent (0), or

heterozygous (0.5) for each of two alleles observed per locus.

Statistical analysis

In order to describe genetic relationships among the Japanese accessions and between Japanese and RA accessions (Table 2), RAPD and SSR marker data were used to calculate genetic distance estimates and provide for matrix comparisons between both marker systems (Mantel 1967). The binary data matrix obtained from scoring polymorphic RAPD bands was used to calculate Jaccard similarity coefficients (Jaccard 1908), and to convert data to individual pairwise genetic distances (GD) matrices by calculating 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 were performed on genetic distance matrices, and relationships among accessions were visualized as dendrograms using the NTSYS-pc program version 1.8 (Rohlf 1997).

Concordances among different GD estimators [i.e., Jaccard's coefficient (Jaccard 1908), simple matching coefficient (Sokal and Sneath 1963), and Nei's distance D (Nei 1973, 1978)] were previously compared by Staub et al. (2000) (Spearman rank correlation coefficient, $r_s = 0.64$ to 0.99, $p > 0.0001$). The correlation between RAPD and SSR cluster analyses in this study and in previous work were positive correlated ($p < 0.05$). Therefore, based on the simplicity and minimal assumptions leading to GD estimation using Jaccard's coefficient (Jackson et al. 1989), and its use in previous melon diversity analyses (García et al. 1998; Staub et al. 2000; Mliki et al. 2001), we used Jaccard's coefficient for analyses described herein.

Comparisons of genetic distances and similarities among melon groups according to origin and horticultural group were made among Japanese melon market classes, and between these Japanese melons and African landraces and European and US market classes with the computer program POPGENE (Yeh et al. 1997). For RAPD band frequency comparisons, data of African, European and US melon accessions were taken from previous studies from which the RA were drawn (Staub et al. 2000; Mliki et al. 2001).

Results

Genetic relationships among Japanese germplasm

The 25 RAPD primers used to assess genetic diversity among 67 Japanese melon accessions provided 56 polymorphic reproducible bands for examination in this germplasm. The amplified fragments ranged in size from approximately 300 to 2300 bp, and were those used by Staub et al. (2000) and Mliki et al. (2001). The mean number of bands per primer was 2.4.

The corresponding allelic number (in parenthesis) of the nine polymorphic loci (total of 36 alleles, mean number of four alleles per locus) were designated: CMGA15 (4), CMCT44 (3), CMGA104 (5), CMGT108 (3), CMCTT144 (6), CMAT141 (4), CMCCA145 (5), CMACC146 (3), and CMTC160a+b (3).

Genetic distance (GD) matrices obtained independently for each marker system (RAPD and SSR) were significantly correlated ($r = 0.6, p < 0.01$). Based on this correlation, and the significant positive correlations and lower variation in RAPD-based GD estimates (standard errors of mean GDs for SSR and RAPD of 0.07–0.12 and 0.04–0.09, respectively) obtained in previous studies (Staub et al. 2000), specific GD comparisons and cluster analyses stated hereafter employ RAPD data matrices.

Cluster analysis (UPGMA) employing RAPD data resulted in a dendrogram with two main branches (Table 2; Figure 1A, nodes 1 and 2). Eleven of 15 Group Conomon accessions formed one cluster grouping (node 2). The other Group Conomon accessions grouped either with Casaba [node 5; nos. 16 and 17 (Sakata)] or with Honeydew [node 5; nos. 66 and 67 (Kobayashi)] cultivars. A House (no. 24) and Ogen (no. 28) accession grouped together with five Galia accessions (node 3). A major branch at node 7 consisted of a mixture of Charentais (5), US cantaloupe (1), and House (1) cultivars. While Earl's accession no. 40 was partitioned to a single cluster grouping (node 8), the remaining Earl's accessions (8) clustered into one group (node 10). Seven House accessions were separated from four other House accessions at node 9. That array of four House accessions (nos. 4, 5, 7, and 27) was more similar to three Galia (nos. 51–53, and 43) and one US cantaloupe type

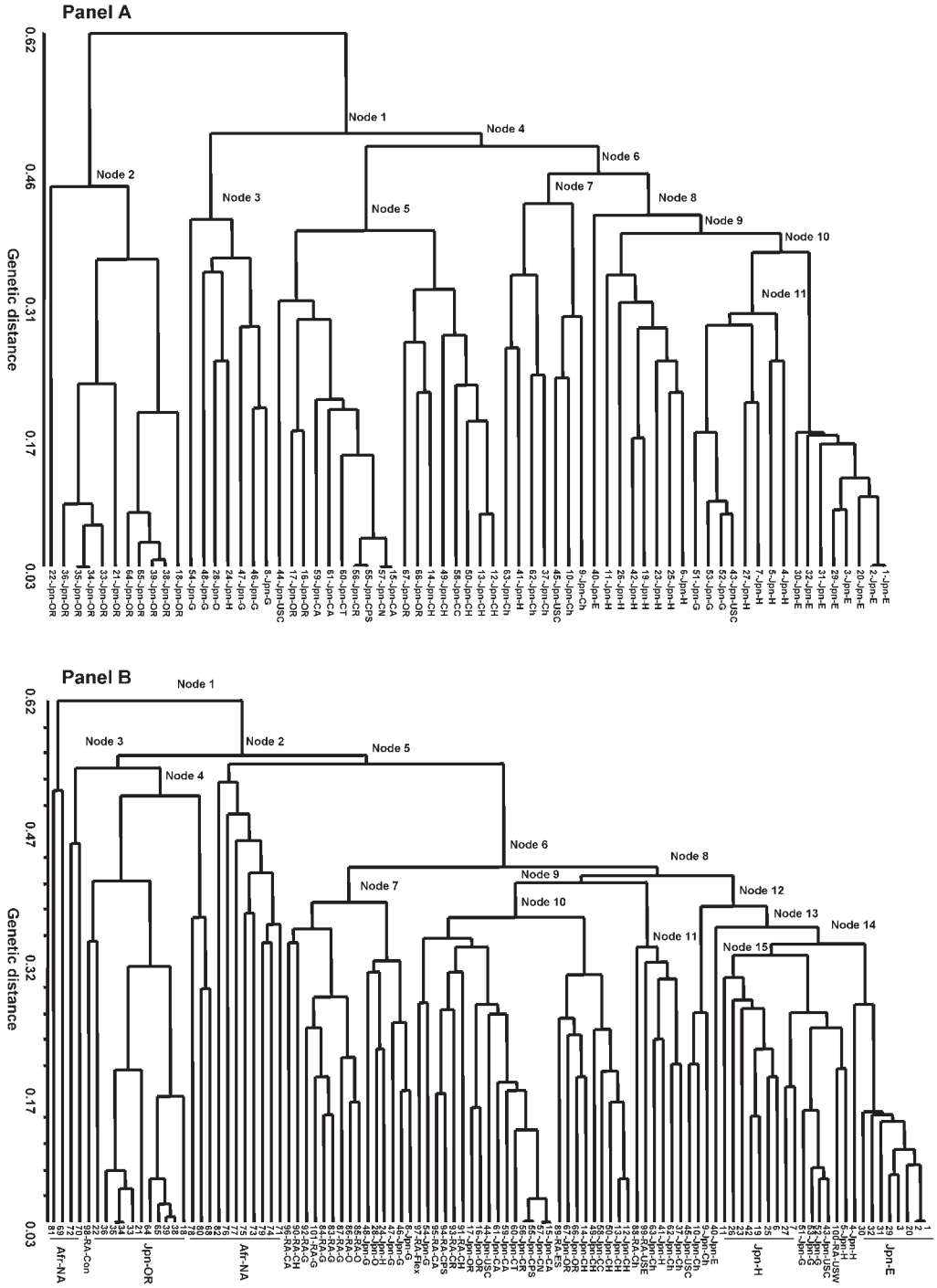

Figure 1. UPGMA Cluster analyses (Jaccard's coefficient) of *Cucumis melo* accessions as estimated by 56 RAPD bands resulting from 25 primers. (A) Analysis of 67 Japanese (Jpn) *C. melo* L. accessions of Earl's (E), House (H), US cantaloupe (USC), Galia (G), Charentais (Ch), Casaba-Honeydew (CH), Casaba-Piel de sapo (CPS), Casaba-Rochet (CR), Casaba-Negro (CN), Casaba-Crenshaw (CC), Casaba-Amarillo (CA), and Oriental (OR) cultivar-groups. (B) Analysis of Japanese and 34 reference array (RA) accessions to include Africa (15, NA), Cononom (Con), Flexuosus (Flex), and European (Ogen, O; European Shipper, ES) and US (US western shipper, USW; US eastern cultivars, USE) cultivar-groups (Tables 1 and 2).

(no. 43) than the other House accessions examined (node 9 *versus* node 11).

The average GD between any two pairs of accessions as estimated by RAPD variation was 0.63 ± 0.04 (data not presented). The average minimum and maximum average genetic distance between any paired contrast was 0.42 ± 0.06 and 0.89 ± 0.06, respectively. Genetic distances ranged between 0.02 [most related lines; accessions no. 1 *versus* no. 2 (Earl's), no. 15 *versus* no. 57 (Casaba), no. 34 *versus* no. 35 (Oriental), no. 38 *versus* no. 39 (Oriental), no. 55 *versus* 56 (Casaba), and no. 55 *versus* 57 (Casaba)] to 0.66 [distantly related lines; no. 9 (Charentais) *versus* no. 65 (Oriental), no. 12 (Honeydew) *versus* no. 64 (Oriental), and no. 64 *versus* no. 65]. The average GD distance between Earl's no. 40 (node 8) and the other Earl's accessions examined was 0.73 ± 0.04 (Figure 1A, GD data not presented). The mean GD between the House type accessions that clustered in node 9 and those House accessions that clustered in node 11 was 0.73 ± 0.06.

Genetic relationships between Japanese and reference accessions

A cluster analysis of Japanese and reference accessions resulted in a dendrogram with four major branches (Table 2; Figure 1B, nodes 1–3, and 5). At node 1, African accessions Zimbabwe nos. 69 and 81 were separated from the rest of the accessions. At node 2, all Oriental accessions (Group Conomon), the Group Conomon RA no. 98, and African accessions 68, 70, 72, 78, and 80 were grouped together, and were genetically distinct from the rest of the accessions examined. These accessions were further partitioned at node 3 [nos. 70 (Zimbabwe) and 72 (Zambia)] and node 4 [all Oriental accessions, RA no. 98, and African accessions from Senegal (nos. 68, 78, and 80)]. The North African RA (nos. 71, 73–77, and 82) formed a unique cluster at node 5. The Japanese House no. 24, Casaba RA no. 90 (Honeydew) and no. 96 (Amarillo), and all Ogen and Galia (except no. 54) accessions were partitioned at node 6. The reference accessions in this cluster grouping were separated from Japanese accessions at node 7.

A Group Flexuosus accession (RA no. 97) and an array of Japanese and Group Inodorous (Casaba and Honeydew), Oriental market class, and Group Cantalupensis (Charentais, House, and US Eastern market classes) RA accessions grouped together in a relatively large super cluster (31 accessions; node 9). This super cluster was further partitioned into two major clusters (nodes 10 and 11). Although the 25 accessions clustering together in node 10 were primarily Japanese and RA Casaba types, the cluster also contained the RA Flexuosus accession (no. 97), a Japanese Galia (no. 54), a US cantaloupe type (no. 44) originating from Japan, and two Japanese Oriental (nos. 16 and 17; Kobayashi Seed Company). The remaining six accessions in this super cluster grouped in node 11 and included Japanese and RA Charentais accessions, a US Eastern melon accession (no. 99), and a Japanese House accession (no. 41). The accessions clustering at node 9 differed genetically from the Earl's (except no. 41) and House market class accessions that clustered at node 12. Two Japanese Charentais accessions (nos. 9 and 10), a US cantaloupe originating from Japan (no. 45) and the US Western Shipping RA no. 100 also clustered in this super cluster consisting of 28 accessions.

Genetic affinities and variation within and among market classes

Comparative analysis of the frequency of polymorphisms (band presence *versus* band absence) among RAPD primers common to this study and that of Staub et al. (2000; US and European germplasm) and Mliki et al. (2001; African germplasm) is presented in Table 3. There were 21 primers used in common providing 50 polymorphic bands. In several cases the level of polymorphism detected in African landraces was appreciably lower than the other populations examined (e.g., $C1_{200}$, $I41_{200}$, $I16_{1600}$, $L18_{1700}$, $AG15_{110}$, $AG15_{750}$, $AK16_{1200}$, $AK16_{700}$, $AT5_{800}$, $AU2_{620}$, $AW10_{2200}$, and $AX16_{2000}$). In some instances the level of polymorphism in African landraces was dramatically higher than the other populations evaluated (e.g., $L18_{500}$, $AF14_{400}$, $AJ18_{1250}$, $AX16_{1200}$, $AX16_{1600}$, $BC299_{750}$, and $BC551_{550}$). In two instances ($C1_{600}$ and $AG15_{950}$) the frequency of bands present was notably higher in the Japanese population than recorded in the other populations examined. In five cases ($D7_{1350}$, $D7_{1250}$, $AT1_{1300}$, $BC299_{750}$, and

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]				No.	Primer designation	RAPD frequency % (band presence)			
		Japan	Africa[c]	Europe[d]	USA[d]			Japan	Africa	Europe	USA
1	$C1_{1200}$	69	14	54	50	26	$AT1_{1300}$	13	27	38	33
2	$C1_{920}$	91	71	100	83	27	$AT1_{1100}$	100	87	100	100
3	$C1_{600}$	85	100	100	83	28	$AT1_{650}$	85	73	46	50
4	$C1_{300}$	25	0	0	0	29	$AT5_{800}$	87	0	69	67
5	$D7_{1350}$	18	64	62	100	30	$AT5_{500}$	72	40	54	83
6	$D7_{1250}$	60	82	92	83	31	$AT15_{850}$	91	47	92	83
7	$D7_{1050}$	91	100	62	100	32	$AT15_{300}$	78	40	100	100
8	$I4_{1200}$	75	27	62	67	33	$AU2_{850}$	7	40	0	17
9	$I4_{900}$	69	53	62	100	34	$AU2_{650}$	46	73	62	50
10	$I16_{1850}$	57	53	46	100	35	$AU2_{620}$	100	53	100	100
11	$I16_{1600}$	51	13	85	80	36	$AW10_{2200}$	25	13	15	17
12	$I16_{950}$	60	60	92	40	37	$AW10_{1200}$	91	87	100	83
13	$L18_{1700}$	48	7	100	67	38	$AW14_{1000}$	27	20	8	50
14	$L18_{500}$	57	80	46	50	39	$AX16_{2000}$	73	47	92	83
15	$AF14_{2200}$	85	60	77	100	40	$AX16_{1600}$	64	93	31	33
16	$AF14_{750}$	100	87	85	67	41	$AX16_{1200}$	57	87	23	50
17	$AF14_{400}$	37	53	15	20	42	$BC231_{2100}$	55	67	83	40
18	$AG15_{1100}$	57	20	100	100	43	$BC299_{750}$	18	80	62	40
19	$AG15_{950}$	99	73	38	0	44	$BC318_{2200}$	81	53	100	100
20	$AG15_{750}$	45	20	42	67	45	$BC318_{900}$	100	93	100	100
21	$AJ18_{1250}$	15	40	0	0	46	$BC388_{1200}$	45	73	77	83
22	$AJ18_{850}$	54	47	38	33	47	$BC526_{1500}$	4	20	0	0
23	$AK16_{1200}$	93	27	100	83	48	$BC551_{700}$	60	53	46	67
24	$AK16_{700}$	100	27	100	100	49	$BC551_{550}$	21	53	0	17
25	$AK16_{650}$	85	73	92	83	50	$BC551_{300}$	33	20	15	33

[a] Primers B to AX obtained from Operon Technologies Inc., Alameda, CA, USA, 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 in as a subscript after a primers (e.g., $B12_{500}$ designates a 500-base pair band amplified from the B12 primer).
[c] African accessions used previously in Mliki et al. 2001.
[d] European and USA accessions used previously in Staub et al. (2000).

$BC388_{1200}$) the frequency of bands were lower than other populations. Although dramatic differences were observed between the frequency of the band presence in European and US germplasm, neither population demonstrated a distinct trend in this regard.

The level of molecular polymorphism within any Japanese market class was highest in the Oriental class [79 (RAPD) to 89% (SSR)]. Japanese House and Charentais market classes were also moderately polymorphic [56 (RAPD) to 67% (SSR)] (data not presented). In contrast, Earl's type accessions possessed relatively few polymorphisms [11 (SSR) to 38% (RAPD)]. In certain instances the polymorphism level detected by RAPD markers was higher than that recorded in SSR markers (Japanese Galia, Earl's, and House types), possibly due to the number of loci examined. There were, however, also cases in certain market classes where the genetic variation detected by SSR markers was higher than RAPD markers (i.e., Japanese Casaba, and Honeydew).

The highest estimates for number of alleles per locus [1.9 (RAPD) to 3.4 (SSR)] and polymorphism level [87 (RAPD) to 100 (SSR) %] were recorded in African accessions (data not presented). The observed number of homozygotes was higher than expected for the SSR loci examined.

RAPD analysis indicated that Galia accessions were the most genetically diverse of the European

and US market classes examined. This observation was supported by the assessment of these Galia accessions using SSR markers (data not presented). Genetic variation in Casaba and Charentais was relatively high (polymorphism ranged between 51 and 59%) as measured by both marker systems when compared to the other market classes examined. A notable exception was the high level of polymorphism detected in US Western Shipping types (94%) by SSR analysis. Genetic diversity in European Shipper accessions was low (~28%) when compared to other European and US market classes examined regardless of marker type.

Genetic distances between Earl's and other market classes (GD = 0.49 ± 0.11) examined were comparatively large (Table 4). The GD (RAPD-based estimates in parentheses) between African landraces and European RA (0.51), African landraces and Japanese Galia (0.48), Japanese Honeydew and Group Flexuosus (0.54), Japanese House and Group Flexuosus (0.48), US Western Shipping and Group Flexusous (0.48), European RA and Group Flexuosus (0.50), Ogen RA and Group Flexuosus (0.50), Japanese Honeydew and Charentais RA (0.47), and US Eastern RA and Honedew RA (0.50) was relatively large. In contrast, the GD between Japanese Casaba and Japanese House (0.11), Japanese Casaba and Japanese Ogen (0.13), Japanese House and Ogen RA (0.05), Japanese Oriental and Galia RA (0.10), Japanese Oriental and Conomon RA (0.11), Japanese House and Japanese Ogen (0.14), Japanese US Cantaloupe and Conomon RA (0.14), Casaba RA and Conomon RA (0.14) was relatively small.

Discussion

Initial estimates of genetic variation detected by RAPDs was relatively low (18.3%) (Baudracco-Arnas and Pitrat 1996). However, more recent studies have identified higher levels of polymorphism in elite commercial germplasm from a restricted origin (49%) (García et al. 1998), and Spanish Group Inodorous market classes (25%) (López-Sesé et al. 2002). In our study, we detected high levels of polymorphism in African landraces (86%) and in several commercial market classes of European and Middle East origin (>51%) (Casaba, Charentais, and Galia). Even though the diversity among European Ogen, European Honeydew and US accessions was comparatively low (19 to 27%), the genetic variation detected by either marker type adequately allowed for discrimination among market classes (Figure 1B).

South and North Africa landraces differ in fruit morphologies and are genetically distinct (Mliki et al. 2001). Our data confirm the results of studies by García et al. (1998), Mliki et al. (2001), Staub et al. (1997, 2000), and Stepansky et al. (1999) regarding genetic relationships among commercial melon types and African landraces (Figure 1). In addition, our study expands this genetic appraisal by defining major Japanese cultivars and their relationship to previously reported US and European commercial market classes described by Staub et al. (2000) and African landraces characterized by Mliki et al. (2001). Moreover, our results clearly show the distinctions between types domesticated from wild subspecies *agrestis* (Figure 1A node 2, Figure 1B node 1–4) and from wild subspecies *melo* (Figure 1B nodes 3–11, and Figure 1, nodes 5–14).

Genetically similar House and Earl's types are unique, and differ from Oriental types (Table 4; Figure 1A). Oriental types were in turn more similar to RA accessions of South African origin than they were to all other accessions examined (Figure 1B). The fruits of South African landrace RA accessions are relatively small (8–10 cm in diameter), and are morphologically similar to Group Conomon (e.g., RA no. 98) and Japanese Oriental cultivars examined (Table 1; Mliki et al. 2001). This observation and the results of RAPD analysis preformed (Figure 1B) suggests that this germplasm shares genetic affinities that would not have been predicted based on the proposed Asiatic origin (China and Japan) of Group Conomon germplasm (Robinson and Decker-Walters 1997), and the genetic relationships between Indian and Asiatic melon landraces and market classes (Akashi et al. 2002). If in fact the lineage of Asiatic Group Conomon cultivars originated in China and/or Japan about 1000 years ago, the presence of Group Conomon-like germplasm in South African might be explained by the introduction of Asiatic Group Conomon germplasm (cultivars) via early trade routes (i.e., China/Japan and/or India) and their subsequent escape from cultivation to evolve into different landraces. This

Table 4. Genetic distance measures among 20 groups including Japanese, African, and European and US melons (*Cucumis melo* L.) measured by RAPD markers [Jaccard's coefficient genetic identity (above diagonal) and genetic distance (below diagonal) (Jaccard 1908)].

	1[a]	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	****	0.66	0.68	0.64	0.62	0.63	0.70	0.70	0.75	0.72	0.71	0.65	0.71	0.60	0.79	0.70	0.69	0.67	0.65	0.61
2	0.41	****	0.80	0.66	0.82	0.81	0.90	0.87	0.73	0.81	0.75	0.72	0.78	0.70	0.65	0.78	0.75	0.83	0.80	0.83
3	0.38	0.22	****	0.69	0.74	0.74	0.79	0.78	0.76	0.69	0.70	0.66	0.72	0.66	0.68	0.73	0.64	0.79	0.79	0.74
4	0.44	0.41	0.37	****	0.66	0.66	0.68	0.73	0.54	0.64	0.56	0.49	0.62	0.54	0.71	0.55	0.54	0.63	0.64	0.62
5	0.48	0.19	0.30	0.42	****	0.74	0.76	0.83	0.75	0.76	0.72	0.74	0.76	0.69	0.67	0.75	0.72	0.76	0.79	0.84
6	0.46	0.21	0.30	0.42	0.30	****	0.82	0.80	0.66	0.67	0.68	0.62	0.67	0.69	0.58	0.68	0.69	0.75	0.75	0.77
7	0.36	0.11	0.23	0.39	0.28	0.20	****	0.87	0.70	0.78	0.78	0.63	0.74	0.75	0.62	0.71	0.69	0.95	0.83	0.81
8	0.36	0.13	0.25	0.32	0.19	0.23	0.14	****	0.71	0.83	0.75	0.69	0.78	0.66	0.70	0.77	0.73	0.85	0.75	0.78
9	0.28	0.32	0.27	0.61	0.29	0.42	0.36	0.34	****	0.81	0.85	0.79	0.89	0.72	0.81	0.91	0.80	0.68	0.74	0.74
10	0.32	0.21	0.36	0.44	0.28	0.40	0.24	0.19	0.22	****	0.77	0.68	0.87	0.67	0.70	0.82	0.73	0.74	0.70	0.77
11	0.34	0.28	0.35	0.58	0.33	0.39	0.25	0.28	0.16	0.26	****	0.74	0.87	0.80	0.76	0.80	0.79	0.78	0.68	0.70
12	0.43	0.33	0.42	0.71	0.31	0.47	0.46	0.37	0.23	0.38	0.31	****	0.76	0.64	0.72	0.78	0.85	0.66	0.62	0.66
13	0.34	0.24	0.33	0.47	0.27	0.40	0.30	0.25	0.11	0.14	0.14	0.28	****	0.80	0.82	0.85	0.78	0.72	0.71	0.76
14	0.51	0.36	0.42	0.61	0.37	0.37	0.29	0.41	0.33	0.40	0.22	0.44	0.23	****	0.61	0.65	0.69	0.76	0.67	0.66
15	0.24	0.43	0.39	0.34	0.40	0.54	0.48	0.36	0.21	0.36	0.28	0.33	0.20	0.50	****	0.73	0.74	0.61	0.63	0.62
16	0.35	0.25	0.31	0.60	0.28	0.39	0.34	0.27	0.10	0.20	0.22	0.25	0.16	0.43	0.31	****	0.80	0.68	0.69	0.78
17	0.38	0.29	0.45	0.62	0.32	0.37	0.37	0.31	0.22	0.31	0.24	0.16	0.25	0.37	0.31	0.23	****	0.67	0.61	0.70
18	0.40	0.19	0.24	0.47	0.27	0.29	0.05	0.16	0.39	0.30	0.25	0.41	0.32	0.28	0.50	0.38	0.40	****	0.76	0.77
19	0.43	0.22	0.24	0.45	0.24	0.28	0.19	0.29	0.31	0.35	0.38	0.48	0.34	0.40	0.46	0.37	0.50	0.28	****	0.82
20	0.50	0.19	0.30	0.47	0.18	0.26	0.21	0.25	0.31	0.26	0.36	0.42	0.28	0.42	0.48	0.25	0.35	0.26	0.20	****

[a] Group number: (1) African (NA); (2) Casaba (Japan); (3) Charentais (Japan); (4) Earl's (Japan); (5) Galia (Japan); (6) Honeydew (Japan); (7) House (Japan); (8) Ogen (Japan); (9) Oriental (Japan); (10) US Cantaloupe (Japan); (11) Casaba (Europe); (12) Charentais (Europe); (13) Conomon; (14) European Shipper; (15) Flexuosus; (16) Galia (Europe); (17) Honeydew (Europe); (18) Ogen (Europe); (19) US Eastern Market; and (20) US Western Shipper.

similarity, however, could be also the result of independent domestication from the same subspecies, i.e., *agrestis*.

North African RA accessions were genetically distinct from the South African RA accessions, the Group Conomon RA accession, and the Japanese Oriental market classes examined (Figure 1B). Although North African RA accessions differed from the other accessions examined, they did share some genetic affinities with US, European, and Japanese Earl's and House market classes. The shared genetic similarities between African and US/European market classes agrees with the results of Mliki et al. (2001), and defines the genetic relationship between this germplasm and the Japanese market classes examined. It is likely that North African and European germplasm share a common lineage which is not of Asiatic origin, possibly due to domestication from wild forms of subspecies *melo*.

Relatively large genetic differences exist between the Group Inodorus and Group Cantalupensis accessions examined (Figure 1B). Our results agree with those of Staub et al. (1997, 2000) and provide information for the development of hypotheses regarding their evolution as distinct market classes. Casaba types originated in Asia Minor and were transported to central and southern Europe where they subsequently refined to form the modern Casaba cultivars known today.

Genetic affinities were also detected among the Group Flexuous RA accession, Japanese Casaba cultivars, and Japanese Group Conomon Oriental market class accessions from Sakata ['Resort' (no. 16) and 'Kinsyou 2' (no. 17)] and Kobayashi ['Akapuruko' (no. 66) and 'Sankyuu (no. 67)] seed companies (Figure 1B, node 10). Those Conomon accessions are unique and likely have received introgression from the Inodorus Group. It is clear that the Kobayashi and Sakata Oriental accessions studied are genetically different, and might be used in breeding to increase the diversity of the Japanese Oriental germplasm pools examined.

Accessions in these germplasm pools (Figure 1B, node 10) differed genetically from the Japanese and RA Galia, Ogen market class accessions, and the RA Honeydew and Casaba accessions used in this analysis (node 7). Given the disparate genetic relationship between Japanese and Israeli Galia accessions, it is unlikely that the Japanese Galia types examined were derived directly by intensive backcrossing using Israeli Galia types as recurrent parents. This hypothesis is supported by the fact that Galia types are themselves genetically diverse (Staub et al. 2000) and the development of Japanese Galia types has historically involved complex crossing with Ogen, Earl's and House market class germplasm.

In our study, the Japanese House and Oriental market class accessions examined were relatively rich in genetic variation, and were followed closely by Charentais and Galia types in this regard. There is, in fact, as much variation within Japanese Charentais and Galia as there is within their European counterparts. In stark contrast is the relative lack of genetic variation within Casaba, European Shipper, and US cantaloupe market classes regardless of origin. Thus, consideration should be given to increasing the genetic diversity of these market classes by the introgression of genes from Charentais and Galia germplasm. Moreover, the genetic diversity of European, US and Japanese commercial market classes could be increased by strategic matings among market classes of different origins.

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