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ORIGINAL PAPER

A. I. López-Sesé · J. E. Staub · M. L. Gómez-Guillamón

Genetic analysis of Spanish melon (*Cucumis melo* L.) germplasm using a standardized molecular-marker array and geographically diverse reference accessions

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Abstract Genetic relationships among 125 Spanish melon (*Cucumis melo* L.) accessions from a Spanish germplasm collection were assessed using a standard molecular-marker array consisting of 34 random amplified polymorphic DNA (RAPD) markers bands (19 primers) and 72 reference accessions drawn from previous studies. The reference accession array consisted of a broad range [Japanese (19) Crete (17), African (15), and USA and Europe (US/EU, 21)] of horticultural groupings (Group Cantalupensis, Group Conomon, Group Inodorus, Group Flexuosus, and Group Chito), and of melon market classes (e.g., Charentais, U.S. Western and European Shipper types, Ogen, and Galia, Honeydew, and Casaba). Spanish melon accessions (largely Casaba, Group Inodorus) were genetically distinct from the reference accessions and other Group Inodorus melons of different origins. Most African accessions showed common genetic affinities, and grouped with the Group Chito and the Group Conomon accessions examined. Those accession groupings were distinct from all other accessions belonging to Group Cantalupensis, Flexuosus, and Inodorus accessions originating from Crete, Japan, Europe, and the U.S. Genetic diversity was highest in accessions of African origin and lowest in accessions of Spanish origin. Additional RAPD markers (49 primers, 141 bands) and 22 selected agronomic traits (quantitative and qualitative) were then used to assess the genetic diversity among Spanish accessions. While cluster analysis using fruit characteristics grouped accessions into cultivars, RAPD-based genetic-distance estimate did not provide consistent accession groupings either by cultivar or geographic origin. While the highest level of polymorphism was detected among melons originating from the central region of Spain, and in the Rochet cultivar, accessions from the Andalucía region and Green cultivars were comparatively less diverse. These results indicate that the Spanish melon accessions could be used to broaden the genetic base of local and foreign Casaba germplasm, to enhance the genetic diversity of U.S and European commercial melon germplasm, and to delineate collection strategies for acquisition of additional Spanish landraces.

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A. I. López-Sesé · J. E. Staub (✉)
USDA Agricultural Research Station/ Dept. of Horticulture,
University of Wisconsin-Madison,
Madison, WI, 53706-1590, USA
e-mail: jestaub@facstaff.wisc.edu
Tel.: +1-608-2620028

M. L. Gómez-Guillamón
Experimental Station 'La Mayora',
Consejo Superior de Investigaciones Científicas (CSIC),
29750 Algarrobo (Málaga), Spain

Introduction

Melon (*Cucumis melo* L.; 2n=2*x*=24) is an outcrossing horticultural crop of economic importance belonging to subsp. *melo* in the Cucurbitaceae family (Kirkbride 1993). Based on vegetative and fruit variation, Naudin (1859) subdivided this species into ten groups that were later revised by Munger and Robinson (1991), proposing trinomial names (i.e., *C. melo agrestis, flexuosus*, etc.). This revision includes the groups: *agrestis* (wild melon), *flexuosus* (snake melon), *conomon* (pickling melon, Chinese white cucumber), *cantalupensis* (cantaloupe or muskmelon), *inodorus* (winter melons, honeydew, Casaba), *chito* (mango melon) and *dudaim* (Queen's pocket melon), and *momordica* (Phoot or snap melon). More recently, Pitrat et al. (2000) proposed a synthesis of the infra-specific classification of melons based on the identification of the different synonymous epithets used in the literature. They and others (Greuter et al. 2000) identified 16 groups (five assigned to subsp. *agrestis* and 11 to the subsp. *melo*) denominated as *varietas* or *variety*. In this provisionary grouping, subspecies *agrestis* include, among others, the varieties *conomon* and *momordica*, and

subspecies *melo* include the varieties *cantalupensis*, *inodorus*, *flexuosus*, *dudaim*, and *chito*. In order to simplify terminology according to groupings in previous work (Staub et al. 2000; Mliki et al. 2001) and to allow for comparative analysis of reference accessions from these studies, we designate these varieties herein as botanical groups (i.e., Group Cantalupensis, Inodorus, etc.).

While the primary center of melon diversification is India, Spain is an important secondary center, and is a major world producer of both Group Cantalupensis and Group Inodorus cultivars (McCreight et al. 1993). The morphologically diverse Cantalupensis and Inodorus groups are commercially important in the United States, Europe, and Mediterranean and Asian countries. Group Inodorus consists mainly of the Honeydew and Casaba types, being cultured in Spain as uniform and distinct cultivars (e.g., Rochet and Piel de Sapo). Some commercial melon types belonging to Group Flexuosus are also cultivated in small areas in Spain for regional domestic markets. The horticultural features of Spanish melon landraces and inbred germplasm (i.e., Casaba) grown for domestic markets differ markedly from other European cultivars (Staub et al. 2000). The culinary attributes (mainly taste) of these Spanish melon types appear to have been a reason for their historical retention as unique Casaba cultivars (Esquinas-Alcázar 1977).

Esquinas-Alcázar (1977) used six isozyme systems to assess the genetic diversity of Spanish and U.S. melon accessions. These data were used by the Food and Agriculture Organization (FAO) to define the high degree of genetic erosion in this species as reported by Esquinas-Alcázar and Gulick (1983). This report subsequently led to the planning and execution of several botanical surveys and an evaluation of the major national Spanish vegetable germplasm collections housed at regional experimental stations (Gómez-Guillamón et al. 1985; Nuez et al. 1986, 1988). These activities resulted in a morphological description of Spanish melons, and the grouping of landrace accessions into unique Casaba cultivars (Gómez-Guillamón et al. 1983a, b, 1985, 1995, 1998; Anastasio et al. 1986; Molina et al. 1986; Nuez et al. 1986, 1988, 1994; Costa et al. 1989). More recently, García et al. (1998) successfully used variation at random amplified polymorphic DNA (RAPD) loci to differentiate elite melon germplasm belonging primarily to Galia and Piel de Sapo cultivars, and found a relatively high correspondence between RAPDs and agronomic traits in those cultivated lines (r^2=0.79). It would be useful for germplasm curators and plant geneticists to know if such associations were present in diverse commercial cultivars and landraces of Spanish origin. Likewise, a rigorous molecular and morphological appraisal of regional collections could be used to define accessions by geographic regions and provide historical baseline data for future genetic studies (e.g., genetic erosion). Therefore, a study was designed to assess the genetic diversity of the Spanish landrace melon collection housed at the Experimental Station 'La Mayora', Spain, and compare this variation with that of other melon diversity analyses of worldwide scope (Staub et al. 2000; Mliki et al. 2001). This collection is representative of other regional Spanish collections that have their origins from the comprehensive botanical surveys conducted between 1984 and 1985 (Nuez et al. 1986, 1988). Landraces of this collection are regionally diverse and comprise several Spanish cultivars. The variation detected among Spanish melons in this study was compared to standard reference accessions (Africa, Japan, Crete, U.S., and Europe) (Staub et al. 1997, 2000; Mliki et al. 2001; Nakata et al. 2003) using a uniform set of RAPD markers (Staub et al. 2000).

Materials and methods

Germplasm

The genetic diversity of 125 Spanish melon accessions was examined using a standard reference array consisting of 72 accessions [Japanese (19), Crete (17), African (15), USA and Europe and other additional melons (US/EU, 21)] (Table 1). This reference array represented accessions of differing geographical origin and the cultivar in *C. melo* L. subsp. *melo* accessions belonging to diverse botanical groups (Cantalupensis, Conomon, Inodorus, Flexuosus, and Chito) (Tables 1 and 2) (Staub et al. 2000; Mliki et al. 2001; Nakata et al. 2003). The accessions were obtained from seed companies, public germplasm collections, and from the U.S. National Plant Germplasm Collection, U.S. Department of Agriculture (Table 1). Spanish melon accessions were obtained from the Experimental Station La Mayora, Consejo Superior de Investigaciones Científicas (CSIC), Málaga, Spain. Most of these Spanish landraces were obtained originally from tenant farmers during a national survey (1984 to 1985) (Table 2; Fig. 1), and thereafter had been historically maintained under controlled pollination. All Spanish accessions belonged to Group Inodorus, except for one accession classified as Group Flexuosus (6W_444) (Table 2). These accessions consisted of several Casaba cultivars originating from different geographic regions (Fig. 1).

DNA extraction

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

RAPD amplification

Forty nine, 10-mer primers were purchased either from Operon Technologies (OP; Alameda, Calif.) 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; Mliki et al. 2001; López-Sesé et al. 2002). All polymerase chain reaction (PCR) solutions were purchased from Promega (Madison, Wis.), and PCR was performed according to Staub et al. (1996). The optimized reaction contained 15 ng of DNA, 0.3 mM of primer, 0.3 mM of

Table 1 The 72 melon (*C. melo* L.) reference array accessions from different countries of origin used for diversity analysis

Origin[a]	ID[b]	Seed source[c]	Accession name[d]	Group	Market class[f]	Cn[g]
US/EU	1	RZ	1	Cantalupensis	Galia	11
	3	RZ	3	Cantalupensis	Galia	11
	4	RZ	4	Cantalupensis	Ogen	8
	6	RZ	6	Cantalupensis	Ogen	8
	10	RZ	10	Cantalupensis	Galia	8
	11	LM	'Printadoux' 23840	Cantalupensis	Charentais	7
	16	LM	'Mango' 23848	Cantalupensis	European shipper	11
	19	LM	23851	Inodorus	Honey dew (RA)	11
	22	LM	24138	Inodorus	Honey dew (RA)	11
	24	Zu	M-473	Cantalupensis	Galia	11
	25	Zu	M-442	Inodorus	Casaba (Rochet)	8
	27	Zu	M-126	Inodorus	Casaba (Piel de Sapo)	11
	29	Zu	MY-124	Inodorus	Casaba (Yellow Canari)	11
	38	Peto	Dorado	Inodorus	Casaba (Yellow Canari) (RA)	9
	39	Peto	Snake melon	Flexuosus	(RA)	11
	43	Peto	Freeman cucumber	Conomon	(RA)	4
	45	HM	HMX 2608	Cantalupensis	U.S. eastern market (RA)	7
	46	USDA	Top Mark	Cantalupensis	U.S. western market (RA)	11
Africa	1	Morocco	PI102077	NA	NA	4
	13	Senegal	PI274954	NA	NA	4
	15	Egypt	PI288233	NA	NA	4
	26	Kenya	PI385966	NA	Ein Dor	4
	27	Senegal	PI436532	NA	G22841	4
	28	Senegal	PI436534	NA	G22843	4
	36	Zimbabwe	PI482400	NA	TGR234	4
	43	Zimbabwe	PI482411	NA	TGR554	4
	47	Zimbabwe	PI482422	NA	TGR1687	4
	55	Mali	PI490388	NA	Kankani	4
	60	Zambia	PI505602	NA	ZM/A5384	1
	64	Egypt	PI525103	NA	NO.22	6
	100	Libya	NA[e]	NA	Adzhur	9
	102	Tunisia	NA	NA	Galaoui	2
	104	Tunisia	NA	NA	Chaman/Machmoum	6
Japan	45	Yokohama Ueki	Earl's Miyabi	Cantalupensis	Earl's type	5
	48	Sakata	SK5–167	Cantalupensis	House type	11
	50	Sakata	SK5–326	Cantalupensis	Galia	8
	52	Sakata	CASALS	Cantalupensis	Charentais	10
	57	Sakata	Utopia (Spain)	Inodorus	Casaba (Amarillo)	11
	59	Sakata	Kinsyou2	Conomon	Oriental	10
	67	Sakata	Columbus	Conomon	Oriental	4
	75	Sakata	A-one	Cantalupensis	Ogen	10
	91	Sakata	Sweet surprise (America)	Cantalupensis	U.S. Cantaloupe	11
	92	Sakata	Rugger (America)	Cantalupensis	U.S. Cantaloupe	6
	96	Sakata	White rosa (America)	Inodorus	Honey dew	6
	99	Sakata	Harvest king (Spain)	Cantalupensis	Galia	11
	102	Sakata	Pegasus (Spain)	Inodorus	Casaba (Piel de sapo)	11
	103	Sakata	Sonic (Spain)	Inodorus	Casaba (Rochet)	11
	104	Sakata	Pelayo (Spain)	Inodorus	Casaba (Negro Tendral)	11
	105	Sakata	Carnival (Spain)	Inodorus	Casaba (Crenshaw)	9
	107	Sakata	Kinka (Spain)	Inodorus	Casaba (Tendral Amarillo)	11
	108	Sakata	Eagle (Spain)	Inodorus	Casaba (Amarillo)	11
	110	Sakata	Artix (Spain)	Cantalupensis	Charentais	11
Crete	241	TEI	Agiou Vassiliou	Inodorus	NA	11
	242	TEI	Adzouri 1	Flexuosus	NA	10
	243	TEI	Adzouri 2	Flexuosus	NA	10
	244	TEI	Adzouri 3	Flexuosus	NA	10
	245	TEI	Adzouri 4	Flexuosus	NA	10
	246	TEI	Adzouri 5	Flexuosus	NA	11
	247	TEI	Argous	Inodorus	NA	11
	248	TEI	Bascavas	Cantalupensis	NA	10
	249	TEI	Klossoudi 1	Flexuosus	NA	11
	251	TEI	Messara 1	Inodorus	NA	11
	252	TEI	Messara 2	Inodorus	NA	11
	253	TEI	Peza	Inodorus	NA	11
	254	TEI	Samos	Inodorus	NA	11
	255	TEI	Ksilagouro 2	Flexuosus	NA	10
	256	TEI	Ksilagouro 3	Inodorus	NA	11
	257	TEI	Ksilagouro 4	Flexuosus	NA	11
	258	TEI	Zakynthos	Cantalupensis	NA	11

Table 1 (continued)

Origin[a]	ID[b]	Seed source[c]	Accession name[d]	Group	Market class[f]	Cn[g]
Other	GO	ARO	Galia (original)	Cantalupensis	Galia	11
	893	CN	NA	Chito	NA	2
	925	CN	NA	Chito	NA	3

[a] Accessions selected from previous and current studies, US/EU=U.S. and European market reference array (Staub et al. 1997, 2000), Africa = African landraces (Mliki et al. 2001), Japan = Japanese accessions (Nakata et al. 2003), Crete = Greek accessions (Fanourakis et al. 2003)
[b] Identification by accession number or name described by origin designation
[c] RZ = Rijk Zwaan Seeds, De Lier, The Netherlands, LM = Leen de Mos BV, Granvendzade, The Netherlands, Zu = Zaadunie BV, Enkuizen (now Syngenta), Peto = Peto Seed Company (now Seminis), Woodland, Calif., HM = Harris Moran Seed, Modesto, Calif., and USDA = United States Department of Agriculture, Agricultural Research Service, Salinas, Calif., ARO = Agricultural Research Organization, Israel; TEI = Technological Education Institute, Heraklion, Crete, Greece; ARO = Agricultural Research Organization, Newe Ya'ar Research Center, P.O.B. 1021, Ramat Yishay, 30095, Israel; CN = The Cucurbit Network, P.O. Box 560483, Miami, Fla. 33256, USA
[d] PI = Plant Introduction, USDA, North Central Plant Introduction Station, Ames, Iowa, INRST = Institute National de Recherche Scientifique et Technique, Hammam-Lif, Tunisa, Yokohama Ueki Seed Corporation, Yokohama, Japan, Sakata = Sakata Seed Corporation, Yokohama, Japan
[e] NA = information not-available or not applicable
[f] Market classes except for RA (reference accessions) according to Staub et al. (2000)
[g] Cn = Cluster node of molecular analysis as depicted in Fig. 2. All Spanish accessions are included in node 10, except 444 in node 7

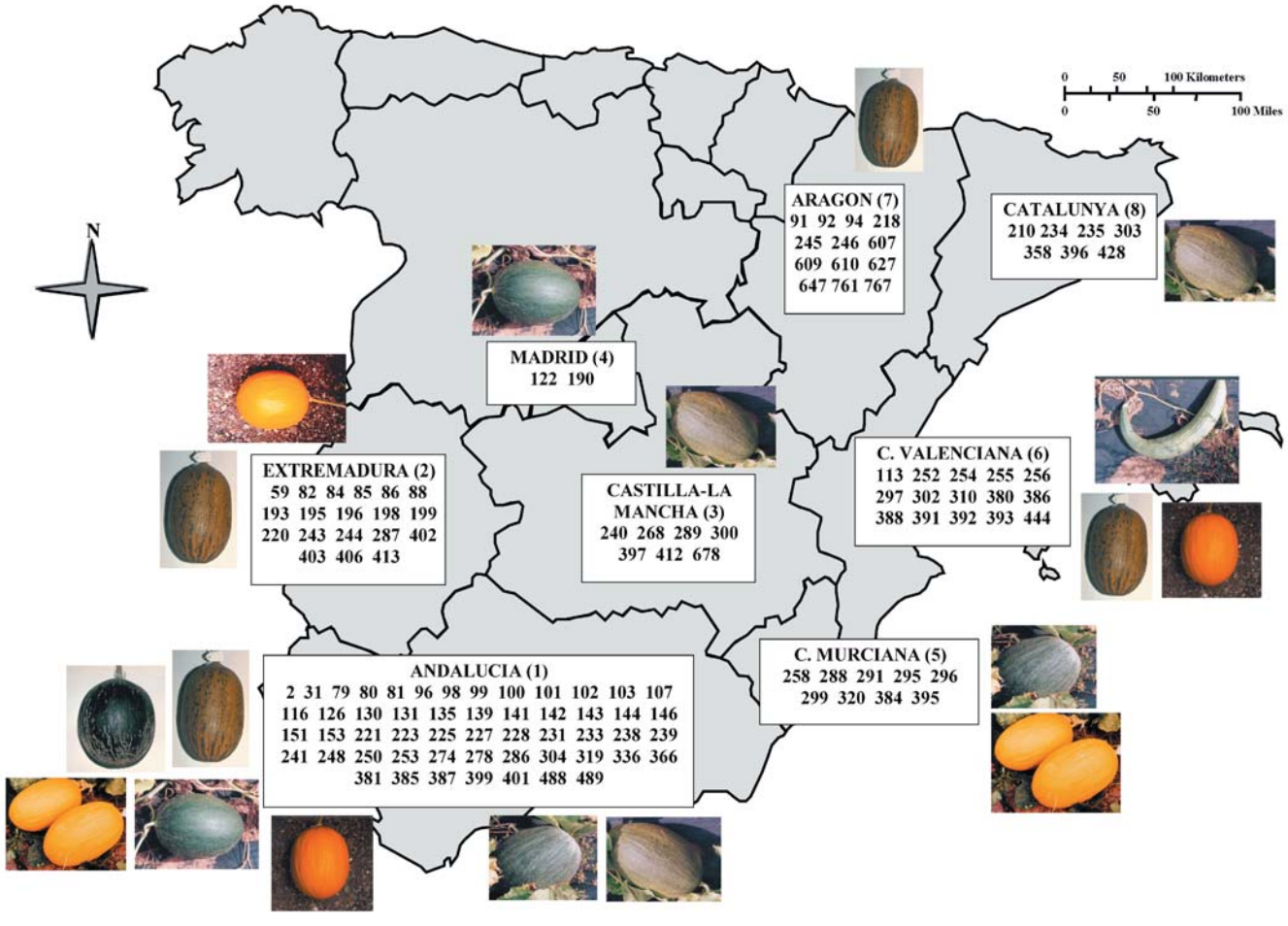


Fig. 1 Origin of Spanish melon (*Cucumis melo* L.) accessions from the Germplasm Bank at Experimental Station 'La Mayora' (CSIC), Málaga (Spain) by geographic regions (autonomous community), and numbered according to Table 2

Table 2 The 125 Spanish melon accessions (*C. melo* L.) from the Germplasm Bank at Experimental Station 'La Mayora' (CSIC), Andalucía (Spain), used for diversity analysis

Org.[a]	Mc[b]	Reg.[c]	Accession name[d]	Cn[e]	Org.	Mc	Reg.	Accession name	Cn	Org.	Mc	Reg.	Accession name	Cn
1	G	96	CA-071084–1-C	5	1	Y	130	Ardales-3–1	2	5	G	299	MUC-15	4
1	G	100	CA-111084–3-C	5	1	Y	135	Ardales-6–2	2	5	G	320	MU-C-44	5
1	G	102	MA-280984–1-C	5	1	Y	141	Ardales-10	2	5	G	384	MUC-41	4
1	G	103	GR-091084–1-C	5	1	Y	142	Ardales-11–1	3	5	PS	395	MU-C-18	4
1	G	139	Ardales-9–1	5	1	Y	144	Ardales-13	2	5	Y	258	MU-C-1	2
1	G	143	Ardales-11–2	4	1	Y	146	Ardales-15–1	2	5	Y	288	MUC-35	3
1	G	151	Ardales-19–1	5	1	Y	221	CA-1311–2-C	3	6	G	256	V-C-11	4
1	G	153	Ardales-20	5	1	Y	223	CA-1311–4-C	3	6	G	297	VC-66	4
1	G	225	CA-1311–1-C	5	1	Y	250	ANC-29	3	6	G	310	VC-122	4
1	G	241	SE-2811–1-C	4	1	Y	278	ANC-57	3	6	G	386	VC-117	4
1	G	248	ANC-48	5	1	Y	319	ANC-42	2	6	G	388	VC-108	4
1	G	274	Tendral	5	2	G	82	CC-140884–1-C	5	6	G	391	VC-109	5
1	G	286	ANC-68	4	2	G	84	CC-190884–3-C	5	6	PS	254	V-C-6	4
1	G	304	ANC-120	4	2	G	85	CC-220884–1-C	4	6	PS	380	VC-19	5
1	G	381	ANC-71	4	2	G	243	E-C-1	4	6	PS	393	VC-9	4
1	G	385	ANC-110	5	2	G	287	EC-33	4	6	W	255	V-C-15	3
1	G	399	ANC-20	4	2	PS	403	EC-19	5	6	W	444	VC-51	3
1	G	488	SH-021–2	5	2	R	402	EC-5	5	6	Y	113	Amarillo Exportacion	2
1	PS	31	ANC-46 P.Sapo	5	2	O	198	Hilo Carrete	3	6	Y	252	V-C-2	3
1	PS	107	ANC-116	5	2	W	195	Punteado amarillo	3	6	Y	302	VC-126	2
1	PS	228	J-2211–7-C	5	2	W	199	Blanco liso	2	6	Y	392	VC-116	2
1	PS	233	J-2811–1-C	5	2	W	220	Blanco liso	3	7	G	94	Z-120884–2-C	4
1	PS	238	CO-2811–2-C	4	2	W	406	EC-23	3	7	G	245	A-C-11	4
1	PS	239	CO-2811–1-C	4	2	Y	59	Maduro negro	3	7	G	246	A-C-4	5
1	PS	401	ANC-85–1	4	2	Y	86	CC-220884–2-C	2	7	G	767	Casero Rayado	1
1	R	116	Severiano de Jata	4	2	Y	88	CC-220884–2-C (2)	2	7	PS	647	T-111	4
1	R	336	ANC-45-seleccionado	3	2	Y	193	Melona amarilla	4	7	O	218	Tendral blanco-83–12	5
1	R	366	ANC-7	5	2	Y	196	Fondo amarillo	3	7	O	607	Negro Zaragoza	2
1	O	2	Tendral negro	5	2	Y	244	E-C-12	3	7	O	609	Invernizo	3
1	W	79	CA-060884–2-C	3	2	Y	413	EC-16	3	7	W	91	Z-100884–1-C	3
1	W	80	CA-080884–1-C	3	3	G	289	CM-C-54	5	7	W	92	Z-100884–2-C	3
1	W	81	CA-060884–1-C	3	3	PS	397	CM-C-30	4	7	Y	610	Amarillo	2
1	W	131	Ardales-3–2	3	3	W	268	CM-C-1	2	7	Y	627	Roget	3
1	W	227	J-2211–13-C	2	3	W	300	CM-C-1	2	7	Y	761	Rajado	3
1	W	231	J.1911–1-C	2	3	W	412	CMC-23 Seleccionado	3	8	G	303	CC-23	5
1	W	253	ANC-22	2	3	Y	240	CM-C-7	3	8	G	396	CC-33	4
1	W	387	ANC-93	2	3	Y	678	CM-C-7 (2)	3	8	PS	235	C-C-13	4
1	W	489	SH-021–1	2	4	G	190	Mochuelo	4	8	R	358	CC-22	5
1	Y	98	CA-101084–1-C	2	4	R	122	Mochuelo	4	8	R	428	CC-37	4
1	Y	99	CA-111084–1-C (2)	2	5	G	291	MUC-34	4	8	O	210	Tendral amarillo	3
1	Y	101	MA-091084–1-C	3	5	G	295	MUC-6	4	8	O	234	C-C-5	2
1	Y	126	Ardales-1–2	3	5	G	296	MUC-20	4					

[a] Geographic origin in Spain (region), where 1 = Andalucía, 2 = Extremadura; 3 = Castilla-La Mancha, 4 = Madrid, 5 = C. Murciana, 6 = C. Valenciana, 7 = Aragón and 8 = Cantalunya
[b] Market class, where G = green type, PS = Piel de Sapo, R = Rochet, W = White type (Blanco), Y = Yellow type (Amarillo) and O = Others (Tendral, Hilo Carrete, etc.)
[c] Registration number given at the Melon Germplasm Bank
[d] Designation given at the Melon Germplasm Bank according to origin and/or traditional name. All accessions belong to the botanical group Inodorus (Casaba type), except 444 (Flexuosus)
[e] Cluster node of morphological analysis as depicted in Fig. 3

dNTPs, 4.0 mM of $MgCl_2$, 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, Conn.). After 1 min of heating at 94°C, amplifications were performed under the following regime: 50 cycles of 93°C, 15 s for denaturing, 36°C, 90 s annealing, and 72°C, 120 s for extension. After amplification, 5 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 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 180 V using a 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, Calif.). *Hin*dIII+*Eco*RI digested lambda-phage DNA was used as a standard marker for estimating the size of PCR products by a 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 bands (loci) having Mendelian, dominant-marker inheritance were scored (Staub et al. 2000, 2002; López-Sesé et al. 2002) such that RAPD banding morphotypes were scored as either present (1) or absent (0).

Morphological evaluation

The nomenclature of mature fruit morphology of Spanish accessions used herein was previously articulated by Esquinas-Alcázar and Gulik (1983). Some vegetative characters recommended for use as descriptors for characterization (Esquinas-Alcázar and Gulick 1983) by the IBPGR (e.g., leaf shape and leaf color) are difficult to evaluate and were not considered. Thirteen qualitative and nine quantitative fruit agronomic characters were measured in 30 fruits of each accession and are available at the web site location as described by evaluation at the Experimental Station La Mayora (CSIC), Málaga, Spain (http://www.hort.wisc.edu/usdavcru/staub/default.htm). The qualitative traits characterized included: (1) exocarp skin color as white, green, yellow, and orange; (2) ease of peduncle separation abscission from fruit; (3) shape as flattened, globular, oblate, elliptical, or other; (4) presence or absence of surface design produced by secondary skin color and design type as speckled, spotted or striped; (5) skin corking as absent, sparse, intermediate or dense; (6) relative degree of netting as absent or present; (7) ribs or sutures as absent, intermediate or deep; (8) skin texture as smooth or wrinkled; (9) blossom scar presence by visual inspection as small to large; (10) presence or absence of aroma; (11) mesocarp flesh color as green, white or orange; (12) seed coat color in a gradient from white to brown; and (13) seed shape as piñonet (pine nut-like) or flat. The quantitative fruit traits examined included: (1) days to maturity; (2) weight; (3) length (cm) as measured in longitudinal section; (4) width (cm) as measured in equatorial section; (5) inner cavity size; (6) epidermal thickness (cm); (7) soluble solid content as measured by Brix grade; (8) 100 seed weight, and; (9) seed number per 100 g.

Data analysis

A binary data matrix obtained from scoring polymorphic RAPD bands was used to calculate Jaccard similarity coefficients (Jaccard 1908) among individual accessions in order to estimate relationships between Spanish melon landraces and reference array melons, as well as the molecular diversity within Spanish melon accessions. The estimator choice was based on the concordance of previously recorded genetic distance (GD) estimators (Staub et al. 2000), the simplicity of estimation using Jaccard's coefficient (Jackson et al. 1989), and the use of this estimator in melon diversity studies (García et al. 1998; Staub et al. 2000; Mliki et al. 2001). Subsequently, these coefficients were transformed to individual pair-wise GD estimates and then used for comparative analyses. This conversion was made by calculating the complement of each coefficient ($1-J_{ij}$) as described by Spooner et al. (1996).

Means, standard deviations and the ranges in quantitative morphological values as well as class frequencies of the qualitative variables (e.g., color determination), were used to describe the variation among and between cultivars. These quantitative and qualitative morphological variables were used in data-matrix construction in order to estimate the morphological diversity of Spanish melon landraces. To provide for a joint analysis of qualitative and quantitative variables (i.e., values of comparable magnitude), quantitative data were transformed to fit 0 to 1-interval values by dividing each value by the maximum value in each variable. Qualitative data were represented as absence (0) or presence (1) for each class of each variable separately (e.g., green, white or orange for flesh color, each given either 0 or 1). The resulting binary data matrix was used for calculation of pair-wise Euclidean distances. Morphological and RAPD data, as well as the genetic, and Euclidean distance matrices utilized in the analyses, are provided at the web site location http://www.hort.wisc.edu/usdavcru/staub/default.htm. An unweighted pair-group method of the arithmetic average clustering procedure (UPGMA) employed GD and Euclidean distance estimates to construct dendrograms (RAPD and morphology) using the computer program NTSYS-pc vs 1.80 (Rohlf 1997).

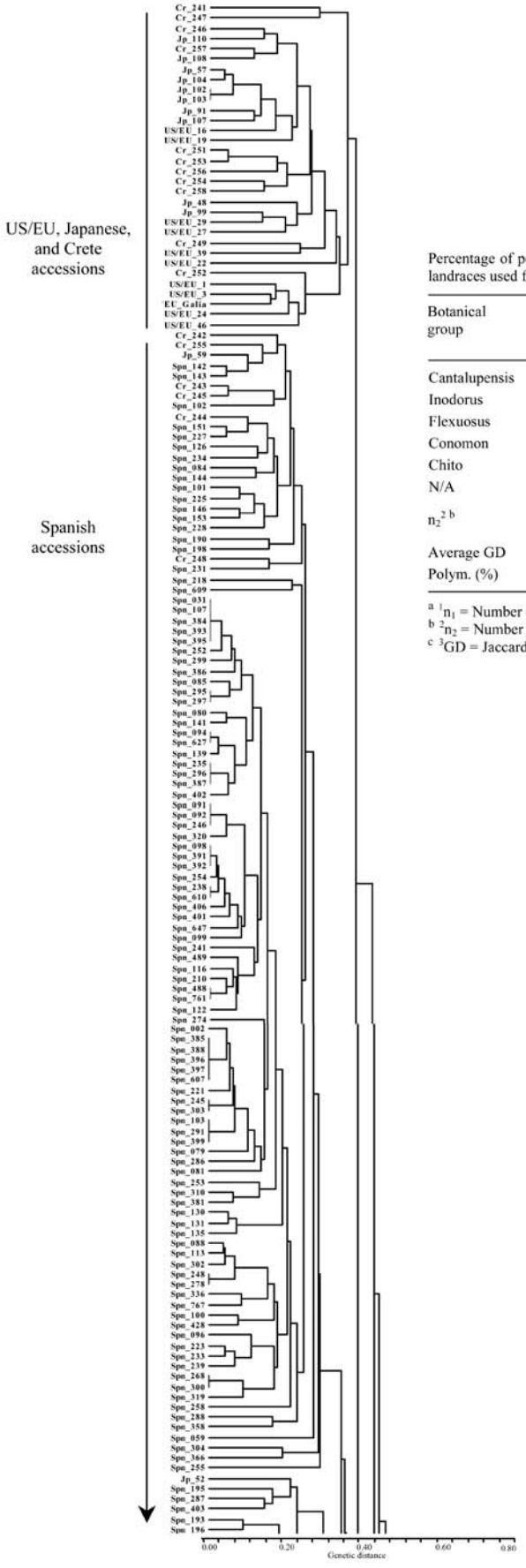
The landrace populations were identified and grouped based on their common geographic origin or the melon cultivar (Table 2, Fig. 1). The percentage of polymorphism (PP) for each population was calculated using the computer program POPGENE (Yeh et al. 1997), and the relative polymorphism level was estimated as a ratio of polymorphism per accession number (percent polymorphism to the number of accessions within a horticultural group; RP). Average genetic- and Euclidean distance-estimates between accessions and the percentage of polymorphism within and between groups obtained were specific to geographic origin and cultivar groupings, and were then used for an appraisal of heterogeneity and diversity.

Results

Relationships among Spanish melon landraces and reference-array accessions

The average GD between any two pairs of accessions as estimated by RAPD variation was 0.36±0.15. Distances ranged between 0.00 (most related lines) among several Spanish accessions, among three Crete Inodorus accessions and among several Japanese Inodorus accessions, to virtually 1.00 (>0.90, distantly related) among the Group Chito accession US/EU_893 and accessions from Africa, Spain, and the Group Flexuosus accession US/EU_39 examined. In fact, accession US/EU_893 and the African accession from Zambia (Af_60) were most distant from other accessions (GD=0.70±0.11 and 0.71±0.07, respectively). In contrast, several Spanish accessions were most similar to all other accessions (GD=0.25±0.16).

RAPD-based cluster revealed that the Spanish melon accessions examined (largely Group Inodorus) were genetically distinct from the reference accessions (Fig. 2). Most of the African accessions possessed genetic affinities, and formed a cluster grouping associated with the Group Chito and the Group Conomon melon accessions examined. These accessions (i.e., African, Chito, and Conomon) grouped in four clusters, and were



Percentage of polymorphism and average genetic distance estimates among melon (*C. melo* L.) landraces used for diversity analysis by origin and melon group

Botanical group	n_1 [1 a]	Geographic origin					Average GD[3 c]	Polym. (%)
		US/EU	Africa	Japan	Crete	Spain		
Cantalupensis	22	11	0	9	2	0	0.38	79.4
Inodorus	145	6	0	8	7	124	0.28	91.2
Flexuosus	10	1	0	0	8	1	0.28	64.7
Conomon	3	1	0	2	0	0	0.47	59.9
Chito	2	2	0	0	0	0	0.78	20.6
N/A	15	0	15	0	0	0	0.49	85.3
n_2 [2 b]		21	15	19	17	125		
Average GD		0.44	0.49	0.34	0.30	0.24		
Polym. (%)		79.4	85.3	82.4	67.7	88.2		

[a] [1]n_1 = Number of accessions by melon group
[b] [2]n_2 = Number of accessions by origin
[c] [3]GD = Jaccard's genetic distance as estimates (Jaccard 1908) from RAPD analysis

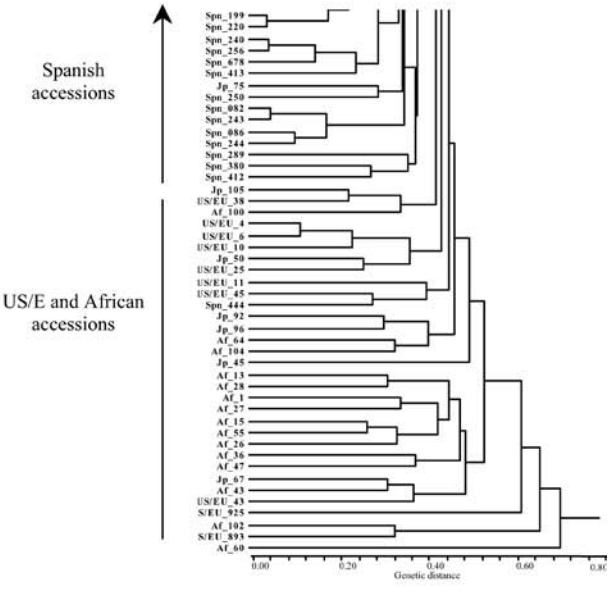


Fig. 2 Cluster analysis of 197 worldwide melon accessions (see Table 1) by UPGMA grouped using genetic distances as estimated by RAPD (Jaccard's coefficient)

separated from all other accessions at node 4 (Fig. 2, Table 1) (GD>0.50).

Accessions belonging to Groups Cantalupensis, Flexuosus, and Inodorus, originating from Crete, Japan, and US/EU accessions, are localized in different nodes. In one group, accessions were associated by GD ranging between 0.50 and 0.30 to include the remaining African accessions (Fig. 2, node 5–9). A second cluster grouping included all Spanish accessions and three Japanese accessions, which were distinct from the Group Cantalupensis, Flexuosus, and Inodorus accessions originating from Crete, Japan, and US/EU accessions (Fig. 2, node 10–11). Genetic distances among Spanish melons ranged between 0.25 and 0.00. In the main, Crete accessions formed a distinct cluster that was closely associated with ten Japanese melons that were mostly of Group Inodorus.

Average GD estimates and the percentage of polymorphism were obtained for groups that were constructed based on their geographic origin and melon market classes. The average GD among accessions within a particular geographic group ranged from 0.24 (Spanish melons) to 0.49 (African landraces) (Fig. 2). Polymorphism level (%) was higher among Spanish melon accessions (88.2%) and lowest among Crete accessions (67.6%). An analysis of horticultural groups indicated that Group Chito (GD=0.78) and Group Conomon (GD=0.47) accessions, and the horticulturally non-ascribed African accessions (N/A; GD=0.49), were genetically most diverse, while Group Flexuosus and Group Inodorus accessions were most similar (GD=0.28). Polymorphism was relatively high among Group Inodorus (91.1%) and African accessions (85.3%), and, by comparison, markedly lower among Group Chito accessions (20.6%) (Fig. 2).

When genetic diversity is estimated by relative polymorphism level (i.e., the ratio of percent polymorphism to the number of accessions within a group), African (5.7), Group Conomon (19.9) and Group Chito (10.3) accessions must be considered most diverse, and Group Inodorus (taken collectively) (0.6) and Spanish (0.7) accessions were the least diverse. Analysis based on polymorphism (PP and RP) by origin and melon market classes revealed that Japanese Group Conomon accessions (41.2%, 20.6), Chito (20.6%, 10.3), and Cantalupensis accessions from Crete (26.5%, 13.2), were comparatively more-diverse than any other group examined. Among the Group Inodorus populations from different origins, those originating from Crete were most diverse (47.1%, 16.7), while Spanish Inodorus (88.2%, 0.7) accessions were the least diverse (Fig. 2).

Genetic diversity within Spanish melon landraces

Morphological diversity

In general, Spanish melon accessions were andromonoecious and leaf lobbing was absent or relatively shallow. Although diverse fruit shapes (i.e., globular to elongate fruits) were detected, the majority of fruit of the Spanish Inodorus accessions were elliptical or oblong, and fruits of the Group Flexuosus accession were relatively long (86 cm). Spanish melon accessions were largely non-aromatic and possessed a white and crispy interior flesh.

Primary fruit skin color (i.e., yellow, orange, green, grey or white) was a distinguishing feature among accessions, and secondary skin color often varied resulting in different designs (given at web site http://www.hort.wisc.edu/usdavcru/staub/default.htm). For instance, fruit of some accessions possessed yellow spots (secondary color) speckled on a green-skinned background (primary color) commonly observed in the Rochet cultivar. Likewise, green-spotted or stripped designs in green-skin fruits were observed and are typical of the Piel de Sapo melon. In a few accessions, secondary fruit color was manifested as white in speckled or stripped designs on fruits possessing a yellow skin color. Fruit ribbing was absent in most of the accessions evaluated, and the skin texture in these entries ranged from smooth to wrinkled. Longitudinal skin-corking patterns, were sparse or intermediate, and are a mark of quality in Spanish Green cultivars. Peduncle separation in most of these accessions was difficult, and a relatively small blossom scar was observed in most accessions. While the mean fruit weight for Spanish melon was 1.5±0.5 kg, the highest variability in fruit weight was observed in the white (Blanco) cultivars (0.8 to 2.9 kg) (data not presented). The mean fruit length among Inodorus accessions was 19±6.6 cm. The average fruit width for Spanish landrace melons was 12±1.2 cm. Considerable variability among landraces in epidermal thickness (0.1 to 0.5 cm) and refractive index (average=11.3±1.9°Brix) was observed. Seed coat-color variability ranged from white to brown in each cultivar.

The average Euclidean distance between Spanish accessions, grouped based on their morphological characteristics, was 1.80±0.52, ranging between 0.00 (most related) to 3.17 (distantly related). The Green melon 7G_767 was most distant from the other accessions examined (average GD among any other accession=2.48±0.29). Accessions that were, on average, closest to the other accessions examined were the Green cultivars 2G_85 and 2G_287, with an average distance among the other accessions of 1.49±0.61.

Cluster analysis based on Euclidean distance allowed for the detection of relationships among accessions (Fig. 3, Panel A). These cluster groupings were mainly due to differences in primary and secondary skin color, and showed no correspondence with geographic origin (Fig. 1). The cluster analysis defined an initial principal node that separated the unique Group Inodorus accession 7G_767 from all other accessions. Euclidean distances among the remaining accessions are less than 2.20 (Fig. 3, Panel A). Partitioning of accessions at nodes 2 to 4 allowed for their further separation based on Euclidean distances less than 1.75. Accession groupings in each of those clusters were the result of partitioning of the White and Yellow, as well as Green and Piel de Sapo, cultivars. The level of polymorphism (RP and PP) was relatively high in all Green cultivar melons (73.1 and 69.6%). The

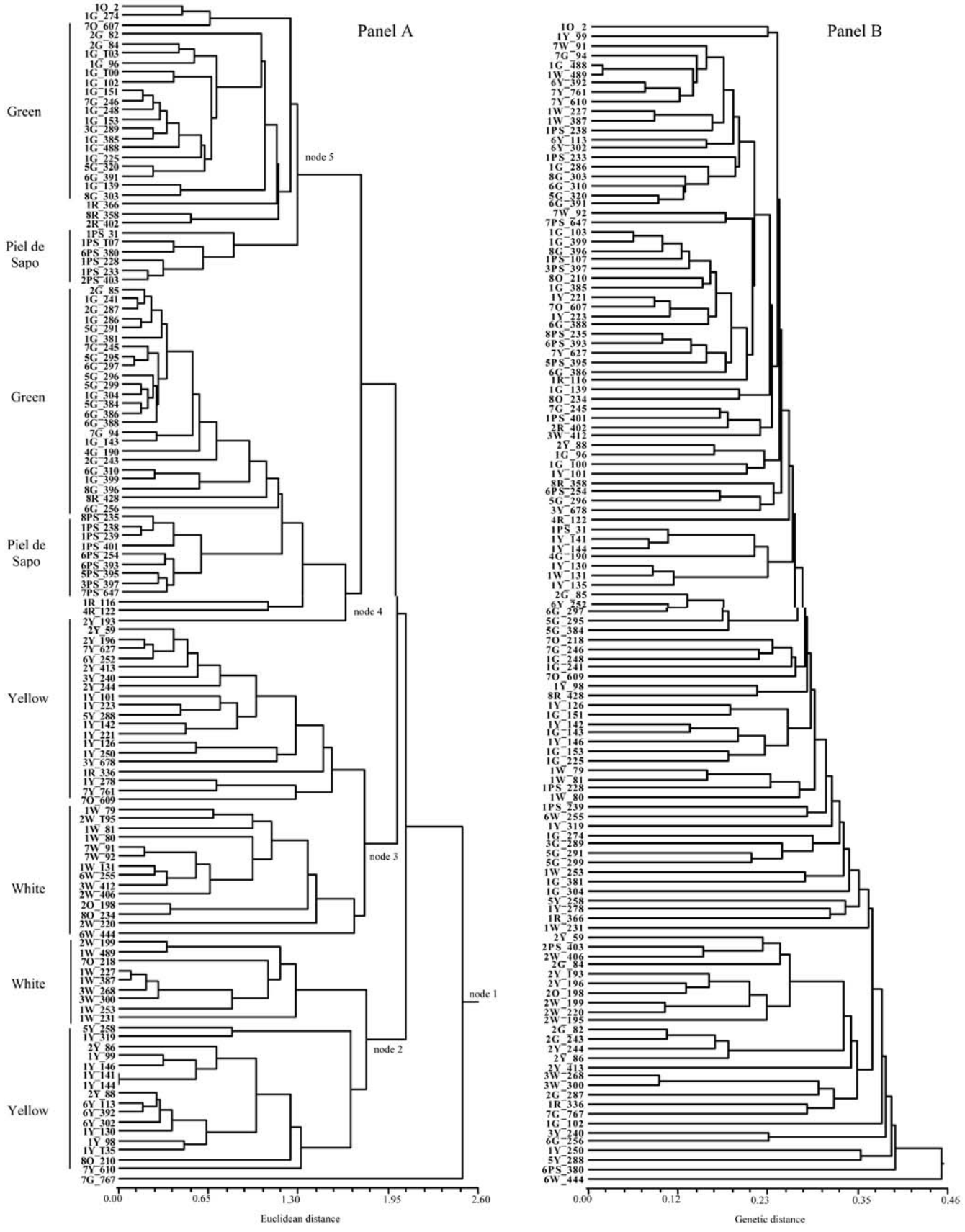


Fig. 3 Cluster analysis of 125 Spanish melon accessions (see Table 2) by UPGMA, grouped using Euclidean distances as estimated by morphological traits (*Panel A*) and using GD as estimated by RAPD (Jaccard's coefficient) (*Panel B*)

lowest polymorphism level was detected in both Piel de Sapo sub-groups (41.1 and 43.3%). However, polymorphism level based on population size (RP) was highest in both Piel de Sapo and White cultivar accessions, and lowest in Green cultivar accessions (data not presented).

Molecular diversity

The 42 RAPD primers used in GD estimation provided for variation at 141 polymorphic loci such that the mean number of polymorphic bands per primer was 3.7. The average GD between any two pairs of accessions was 0.31±0.08. Genetic distances ranged between 0.01 (most related; accessions 1G_488 vs 1W_489) to 0.58 (distantly related; 5G_384 vs 6W_444). The accession most distant from other accessions was 6W_444 (average GD=0.46). In contrast, several accessions (e.g., 1W_387, 6G_391, 6Y_392, 7Y_610 and 7Y_761) possessed relatively close genetic affinities (average GD$\leq$0.25). Within Casaba accessions (excluding 6W_444, Group Flexuosus), the average GD between any two pairs of accessions was 0.31±0.08. Genetic distances ranged between 0.01, based on a whole-collection comparison, to 0.56 by pairwise accession examination (5Y_288 vs 1G_102). The Inodorus accessions 5Y_288, 2Y_244, and 2G_243 were most distant from the other accessions examined (average GD=0.41). In contrast, the accession Group Inodorus accession 6Y_392 possessed common affinities to most accessions (average GD=0.22; data not presented).

RAPD-based cluster analyses resulted in four similar trees. Dendrograms possessed two distinct branches (GD>0.45), one containing the Flexuosus accession 6W_444 and the another containing all Group Inodorus accessions (Fig. 3, Panel B). Within Casaba melons (Group Inodorus), groupings were not related to either the melon cultivar type or to geographic origin. When grouped by geographic origin, the average GD among accessions ranged from 0.23±0.04 (Cantalunya) to 0.32±0.09 (Castilla-La Mancha). The percentage of polymorphic markers (PP) was highest in the Andalusian group (84.4%). When grouped by cultivars, White melons were most dissimilar (average GD=0.34±0.09) and Piel de Sapo melons were most similar (GD=0.25± 0.07). The level of polymorphism was relatively high in green-melon types (80.1%). White (74.5%) and Yellow (77.3%) melons were also highly polymorphic (data not presented).

Genetic diversity as estimated by the polymorphism level calculated as a function of the population size (RP) was highest (7.1) in accessions originating from central Spain (Madrid and Castilla-La Mancha) and lowest (1.6) in Andalusian accessions (data not shown). In this regard, the cultivar Rochet (7.6) was more diverse than other cultivars (1.7 to 6.5), and Green cultivar melons were highly homogeneous (1.2) (data not presented).

Discussion

RAPD loci are widely distributed across the melon genome (Baudracco-Arnas and Pitrat 1996), and variation in this marker system is higher than that resolved by other systems (Perl-Treves et al. 1985; Neuhausen 1992; Staub et al. 1997, 2000), allowing for the descriptive analysis of this cultivated species (Staub et al. 1997, 2000; García et al. 1998). The strategic use of 'pre-selected' RAPD primers for genetic diversity analysis, based on their reproducibility and relatively high level of polymorphism across diverse melon accessions, could enhance the efficiency of screening large germplasm collections for genetic resource management (Bretting and Wirdlechner 1995; Lanaud and Lebot 1997; Staub et al. 2000).

In previous studies a highly polymorphic array of RAPD primers was used to characterize genetic differences among U.S. and European-commercial melon cultivars (Staub et al. 1997, 2000). More recently, this RAPD primer array was used to determine the genetic diversity and inherent relationships among melon accessions of diverse geographic origin (i.e., Africa, Japan and Greece) (Mliki et al. 2001; Nakata et al. 2003; Staub et al. 2003). Thus, by using the molecular data in combination with previously collected morphological and disease information, the National Spanish Melon collection might be adequately characterized to allow for the construction of a core collection and test arrays (Staub et al. 2002). Such an analysis is essential for designing curation strategies (Zhang et al. 1995), and the construction of situation-specific accession arrays for germplasm enhancement (Staub et al. 2002).

The distinctive morphological characteristics among Spanish melon cultivars and their concomitant culinary uniqueness (texture and specialized taste) has prevented the introgression of genes from other germplasm of diverse origin, despite the lack of geographic isolation (Esquinas-Alcázar 1977). Allozyme (Esquinas-Alcázar 1977) and, more recently, morphological (Nuez et al. 1986, 1988) analysis of a restricted number of Spanish landraces has led to the characterization of several cultivar types. Morphological characterization and a standardized, historically based molecular marker array and reference accessions of worldwide origin (Staub et al. 2000; Mliki et al. 2001), confirmed these relationships and defined more clearly market-class differences among Spanish Casaba landraces and their relationship to other prominent market classes of worldwide origin.

Spanish landrace melons clustered together forming a genetically unique assemblage of Group Inodorus accessions, which were distinct from the reference accessions employed (Figs. 2). These landrace accessions possessed genetic affinities with the other previously studied Casaba accessions in the reference array (Staub et al. 1997; Mliki et al. 2001). Nevertheless, Spanish accessions possessed some affinity to the Group Cantalupensis types of European and U.S. origin (Staub et al. 2000; Mliki et al. 2001). Group Conomon accessions and African accessions were, by comparison, more distantly related

to the Group Inodorus examined (Staub et al. 2000; Mliki et al. 2001). African accessions could not be placed into typical U.S.- or European-melon cultivar types, and were morphologically and biochemically distinct to the other reference accessions (Mliki et al. 2001). Genetic diversity as estimated by the polymorphism level, calculated as a function of population size (RP), indicated that variation was highest in African, Group Conomon, and Group Chito accessions. This result might have been predicted since these accessions originated close to the center of diversity for this species (Stepansky et al. 1999).

Although the polymorphism level in the Group Inodorus accessions examined herein was relatively high (Fig. 2), the genetic diversity is the lowest among Inodorus and Spanish accessions (RP, data not presented). Group Inodorus accessions originating from Crete were more diverse than Spanish accessions. Moreover, molecular analysis of Flexuosus and Inodorus accessions of Mediterranean origin (e.g., Crete and Spain) are congruent with previously described morphology and agronomic similarities (Fanourakis et al. 2000). It is possible that some Group Flexuosus accessions from Crete (e.g., "Adzouri") having a similar shape and use as the Spanish Group Flexuosus accession, may originate from or have ancestral relationships with melons from Western Asia. Crete Group Inodorus landraces such as "Messara", "Peza", "Agiou Vassiliou" and "Argous" are similar in fruit shape, size and color to that of Piel de Sapo, and other Green Spanish melon cultivar types (data not presented), which suggests that ancestral ties may exist between these melons and others from Mediterranean regions such as Spain (Fanourakis, personal communication). However, no consistent and close genetic affinities were detected among these accessions (Fig. 2).

We found no association between geographic origin and either molecular or morphological analysis. Only groupings by several economically important fruit traits (skin color and surface design by secondary color) allowed for discrimination of cultivars and comparative analysis (Table 2, Fig. 3). García et al. (1998) detected high correspondence between RAPDs and agronomic traits in cultivated Spanish lines. As in Costa et al. (1989), the lack of agreement in molecular data (Fig. 3, Panels A and B), the morphological trait association with specific cultivar types, and the partition in different clusters of those cultivars (Table 2, Fig. 3) reported herein, suggest that cultivars and melons originating in a particular region are heterogeneous and retain potentially important genetic variation.

Average genetic distances among accessions of certain horticultural groups (e.g., Groups Cantalupensis and Inodorus) detected herein (Fig. 2) are similar to those obtained in previous studies (Staub et al. 2000). The Group Cantalupensis accessions were, on average, distantly related (average GD among accessions=0.55) from Group Inodorus accessions with average GD ranging between 0.19 and 0.28 (García et al. 1998; Staub et al. 2000). Some cultivar-associated accession groupings possessed lower variability (Spanish Group Inodorus accessions, GD=0.31; Fig. 3, Panel B) than predicted, given their overall agronomic differences (García et al. 1998). Likewise, discrimination between Group Inodorus and Group Flexuosus accessions could be clearly detected herein by RAPD-based cluster analysis (Fig. 3, panel B), indicating that this Group Flexuosus accession is genetically distant from all other accessions (López-Sesé et al. 2002). Thus, our results support the use of this standard marker array for comprehensive genetic comparisons and the large collection analysis in *C. melo*.

The relationship analyses provided herein, provide support for the hypothesis that Spanish melon germplasm is genetically unique, and indicate that US and European commercial germplasm could be enhanced by the introduction of allelic diversity from heterogeneous Spanish landrace accessions. Moreover, there would be great utility in developing an extended database that circumscribes the genetic diversity of accessions housed in regional Spanish collections and the acquisition of additional accessions to ensure retention of existing genetic diversity, and reduce genetic erosion.

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