Morphological and Molecular Variation in a Collection of Eggplants from a Secondary Center of Diversity: Implications for Conservation and Breeding

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ABSTRACT. Eggplant (*Solanum melongena* L.) was introduced by the Arabs into Spain. Since then, many local cultivars have arisen. These materials are grouped in four cultivar groups: “round,” “semi-long,” “long,” and “listada de Gandía.” We studied the morphological and molecular [amplified fragment length polymorphism (AFLP)] diversity of a collection of 28 Spanish traditional cultivars of eggplant. Four eggplant accessions from different origins were used as controls and three scarlet eggplant (*Solanum aethiopicum* L.) accessions as outgroups. Morphology and AFLP markers showed that *S. melongena* and *S. aethiopicum* are separate taxonomic entities, and that, compared to controls, Spanish eggplants are very variable, indicating that the Iberian Peninsula can be regarded as a secondary center of diversity. Morphological differences were found among cultivar groups in traits other than those used for the grouping although, in some cases, accessions from different cultivar groups shared a similar general morphology. Eggplant cultivar groups also showed some genetic differences, which are revealed in the gene diversity statistics ($G_{ST} = 0.30$). Nonetheless, no individual AFLP markers specific and universal to one cultivar group could be found. “Round” cultivars were genetically more diverse than the other cultivar groups. A positive correlation ($r = 0.68$) was found between morphological and molecular distances. However, correlations between geographical and either morphological or molecular distances were low. Results suggest that evolution of eggplants in Spain has involved frequent hybridizations and a frequent movement and exchange of seeds. Structure of diversity among regions indicates that most of the diversity can be collected in single selected regions. All these results have important implications in eggplant germplasm conservation and breeding.

The “eggplant complex” includes the cultivated and weedy forms of eggplant and wild species closely related to the crop (Pearce and Lester, 1979). Within this complex, the cultivated forms are represented by groups G and H (Lester and Hasan, 1991). Group G is widespread in Southeast Asia and corresponds to primitive cultivars, slightly prickly, with small fruits (3–4 cm in diameter), which are green with white stripes. Group H comprises advanced cultivars, unarmed or with just a few tiny prickles, and that yield large (usually >100 g) fruits that may be very variable in color.

The cultivated eggplant was brought from Indochina to North Africa and to the Iberian Peninsula by the Arabs before the 10th century (Prohens and Nuez, 2001; Watson, 1998). From there, although much later and perhaps not before the 14th century, it spread to other European countries like Italy and France (Daunay, 1996). From Spain it was also introduced into America soon after its discovery in 1492 (Nuez et al., 2002). Descriptions and depictions of eggplants found in al-Andalus (Muslim Spain) and other European Middle Age sources match the characteristics of group H, suggesting that eggplants introduced by Arabs into Europe belonged to this group (Nuez et al., 2002; Prohens and Nuez, 2001; Watson, 1998).

Because of the intense commercial trade between al-Andalus and the rest of North Africa and the Middle East, very likely many new eggplant populations were introduced into Spain until the 15th century, when Muslim domination in Spain arrived at an end. These populations were subjected to natural and artificial selection, mutation, genetic drift, and migration, which together with recombination and segregation resulting from occasional hybridization (Kalloo, 1993; Sandambam, 1964) probably led to an increase of variation. The result was the development of local cultivars with adaptation to specific environmental conditions and suited to different uses (Prohens and Nuez, 2001). In this way in the 12th century, on the basis of fruit and flower color, al-Andalus agronomist Abú-Zacaria (1802) described four cultivars of eggplant in Spain, while at the end of 19th century, and as a consequence of the evolution of local materials and new introductions, Navarro (1880) reported on the existence of more than 30 Spanish traditional cultivars that differed in shape, size, and color, each of which might have had several local variants.

Spanish eggplants are classified in four exclusive groups of cultivars (“round,” “semi-long,” “long,” and “listada de Gandía”), basically distinguished by fruit shape and coloration (Nuez et al., 2002; Prohens et al., 2003). The first three types are distinguished by the length : breadth ratio of the fruit (~1 for “round”; >1.2 and <2 for “semi-long”; and >2 for “long”) and are characterized by presenting either uniform fruit color or a secondary color distributed in broad strips. The “listada de Gandía” type is typical of the Spanish Mediterranean region, and is characterized by a white or yellowish background color covered by narrow purple stripes.

Despite the substantial morphological variation of the cultivated

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eggplant, this crop has suffered several genetic bottlenecks during its evolution, which probably restricted its genetic diversity (Spillane and Gepts, 2001). As in the tomato (*Lycopersicon esculentum* Mill.) (Jenkins, 1948), eggplant was not domesticated in the region where its putative wild ancestors are distributed (Africa and Near East), but in Indochina (Lester and Hasan, 1991). Therefore, domestication probably led to an important founder effect, which was accompanied by isolation from wild relatives, preventing introgression from these materials into the domesticate. Introduction into Europe probably represented an additional bottleneck in the genetic diversity (Prohens and Nuez, 2001).

Studies of the molecular diversity of the “eggplant complex” with seed proteins (Karihaloo et al., 2002; Lester and Hasan, 1991; Pearce and Lester, 1979), isozymes (Lester and Hasan, 1991), allozymes (Isshiki et al., 1994; Karihaloo and Gottlieb, 1995), mitochondrial DNA (Isshiki et al., 2003), chloroplast DNA (Isshiki et al., 1998; Sakata and Lester, 1994, 1997; Sakata et al., 1991), random amplified polymorphic DNA (RAPD) markers (Karihaloo et al., 1995; Nunome et al., 2001), amplified fragment length polymorphism (AFLP) markers (Furini and Wunder, 2004; Mace et al., 1999), and microsatellites (Nunome et al., 2001, 2003) show that cultivated eggplant has a narrow molecular diversity. However, these works have been aimed mainly at examining the relationships among the crop and related wild species and weedy forms, and the number of accessions of group H (especially of those from the Mediterranean region) studied has been very limited. In addition, the simultaneous evaluation of the morphological and molecular diversity of group H eggplants would be of great interest in eggplant breeding and conservation of germplasm, as it would give information on the variation and genetic base of the crop, evolution and differentiation among groups of cultivars and regions, and the association between molecular and morphological variation.

AFLP markers have been successfully used to examine the relationships of eggplant with related species (Furini and Wunder, 2004; Mace et al., 1999) and in the development of a genetic map of eggplant (Nunome et al., 2001). In addition, AFLP markers allow a large number of loci to be scored in a single reaction, and have a much better repeatability among laboratories than RAPDs (Jones et al., 1997). It is because of these reasons that we consider that AFLP markers may be a useful tool to study the diversity of group H eggplants.

In this work we study the morphological and molecular (AFLP) diversity of a collection of *Solanum melongena* group H. Implications of the results in the conservation of germplasm and in eggplant breeding are discussed.

Material and Methods

PLANT MATERIAL. Thirty-two accessions corresponding to *S. melongena* group H were used for the present study. Twenty-eight *S. melongena* accessions corresponded to local Spanish cultivars of the “round,” “semi-long,” “long,” or “listada de Gandía” groups from the main producing regions (Table 1). Four accessions, one from China (“round”), one from Cuba (“semi-long”), one from France (“long”), and a Spanish commercial selection of “listada de Gandía” (semillas Clemente, Vitoria, Spain) were used as *S. melongena* controls. Three accessions of the scarlet eggplant (*Solanum aethiopicum* L. Gilo group) were used as outgroups. All the plant material is part of the collection of the Centro de Conservación y Mejora de la Agrodiversidad Valenciana (Valencia, Spain).

MORPHOLOGICAL CHARACTERIZATION. Five plants per accession were grown in the open air in Valencia (Spain) and were characterized using the primary characterization descriptors developed by the European Eggplant Genetic Resources Network (EGGNET). These descriptors include seven vegetative traits and 28 inflorescence and fruit traits. The states of the descriptors, a complete description on how each trait is measured as well as explicative figures, can be obtained from the authors or consulted in the International Board for Plant Genetic Resources (IBPGR) (1990) and Nuez et al. (2002). Some traits are metric: plant height (cm; P-Height), flowering time (days from sowing; Fl-Time), fruit pedicel length (cm; F-Pedicel), fruit length (cm; F-Length), fruit breadth (cm; F-Breadth), and fruit weight (g; F-Weight); others are measured in an arbitrary scale and are treated as metric (see Table 2 for a list of traits); others are meristic: leaves to first flower (L-First), flowers per inflorescence (Fl-Inflorescence), and fruits per plant (F-Plant); finally, several fruit color traits are qualitative: fruit predominant color (at commercial ripeness), fruit additional color (at commercial ripeness), fruit additional color distribution (at commercial ripeness), and fruit color at physiological ripeness. Each plant was treated as a replication and one measurement was made for whole plant traits (like plant height) and five for the rest of vegetative, inflorescence, and fruit traits. When possible, all measurements of a given trait were made on the same date to avoid differences in the environment or developmental stage of the plants. Traits corresponding to fruit shape and color were used to assign the accessions to cultivar groups.

DNA EXTRACTION AND AFLP ANALYSIS. For each accession, genomic DNA was isolated from young leaves using the DNeasy Plant Mini Kit (Qiagen Inc., Valencia, Calif.) following the protocol of the manufacturer. DNA concentration was quantified on agarose, and a 0.25- μ g DNA sample was digested by the enzyme combination *Eco*RI and *Mse*I at 37 °C for 2 h. Ligation was performed with the AFLP Core Reagent Kit (Invitrogen Corp., Carlsbad, Calif.) following the instructions of the manufacturer. After ligation, the reaction mixture was diluted 1:10 in TE buffer.

For the preselective amplification, a 5- μ L aliquot from the DNA dilution was added to a 25- μ L solution containing 2.5 μ L of 10x buffer, 0.5 μ L of primer EcoA (10 mM), 0.5 μ L of primer MseC (10 μ M), 1.0 μ L of dNTPs (10 mM), and 0.8 units of *Taq* polymerase (Roche, Basel, Switzerland). After preamplification, DNA was diluted again 1:10 in TE buffer. The selective amplification was performed on 2- μ L aliquots using four combinations of primers (Table 3). The resulting DNA fragments were separated in a capillary ABI Prism 310 genetic analyser (Applied Biosystems, Foster City, Calif.). Resulting fragments were visualized using Genographer 1.6 software (Benham, 2001) and scored as binary traits (1 = present; 0 = absent). Only those bands falling within the range of 80 to 380 base pairs (bp) were considered.

DATA ANALYSIS. Standardized quantitative and meristic morphological data were used to calculate pairwise Euclidean distances among the accessions using Phyltools software (Buntjer, 2001). For the AFLP data, pairwise genetic similarities were estimated with the Dice (Sorensen) similarity coefficient $S_{ij} = 2a/(2a + b + c)$, where a is the number of bands shared by i and j , b is the number of bands present in i and absent in j , and c is the number of bands present in j and absent in i . The resulting morphological and genetic similarity matrices were used to generate UPGMA (unweighted pair group method using arithmetic means) phenograms using the Phylip software package (Felsenstein, 1993). As recommended by Mohammadi and Prassana (2003), principal components analysis (PCA) was performed for morphological

Table 1. Source of seed, geographical coordinates, and cultivar group of the materials used for the study of morphological and molecular (AFLP) variation in a collection of Spanish eggplants.

Accessions	Locality	Province	Region/country	Longitude	Latitude	Altitude (m)	Cultivar group
<i>Solanum melongena</i> Spanish traditional types							
ANS3	Castro del Río	Córdoba	Andalusia	4°30'W	37°45'N	30	Semi-long
ANS6	Uguíjar	Granada	Andalusia	2°42'W	36°55'N	120	Round
ANS24	Carena	Jaén	Andalusia	3°30'W	38°05'N	n.a. ^z	Semi-long
ANS26	Santa Fe	Granada	Andalusia	2°42'W	37°11'N	20	Semi-long
ANS37	Uguíjar	Granada	Andalusia	2°42'W	36°55'N	120	Long
BS3	Porrires	Baleares	Baleares	2°40'E	39°35'N	150	Long
CS5	Bítem	Tarragona	Catalonia	0°30'E	40°50'N	40	Long
CS16	Vilafranca del Penedés	Barcelona	Catalonia	1°41'E	41°22'N	30	Long
CS21	El Vendrell	Tarragona	Catalonia	1°32'E	41°13'N	22	Long
CMS2	Belvis de la Jara	Toledo	Castilla-La Mancha	4°57'W	39°45'N	505	Long
IVIA25	n.a.	Valencia	Valencia	n.a.	n.a.	n.a.	Listada de Gandía
IVIA178	n.a.	Valencia	Valencia	n.a.	n.a.	n.a.	Listada de Gandía
IVIA371	n.a.	Valencia	Valencia	n.a.	n.a.	n.a.	Listada de Gandía
IVIA400	n.a.	Valencia	Valencia	n.a.	n.a.	n.a.	Listada de Gandía
IVIA604	n.a.	Valencia	Valencia	n.a.	n.a.	n.a.	Listada de Gandía
MUS3	Monteagudo	Murcia	Murcia	1°08'W	38°01'N	40	Listada de Gandía
MUS4	Orilla del Azarbe	Murcia	Murcia	1°05'W	38°03'N	30	Long
MUS5	Zeneta	Murcia	Murcia	1°00'W	38°01'N	30	Semi-long
MUS6	Patiño	Murcia	Murcia	1°09'W	37°58'N	43	Round
MUS7	Beniaján	Murcia	Murcia	1°06'W	37°59'N	50	Long
MUS8	Media Legua	Murcia	Murcia	1°11'W	37°58'N	60	Round
MUS11	Totana	Murcia	Murcia	1°23'W	37°43'N	250	Round
VS2	Gandía	Valencia	Valencia	0°11'W	38°59'N	22	Listada de Gandía
VS8	Valencia	Valencia	Valencia	0°19'W	39°25'N	15	Listada de Gandía
VS9	La Aparecida	Alicante	Valencia	1°00'W	38°04'N	23	Round
VS10	La Aparecida	Alicante	Valencia	1°00'W	38°04'N	23	Semi-long
VS19	Mutxamel	Alicante	Valencia	0°44'W	38°23'N	63	Long
VS21	Gandía	Valencia	Valencia	0°12'W	38°59'N	22	Long
<i>Solanum melongena</i> controls							
ASIS1	Beijing	Beijing	China	99°03'E	47°08'N	45	Round
LF324	Breeding line developed by INRA (France)						Long
'Listada de Gandía'	Commercial cultivar (Semillas Clemente, Spain)						Listada de Gandía
SUDS5	La Habana	La Habana	Cuba	82°21'W	23°08'N	10	Semi-long
<i>Solanum aethiopicum</i>							
AFRS5	Luanda	n.a.	Angola	13°13'E	8°48'S	74	Gilo
IVIA26	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Gilo
IVIA94	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Gilo

^zn.a. = not available.

data, and principal coordinates analysis (PCoA) for AFLP data. Calculations were performed using the NTSYSpc2.0 software package (Applied Biostatistics Inc., Setauket, N.Y.). Correlations between morphological, molecular, and geographical distance matrices were investigated with a Mantel (1967) test.

Genetic diversity was estimated with Popgene software (Yeh and Boyle, 1997). The proportion of polymorphic fragments (P) and total diversity (H_T) was calculated, and total diversity was partitioned into diversity among (D_{ST}) and within (H_S) groups (Nei, 1973). The relative magnitude of gene differentiation among groups (G_{ST}) was calculated as the ratio $D_{ST}:H_S$.

Results

MORPHOLOGICAL CHARACTERIZATION. Spanish traditional cultivars of eggplant studied present the typical characteristics of the group H of the "eggplant complex" (Lester and Hasan, 1991):

plants vigorous, with relatively few prickles, and with large fruits (>100 g), but exhibit considerable variation for many other morphological traits, in particular for those related to the fruit (Table 4). Despite the diversity of origins of the *S. melongena* controls used, the variation for most traits studied in these materials is comprised within the range observed for Spanish eggplant landraces (Table 4). *Solanum aethiopicum* accessions studied belong to the Gilo cultivar group, as they have hairy leaves and are not prickly (Lester et al., 1986). *S. aethiopicum* can be distinguished from *S. melongena* by many morphological features (Table 4).

On the basis of fruit shape and color, six *S. melongena* accessions corresponded to the "round" group, six to the "semi-long," 11 to the "long," and nine to the "listada de Gandía" (Table 1). "Round" and "semi-long" groups presented a greater variation in the main color (white, green, and a spectrum of colors from lilac gray to black) as well as in the distribution of the color (uniform or with secondary coloration). No "long" cultivars with second-

Table 2. Morphological quantitative traits measured in an arbitrary scale, codes for the traits, range of values, and description of the scale used for the study of the morphological variation in a collection of Spanish eggplants.

Trait	Codes	Range [scale]
Plant growth habit	P-Habit	1–9 [3 = upright, 7 = prostrate]
Shoot tip anthocyanins intensity	S-Anthocyanins	0–9 [0 = absent, 9 = very strong]
Leaf blade lobing	L-Lobing	1–9 [1 = very weak, 9 = very strong]
Leaf prickles (upper surface)	L-Prickles	0–9 [0 = none, 9 = very many (>20)]
Leaf hairs (lower surface)	L-Hairs	1–9 [1 = very few, 9 = very many]
Leaf surface shape	L-Surface	1–9 [1 = flat, 9 = very convex]
Corolla color	C-Color	1–9 [1 = greenish white, 9 = bluish violet]
Fruit shape	F-Shape	1–9 [1 = broader than long, 9 = several times broader than long]
Fruit curvature	F-Curvature	1–9 [1 = None, 9 = U shaped]
Fruit cross-section	F-Section	1–9 [1 = circular, 9 = very irregular]
Fruit widest part	F-Widest	1–9 [3 = about 1/4 way from base to tip, 7 = about 3/4 way from base to tip]
Fruit apex shape	F-Apex	1–9 [3 = protruded, 7 = depressed]
Fruit predominant color intensity	F-Color	1–9 [1 = very light, 9 = very dark]
Fruit flesh color	F-Flesh	1–9 [3 = white, 7 = green]
Fruit epidermis shininess	F-Shininess	1–9 [3 = dull, 7 = shiny]
Fruit epidermis surface	F-Surface	1–9 [3 = smooth, 7 = rough]
Fruit color intensity under calyx	FC-Intensity	0–9 [0 = none, 9 = very strong]
Fruit calyx color (amount of anthocyanins)	FC-Color	0–9 [0 = none, 9 = very strong]
Fruit firmness in broadest part	F-Firmness	1–9 [1 = very soft, 9 = very hard]
Fruit calyx length (relative to the fruit length)	FC-Length	1–9 [1 = very short (<10%), 9 = very long (>75%)]
Fruit calyx prickles	FC-Prickles	0–9 [0 = none, 9 = very many (>30)]

Table 3. Restriction enzyme, sequence and fluorescent label for the oligonucleotide pre-selective (E-A and M-C) and selective (E-ACG, E-ACT, M-CAA, and M-CTA) primers used for the AFLP analysis.

Primers	Restriction enzyme	Sequence	Label
E-A	<i>Eco</i> RI	5'-AGACTGCGTACCAATTCA-3'	---
M-C	<i>Mse</i> I	5'-GATGAGTCCTGAGTAAC-3'	---
E-ACG	<i>Eco</i> RI	5'-AGACTGCGTACCAATTACACG-3'	HEX or NED
E-ACT	<i>Eco</i> RI	5'-AGACTGCGTACCAATTCAAC-3'	FAM
M-CAA	<i>Mse</i> I	5'-GATGAGTCCTGAGTAACAA-3'	---
M-CTA	<i>Mse</i> I	5'-GATGAGTCCTGAGTAACTA-3'	---

ary color were found, and their color ranged from lilac gray to black. All “listada de Gandía” accessions were lilac gray or purple covered with yellowish or white narrow strips.

Differences among groups of cultivars were not limited to fruit color and shape, as there were differences among them in other vegetative, inflorescence, and fruit traits (Table 5). In this way, “listada de Gandía” cultivars have lower anthocyanin intensity in the shoot tips and a greater leaf blade lobing than the other cultivars. Regarding fruit traits, “round” cultivars are more irregular in fruit cross-section, have a lower fruit color intensity under calyx, and a more intense fruit calyx color than the rest of the cultivars, while the “long” are the most curved and have the firmest fruits, and the “listada de Gandía” have fewer fruits per plant, but of greater weight, and have more prickles on the calyx than the other cultivar groups (Table 5).

Differences among cultivars from the regions with a greater number of accessions (Valencia, Murcia, Andalusia, and Catalonia) were nonsignificant for most of the traits studied, with the exception of some fruit traits (fruit length, breadth, weight, and curvature; data not shown). However, these differences are a consequence of an uneven distribution among regions of the groups of cultivars; when the differences among regions are studied cultivar group-by-cultivar group, no differences are found.

Phenograms performed with morphological data (not shown) show that *S. melongena* and *S. aethiopicum* group separately in two consistent clusters. However, when the relationships among the *S. melongena* accessions are studied, the correlation coefficient between the cophenetic and the genetic distances matrices is 0.79, a value considered to be relatively low (Rohlf, 1992). This low value indicates that some distortion might have occurred, and that the analysis of the relationships among accessions through this method may produce misleading conclusions (Mohammadi and Prasanna, 2003). Therefore, we studied the relationships among *S. melongena* accessions through a PCA. In this analysis,

the first and second components (Fig. 1) account for 23.0% and 15.0%, respectively. The most important traits separating accessions in the first component are fruit shape traits (fruit curvature, length, and breadth), while in the second are traits related to coloration (fruit calyx intensity, stem anthocyanins, calyx color, and fruit calyx color). In general, the projections of the accessions on the fruit traits plot agree with the data on the differences among groups of cultivars (Table 5). However, there are some accessions that are closer to accessions of other cultivar groups than to those of their own group (Fig. 1). Data corresponding to pairwise distances based on morphological data confirm these results. One example is given by accessions ANS3, MUS4, and MUS3, which, despite belonging to different cultivar groups (“semi-long,” “long,” and “listada de Gandía,” respectively) based on fruit shapes, are otherwise morphologically very similar.

MOLECULAR CHARACTERIZATION. A total of 339 fragments ranging from 80 to 380 bp were scored, of which 269 (79.4%) were polymorphic. However, this high degree of polymorphism is mostly due to differences between *S. melongena* and *S. aethiopicum* (Fig. 2). There are 145 fragments specific of *S. melongena* (92 present in all accessions) and 112 fragments specific of *S. aethiopicum*. Apart from the 70 monomorphic fragments, these species only share 12 polymorphic markers.

Table 4. Range and mean for the traits measured in the Spanish traditional cultivars and controls of *S. melongena* and in *S. aethiopicum*.

Trait	Spanish traditional <i>S. melongena</i>		<i>S. melongena</i> controls		<i>S. aethiopicum</i>	
	Range	Mean	Range	Mean	Range	Mean
No. of accessions	28		4		3	
Plant growth habit	1–5 ^z	2.93 ^y	3–3	3.00	5–5	5.00
Plant height (cm)	50–85	67.92	44.6–73.8	57.3	76.8–110.6	91.2
Shoot tip anthocyanins intensity	1–9	4.96	1–9	4.50	0–0	0.00
Leaf blade lobbing	1–7	4.43	3–7	5.50	9–9	9.00
Leaf prickles	0–3	0.54	0–3	0.75	0–0	0.00
Leaf hairs	3–9	6.36	5–7	6.50	5–5	5.00
Leaf surface shape	1–5	2.00	1–1	1.00	5–5	5.00
Flowering time (d)	53–62	59.25	61–62	61.75	56–56	56.00
No. of leaves to first flower	6–10	7.79	7–8	7.75	8–10	9.00
No. of flowers per inflorescence	1–3	1.25	1–2	1.50	2–3	2.67
Corolla color	5–9	7.07	3–7	6.00	3–3	3.00
Fruit pedicel length	4.4–15.2	7.58	3.7–9.5	6.63	3.4–4.6	4.20
Fruit length (cm)	9.3–25.8	18.36	8.0–21.6	17.13	4.7–7.2	6.23
Fruit breadth (cm)	4.5–12.0	8.37	4.9–10.8	8.00	3.7–4.3	3.93
Fruit shape	1–9	6.50	1–9	6.00	3–7	5.00
Fruit weight	143–611	322.68	191–413	284.00	46–97	74.30
Fruit curvature	1–5	1.86	1–3	1.50	1–1	1.00
Fruit cross-section	1–7	2.43	1–3	1.50	1–1	1.00
Fruit widest part	5–7	6.50	5–7	6.00	5–5	5.00
Fruit apex shape	3–7	6.29	5–7	6.00	3–7	4.33
Fruit predominant color intensity	3–9	5.93	3–9	6.50	5–7	6.33
Fruit flesh color	1–7	3.36	3–7	4.50	5–5	5.00
Fruit epidermis shininess	3–7	5.79	5–7	6.50	7–7	7.00
Fruit epidermis surface	3–5	3.36	3–5	3.50	3–3	3.00
Fruit color intensity under calyx	0–9	4.50	0–7	4.25	9–9	9.00
Fruit calyx color	0–9	1.64	0–9	2.50	0–0	0.00
Fruit firmness in broadest part	3–9	5.14	3–9	6.50	5–9	7.00
Fruit calyx length	1–3	2.07	1–3	2.50	1–1	1.00
Fruit calyx prickles	0–5	2.00	1–7	3.00	0–0	0.00
Fruits per inflorescence	1–1	1.00	1–1	1.00	2–2	2.00
Fruits per plant	2–11	4.96	2–7	4.75	10–30	17.00

^zRange expresses the minimum and maximum modal value for each of the accessions studied.^yMeans were calculated as the average of mean values of all accessions included in each group.^xRange and mean values based on five plants per accessions and one measurement per plant for whole plant traits and five measurements per plant for the rest of vegetative, inflorescence, and fruit traits.Table 5. Means for each *S. melongena* cultivar group for which differences in quantitative traits have been detected.

Trait	Round	Semi-long	Long	Listada de Gandía
Shoot tips anthocyanin intensity	7.33 a ^{z, y}	5.17 a	5.36 a	2.78 b
Leaf blade lobbing	4.67 b	4.33 b	2.82 c	6.56 a
Leaf hairs	6.67 ab	6.00 ab	5.36 b	7.22 a
Flowering time (d)	59.83 ab	58.00 b	61.81 a	57.67 b
Corolla color	7.67 a	7.00 ab	7.00 ab	6.33 b
Fruit length (cm)	10.57 d	16.05 c	22.92 a	18.74 b
Fruit breadth (cm)	10.28 a	8.31 b	5.50 c	9.37 ab
Fruit shape	2.67 c	7.00 b	8.91 a	6.33 b
Fruit weight (g)	226.67 b	312.00 b	241.55 b	449.56 a
Fruit curvature	1.00 b	1.33 b	3.18 a	1.00 b
Fruit cross section	4.67 a	1.67 b	1.37 b	2.33 b
Fruit widest part	5.33 b	6.00 b	7.00 a	6.78 a
Fruit color intensity under calyx	1.00 b	5.00 a	4.64 a	5.67 a
Fruit calyx color	5.00 a	1.67 b	0.45 b	0.44 b
Fruit firmness in broadest part	7.67 a	5.00 b	3.91 b	5.67 b
Fruit calyx prickles	1.17 b	1.50 b	1.27 b	3.89 a
Fruit number	6.83 a	5.00 a	7.55 a	3.33 b

^zMeans were calculated as the average of mean values (five plants per accessions and one measurement per plant for whole plant traits and five measurements per plant for the rest of vegetative, inflorescence, and fruit traits) of all accessions included in each group.

Fig. 1. Diagram showing relationships among quantitative traits used (see Table 2) for the characterization of 32 *S. melongena* accessions based on the two first principal components of PCA (23.0% and 15.0% of the total variation, respectively) (above). Morphological-based similarities among the 32 *S. melongena* accessions based on the PCA (below). × = round type (thick continuous line); | = semi-long type (thick discontinuous line); _ = long type (thin continuous line); + = listada de Gandía type (thin discontinuous line).

Fig. 2. Computer-generated (Genographer software) representation of a gel-like image corresponding to the fluorescent traces recorded after separation of the AFLP fragments in a capillary genetic analyser of 32 *S. melongena* accessions corresponding to the “round”, “semi-long”, “long,” and “listada de Gandía” (listada) cultivar groups and to 3 *S. aethiopicum* (Gilo group) accessions generated by the Mse CAA-Eco ACT FAM combination.

Table 6. Gene diversity statistics (Nei, 1973) estimated from AFLP markers data for the materials studied grouped by species (*S. melongena* or *S. aethiopicum*), cultivar group (“round,” “semi-long,” “long,” or “listada de Gandía”) or region of origin within Spain (Catalonia, Valencia, Murcia, or Andalusia).

Groups	No. of accessions	H^z	H_s^z	G_{ST}^z
All accessions	35	0.137	0.043	0.86
<i>S. melongena</i>	32	0.046		
<i>S. aethiopicum</i>	3	0.040		
<i>S. melongena</i>	32	0.056	0.042	0.30
Round	6	0.071		
Semi-long	6	0.041		
Long	11	0.030		
Listada de Gandía	9	0.026		
<i>S. melongena</i>				
(selected regions)	26	0.037	0.028	0.26
Catalonia	3	0.012		
Valencia	11	0.037		
Murcia	7	0.034		
Andalusia	5	0.039		

zH = gene diversity; H_s = average gene diversity within subpopulations; G_{ST} = relative magnitude of gene differentiation among groups (H_s/H_T).

When considering *S. melongena*, 65 (19.2%) markers were polymorphic and allowed distinguishing morphologically similar cultivars (Fig. 2). Most of the polymorphisms (52.3%) are the result of the presence/absence of one fragment in a single accession. No fragments were found to be present in all the accessions of one group and absent in the other groups. The same occurred when studying accessions by region of origin. For the three accessions of *S. aethiopicum*, there were 33 (9.7%) polymorphic markers.

Despite the uneven number of accessions studied for each species (32 for *S. melongena* and three for *S. aethiopicum*), total diversity (H_T) of the *S. melongena* accessions was quite similar to that of *S. aethiopicum* (Table 6). Genetic differentiation (G_{ST}) among these species was very high (0.86). Regarding the cultivar groups of *S. melongena*, the “round” group has the greatest genetic diversity, with values 2.7, 2.4, and 1.7 times greater than those found in “listada de Gandía,” “long,” and “semi-long,” respectively. When considering regions, diversity is much greater in Murcia, Valencia, and Andalusia than in Catalonia (Table 6). Values of genetic differentiation among groups and among regions have been moderate (Table 6).

Cluster analysis (not shown) was performed with genetic distance data groups *S. aethiopicum* and *S. melongena* in two main clusters (bootstrap of 100%). How-

ever, within the *S. melongena* cluster it is not possible to find subclusters grouping a substantial number of accessions supported by bootstrap values of 50% or greater. In addition, the value of the coefficient of correlation between the cophenetic matrix and the genetic distances matrix is 0.77, which is low (Rohlf, 1992). Therefore, as for the morphological data, we chose to study the relationships among accessions through a PCoA.

In the PCoA with all accessions (not shown), the first coordinate explains most of the variation (70%) and mostly accounts for the separation between *S. aethiopicum* and *S. melongena*. Therefore, we performed a PCoA using only the *S. melongena* accessions (Fig. 3). In this analysis, the first and second coordinates explain, respectively, 20.2% and 18.5% of the observed variation.

The PCoA scatter plot shows that each of “long,” “semi-long,” and “listada de Gandía” groups of accessions are plotted in a different area. On the contrary, the “round” accessions, with the exception of a subgroup constituted by three accessions that are clustered together, are spread in different areas of the plot. No regional clustering is observed, with the exception of the accessions from Catalonia, all of which are of the “long” type and are plotted together. Control cultivars used in the present study are genetically similar to the Spanish traditional cultivars (Fig. 3). The Asian cultivar ASIS1 (“round”) is genetically close to two Spanish “round” cultivars; the American cultivar SUDS5 (“semi-long”) is similar to a “round” cultivar; French cultivar LF324 (“long”) is included in the “long” cluster; and the commercial selection ‘Listada de Gandía’ is genetically similar to other Spanish traditional cultivars of the “listada de Gandía” type.

Fig. 3. Relationships between 32 eggplant cultivars based on principal coordinates analysis (first and second coordinates account for 20.2% and 18.5% of variation, respectively) using AFLP-based genetic similarities. × = round type (thick continuous line); | = semi-long type (thick discontinuous line); _ = long type (thin continuous line); + = listada de Gandía type (thin discontinuous line).

CORRELATIONS BETWEEN DISTANCES. Correlation between the morphological and molecular distances of Spanish accessions of *S. melongena* is 0.68 ($P < 0.01$), revealing the existence of a moderate association between both measures. Correlations between the geographic distance of the collection sites and either the morphological or molecular distances are low, with values of 0.25 ($P < 0.01$) and 0.11 ($P < 0.10$), respectively.

Discussion

Spanish eggplant cultivars are neither morphologically nor molecularly differentiated from other *S. melongena* group H accessions from other countries, and exhibit a high degree of morphological variability, comparable to that existing in wild and weedy materials from the primary center of diversity of this crop (Karihaloo and Rai, 1995). This high diversity suggests that the Iberian Peninsula might be regarded as a secondary center of diversity for *S. melongena* group H. Also, despite the genetic bottlenecks suffered during domestication and migration to the Iberian Peninsula, almost 20% of the AFLP markers scored are polymorphic. The role of Spain as a bridge between Europe and North Africa, from where eggplants were introduced into western Europe, the diversity of environmental conditions, and the different uses for which eggplants have been selected in this country have contributed to the buildup of this diversity (Prohens and Nuez, 2001).

Artificial classification of cultivars of a crop species may result in groups morphologically very different one from each other because they display a characteristic syndrome for many

morphological traits other than those used for the grouping; or, on the contrary, they may show a heterogeneous assortment among groups of traits other than those that allow the classification into groups (Parker, 1986; Spooner et al., 2003). The case of Spanish eggplants seems to be intermediate between these extremes. This situation can be observed in the morphological PCA plots, in which there is a clustering of accessions of the same group, although in some cases, accessions belonging to different cultivar groups show more similarities among themselves than to accessions of their respective cultivar groups.

Although, as is common in any domesticate used for its fruit (Harlan, 1992), considerable morphological variation is found in this organ, important variation has also been detected for many other traits in the eggplant collection studied. Variation in vegetative characters might be the result of an accumulation of variation on neutral traits, a consequence of selection for adaptation to specific environments, or a combination of both. In this way, high anthocyanin content may confer a greater tolerance to some stresses (Atanassova et al., 2001).

As in the case of morphological traits, there is a differentiation among cultivar groups at the molecular level, although one or more accessions of one group are more similar to accessions of other groups than to those of its own group. Additionally, some of the "round" accessions are scattered in the molecular PCoA graph. Despite the molecular similarity of the accessions of a given cultivar group, which indicates the existence of a shared genetic background, no AFLP markers are present in all accessions of a cultivar group and absent in all the other accessions, which suggests that hybridization (Sandambam, 1964) and in-

trogression among the different groups may have been common events in the evolution of this crop. In fact, the low bootstrap values for the nodes of the tree generated with the AFLP data indicates that evolution of Spanish cultivars of eggplant, rather than a tree-model, has followed a network-model, as it is common for the genealogical relationships within a species (Crandall and Templeton, 1996). This hypothesis would be supported by the moderate values of genetic differentiation among cultivar groups. The fact that the traits for which the different exclusive cultivar groups are distinguished, like fruit shape and color, are under the control of a few genes or QTLs (Doganlar et al., 2002; Frary et al., 2003; Kalloo, 1993) indicates that hybridization among individuals belonging to different groups could lead, after fixation in the subsequent generations, to new lines with traits corresponding to different agronomic groups that would share, as an average and under no selection, 50% of the genes for which the parents differ. This could explain the existence of accessions that are molecularly similar but belong to different agronomic groups. Because of these arguments we consider that it would be difficult to find individual AFLP markers that are able to distinguish the four cultivar groups.

The historical records (Nuez et al., 2002; Prohens and Nuez, 2001; Watson, 1998) indicate that the eggplants introduced by the Arabs, at least in the earlier times, were not of the “long” type. As far as we know, al-Andalus agronomists and other European authors of the Middle Ages do not depict or make reference to this type of eggplant. Therefore, it seems that the “long” types were introduced later than “round” and “semi-long” types. Alternatively, these cultivars might have arisen recently and independently of other “long” cultivars from other places, a process that might have taken place by loss of existing gene function or regulation (Lester and Daunay, 2003). Any of these processes could explain its lower genetic diversity when compared to the “long” and “semi-long” types. In the same way, the lower diversity of the “listada de Gandía” group may reflect a common origin, a restricted geographical distribution, or both.

The low level of correlation between the geographic and the morphological or molecular distances probably is a consequence of the frequent movements and exchanges of seeds between farmers. Nonetheless, the ‘listada de Gandía’ type is widespread only in the coastal area of Valencia and Murcia, and seems to have a good adaptation to these specific conditions. For the other groups, there are representatives in each of the main regions studied, except for Catalonia, where all the cultivars studied are of the “long” type. Nuez et al. (2002) and Prohens and Nuez (2001) already described that in Catalonia most of the cultivars are “long,” which is probably linked to a specific use of the eggplant in the gastronomy of the area (longitudinally cut, stuffed halves of eggplant) for which cultivars of this type are especially suited.

The positive correlation between the morphological and molecular diversity is useful in eggplant germplasm management and breeding. In this way, the establishment of nuclear collections based on morphological data will probably encompass an important part of the molecular diversity, and vice versa. Also, the fact that most of the morphological and molecular diversity can be found in accessions from a single region can reduce the costs of germplasm collection and conservation (Hawkes et al., 2000). In this way, almost all of the genetic diversity of Spanish eggplants is represented in each of the Andalusia, Murcia, or Valencia regions. The greater genetic diversity found in the “round” cultivar group indicates that these cultivars should be more represented than others in the screening for sources of varia-

tion for traits for which there is no previous information on the distribution of diversity among groups. In addition, the degree of polymorphism detected with AFLP markers suggests that they may be adequate to detect duplicates or accessions genetically very similar in the collections.

Regarding eggplant breeding, the correlation between morphological and molecular traits indicates that selection of morphologically different individuals may lead to the identification of genetically distant individuals and vice versa. As in other Solanaceae (Paz and Veilleux, 1997; Rodríguez-Burruezo et al., 2003), choosing genetically distant parents may be helpful for obtaining hybrids heterotic for yield. In fact, heterosis after crossing unrelated individuals has already been described in eggplant (Kalloo, 1993; Sandambam, 1962). Also, AFLP markers, because of their levels of polymorphism in eggplant, could be used to obtain fingerprints of cultivars, even when they are morphologically similar. Therefore, these markers can be used for the protection of cultivars and for detecting “essentially derived” cultivars.

In conclusion, this work shows that Spanish cultivars of eggplant display a considerable diversity, that the evolution of the crop probably has involved frequent hybridizations and introgressions among cultivar groups, and that there is no clear regional differentiation at a morphological or molecular level. All this information is useful for the conservation of germplasm and for the genetic improvement of this crop.

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