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Characterization, diversity, and relationships of the Spanish striped (*Listada*) eggplants: a model for the enhancement and protection of local heirlooms

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Abstract Spanish striped eggplants (*Solanum melongena* L.) are generally known as *Listada*. Among them, the most internationally renowned is the *Listada de Gandía* heirloom. Enhancement and legal protection of these local materials requires the analysis of their characteristics, diversity and relationships with similar accessions. We performed a morphological, agronomic and molecular (amplified fragment length polymorphism; AFLP) characterization of 33 eggplant accessions, which included 20 *Striped* accessions, of which nine were Spanish (five *Listada de Gandía* and four of *Other Spanish Listada*), 11 non-Spanish (six of *Non-Spanish Listada* and five of *Other Non-Spanish Striped*) and 13 *Non-Striped* accessions. The *Striped* accessions presented a range of morphological, agronomic and genetic diversity comparable to the one observed in the *Non-Striped* accessions. Multivariate PCA (morphological) and PCoA (molecular) analyzes group together the *Listada* accessions, and clearly separate the three *Listada* subgroups (*Listada de Gandía*, *Other Spanish Listada* and *Non-Spanish Listada*). On the other hand, the *Other Non-Spanish Striped* accessions are closer to *Non-Striped* than to *Listada* accessions. *Listada* eggplants, as well as each of their subgroups, are characterized by a syndrome of morphological traits that allows distinguishing them from other accessions. Furthermore, AFLP markers which allow distinguishing *Listada* accessions and the *Listada de Gandía* heirloom have been found. The agronomic characterization shows that despite the low diversity within *Listada de Gandía* group, it is possible to select accessions with improved agronomic performance. The results obtained have important implications for the conservation, improvement and legal protection of Spanish striped eggplants, and in particular of the *Listada de Gandía* heirloom. The methodology and approaches used may provide a model for the enhancement and protection of other vegetable crops heirlooms.

Keywords AFLPs · Characterization · Eggplant · Genetic resources · Germplasm · *Listada de Gandía* · Multivariate analysis · *Solanum melongena*

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Introduction

Eggplant (*Solanum melongena* L.) was introduced in Spain by the Arabs before the tenth century (Prohens and Nuez 2001; Watson 1998). Since then, selection and adaptation to local conditions has resulted in the accumulation of an important diversity, with the consequence that Spain is considered as a secondary center of diversity for eggplant (Prohens et al. 2005).

The commercial and local materials of eggplants are classified into varietal groups depending on their resemblance for several fruit characters (Muñoz-Falcón et al. 2005; Marín 2007; Daunay 2008). At present, the "black" and "striped" types are the most important groups in Spain. The "black" eggplant group is characterized by dark uniformly pigmented fruits, and is by far the commercially most important group in this region (Muñoz-Falcón et al. 2005; Aguilar et al. 2006; Daunay 2008). "Black" eggplants have been subjected to intensive breeding, and many hybrid varieties adapted to greenhouse cultivation and destined to export markets have been developed (Marín 2007). On the other hand, "striped" eggplants, compared to the "black" type, have undergone few breeding efforts and have mostly been commercialized through local markets, where the fruits of the heirloom local varieties are highly appreciated (Prohens and Nuez 2001).

Heirloom striped Spanish eggplants are locally and internationally known as *Listada* (which means "striped" in Spanish). Typically, the Spanish *Listada* eggplant fruits are characterized for having obovate to oblong shape and white background color covered by narrow purple stripes (Prohens et al. 2005). Among the different *Listada* local materials grown in Spain, one of the most renowned is the so-called *Listada de Gandía*, whose putative origin is considered to be the area around the city of Gandía, situated on the Mediterranean coast some 60 km south from Valencia (Prohens and Nuez 2001). The *Listada de Gandía* heirloom variety is widely grown in the region of Valencia, and is reputed for having fruits with intense purple stripes, luminous white flesh, lack of bitterness, and excellent texture and cooking quality. Because of these characteristics, the *Listada de Gandía* heirloom has acquired international recognition and given that this name is associated to high quality, in some cases, materials that did not originate in the region of Valencia or do not conform to the typical *Listada de Gandía* characteristics are marketed under this name by some seed companies.

During the last years there has been an increasing interest for heirloom vegetables produced in their regions of origin, and which may acquire an added value in the market if protected by geographical indications or designations of origin (Gracia and Albisu 2001; Babcock and Clemens 2004). In the last years, 19 protected geographical indications and designations of origin have been established in Spain for vegetable crops (MAPA 2007). Among these, there is already one eggplant, the "Almagro eggplant", a local variety used in the center of Spain for making pickles, which was granted a protected geographical indication in 1994 (Prohens et al. 2007). In consequence, Spanish *Listada* accessions, and in particular the *Listada de Gandía* eggplant, might be candidates for some legal protection that increases the prospects of benefits for the farmers.

Morphological and agronomic characterization of the accessions, the analysis of their genetic diversity, and the study of their relationships with other similar accessions, is of great relevance for developing strategies for the enhancement of local landraces and their legal protection (Frankel 1989; Frison and Hodgkin 2005). Morphological characterization is essential to describe the distinctive characteristics of cultivars and landraces (UPOV 1991). To this respect, the European Eggplant Genetic Resources Network (EGGNET; Contract UE RESGEN PL98-113 for the period 2000–2004) developed primary characterization descriptors for eggplant, which have been used for describing the main morphological traits of eggplant accessions (Prohens et al. 2005; van der Weerden and Barendse 2007; Daunay 2008).

Molecular markers provide complementary information to the conventional morphological characterization, and the use of both types of data provides extra information that cannot be obtained by using separately each type of data (Waycott and Fort 1994; Bretting and Widlechner 1995; Karp et al. 1997). Among the molecular markers used for studying the diversity of eggplant, amplified fragment length polymorphisms (AFLPs) have been successfully used in this crop for studying the diversity among traditional varieties (Prohens et al. 2005), examining the relationships of cultivated and wild relatives (Furini and Wunder 2004; Mace et al. 1999), developing genetic maps (Nunome et al. 2001; Sunseri et al. 2003), and also for selecting parents of hybrids (Rodríguez-Burruezo et al. 2008). In other Solanaceae related crops, like pepper (Portis et al. 2006) and pepino (Blanca et al. 2007), AFLPs have also been used to study the diversity and relationships of accessions within varietal groups. Also, AFLPs allow many loci to be scored in a single reaction (Vos et al. 1995). Because of all these reasons we decided to use AFLP markers for our study.

Here we study the morphological and molecular diversity of the Spanish striped eggplants, and compare these accessions with other striped and non-striped accessions. The final objective is identify the specific characteristics, diversity, agronomic performance and relationships of the *Listada de Gandía* group. This information will be of utility for the enhancement of these local accessions and their eventual legal protection and may also constitute a model for other crops.

Material and methods

Plant material

A total of 33 accessions, belonging to five varietal groups, were used (Table 1; Fig. 1):

(i) Group *Listada de Gandía* (five accessions): Spanish striped cultivars traditionally grown in the region of Valencia and locally known under this name.
(ii) Group *Other Spanish Listada* (four accessions): Spanish striped cultivars known as *Listada*, but traditionally grown outside the region of Valencia.
(iii) Group *Non-Spanish Listada* (six accessions): Striped cultivars that presumably do not originate from Spain (Table 1) and are sold by heirloom seed companies. Morphologically, they conform to the "Listada" (i.e., striped) type. However, these cultivars are sometimes mis-labeled as "Listada de Gandía".
(iv) Group *Other Non-Spanish Striped* (five accessions): Striped cultivars that do not originate from Spain.
(v) Group *Non-Striped* (13 accessions): Set of cultivars that are not striped, and display a diversity of geographical origins, fruits size, shape and color.

The plant material used in this study is either part of the germplasm collection of the Instituto de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV) or was purchased from heirloom seed companies (Table 1). All accessions, with the exception of F1-hybrid varieties 'Zebra' (*Non-Spanish Listada*) and 'Little Purple Tiger' (*Other Non-Spanish Striped*), are open-pollinated.

Morphological and agronomic characterization

Six plants per accession were grown in an open air field plot (GPS coordinates of the field plot: lat. 39°28′56″ N, long. 0°20′11″ W) with sandy loams soil in Valencia (Spain) using a completely randomized design. Plants were spaced 1 m between the rows and 0.8 m apart in the row. The standard horticultural practices for eggplant production in the area of Valencia were followed (Baixauli 2001). Plants were drip irrigated and fertilization was applied with the drip irrigation system. Phytosanitary treatments against spider mites were performed when necessary. Plants were characterized using primary characterization descriptors developed by EGGNET (Prohens et al. 2005; van der Weerden and Barendse 2007) as well as with some additional characterization and agronomic evaluation descriptors considered by the authors as important. These descriptors include vegetative, inflorescence and fruit traits, as well as developmental stage and agronomic traits. Eighteen traits are quantitative: plant heigth (cm), leaf pedicel length (cm), leaf blade length (cm), leaf blade breadth (cm), leaf blade length/breadth ratio, flowering time (days form planting to anthesis of the first flower), first fruit harvest (days form planting), fruit length (cm), fruit breadth (cm), fruit length/breadth ratio, L*, a*, and b* color coordinates of the primary color (for striped accessions this is the purple color, and its measurement was performed in an area where the stripes cover completely the background color, usually just below the calyx end), puncture force (N), fruit deformation at puncture (mm), fruits per plant, fruit weight (g), and yield (kg/m^2). L*, a*, and b* Hunter color coordinates of the primary color were assessed with a HunterLab color meter (model CR300, Minolta Co Ltd., Osaka, Japan) fitted with a 8 mm diameter aperture. Puncture force and fruit deformation at puncture was measured with a materials testing machine (model 5540, Instron, Canton, MA) fitted with a 5.9 mm diameter cylindrical probe at a speed of 20 mm/min. The other 17 traits are measured in a scale with predetermined values of the descriptor states (Table 2). When possible all measurements of a trait were made on a same date to avoid differences in the environment or developmental stages of the plant.

Molecular characterization

Genomic DNA from each accession was extracted from a mixture of young leaves from the six plants evaluated using the DNeasy Plant Mini Kit (Quiagen Inc., Valencia, California) using the protocol recommended by the manufacturer. The DNA concentration

Table 1 The five groups of eggplant cultivars used for morphological and molecular variation, with the origin of each accession

Accesion name	Code	Origin
Spanish Listada de Gandía		
IVIA-25	I25	Moncada,Valencia, Spain
IVIA-371	I371	Moncada, Valencia, Spain
Listada de Gandía	LGA	Valencia, Valencia, Spain
V-S-1	VS1	Alcira, Valencia, Spain
V-S-8	VS8	La Punta, Valencia, Spain
Other Spanish Listada		
AN-S-4	ANS4	Castro del Río, Cordoba, Spain
C-S-10	CS10	Barcelona, Barcelona, Spain
C-S-23	CS23	Gavá, Barcelona, Spain
C-S-7	CS7	Villabertrán, Gerona, Spain
Non-Spanish Listada		
Antigua	ANT	Unknown (Tomato Growers Seeds, USA)
Listada de Gandía[a]	LBCS	Italy (Baker Creek Seeds, USA)
Listada de Gandía[a]	LRS	Italy (Reimer Seeds, USA)
Listada de Gandía[a]	LTGS	Italy (Tomato Growers Seeds, USA)
Pandora Striped Rose	PAN	Italy (Baker Creek Seeds, USA)
Zebra	ZEB	Unknown (Tomato Growers Seeds, USA)
Other Non-Spanish Striped		
Little Purple Tiger	LPT	Unknown (Reimer Seeds, USA)
Manjri Gota	MAN	India (Reimer Seeds, USA)
PI-169659	P169	Edirme, Turkey
PI-491260	P491	Tsakoniki, Greece
RNL-580	R580	Homs, Syria
Non-Striped		
AFR-S-1	AFR1	El Kelaa, Morocco
Almagro	ALM	Almagro, Ciudad Real, Spain
ASI-S-1	ASI1	Beijing, China
BBS-189	B189	Abidjan, Adzope, Ivory Coast
Balady	BAL	Egypt
Bellezza Nera	BEN	Italy (Semillas Vilmorin, Spain)
B-S-3	BS3	Porreres, Mallorca, Spain
C-S-16	CS16	Villafranca del Penedés, Barcelona, Spain
GR-S-19	GR19	Thessaloniki, Macedonia, Greece
INRA-11	IN11	INRA, France
Larga de Barbetane	LDB	France (Semillas Vilmorin, Spain)
Ping Tung	PIT	Taiwan (Evergreen Seeds, USA)
Thai Long Green	TLG	Thailand (Evergreen Seeds, USA)

[a] Accessions labeled as *Listada de Gandía* but which do not conform to the typical characteristics of the Spainsh *Listada de Gandía* heirloom

For those accessions obtained from heirloom seed companies, the name of the company is indicated between brackets

was quantified on an agarose gel, and a 0.1 μg DNA sample was digested by the enzyme combination *Eco*RI and *Mse*I at 37°C for 2.5 h. Ligation was performed with the AFLP Core Reagent Kit (Invitrogen Corp., Carlsbad, California) following the instructions of the manufacturer. After ligation, the reaction mixture was diluted 1:10 in Tris–EDTA (TE) buffer.

For the preselective amplification, a 5 μl aliquot from the 1:10 DNA dilution was added to a 25 μl solution containing 2.5 μl of 10× buffer, 1 μl of MgCl (25 mM), 0.5 μl of primer *Eco*A (10 mM), 0.5 of

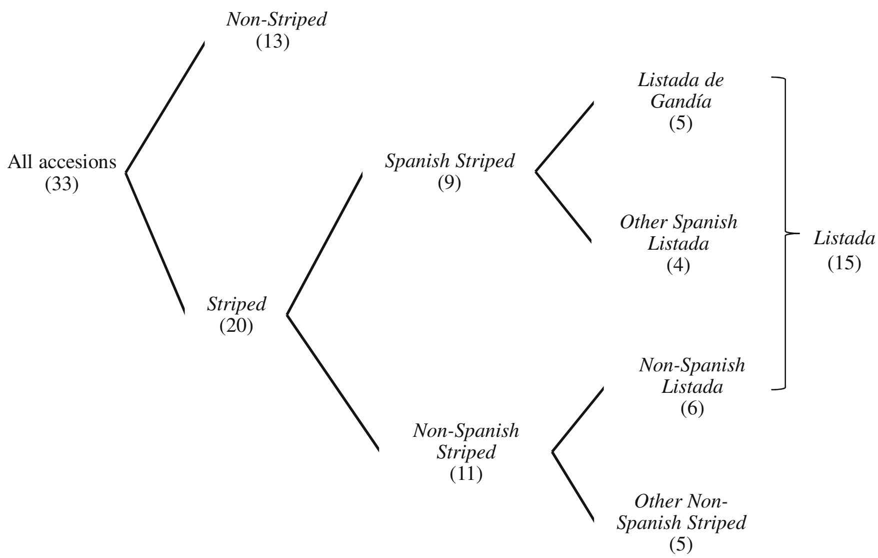

Fig. 1 Diagrammatic representation and nomenclature used for the groups of eggplant accessions at the different levels considered

primer *Mse*C (10 mM), 1 µl of dNTPs (10 mM), and 0.8 units of *Taq* polimerase (Roche, Basel, Switzerland). After preamplification, DNA was diluted again 1:10 in TE buffer. The selective amplification was performed on 2 µl aliquots of the former solution using three combinations of primers (Table 3). DNA fragments were separated in an ABI Prism 310 genetic analyzer (Applied Biosystems, Foster City, California). Resulting fragments were visualized using the Genographer 1.6 software (Benham 2007) and scored as binary traits (1 = present; 0 = absent). Only fragments falling within the range of 80–380 base pairs (bp) were considered, as this is the range in which fragments can be clearly resolved under our conditions.

Table 2 Morphological quantitative traits measured in a scale with pre-determined values of the descriptor states, and description of the scale used for the study of morphological variation in the eggplant accessions studied

Traits	Range (scale)
Plant growth habit	1–9 (1 = upright, 7 = prostrate)
Shoot tip anthocyanins intensity	0–9 (0 = absent, 9 = very strong)
Leaf blade lobing	1–9 (1 = very weak, 9 = very strong)
Leaf prickles	0–9 (0 = none, 9 = very many)
Leaf blade tip angle	1–9 (1 = very acute, 9 = very obtuse)
Fruit curvature	1–9 (1 = none, 9 = U shape)
Fruit cross-section	1–9 (1 = circular, 9 = very irregular)
Fruit widest part	1–9 (3 = about 1/4 way from base to tip, 7 = about 3/4 way from base to tip)
Fruit apex shape	1–9 (3 = protruded, 7 = depressed)
Fruit predominant color intensity	1–9 (1 = very light, 9 = very dark)
Fruit epidermis shininess	1–9 (3 = dull, 7 = shiny)
Fruit color intensity under calyx	0–9 (0 = none, 9 = very strong)
Fruit surface covered by secondary color	0–9 (0 = 0%, 5 = aprox. 50%)
Relative fruit calyx length	1–9 (1 = very short (<10%), 9 = very long (>75%))
Fruit calyx prickles	0–9 (0 = none, 9 = very many (>30))
Fruit skin chlorophyll	0–1 (0 = none, 1 = presence)
Fruit stylar scar	1–9 (3 = small, 7 = big)

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

Primers	Restriction enzyme	Sequence	Label
E-A	*Eco*RI	5′-AGACTGCGTACCAATTCA-3′	–
M-C	*Mse*I	5′-GATGAGTCCTGAGTAAC-3′	–
E-ACT	*Eco*RI	5′-AGACTGCGTACCAATTCACT-3′	FAM
E-ACG	*Eco*RI	5′-AGACTGCGTACCAATTCACG-3′	NED
E-AGC	*Eco*RI	5′-AGACTGCGTACCAATTCAGC-3′	HEX
M-CTA	*Mse*I	5′-GATGAGTCCTGAGTAACTA-3′	–
M-CAA	*Mse*I	5′-GATGAGTCCTGAGTAACAA-3′	–

The three combinations of selective primers used were: E-ACT + M-CTA, E-ACG + M-CTA and E-AGC + M-CAA

Data analysis

In order to compare the diversity for morphological, development, and agronomic traits of *Spanish Striped* with *Non-Spanish Striped*, and *Non-Striped* accessions, means and ranges of variation for these groups were calculated for each of these 35 traits. Also, with the objective of detecting differences among individual accessions for traits with agronomic interest, ANOVA tests were performed on the agronomic traits, and the average standard error of the mean for each accession was calculated from these ANOVA. The coefficient of variation (CV, %) for these agronomic traits was also obtained for each varietal group. Principal components analysis (PCA) was performed using pairwise Euclidean distances obtained from standarized morphological, development, and agronomic data (Mohammadi and Prassana 2003). PCA traits with a correlation >0.7 were considered as highly relevant for that component.

For the AFLP data, principal coordinates analysis (PCoA) was performed using pairwise genetic similarities, which were estimated with the Dice 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 (Mohammadi and Prassana 2003). Genetic diversity (Nei 1973) was estimated with the Popgene software (Yeh and Boyle 1997).

Results

Morphological and agronomic characterization

The eggplant accessions studied displayed considerable diversity for the morphological and agronomic traits studied (Table 4). Furthermore, within the *Spanish Striped*, *Non-Spanish Striped* and *Non-Striped* groups, considerable diversity was found for many traits. For all traits (except for the fruit weight between *Spanish Striped* and *Non-Spanish Striped* groups) the range of variation among these three main groups overlaps (Table 4). Nonetheless, when compared with the *Non-Striped* group, *Striped* eggplants, in general, have a low shoot tips anthocyanins intensity, low to medium fruit length/breadth ratio, low fruit curvature, and low frequency of chlorophyll presence in the skin (Table 4). When comparing the differences between the *Spanish Striped* and *Non-Spanish Striped* eggplants, the former have, in general, lower plant heigth, lower shoot tips anthocyanins intensity, delayed flowering and first fruit harvest times, more calyx prickles, lower puncture force and fruit deformation at puncture, lower number of fruits per plant, and higher fruit weight than the latter (Table 4).

Multivariate PCA analysis of the morphological and agronomic traits revealed that the first two principal components cumulatively accounted for 36.6% of the total variance (Table 5). The first component explained 20.3% of the total variance, and was positively correlated with the fruit length/breadth ratio and fruit curvature, and negatively with the fruit breadth, fruit apex shape, and fruit weight (Table 5). The second component accounted for 16.3% of the total variance and was positively correlated with leaf blade breadth and leaf pedicel length, and negatively with the leaf blade length/breadth ratio (Table 5). The PCA plot (Fig. 2) shows that most of the striped accessions plot together, with a combination of low values for the first component and moderately high values for the second. The group of striped accessions,

Table 4 Range and mean for the traits measured in the *Spanish Striped*, *Non-Spanish Striped* and *Non-Striped* eggplant accessions used for the present study

Trait	*Spanish Striped*		*Non-Spanish Striped*		*Non-Striped*	
	Mean	Range[a]	Mean	Range	Mean	Range
No. of accessions	9		11		13	
Plant growth habit	3.89	1–7	2.82	1–5	2.85	1–5
Plant height (cm)	82.37	42–98	91.87	78–104	99.68	83–117
Shoot tips anthocyanins intensity	0.33	0–3	1.18	0–5	4.38	0–7
Leaf pedicel length (cm)	5.76	3.5–8.5	4.75	3–7	4.48	1.5–9.5
Leaf blade length (cm)	15.00	13.5–16.6	14.71	11.1–19.8	15.18	11.4–19.0
Leaf blade breadth (cm)	10.67	9.5–11.7	9.54	5.6–14.9	9.59	6.2–15.2
Leaf blade length/breadth ratio	1.41	1.1–1.5	1.59	1.3–2.0	1.63	1.2–5.0
Leaf blade lobing	4.78	3–5	4.45	3–7	4.23	3–7
Leaf prickles	0.00	0–0	0.27	0–3	0.31	0–3
Leaf blade tip angle	3.89	3–5	3.55	3–5	3.62	1–7
Flowering time (d)	35.00	30–39	32.24	24–42	32.87	22–43
First fruit harvest (d)	56.61	41–66	48.31	42–59	51.18	43–68
Fruit length (cm)	13.63	11.5–19.0	12.20	8.6–14.9	15.68	7.2–26.1
Fruit breadth (cm)	7.74	6.5–8.5	6.63	5.1–8.1	6.19	3.3–9.8
Fruit length/breadth ratio	1.78	1.5–2.5	1.95	1.1–2.9	3.09	0.7–7.4
Fruit curvature	1.00	1–1	1.18	1–3	1.54	1–3
Fruit cross-section	1.89	1–3	1.00	1–1	2.23	1–7
Fruit widest part	5.00	5–5	5.00	5–5	4.23	3–5
Fruit apex shape	7.00	7–7	6.45	5–7	5.46	3–7
Fruit predominant color intensity	6.33	5–9	6.45	3–9	6.85	3–9
Fruit epidermis shininess	6.33	5–7	6.09	5–7	6.31	7–3
L* primary color	42.69	27–63	42.03	28–51	35.68	21–83
a* primary color	17.50	11–23	17.79	12.5–22.0	4.18	−19.8–24.2
b* primary color	0.15	−2.3–3.1	0.47	−0.5–1.8	4.69	−6.0–35.8
Fruit color intensity under calyx	6.33	5–9	6.45	3–7	4.62	0–9
Fruit surface covered by secondary color	3.33	3–5	2.91	2–3	1.00	0–2
Relative fruit calyx length	2.33	1–3	2.27	1–3	2.23	1–9
Fruit calyx prickles	6.44	5.0–8.3	2.97	0.3–6.3	2.18	0.0–4.7
Fruit puncture force (N)	37.00	28–46	51.29	35–72	41.84	22–59
Fruit deformation at puncture (mm)	9.76	7.7–10.5	11.52	8.3–15.0	10.51	7.7–14.2
Fruit skin chlorophyll	1.00	1–1	1.09	1–2	1.69	1–2
Fruit stylar scar	3.67	1–5	3.00	1–5	3.31	1–5
Number of fruits per plant	14.03	8.8–18.0	19.47	13.1–31.6	16.28	7.8–25.4
Fruit weight (g)	406.22	350–442	250.62	142–332	256.34	118–469
Yield (kg/m^2)	5.86	3.2–7.7	5.09	2.6–8.1	4.05	2.3–7.5

[a] Range expresses the minimum and maximum mean (metric traits) and modal (arbitrary scale traits) values for each of the accessions studied

which plots together, consist of the *Listada* accessions (Spanish *Listada de Gandía*, *Other Spanish Listada* and *Non-Spanish Listada*) and the Indian variety MAN (*Other Non-Spanish Striped*). The other four accessions corresponding *Other Non-Spanish Striped* accessions plot intermingled with *Non-Striped* eggplants and are situated closer to the latter varieties than to other *Striped* varieties. These accessions come from

Table 5 Correlation coefficients with values above 0.7 for morphological traits contributing to the first two principal components, and proportion of total variance explained by the principal components analyzes including all (33) accessions, or only the "Listada" (15) accessions

Traits	Principal components correlation coefficients	
	First component	Second component
All accessions (33)		
Fruit breadth	−0.895	−0.047
Fruit length/breadth ratio	0.849	0.117
Fruit apex shape	−0.776	−0.177
Fruit weight	−0.770	0.229
Fruit curvature	0.739	−0.098
Leaf blade breadth	−0.058	0.862
Leaf pedicel length	−0.125	0.786
Leaf blade length/breadth ratio	0.361	−0.776
Variability (%)	20.3	16.3
Listada accessions (15)		
Fruit cross-section	0.801	−0.233
Fruit stylar scar	0.779	−0.100
Fruit length	0.775	0.218
b* primary color	−0.778	0.007
L* primary color	−0.748	−0.298
Fruit calyx prickles	0.737	−0.570
Fruit deformation	0.072	0.825
Fruit breadth	0.110	−0.810
Fruit weight	0.357	−0.796
Variability (%)	24.4	16.0

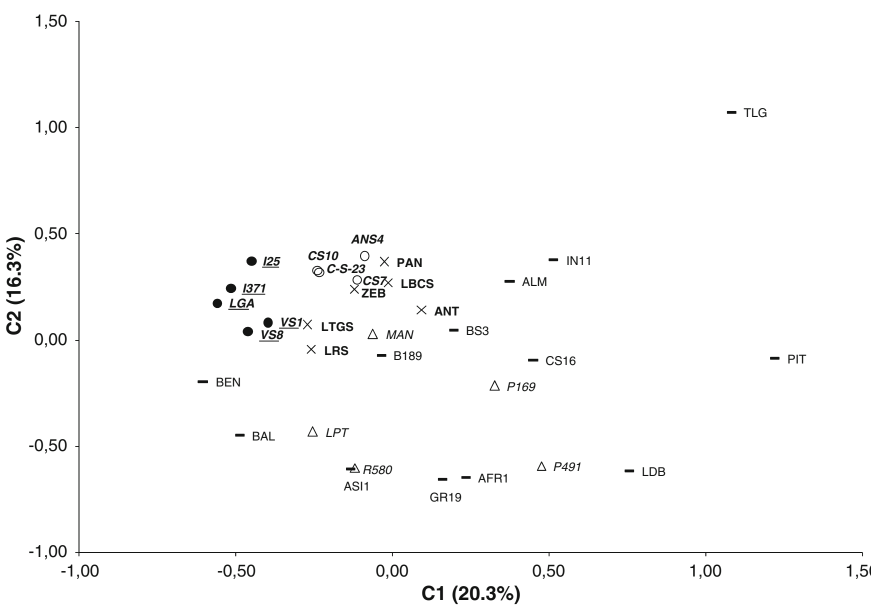

Fig. 2 Relationships between all the eggplant groups studied (33 accessions) based on principal components analysis (20.3% and 16.3% of the total variation explained by the first and second component, C1 and C2, respectively) using data on 35 morphological, developmental and agronomic traits (see text and Table 2). ● = *Listada de Gandía* (in bold, italics, underlined font); ○ = *Other Spanish Listada* (in bold, italics font); × = *Non-Spanish Listada* (in bold font); Δ = *Other Non-Spanish Striped* (in italics font); ▬ = *Non-Striped* (in normal font)

the Eastern Mediterranean region (P169, P491 and R580), or have an unknown origin (LPT).

Despite the fact that all the 15 *Listada* accessions plot in the same part of the graph, the accessions corresponding to each of the Spanish *Listada de Gandía*, *Other Spanish Listada*, and *Non-Spanish Listada* groups are not intermingled (Fig. 2). In order to have a closer examination of the relationships among these *Listada* accessions we performed an additional PCA taking into account only the accessions of this group (Fig. 3). In this case, the two first components accounted for 40.4% of the total variation, with a 24.4% explained by the first component and an additional 16.0% by the second component (Table 5). The first component, which separates the Spanish *Listada de Gandía* (positive values) from the *Other Spanish Listada* (negative values) accessions, was positively correlated with the fruit cross-section, fruit stylar scar, fruit length, and fruit calyx prickles, and negatively with the L* and b* values of the primary color (i.e., indicating a darker blue color of the stripes in the *Listada de Gandía*). The second component separates some of the *Non-Spanish Listada* (ANT, PAN and ZEB) from the rest of accessions (Fig. 3), and was positively correlated with the fruit deformation at puncture and negatively with the fruit breadth and with the fruit weight (Table 5).

An important variation among the accessions studied was found for most of these traits of agronomic interest, both within the *Striped* and *Non-Striped* accessions (Table 6). Although the variation within each of the *Listada* groups is much more reduced, it is possible to find significant differences among varieties within each group. For example, within the *Listada de Gandía* group accession VS8 has a greater yield (>7 kg/m^2) and earlier flowering than other accessions. Similarly, in the *Other Spanish Listada* group, one of the accessions (ANS4) is much less productive and enters into production later than the others (Table 6). Variation also exists within the *Non-Spanish Listada* group; for example, accessions LBCS, LRS, and LTGS give good yields and have high resistance to puncture. Finally, an important variation for traits of agronomic interest has been found within the other *Non-Spanish Striped* and in the *Non-Striped* groups (Table 6).

Molecular characterization

A total of 109 AFLP fragments were scored, of which 27 (24.8%) were polymorphic. No AFLP fragments specific and universal to all *Striped* accessions was found. However, there was one AFLP fragment (360 bp in size with the combination of primers E-ACT plus M-CTA) specific and universal to all 15 *Listada* accessions and absent both in the *Other Non-Spanish Striped* and in the *Non-Striped* accessions. One fragment was also present in all the *Spanish Listada* accessions and absent in the rest of accessions. Three AFLP fragments were present in all the

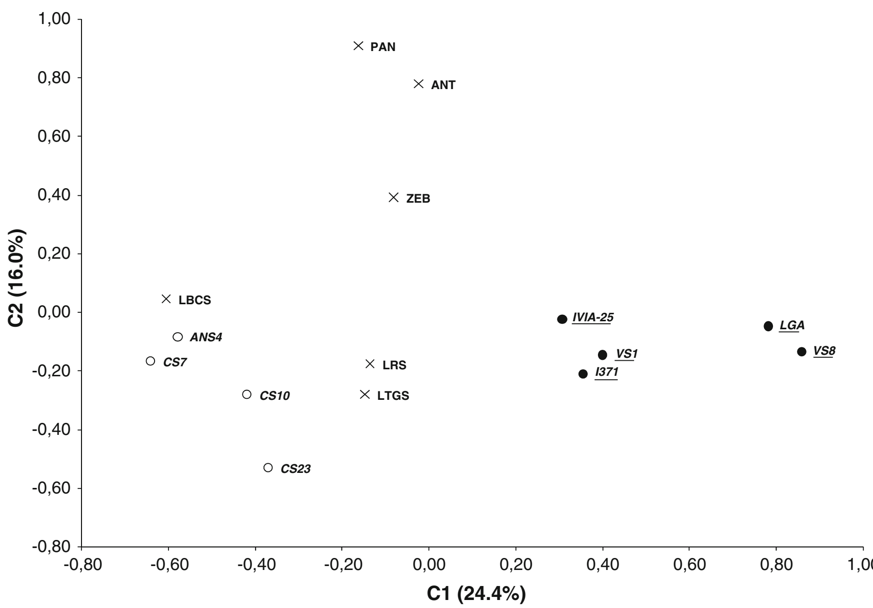

Fig. 3 Relationships between the *Listada* groups studied (15 accessions) based on principal components analysis (24.4% and 16.0% of the total variation explained by the first and second component, C1 and C2, respectively) using data on 35 morphological, developmental and agronomic traits (see text and Table 2). ● = *Listada de Gandía* (in bold, italics, underlined font); ○ = *Other Spanish Listada* (in bold, italics font); × = *Non-Spanish Listada* (in bold font)

Table 6 Mean values for each accession and group of accessions, coefficient of variation within each group of accessions (CV), and average standard error (SE; see last line of the table) for traits of agronomic interest in the eggplant accessions studied

Accession	Yield (kg/m^2)	Fruit weight (g)	Fruit length (cm)	Fruit calyx prickles[a]	Flowering time (d)	First fruit harvest (d)	Fruit puncture force (N)	Fruit deformation at puncture (mm)
Spanish Listada de Gandia								
I25	5.72	420.5	12.9	6.3	38.8	58.5	32.0	9.7
I371	5.69	442.6	12.5	7.0	39.2	57.8	33.0	10.0
LGA	4.90	436.4	15.8	8.0	36.1	66.3	34.6	9.7
VS1	6.37	413.3	13.6	6.2	35.3	55.5	31.8	10.2
VS8	7.76	413.9	18.9	8.3	34.0	56.0	28.1	10.5
Mean	6.09	425.3	14.7	7.2	36.7	58.8	31.9	10.0
CV (%)	17.6	3.2	18.0	13.4	6.1	7.4	7.5	3.4
Other Spanish Listada								
ANS4	3.26	350.0	11.5	5.2	33.8	63.4	40.7	10.0
CS10	6.17	387.4	13.0	5.0	30.5	52.5	41.4	9.6
CS23	6.76	404.6	12.7	7.0	36.8	57.6	46.3	7.8
CS7	6.10	386.9	11.8	5.0	30.3	41.8	44.6	10.0
Mean	5.57	382.2	12.2	5.6	32.9	53.8	43.3	9.4
CV (%)	28.2	6.0	5.8	17.5	9.4	17.0	6.1	11.2
Non-Spanish Listada								
ANT	4.82	185.5	14.8	4.0	42.6	59.6	35.0	10.8
LBCS	5.84	305.2	11.7	2.3	31.5	43.3	52.9	10.2
LRS	6.11	324.7	11.2	4.3	33.3	53.1	57.7	10.0
LTGS	5.82	332.4	11.1	5.0	31.0	44.3	51.0	8.3
PAN	4.42	231.8	13.9	2.3	34.8	53.6	38.1	12.5
ZEB	5.74	305.5	13.2	4.0	37.3	55.3	40.7	12.0
Mean	5.46	280.9	12.7	3.7	35.1	51.5	45.9	10.6
CV (%)	12.3	20.9	12.2	30.3	12.4	12.5	20.0	14.2
Other Non-Spanish Striped								
LPT	8.16	246.3	9.0	0.3	32.5	42.8	72.2	15.0
MAN	2.89	199.6	8.7	6.3	28.3	47.5	60.8	12.4
P169	2.64	142.4	13.2	1.8	28.1	47.3	50.0	11.2
P491	5.48	239.8	15.0	1.7	24.8	42.1	41.7	11.2
R580	4.10	243.1	12.3	0.7	30.1	42.1	63.6	12.7
Mean	4.66	214.2	11.6	2.2	28.8	44.4	57.7	12.5
CV (%)	43.4	18.5	21.0	99.1	8.8	5.6	18.5	11.1
Non-Striped								
AFR1	3.36	259.6	15.2	3.7	27.0	44.0	44.8	11.0
ALM	4.25	218.0	9.0	1.2	35.1	49.3	59.5	12.4
ASI1	2.31	288.5	7.3	0.0	22.1	44.3	49.2	9.9
B189	4.07	174.8	10.7	4.0	37.6	48.1	54.6	11.5
BAL	5.53	352.6	11.8	2.2	32.8	54.6	55.1	14.2
BEN	7.55	469.0	12.2	4.7	32.8	46.8	45.9	12.3
BS3	2.48	305.5	17.2	1.5	33.7	68.0	34.6	9.7
CS16	3.73	240.7	18.7	2.0	35.0	49.3	33.8	9.6
GR19	4.28	290.9	17.2	1.3	28.6	47.3	44.0	10.5
IN11	4.86	229.9	16.6	1.5	31.3	54.8	43.2	7.7

Table 6 continued

Accession	Yield (kg/m^2)	Fruit weight (g)	Fruit length (cm)	Fruit calyx prickles[a]	Flowering time (d)	First fruit harvest (d)	Fruit puncture force (N)	Fruit deformation at puncture (mm)
LDB	2.50	190.3	14.4	2.6	29.4	43.8	29.4	9.6
PIT	2.69	118.8	24.6	0.0	43.1	59.3	22.8	9.1
TLG	5.04	193.4	26.2	3.7	38.3	55.3	26.4	8.6
Mean	4.05	256.3	15.7	2.2	32.8	51.1	41.8	10.5
CV (%)	36.7	34.8	36.0	68.2	16.4	13.8	27.7	17.0
Average SE	0.80	23.1	1.0	0.7	2.9	4.4	3.0	0.7

[a] Measured in a 0–9 scale with pre-determined values of the descriptor states: 0 = none, 9 = very many (>30)

Other Spanish Listada accessions and absent in all the *Listada de Gandía* accessions.

The 20 accessions of *Striped* accessions studied presented a diversity similar to those of the 13 *Non-Striped* accessions (Table 7). However, the *Non-Spanish Striped* accessions were much more diverse (total genetic diversity index H almost three times greater) than the *Spanish Striped* accessions. When considering the *Spanish Listada* accessions, the *Other Spanish Listada* presented a much greater diversity (more than four times higher) than the *Listada de Gandía* accessions (Table 7).

The first and second coordinates of the PCoA made on AFLP data account respectively for 33.7% and 17.2% of the variation observed. The PCoA graph (Fig. 4) shows that *Listada* accessions, with the exception of the Italian accession 'Pandora Striped Rose' (PAN), plot in the same part of the graph. The five *Other Non-Spanish Striped* accessions (LPT, MAN, P169, P491, and R580) are intermingled with the *Non-Striped* accessions. When focusing only on the *Listada* eggplants (Fig. 5), the first and second coordinates of the PCoA respectively account for 44.5% and 22.4% of the variation observed. In this case, the PCoA graph (Fig. 5) shows that the Spanish *Listada de Gandía*, *Other Spanish Listada*, and *Non-Spanish Listada* accessions plot in different areas of the graph. One odd accession of *Non-Spanish Listada* (ANT) plots closer to the *Listada de Gandía* group that to the rest of *Non-Spanish Listada*.

Table 7 Total genetic diversity (H; Nei 1973) estimated from AFLP markers data for the materials studied considering all accessions (*Striped* versus *Non-Striped*), *Striped* accessions (*Spanish Striped* versus *Non-Spanish Striped*), and the *Spanish Striped* accessions (*Listada de Gandía* versus *Other Spanish Listada*)

Groups	No. of accessions	H
All accessions	33	0.082
Striped	20	0.071
Non-Striped	13	0.081
Striped	20	0.064
Spanish Striped	9	0.027
Non-Spanish Striped	11	0.073
Spanish Striped	9	0.027
Listada de Gandía	5	0.004
Other Spanish Listada	4	0.017
Non-Spanish Striped	11	0.073
Non-Spanish Listada	6	0.054
Other Non-Spanish Striped	5	0.045

Discussion

This research was performed in order to characterize and study the diversity and relationships of the Spanish striped eggplants and to use this information for the enhancement and protection of local heirlooms. The combination of morphology and molecular markers has been successfully applied to study the diversity of eggplants and its implications for breeding and conservation (Prohens et al. 2005). However, to our knowledge, this is the first report of the use of morphology and molecular markers for assessing the diversity and relationships within a varietal group of eggplants.

Despite the genetic bottleneck suffered during the domestication of eggplant (Lester and Hasan 1991), we have found considerable morphological, agronomic and genetic diversity both in the *Striped* and *Non-Striped* accessions. Although both the *Striped* and *Non-Striped* groups contain considerable morphological, agronomic and genetic diversity, the *Listada*

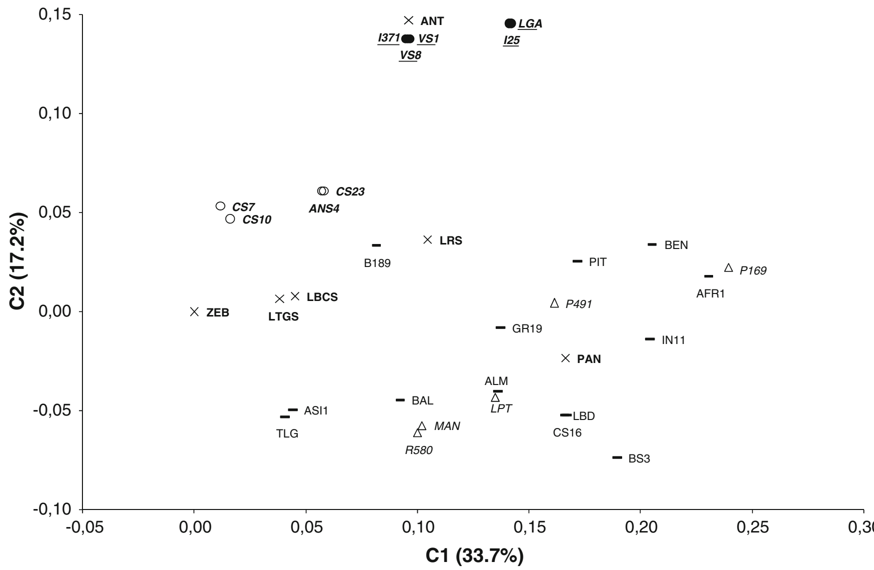

Fig. 4 Relationships between all the eggplant groups studied (33 accessions) based on principal coordinates analysis (33.73% and 17.2% of the total variation explained by the first and second coordinate, C1 and C2, respectively) using AFLP-based genetic similarities. ● = *Listada de Gandía* (in bold, italics, underlined font); ○ = *Other Spanish Listada* (in bold, italics font); × = *Non-Spanish Listada* (in bold font); Δ = *Other Non-Spanish Striped* (in italics font); ▬ = *Non-Striped* (in normal font)

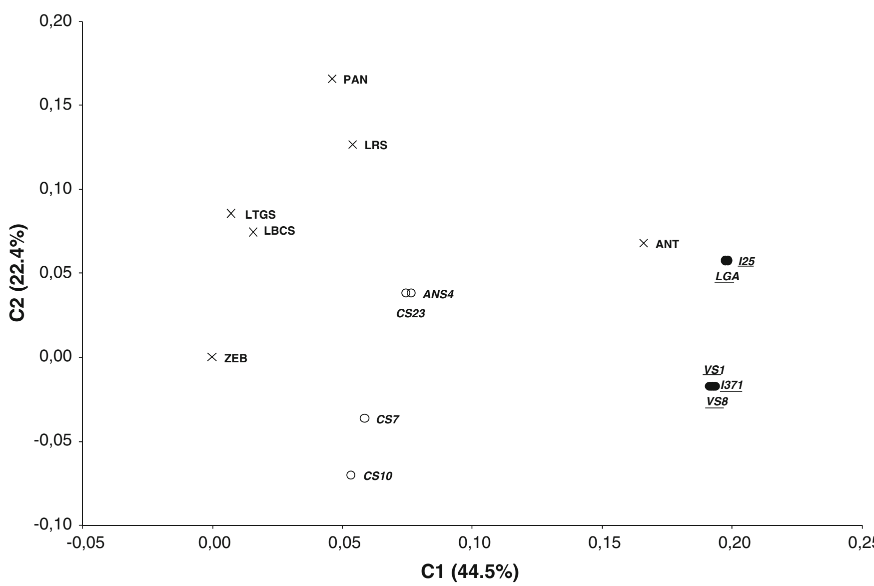

Fig. 5 Relationships between the *Listada* groups studied (15 accessions) based on principal coordinates analysis (44.5% and 22.4%) of the total variation explained by the first and second coordinate, C1 and C2, respectively) using AFLP-based genetic similarities. ● = *Listada de Gandía* (in bold, italics, underlined font); ○ = *Other Spanish Listada* (in bold, italics font); × = *Non-Spanish Listada* (in bold font)

accessions, in particular, are morphologically and molecularly more similar to one another compared to the *Other Non-Spanish Striped* and *Non-Striped* groups. In fact, no AFLP fragments specific and universal to all *Striped* accessions have been found. This may suggest that the striped trait, which seems to have a recessive inheritance (personal observation), may have had more than one independent origins during the evolution of the crop, or that it has been incorporated into different genetic backgrounds from a single origin.

All the *Listada* accessions share one AFLP fragment which is absent from the rest of *Other Non-Spanish Striped* and *Non-Striped* accessions, suggesting a common origin for the *Listada* eggplants. Historical evidence indicates that the *Listada* eggplant may be a varietal group of relatively recent origin. To this respect, in the eleventh century the agronomist Abu-Zacaría describes four varieties of eggplant in Al-Andalus (Muslim Spain), but none of them is striped (Abú-Zacaría 1802). Similarly, in the sixteenth century the Spanish agronomist Alonso de Herrera while describing the eggplant materials grown in Spain does not mention striped eggplants (Alonso de Herrera 1970). Daunay and Janick (2007) and Daunay et al. (2007), in their studies of the iconography of eggplant in Medieval and Renaissance Europe do not mention or display pictures of striped eggplants. This recent origin might explain the relatively low diversity of the *Listada* group.

Despite the morphological and genetic similarities among the Spanish and non-Spanish *Listada* types, the Spanish *Listada* accessions can be distinguished morphologically and molecularly from the *Non-Spanish Listada* accessions. The Spanish *Listada* accessions are characterized by a higher fruit weight and more prickles in the calyx than the *Non-Spanish Listada*, and also many of the Spanish *Listada* accessions have a delayed flowering and fruiting with respect to the *Non-Spanish Listada*. These traits are under the control of a few QTLs accounting for an important part of the variation of these traits (Doganlar et al. 2002; Frary et al. 2003). This may explain why, although genetically close, these accessions display differences for several morphologically important traits. The fact that all the Spanish *Listada* accessions present one AFLP fragment absent in all the *Non-Spanish Listada*, suggests that Spanish *Listada* materials may have undergone some differentiation from other *Listada* types from other origins.

The classification obtained either with the PCA (morphological) and PCoA (AFLP) analyzes has allowed distinguishing two main groups of Spanish *Listada* eggplant. One of them is constituted by the *Listada de Gandía* heirloom, while the other by the *Other Spanish Listada* accessions from other regions of Spain. At this respect, as shown in the PCA analysis and in the means of the groups, the *Listada de Gandía* accessions have a heavier and more elongated fruit, with more prickles in the calyx and darker stripes than the *Other Spanish Listada* accessions. Also, the absence in the *Listada de Gandía* type of three AFLP fragments present in the *Other Spanish Listada* accessions indicates that, genetically, it is possible to differentiate this local heirloom from other similar accessions. This suggests that, as has been found in Italian landraces of pepper (Portis et al. 2006), local conditions and selection by farmers have generated some degree of differentiation among eggplant accessions sharing the same origin. The fact that eggplant is mostly autogamous (Pessarakli and Dris 2004; Daunay 2008) may have favoured the isolation of local variants.

Prohens et al. (2005) found that *Listada de Gandía* accessions can be genetically distinguished from other non-striped Spanish accessions. Here we have found that the *Listada de Gandía* heirloom is both morphologically and genetically distinguishable from the other *Listada* accessions. The fact that the so-called *Listada de Gandía* has been traditionally grown in the region of Valencia and has a unique morphological and AFLP fingerprint may have important implications for its protection and registration of materials under a protected geographical indication or designation of origin as shown already for pepper (Portis et al. 2006) and for tomato (Rao et al. 2006).

Although several of the *Non-Spanish Listada* accessions are marketed as *Listada de Gandía*, their morphological and molecular characteristics do not fit to those of the typical *Listada de Gandía* and probably represent accessions originating from countries other than Spain. For example, three *Non-Spanish Listada* accessions originating in Italy (LBCS, LRS, and LTGS) are sold by the seed companies as *Listada de Gandía*. During the period lasting from the end of the thirteenth century to the beginning of the eighteenth century parts of the South of Italy belonged to the Spanish Crown of Aragon (Bisson 1986), which also included Valencia, and this could have contributed to the exchange of eggplant materials among these regions. During that time, the city and port of Gandía were a flourishing center of trade (Soria and Jarque 2002). One of the accessions of *Non-Spanish Listada* (Antigua) is molecularly similar to the *Listada de Gandía*, but morphologically different, suggesting that they may have a common origin, but that selection for different conditions has resulted in different morphological characteristics.

Despite of the high degree of morphological and genetic similarity within each of the *Listada* groups, the results show that there is some genetic variation and also differences in the agronomic performance among accessions within these groups. For example, *Listada de Gandía* VS8 accession presents a higher yield and enters earlier into production, than other *Listada de Gandía* accessions (Table 6). This indicates that these accessions, even within this relatively homogenous type, are amenable to selection. Because within the *Listada* (i.e., *Listada de Gandía* + *Other Spanish Listada* + *Non-Spanish Listada*) type there is genetic variation, hybrids heterotic for yield and maintaining the characteristics of the *Listada* type might be obtained by crossing *Listada* parents situated at high genetic distance (Rodríguez-Burruezo et al. 2008). In addition, our unpublished results indicate that although the genetic diversity of *Listada de Gandía* accessions is limited, hybrids maintaining the characteristics of this group and having improved yield might be obtained.

The results we have obtained demonstrate that both morphology and molecular markers are useful for determining the diversity and relationships among striped eggplant accessions. Combination of both types of data provides additional information relevant for the conservation, improvement and legal protection of *Listada* eggplants. In particular, by using both types of data, we have been able to demonstrate that the *Listada de Gandía* eggplants, originating from the region of Valencia (Spain), can be distinguished from similar types of eggplant, some of which are erroneously labeled (and sold) as *Listada de Gandía*. Thus, the combination of molecular and morphological characteristics can be appropriate for implementing a legal protection of this heirloom. Also, despite the low genetic diversity found within the *Listada de Gandía* group, variation has been found among accessions for traits of agronomic interest, indicating that accessions with an improved combination of characteristics could be obtained by recombining germplasm collections. The combination of selected *Listada de Gandía* accessions with a protected geographical indication or designation of origin could be of great advantage to farmers of the region and could provide a model for exploiting local landraces for the development of horticulture. The methodologies and approaches used here, which combine morphological, agronomic and molecular data for the characterization, fingerprinting, assessment of diversity, and of relationships with related accessions could be used for the enhancement and protection of other heirlooms.

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