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Additional Information

1 Diversity, relationships, and genetic fingerprinting of the *Listada de*

2 *Gandía* eggplant landrace using genomic SSRs and EST-SSRs

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4 Julio E. Muñoz-Falcón[a], Santiago Vilanova[b], Mariola Plazas[b], Jaime Prohens[b,*]

5

6 [a] *Instituto Nacional de Investigaciones Agrícolas, Carretera Barquisimeto vía Duaca km*

7 *7, Sector El Cují, Estado Lara, Venezuela*

8 [b] *Instituto de Conservación y Mejora de la Agrodiversidad Valenciana, Universidad*

9 *Politécnica de Valencia, Camino de Vera 14, 46022 Valencia, Spain*

10

11 *Corresponding author: Instituto de Conservación y Mejora de la Agrodiversidad

12 Valenciana, Universidad Politécnica de Valencia, Camino de Vera 14, 46022 Valencia,

13 Spain. Tel.: +34 963879424; fax: +34 963879422.

14 E-mail address: jprohens@btc.upv.es (J. Prohens)

15

16   ABSTRACT

17

18   *Listada de Gandía* is one of the most renowned Spanish eggplant (*Solanum melongena*

19   L.) landraces. Assessing its genetic diversity and relationships, as well as devising tools

20   for its identification, is of great relevance for the enhancement and protection of this

21   landrace. Forty-two eggplant accessions, which included 25 *Striped* accessions, of

22   which 19 were of the *Listada* type (six accessions of *Listada de Gandía*, eight of *Other*

23   *Spanish Listada*, and five of *Non-Spanish Listada*) and six of the *Other Non-Spanish*

24   *Striped* group, and 17 *Non-Striped* accessions were characterized with 17 genomic SSRs

25   and 32 EST-SSRs. Genomic SSRs had, as a mean, a greater polymorphism and

26   polymorphic information content (PIC) than EST-SSRs. Although *Listada de Gandía*

27   proved to be genetically diverse, specific and universal alleles for two SSR markers

28   were found for this landrace. All the *Listada* accessions cluster together in the

29   multivariate PCoA and UPGMA phenograms performed, and are separated from the

30   *Other Non-Spanish Striped* and *Non-Striped* accessions. Also, *Listada de Gandía*

31   accessions were clearly differentiated from the *Other Spanish Listada* and *Non-Spanish*

32   *Listada* accessions in these analyses. SSR markers revealed of great utility to obtain a

33   specific fingerprint for the *Listada de Gandía* eggplant as well as to establish the

34   uniqueness and distinctness of this landrace. This information will be very helpful for

35   the enhancement and protection from imitation of *Listada de Gandía*, and contributes to

36   support its potential recognition with a Protected Designation of Origin (PDO) status.

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38   Keywords: EST-SSRs, fingerprinting, genomic SSRs, landraces, *Listada de Gandía*,

39   *Solanum melongena*

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1. Introduction

Amongst the many Spanish local varieties of eggplant (*Solanum melongena* L.), the *Listada de Gandía* landrace is the most internationally known (Muñoz-Falcón et al., 2008a). The *Listada de Gandía* eggplant is native to the area around the city of Gandía, situated some 60 km to the South of Valencia, and is characterized by having large, semi-long fruits covered by a bright skin with intense purple stripes over a white background, luminous flesh, and excellent organoleptic and cooking qualities (Prohens and Nuez, 2001). The *Listada* name refers to the striped ("listada" in Spanish) characteristic of the fruit skin. Given the good reputation of quality of the *Listada de Gandía* eggplant, it is frequent to find striped eggplant materials marketed under the name *Listada de Gandía*, although they do not correspond to this landrace (Muñoz-Falcón et al., 2008a).

Certification of the authenticity of materials marketed as *Listada de Gandía*, as well as detecting fake materials marketed under this name, is essential for the protection and enhancement of this landrace. In the European Union, agricultural produces from a specific region and with a high quality can be protected from imitation by a Protected Designation of Origin (PDO) or a Protected Geographical Indication (PGI) (Commision of the European Communities, 2006). Usually, produces with a PDO or PGI label have an added value in the market (Gracia and Albisu, 2001). A clear example of this situation is the case of the *Almagro* eggplant, which is a local Spanish eggplant landrace used for making pickles, which since achieving a PGI status in 1994 has resulted in an increase in the production and value of the crop (Muñoz-Falcón, et al., 2008b; Prohens et al., 2007, 2009). In this respect, *Listada de Gandía*, might be a candidate to obtain a PDO status (MAPA, 2010). However, it is necessary to devise tools for the certification

66   of the uniqueness and authenticity of this landrace.

67   A previous study performed with morphological and AFLP data revealed that, when

68   grown under a uniform environment, the *Listada de Gandía* eggplant could be

69   distinguished from other striped materials from Spain and other countries by a

70   combination of morphological characteristics (Muñoz-Falcon, et al., 2008a). Also, the

71   study of the genetic diversity with AFLP markers showed that *Listada de Gandia* is

72   genetically different from other striped accessions, including also materials from other

73   origins marketed as *Listada de Gandía* (Muñoz-Falcón et al., 2008a). However, no

74   AFLP markers specific and universal to all *Listada de Gandía* accessions could be

75   found.

76   Morphological characterizations allow describing the phenotypic characteristics

77   of the plant material and, currently, morphological traits constitute the tools used for the

78   distinctness, uniformity and stability (DUS) tests required for protection of plant

79   materials (UPOV, 2002). Despite their evident usefulness, it has been demonstrated that

80   morphological traits used for eggplant characterization may be subjected to

81   environmental variation (Gajewski et al., 2009; Prohens et al., 2004; Raigón et al.,

82   2010). Furthermore, this situation gets more complicated in the cases where only the

83   fruit is available to certificate the authenticity of the produce. In this respect, molecular

84   markers represent an additional tool for protecting and characterizing the plant material,

85   and their utility for the protection of local landraces of vegetables is evident (Mazzucato

86   et al., 2010; Muñoz-Falcón et al., 2008b; Rao et al., 2006).

87   SSR markers present several advantages, like its high reproducibility, co-dominance,

88   hyper-variability, relative abundance, and high genome coverage, (Morgante et al.,

89   2002; Powell et al., 1996), for being used in the characterization of diversity and

90   relationships of plant landraces. SSRs have been successfully applied to the

91   characterization and to obtaining genetic fingerprints of eggplant (Demir et al., 2010;

92   Muñoz-Falcón, et al., 2008b; Nunome et al., 2003; Stàgel, et al., 2008). Furthermore,

93   using *Almagro* eggplant landrace materials, we have found that SSRs are much more

94   adequate than AFLPs to detect relationships among closely related materials as well as

95   to obtain specific genetic fingerprints (Muñoz-Falcón et al., 2008b). In this respect,

96   SSRs seem to be more suited than AFLPs to studying specific sets of genetically related

97   materials, probably because of its sensitivity to neutrality and/or linkage disequilibrium

98   (Tam et al., 2005).

99   SSR markers can be classified in genomic SSRs and EST-SSRs (Chabane et al.,

100   2005; Duran et al., 2009; Morgante et al., 2002). Genomic SSRs are mostly associated

101   with non-coding regions, while EST-SSRs derive from expressed regions of the genome

102   (Morgante, et al., 2002; Li et al., 2004; Panaud et al., 1995). Genomic SSRs usually

103   have a higher degree of polymorphism than EST-SSRs (Lee et al., 2004; Martin et al.,

104   2010; Tehrani et al., 2009; Varshney et al., 2005); the latter, on the other hand, are

105   relatively easier to obtain through *in silico* data mining of EST sequences, have a

106   greater transferability among species, and since they are located within genes, their

107   variation may be related to phenotypic variation (Andersen and Lubberstedt, 2003;

108   Duran et al., 2009; Li et al., 2004)

109   In this work we use genomic SSRs and EST-SSRs to study the diversity of the

110   *Listada de Gandía* landrace, as well as its relationships with other striped and non-

111   striped materials. This information may be useful to establish the genetic differentiation

112   and uniqueness of the *Listada de Gandía* eggplant, as well as to identify markers that

113   can provide a specific genetic fingerprint for this landrace, which can be a useful tool

114   for the certification of authenticity and labeling of the *Listada de Gandía* eggplant.

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2. Materials and Methods

2.1. Plant material

A total of 42 eggplant accessions, belonging to five varietal groups were used (Table 1; Figure 1):

i) Group *Listada de Gandía* (6 accessions): Spanish striped landraces traditionally grown in the region of Valencia and locally known under this name.

ii) Group *Other Spanish Listada* (8 accessions): Spanish striped landraces known as *Listada*, but traditionally grown outside the province of Valencia. They are similar in gross morphology to the *Listada de Gandía.*

iii) Group *Non-Spanish Listada* (5 accessions): Striped landraces and cultivars that presumably do not originate from Spain (Table 1) and are sold by heirloom seed companies. Morphologically, they also resemble in gross morphology to the *Listada de Gandía* Accessions in these group are sometimes mis-labeled and sold under the name *Listada de Gandía*.

iv) Group *Other Non-Spanish Striped* (6 accessions): Striped landraces and cultivars that do not originate from Spain. They are morphologically very distinct to the three former *Listada* groups.

v) Group *Non-Striped* (17 accessions): Set of landraces and cultivars that are not striped, and display a diversity of geographical origins, fruits size, shape and colour.

Groups i) to iv) have in common that all accessions have striped fruits, and are jointly referred as *Striped* (Figure 1). Within this set of accesions, groups i) to iii) are collectively known as *Listada*, as they all share a similar fruit and plant morphology. 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

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141   purchased from heirloom seed companies (Table 1). A detailed description of the

142   morphological characteristics of the different groups can be consulted elsewhere

143   (Muñoz-Falcón et al. 2008a).

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145   *2.2. DNA extraction and SSR analysis*

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147   Genomic DNA from each accession was extracted from a mixture of young leaves

148   from six plants with the DNeasy Plant Mini Kit (Quiagen Inc., Valencia, California,

149   USA) using the protocol recommended by the manufacturer. The DNA concentration

150   was quantified after electrophoresis on a 0.8% agarose gel, and the DNA concentration

151   of each of the samples was determined with a Nanodrop ND-1000 (Nanodrop

152   Technologies, Wilminton, Delaware, USA) spectrophotometer. DNA was diluted to a

153   concentration of 10 ng/µl in order to perform PCRs.

154   Accessions were screened with a total of 49 SSR markers, of which 17 were genomic

155   SSRs and 32 EST-SSRs (Table 2). Eleven of the genomic SSRs were developed by

156   Nunome et al. (2003), while the other six were developed by ourselves from the

157   information available from the National Center for Biotechnology Information database

158   (http://www.ncbi.nlm.nih.gov/) using the TROLL software (Castelo et al. 2002) (Table

159   3). Twenty-two of the EST-SSRs were developed by Stàgel et al. (2008), while the other

160   ten were developed by Tümbilen (2007). SSRs were tested following the M13-tail PCR

161   method of Schuelke (2000), which involves an M13-tailed forward primer used in

162   combination with a standard M13 primer dye-labeled with FAM, NED, PET or VIC

163   fluorophores at its 5'-end.

164   The PCR reaction consisted of $1\times$ PCR buffer, 1.5mM $MgCl_2$, 0,2 mM dNTPs, 0.04

165   units Taq DNA polymerase, 0.05 µM forward primer, 0.25 µM reverse primer, 0.2 µM

166   M13-labeled primer, 10 ng DNA, and distilled H_2O in an 10 µl total reaction volume.

167   Amplifications were carried out in an Eppendorf thermocycler with an initial step at

168   94ºC for 3 min, 35 cycles of 94 ºC for 30 s, 58ºC for 45 s, 72ºC for 1 min and a final 10

169   min extension at 72ºC. PCR products were separated in an ABI Prism 310 genetic

170   analyser (Applied Biosystems, Foster City, California, USA). The analysis was

171   performed using Genscan and Genotyper (Applied Biosystems) software.

172

173   *2.3. Data analyses*

174

175   The polymorphism information content (PIC), defined as PIC=1-$\sum p_{ij}^2$, where p_{ij} is

176   the frecuency of the *j*th allele for marker *i* and the summation extends over *n* alleles

177   (Anderson et al., 1993), and the number of polymorphic alleles for each SSR marker

178   were calculated using the PowerMarker program (Liu and Muse, 2005). Pairwise

179   genetic similarities were estimated with the Dice (Sorensen) similarity coefficient

180   (Mohammadi and Prassana, 2003). Principal coordinates analyses (PCoA) and UPGMA

181   (unweighted pair group method using arithmetic means) phenograms were performed

182   using the pairwise genetic similarities using the NTSYSpc 2.0 software package

183   (Applied Biostatistics, Port Jefferson, New Jersey, USA). Supports for the groups on

184   the phenograms were tested by bootstrap analysis with 1000 replications, using the

185   PHILYP 3.67 program (Felsenstein, 1989). The genetic diversity of the different groups

186   was estimated with the total diversity (H_T) Nei (1973), and the genetic distance among

187   the different goups was estimated using the PopGene 32 program (Yeh et al., 2000).

188

189   **3. Results**

190

191   *3.1. SSRs characterization*

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193   Fourteen out of the 17 genomic SSRs evaluated (82.3%) were polymorphic, while

194   only seven out of the 32 EST-SSR (21.8%) showed polymorphism in the 42 accessions

195   studied. In total, the 21 polymorphic SSRs amplified 85 alleles (mean of 4.05

196   alleles/locus). The mean number of alleles per locus for polymorphic genomic SSRs

197   was 4.50, while for EST-SSR was 3.14 (Table 4). Genomic SSRs PIC ranged from

198   0.086 (BMS33) to 0.819 (EM155), with a mean value of 0.401, while for EST-SSRs

199   PIC varied between 0.094 (EEMS37) and 0.771 (EEMS15), with a mean value of 0.248

200   (Table 4).

201

202   *3.2. Genetic diversity*

203

204   H_T values for the set of *Striped* accessions and *Non-Striped* controls was similar, with

205   values of 0.3739 and 0.4106, which represent 84.5% and 93.2% of the total genetic

206   diversity of the materials studied (H_T=0.4406) (Table 5). Also, the percentage of

207   polymorphic loci of both groups was quite similar, with 18 polymorphic SSRs for the

208   *Striped* group and 19 for the *Non-Striped* group. The *Listada de Gandía* group presented

209   9 polymorphic loci and an H_T value of 0.1945. The greatest diversity and number of

210   polymorphic loci among the different *Striped* subgroups was found in the *Other Non-*

211   *Spanish Striped*, followed by the *Non-Spanish Listada*, *Listada de Gandía*, and *Other*

212   *Spanish Listada* (Table 5). The genetic distance values among the groups considered

213   ranged between 0.1259 between *Other Spanish Listada* and *Non-Spanish Listada* and

214   0.4834 between *Listada de Gandía* and *Other Non-Spanish Striped* (Table 6). The group

215   most similar to the *Listada de Gandía* was the *Other Spanish Listada*, with a genetic

216   distance value of 0.2822.

217

218   *3.3. Genetic fingerprinting*

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220   A unique and distinct fingerprint was obtained for each of the accessions tested with

221   the combination of SSR markers used. However, no SSR alleles were found to be

222   specific and universal to the set of *Striped* or *Non-Striped* accessions. When considering

223   the *Striped* accessions, we found two SSR alleles specific and universal to all *Listada de*

224   *Gandia*, which correspond to allele 204 for SSR marker EEMS37 and to allele 184 of

225   SSR marker EM133 (Table 7). These alleles are absent from the rest of groups of striped

226   eggplants (*Other Spanish Listada*, *Non-Spanish Listada*, and *Other Non-Spanish*

227   *Striped*). In addition, allele 310 for SSR marker EM140 is specific, but not universal, to

228   the *Listada de Gandía* group. Also, we found that for SSR marker EM126, accessions of

229   the three *Listada* groups (*Listada de Gandía*, *Other Spanish Listada*, and *Non-Spanish*

230   *Listada*) present alleles 226 or 230, while the *Non-Spanish Striped* accessions are

231   monomorphic for allele 228 (Table 7).

232

233   *3.4. Genetic relationships*

234

235   The first and second coordinates of the PCoA analysis performed with SSR data

236   account for 13.64% and 7.61% of the total variation, respectively. In this analysis, all

237   the *Listada* accessions (i.e., *Listada de Gandía*, *Other Spanish Listada*, and *Non-*

238   *Spanish Listada*) plot together in the upper central-right part of the graph (Figure 2).

239   The *Other Non-Spanish Striped* and *Non-Striped* groups are intermingled and situated in

240   the lower central-left part of the graph. Although in this general PCoA *Listada de*

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241   *Gandía*, *Other Spanish Listada*, and *Non-Spanish Listada* accessions are not

242   intermingled, an additional PCoA taking into account only these three *Listada* groups of

243   accessions provided a clearer picture of their relationships. In this case, the first and

244   second components of the PCoA accounted for 22.71% and 16.85% of the total

245   variation, respectively, and shows that the *Listada de Gandía*, *Other Spanish Listada*,

246   and *Non-Spanish Listada* plot in different sections of the graph (Figure 3). The only

247   exception is accession LBCS (*Non-Spanish Listada*), which plots close to the *Other*

248   *Spanish Listada*.

249   The UPGMA phenogram performed with all accessions shows that three main

250   clusters can be distinguished (Figure 4). One of them, which is supported by a bootstrap

251   value of 53.3%, includes all the *Listada de Gandía* accessions, a second one includes all

252   the *Other Spanish Listada*, and *Non-Spanish Listada* accessions, and the third one

253   includes all the *Non-Spanish Striped* and *Non-Striped* accessions (Figure 4). When

254   considering the *Listada de Gandía* accessions, a subdivision is evident in two

255   subclusters, one which contains accessions I25 and I371, and which is supported by a

256   bootstrap value of 100%, and another one, which contains the rest of accessions, and

257   which is supported by a bootstrap value of 96.2%. The *Other Spanish Listada* and *Non-*

258   *Spanish Listada* accessions are intermingled within the second major cluster, while the

259   *Non-Spanish Striped* and *Non-Striped* accessions are also intermingled within the third

260   major cluster. The results obtained when obtaining a phenogram including only the

261   *Listada de Gandía*, *Other Spanish Listada*, and *Non-Spanish Listada* accessions does

262   not provide additional information on the relationships of these groups of accessions to

263   the one obtained with the general phenogram with all the accessions (not shown).

264

265   **4. Discusion**

266

267   Determining the diversity, relationships, establishing the uniqueness, and obtaining a

268   genetic fingerprint of the *Listada de Gandía* landrace is of great interest for the

269   protection from imitation and enhancement of this landrace. Agricultural products well

270   characterized and that are perceived as unique usually get a higher price (McLaughlin,

271   2004). Genetic characterization has proved very useful to identify and discriminate

272   materials marketed or that may in a future under specific labels, like PDO, PGI, or

273   Controlled Apellation (Castro et al., 2011; Lanteri et al., 2010; Mazzucatto et al., 2010;

274   Muñoz-Falcón et al., 2008b; Portis et al., 2006; Rao et al., 2005). Also, a good genetic

275   characterization could be a key point in getting a PDO status for *Listada de Gandía*

276   eggplant, which very likely would increase the market value of this landrace (Gracia

277   and Albisu, 2001). In this respect, during the last decades, 20 PGIs and PDOs have been

278   established in Spain for vegetable landraces (MAPA, 2010).

279   In a former study (Muñoz-Falcón et al., 2008a) we found that, when grown in a

280   uniform environment, *Listada de Gandia* can be distinguished from other *Striped*

281   accessions by a combination of morphological markers. However, given the existence of

282   G×E environment for morphological descriptors in eggplant (Prohens et al., 2004), no

283   absolute values of specific morphological descriptors can be used to properly identify

284   the *Listada de Gandía* eggplant with complete confidence. In this same study (Muñoz-

285   Falcón et al., 2008a), we found that while AFLPs were appropriate to study the diversity

286   and relationships of the *Listada de Gandía* eggplant with other genetically distant

287   materials, they did not allow obtaining markers specific and universal to the *Listada de*

288   *Gandía* eggplant. A similar result, was found when studying the diversity of the local

289   *Almagro* eggplant landrace (Muñoz-Falcón et al., 2008b). Furthermore, a recent study

290   by Barchi et al. (2010) has shown that many AFLP markers are clustered in the same

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291 regions of the eggplant genome, and this may result is biased estimations of genetic

292 distances and of the relationships between groups (Lefebvre et al., 2001; Nybom, 2004).

293 Because of these reasons, and also due to the fact that SSRs have a high reproducibility

294 (Morgante et al., 2002; Powell et al., 1996), and have been of greater utility than AFLPs

295 to study the genetic relationships between closely related materials of eggplant (Muñoz-

296 Falcon, et al., 2008b, 2009) we decided to use SSR markers in this study.

297   When working with closely related materials, it is expected that EST-SSRs will be

298 less polymorphic than genomic SSRs, as the former are part of expressed regions of the

299 genome, while the latter are mostly associated to non-coding regions (Lee et al., 2004;

300 Martin et al., 2010; Tehrani et al., 2009; Varshney et al. 2005). In our case, only seven

301 out of 32 EST-SSRs tested were polymorphic, while only three out of 17 genomic SSRs

302 were monomorphic; also, genomic SSRs presented a higher number of polymorphic

303 alleles and a higher PIC value, indicating that genomic SSRs are of greater utility for

304 diversity studies of eggplant materials. Nonetheless, given its higher transferability

305 among species of the Solanaceae family (Ince et al., 2010; Stàgel et al., 2008), EST-

306 SSRs may be very useful for diversity studies that include eggplant relatives.

307   The results of diversity within each of the groups considered are in agreement with

308 those obtained in a previous study with AFLP markers (Muñoz-Falcón et al., 2008a). In

309 this respect, *Striped* and *Non-Striped* eggplants display a similar level of SSR diversity,

310 revealing that a considerable genetic diversity exists within the *Striped* eggplants. Also,

311 we have found that *Listada de Gandía* is a genetically diverse landrace, which includes

312 genetically related materials, and which are differentiated from the rest of *Striped*

313 eggplant groups. This is a typical characteristic of landraces (Zeven, 1998). In this

314 respect, we have found two SSR markers which present alleles that are unique and

315 universal to all *Listada de Gandía* accessions, and which can serve as a fast and reliable

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316 method to certify the authenticity of the materials belonging to the *Listada de Gandía*

317 landrace. In particular, these markers can be useful to distinguish the authentic *Listada*

318 *de Gandía* eggplants from other materials marketed under this name. It is also

319 remarkable to indicate that the three *Listada* groups (*Listada de Gandía*, *Other Spanish*

320 *Listada*, and *Non-Spanish Listada*) are genetically close, and they lack a SSR allele

321 which is universal to the *Other Non-Spanish Striped* accessions, which suggests that the

322 accessions considered under the name *Listada* may have a common origin (Muñoz-

323 Falcon et al.; 2008a). In fact, the accessions corresponding to the three *Listada* groups

324 are clustered together and separated from the rest of accessions in the PCoA and

325 UPGMA phenogram obtained.

326   As occurred with other eggplant materials (Demir et al., 2010; Muñoz-Falcón et al.,

327 2009; Nunome et al., 2003; Stàgel et al., 2008), SSR markers have revealed as very

328 useful for discriminating among closely related groups of eggplant accessions, and have

329 allowed confirming that some accessions marketed by seed companies as *Listada de*

330 *Gandía*, like the LBCS, LRS and LTGS accessions of the *Non-Spanish Listada* group,

331 do not correspond to *Listada de Gandía* materials (Muñoz-Falcón et al., 2008a). This

332 has important implications regarding the protection of *Listada de Gandía* from imitation

333 (Babcock and Clemens, 2004).

334   Both the PCoA and UPGMA phenogram have also shown that all the *Listada de*

335 *Gandía* accessions cluster together and are separated from the rest of accessions. This

336 suggests that, as has occurred with other Solanaceae landraces (Coombs, et al., 2004;

337 Portis et al., 2006; Rao, et al., 2005), microevolutionary forces, including natural

338 selection under local conditions, artificial selection by farmers, genetic drift, migration,

339 and recombination, may have resulted in a certain degree of differentiation of the

340 *Listada de Gandía* eggplant. As a result, *Listada de Gandía* has become a genetically

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341 unique and distinct eggplant landrace.

342

343 **5. Conclusions**

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345   SSR markers have proved of great advantage for the study of the genetic diversity

346 and relationships of the *Listada de Gandía* eggplant, as well as for obtaining a specific

347 genetic fingerprint for this landrace. Genomic SSRs have displayed a higher percentage

348 of polymorphic loci and a greater mean PIC value than EST-SSRs in the eggplant

349 materials used. *Listada de Gandía* is genetically diverse but is differentiated from other

350 similar materials, and for two SSR markers we have found alleles universal and specific

351 to this landrace, which may be very useful for a rapid diagnostic of authenticity of

352 *Listada de Gandía* materials. The information presented here may be of great utility for

353 the protection and enhancement of the *Listada de Gandía*, as well as for promoting the

354 recognition of a PDO status for this renowned eggplant landrace.

355

356 **Acknowledgements**

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362 **References**

363

364 Andersen, J.R., Lubberstedt, T., 2003. Functional markers in plants. Trends Plant Sci. 8,

365   554-560.

366   Anderson, J., Churchill, A., Autrique, J., Tanksley, S., Sorrells, M., 1993. Optimizing

367   parental selection for genetic linkage maps. Genome 36, 181-186.

368   Babcock, B.A., Clemens, R., 2004. Geographical indications and property rights:

369   protecting value-added agricultural products. MATIC Briefing Paper 04-MBP 7,

370   Iowa State University, Iowa, USA.

371   Barchi, L., Lanteri, S., Portis, E., Stàgel, A., Valè, G., Toppino, L., Rotino, G.L., 2010.

372   Segregation distortion and linkage analysis in eggplant (*Solanum melongena* L.).

373   Genome 53, 805-815.

374   Castelo, A.T., Martins, W., Gao, G.R., 2002. TROLL: tandem repeat occurrence locator.

375   Bioinformatics 18, 634-636.

376   Castro, I., Martín, J.P., Ortiz, J.M., Pinto-Carnide, O., 2011. Varietal discrimination and

377   genetic relationships of *Vitis vinifera* L. cultivars from two major Controlled

378   Appellation (DOC) regions in Portugal. Sci. Hort. 127, 507-514.

379   Chabane, K., Abblett, A., Cordeiro, G.M., Valkoun, J., Henry, R.J., 2005. EST versus

380   genomic derived microsatellite markers for genotyping wild and cultivated barley.

381   Genet. Res. Crop Evol. 52, 903-909.

382   Commission of the European Commmunities. 2006. Council Regulation (EC) No.

383   509/2006 of 20 March 2006 on the protection of geographical indications and

384   designations of origin for agricultural products and foodstuffs. Off. J. Eur. Union

385   L93, 12-25.

386   Coombs, J., Frank, L., Douches, D., 2004. An applied fingerprinting system for

387   cultivated potato using simple sequence repeats. Am J Potato Res. 81, 243-250.

388   Demir, K., Bakir, M., Sarikamiş, G., Acunalp, S., 2010. Genetic diversity of eggplant

389   (*Solanum melongena*) germplasm from turkey assessed by SSR and RAPD markers.

390   Genet. Mol. Res. 9, 1568-1576.

391   Duran, C., Appleby, N., Edwards, D., Batley, J., 2009. Molecular genetic markers:

392   discovery, applications, data storage and visualization. Curr. Bioinf. 4, 16-27.

393   Felsenstein, J. ,1989. PHYLIP: phylogeny inference package (version 3.2). Cladistics.

394   5, 164–166.

395   Gajewski, M., Kowalczyk, K., Bajer, M., Radzanowska, J., 2009. Quality of eggplant

396   fruits in relation to growing medium used in greenhouse cultivation and to a cultivar.

397   Notulae Botanicae Horti Agrobotanici Cluj-Napoca 37, 229-234.

398   Gracia, A., Albisu, L.M., 2001. Food consumption in the European Union: main

399   determinants and country differences. Agribusiness 17, 469-488.

400   Ince, A.G., Karaca, M., Onus, A.N., 2010. Polymorphic microsatellite markers

401   trasnferable across species. Plant Mol. Biol. Rpt. 28, 285-291.

402   Lanteri, S., Acquadro, A., Quagliotti, L., Portis, E., 2003. RAPD and AFLP assessment

403   of genetic variation in a landrace of pepper (*Capsicum annuum* L.), grown in North-

404   West Italy. Genet. Res. Crop Evol. 50, 723-735.

405   Lefebvre, V., Goffinet, B., Chauvet, J.C., Caromel, B., Signoret, P., Brand, R., Palloix,

406   A., 2001. Evaluation of genetic distances between pepper inbred lines for cultivar

407   protection purposes: comparison of AFLP, RAPD and phenotypic data. Theor. Appl.

408   Genet. 102, 741-750.

409   Lee, J.M., Nahm, S.H., Kim, Y.M., Kim, B.D., 2004. Characterization and molecular

410   genetic mapping of microsatellite loci in pepper. Theor. Appl. Genet. 108, 619-627.

411   Li, Y.C., Korol, A.B., Fahima, T., Nevo, E., 2004. Microsatellites within genes:

412   structure, function, and evolution. Mol. Biol. Evol. 21, 991-1007.

413   Liu, K., Muse, S., 2005. PowerMarker: an integrated analysis environment for genetic

414   marker analysis. Bioinformatics 21, 2128-2129.

415   MAPA. 2010. Denominaciones de origen e indicaciones geográficas.

416   http://www.mapa.es/es/alimentacion/pags/Denominacion/consulta.asp. Consulted 19

417   Nov. 2010.

418   Martin, M.A., Mattioni, C., Cherubini, M., Taurchini, D., Villani, F., 2010. Genetic

419   diversity in European chestnut populatyions by means of genomic and genic

420   microsatellite markers. Tree Genet. Genom. 6, 735-744.

421   Mazzucato, A., Ficcadenti, N., Caioni, M., Mosconi, P., Piccinini, E., Sanampudi,

422   V.R.R., Sestili, S., Ferrari, V., 2010. Genetic diversity and distinctiveness in tomato

423   (*Solanum lycopersicum* L.) landraces: the Italian case of 'A pera Abruzzese'. Sci.

424   Hort. 125, 55-62.

425   McLaughlin, E.W., 2004. The dynamics of fresh fruit and vegetable pricing in the

426   supermarket channel. Preventive Med. 39:S81-S87.

427   Mohammadi, S.A., Prassana, B.M., 2003. Analysis of genetic diversity in crop plants-

428   salient statistical tools and considerations. Crop Sci. 43, 1235-1248.

429   Morgante, M., Hanafey, M., Powell, W., 2002. Microsatellites are preferentially

430   associated with nonrepetitive DNA in plant genomes. Nature Genet. 30, 194-200.

431   Muñoz-Falcón, J.E., Prohens, J., Vilanova, S., Nuez, F., 2008a. Characterization,

432   diversity, and relationships of the Spanish striped (*Listada*) eggplants: a model for

433   the enhancement and protection of local heirlooms. Euphytica 164, 405-419.

434   Muñoz-Falcón, J.E., Prohens, J., Vilanova, S., Ribas, F., Castro, A., Nuez, F., 2008b.

435   Distinguishing a protected geographical indication vegetable (*Almagro* eggplant)

436   from closely related materials with selected morphological traits and molecular

437   markers. J. Sci. Food Agric. 89, 320-328

438   Nei, M., 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad.

439   Sci. USA 70, 3321-3323.

440   Nunome, T., Suwabe, K., Iketani, H., Hirai, M. 2003. Identification and characterization

441   of microsatellites in eggplant. Plant Breed. 122, 256-262.

442 Nunome, T., Suwabe, K., Iketani, H., Hirai, M. 2003. Identification and characterization

443   of microsatellites in eggplant. Plant Breed. 122, 256-262.

444 Nybom, N., 2004. Comparison of different nuclear DNA markers for estimating

445   intraspecific genetic diversity in plants. Mol. Ecol. 13, 1143-1155.

446 Powell, W., Machray, G., Provan, J., 1996. Polymorphism revealed by simple sequence

447   repeats. Trends Plant Sci. 1, 215-222.

448 Portis, E., Nervo, G., Cavallanti, F., Barchi, L., Lanteri, S., 2006. Multivariate analysis

449   of genetic relationships between Italian pepper landraces. Crop Sci. 46, 2517-2525.

450 Prohens, J., Blanca, J.M., Nuez, F., 2005. Morphological and molecular variation in a

451   collection of eggplant from a secondary center of diversity: implications for

452   conservation and breeding. J. Amer. Soc. Hort. Sci. 130, 54-63.

453 Prohens, J., Blanca, J.M., Rodríguez-Burruezo, A., Nuez, F., 2004. Spanish traditional

454   varieties of eggplant: diversity and interest for plant breeding. Proc. XIIth

455   EUCARPIA Mtg. Genet. Breed. Capsicum Eggplant, 38-43.

456 Prohens, J., Muñoz, J.E., Vilanova, S., Castro, A., Ribas, F., Nuez, F., 2007.

457   Participatory breeding in eggplant: selection and improvement for quality and yield

458   in a local landrace, in: Niemirowicz-Szczytt K (Ed.). Progress in research on

459   Capsicum & eggplant. Warsaw University of Life Sciences Press, Warsaw, Poland,

460   pp. 221-230.

461 Prohens, J., Muñoz-Falcón, J.E., Rodríguez-Burruezo, A., Ribas, F., Castro, A., Nuez,

462   F., 2009. 'H15', an *Almagro*-type pickling eggplant with high yield and reduced

463   prickliness. Hortscience 44, 2017-2019.

464 Prohens J., Nuez F., 2001. Variedades tradicionales de berenjena en España. Vida Rural

465   130, 46-50.

466   Raigón, M.D., Rodríguez-Burruezo, A., Prohens, J., 2010. Effects of organic and

467   conventional cultivation methods on composition of eggplant fruits. J. Food Agric.

468   Chem. 58, 6833-6840.

469   Rao, R., Corrado, G., Bianchi, M., Di Mauro, A., 2006. (GATA)$_4$ DNA fingerprinting

470   identifies morphologically characterized 'San Marzano' tomato plants. Plant Breed.

471   125, 173-176.

472   Schuelke, M., 2000. An economic method for the fluorescent labeling of PCR

473   fragments. Nature Biotechnol. 18, 233-234.

474   Stàgel, A., Portis, E., Toppino, L., Rotino, G.L., Lanteri, S., 2008. Gene-based

475   microsatellite development for mapping and phylogeny studies in eggplant. BMC

476   Genom. 9, 357.

477   Tam, S.M., Mhiri, C., Vogelaar, A., Kerkveld, M., Pearce, S.R., Grandbastien, M.A.,

478   2005. Comparative analyses of genetic diversities within tomato and pepper

479   collections detected by retro-transposon based SSAP, AFLP and SSR. Theor. Appl.

480   Genet. 110, 819-831.

481   Tehrani, M.S., Mardi, M., Sahebi, J., Catalán, P., Díaz-Pérez, A., 2009. Genetic diversity

482   and structure among Iranian tall fescue populations based on genomic-SSR and EST-

483   SSR marker analysis. Plant Syst. Evol. 282, 57-70.

484   Tümbilen, Y., 2007. Determination of genetic diversity between eggplant and its wild

485   relatives. MSc Thesis, Izmir Institute of Technology, Izmir, Turkey.

486   UPOV, 2002. General introduction to the examination of distinctness, uniformity and

487   stability and the development of harmonized descriptors of new varieties of plants.

488   International Union for the Protection of New Varieties of Plants. Geneva,

489   Switzerland.

490   Varshney, R.K., Sigmund, R., Boerner, A., Korzun, V., Stein, N., Sorrells, M.,

491   Langridge, P., Graner, A., 2005. Interspecific transferability and comparative

492   mapping of barley EST-SSR markers in wheat, rye and rice. Plant Sci. 168, 195-202.

493   Yeh, F.C., Yang, R., Boyle, T.J., Yez, Z., Xiyan, J.M. 2000. Popgene 32, Microsoft

494   Windows-based freeware for population genetics analysis. Molecular Biology and

495   Biotechnology Centre, University of Alberta, Edmonton, Canada.

496   Zeven, A.C., 1998. Landraces: a review of definitions and classifications. Euphytica

497   104, 127-139.

498

Table 1

500 The five groups of eggplant accessions (i to v) used for the present study, with the origin

501 of each accession. For those accessions obtained from seed companies, the name of the

502 company is indicated between brackets.

Accesion name	Code	Origin
Listada de Gandía (i)		
IVIA-25	I25	Moncada,Valencia, Spain
IVIA-371	I371	Moncada, Valencia, Spain
Listada de Gandía	LGA1	Valencia, Valencia, Spain
Listada de Gandía	LGA2	Valencia, Valencia, Spain
V-S-1	VS1	Alcira, Valencia, Spain
V-S-8	VS8	La Punta, Valencia, Spain
Other Spanish Listada Gandía (ii)		
AN-S-4	ANS4	Castro del Río, Córdoba, Spain
C-S-10	CS10	Barcelona, Barcelona, Spain
C-S-23	CS23	Gavá, Barcelona, Spain
C-S-7	CS7	Villabertrán, Gerona, Spain
MU-S-3	MUS3	Monteagudo, Murcia, Spain
V-S-11	VS11	Dolores, Alicante, Spain
V-S-15	VS15	Aspe, Alicante, Spain
V-S-22	VS22	Orihuela, Alicante, Spain
Non-Spanish Listada Gandía (iii)		
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 Gandía (iv)		
BBS134	B134	Ivory Coast
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 Gandía (v)		
AFR-S-1	AFR1	El Kelaa, Morocco
ASI-S-1	ASI1	Beijing, China
B-S-3	BS3	Porreres, Mallorca, Spain
Balady	BAL	Egypt
BBS-189	B189	Abidjan, Adzope, Ivory Coast
Bellezza Nera	BEN	Italy (Semillas Vilmorin, Spain)
C-S-16	CS16	Villafranca del Penedés, Barcelona, Spain
Fairy	FAI	Unknown (Tomato Growers Seeds, USA)
GR-S-19	GR19	Thessaloniki, Macedonia, Greece
INRA-11	IN11	INRA, France
Kermit	KER	Unknown (Evergreen Seeds, USA)
Larga de Barbentane	LDB	France (Semillas Vilmorin, Spain)
LF3-24	LF24	INRA, France
Ping Tung	PIT	Taiwan (Evergreen Seeds, USA)
MM1010	MM10	Malaysia
RNL019	R019	Klouekanme, Benin
Thai Long Green	TLG	Thailand (Evergreen Seeds, USA)

503 [a]Accessions labeled as *Listada de Gandía* but which do not conform to the typical

504 characteristics of the Spanish *Listada de Gandía* heirloom.

505

Table 2

SSR markers used in the present study along with their repeat motif and source.

SSR locus	Motif	Source
Genomic SSRs		
BMS 33	$(TC)_9$	GenBank: EU714957.1
BMS 34	$(GA)_7$	GenBank: EU714958.1
BMS 35	$(AG)_{10}$	GenBank: EU714959.1
BMS 39	$(GT)_{12}$	GenBank: EU714963.1
BMS 40	$(GA)_8$	GenBank: EU714964.1
BSMSSR1	$(TAT)_8$	GenBank: EF517791.1
EM114	$(AC)_{13}$	Nunome et al. (2003)
EM117	$(AC)_{19}(AT)_{11}$	Nunome et al. (2003)
EM126	$(AT)_7(GT)_{18}$	Nunome et al. (2003)
EM133	$(AC)_{13}(AT)_4$	Nunome et al. (2003)
EM134	$(GT)_2GC(GT)_6$	Nunome et al. (2003)
EM135	$(CA)_{11}(GA)_{20}$	Nunome et al. (2003)
EM140	$(AC)_4GC(AC)_5T(AC)_3ATGC(AC)_4AT(AC)_6(AT)_5G(TA)_{13}$	Nunome et al. (2003)
EM145	$(TACA)_4TA(TACA)_4(CA)_{37}(TA)_5TG(TA)_3(TTAA)_3$	Nunome et al. (2003)
EM146	$(AC)_{19}(AT)_{11}AC(AT)_2$	Nunome et al. (2003)
EM155	$(CT)_{38}$	Nunome et al. (2003)
EM141	$(AT)_{16}(GT)_{19}$	Nunome et al. (2003)
EST-SSRs		
EEMS06	$(T)_{14}$	Stàgel et al. (2008)
EEMS07	$(T)_{13}$	Stàgel et al. (2008)
EEMS10	$(A)_{20}$	Stàgel et al. (2008)
EEMS12	$(A)_{16}$	Stàgel et al. (2008)
EEMS14	$(A)_{13}$	Stàgel et al. (2008)
EEMS15	$(C)_{12}$	Stàgel et al. (2008)
EEMS16	$(AC)_7$	Stàgel et al. (2008)
EEMS18	$(AG)_7$	Stàgel et al. (2008)
EEMS19	$(AT)_9$	Stàgel et al. (2008)
EEMS21	$(AGA)_5$	Stàgel et al. (2008)
EEMS22	$(AAG)_5$	Stàgel et al. (2008)

EEMS24	$(CTT)_5$	Stàgel et al. (2008)
EEMS25	$(CTT)_5$	Stàgel et al. (2008)
EEMS26	$(CTT)_5$	Stàgel et al. (2008)
EEMS30	$(TAC)_5$	Stàgel et al. (2008)
EEMS31	$(TGG)_5$	Stàgel et al. (2008)
EEMS36	$(TGT)_5$	Stàgel et al. (2008)
EEMS37	$(TCC)_5$	Stàgel et al. (2008)
EEMS45	$(AGAACC)_4$	Stàgel et al. (2008)
EEMS46	$(ACCAGC)_6$	Stàgel et al. (2008)
EEMS47	$(GCT)_5..(TTC)_5$	Stàgel et al. (2008)
EEMS49	$(TA)_{12}(GA)_7$	Stàgel et al. (2008)
smSSR09	$(TTTGC)_3$	Tümbilen (2007)
smSSR11	$(AGC)_6$	Tümbilen (2007)
smSSR12	$(ACCAA)_3$	Tümbilen (2007)
smSSR14	$(ATTA)_4$	Tümbilen (2007)
smSSR15	$(CCTTT)_3$	Tümbilen (2007)
smSSR17	$(ATAC)_4$	Tümbilen (2007)
smSSR18	$(TAAT)_4$	Tümbilen (2007)
smSSR28	$(TCA)_5$	Tümbilen (2007)
smSSR33	$(TCA)_5$	Tümbilen (2007)
smSSR43	$(GCT)_5$	Tümbilen (2007)

508
509

510 **Table 3**

511 Primer sequences and expected size of the six new genomic SSR markers developed by

512 ourselves using information from the National Center for Biotechnology Information

513 database.

SSR locus	Primer sequence (5'-3')	Expected size (bp)
BMS 33	F- AAATGGTCAAGGAGAACAATGG	139
	R- GGCAAGAAGAATGGAGAAGACA	
BMS 34	F- GAGTGGAGAGAGGCGAATTG	153
	R- GTTAGGATTTTGTTGCTATTTTCTATT	
BMS 35	F- CAGAGAAGAGGGAGAAAGGAGG	134
	R- TATACCATAGGATCTGCCACCC	
BMS 39	F- TGCACATGCGGGACTTAATA	155
	R- CGACATACCACCGGAGTACA	
BMS 40	F- AATCTGTGTGTATGCGTGCG	198
	R- ACTGCTTCGCCTTCATGTTC	
BSMSSR1	F- CAGATCAAACGGTTAGTTGAGG	217
	R-TACGGCTGAGATTCATTTGC	

514

515 **Table 4**

516 Polymorphic SSRs, and number of alleles and polymorphic information content (PIC)

517 for each of them.

SSR marker	Number of alleles	PIC
Genomic SSRs		
BMS 33	2	0.0866
BMS 34	2	0.3579
BMS 39	2	0.0712
BSMSSR1	2	0.2970
EM114	2	0.2648
EM117	5	0.6640
EM126	3	0.5500
EM133	4	0.3733
EM134	2	0.1730
EM140	10	0.7411
EM145	5	0.5910
EM146	5	0.4279
EM155	13	0.8191
EM141	6	0.7737
Mean	*4.50*	*0.4006*
EST-SSRs		
EEMS15	7	0.7714
EEMS30	2	0.3589
EEMS36	2	0.0454
EEMS37	3	0.2480
EEMS46	3	0.0940
EEMS49	2	0.2149
smSSR17	3	0.4529
Mean	*3.14*	*0.2480*

518
519

520 **Table 5**

521 Total genetic diversity (H_T, Nei, 1973) estimated from SSR markers for the materials

522 studied considering all the groups of accessions under study.

Groups	Accessions (n)	Polymorphic loci (n)	H_T
All accessions	42	21	0.4406
Striped	25	18	0.3739
Listada de Gandía	6	9	0.1945
Other Spanish Listada	8	7	0.1430
Non-Spanish Listada	5	11	0.2443
Other Non-Spanish Striped	6	15	0.3620
Non-Striped	17	19	0.4106

523
524

28

525 **Table 6**

526 Genetic distances among the different eggplant groups obtained using SSR markers.

	Other Spanish Listada	*Non-Spanish Listada*	*Other Non-Spanish Striped*	*Non-Striped*
Listada de Gandía	0.2822	0.3316	0.4834	0.4670
Other Spanish Listada		0.1259	0.3459	0.3282
Non-Spanish Listada			0.2496	0.2365
Other Non-Spanish Listada				0.1351

527
528

529 **Table 7**

530 Allele sizes in base pairs present in the four *Striped* groups for the SSR loci that were

531 polymorphic in the 42 eggplant accessions tested.

SSR Marker	*Listada de Gandia*	*Other Spanish Listada*	*Non-Spanish Listada*	*Other Non-Spanish Striped*
Genomic SSRs				
BMS 33	155	155	155	155
BMS 34	131	130, 131	131	130, 131
BMS 39	169	169	169	169
BMSSR1	238	238	238	229, 238
EM114	236	234, 236	234, 236	236
EM117	134	134, 138	134, 138	138, 140, 142
EM126[a]	226	226	226, 230	228
EM133[b]	184	192	192	192, 194
EM134	184, 186	184	184	184, 186
EM140[c]	284, 310	320, 284	284, 314, 320	284, 286, 306, 320
EM145	384, 386	384	384	380, 384
EM146	302, 304	304	304	302, 304
EM155	271, 273, 285	281, 285	275, 285, 289	251, 271, 275, 279, 285
EM141	243, 251	198, 241, 243	241, 243, 251	198, 243, 245, 249
EST-SSRs				
EEMS15	295, 297	295	295,294,299	296,299,297, 301
EEMS30	217, 219	219	217, 219	217, 219
EEMS37[b]	204	201	201	201
EEMS46	262	262	262, 264	258, 262
EEMS49	262	262	262, 270	262, 270
smSSR17	130, 131	130, 131	130, 131	121, 130, 131
smSSR27	200	200	200	200

532 [a]SSR marker with alleles specific and universal to the three *Listada* (*Listada de Gandía*,

533 *Other Spanish Listada*, *Non-Spanish Listada*) groups.

534 [b]SSR marker with alleles specific and universal to the *Listada de Gandía* group.

535 [c]SSR marker with alleles specific, but not universal, of *Listada de Gandía* group.
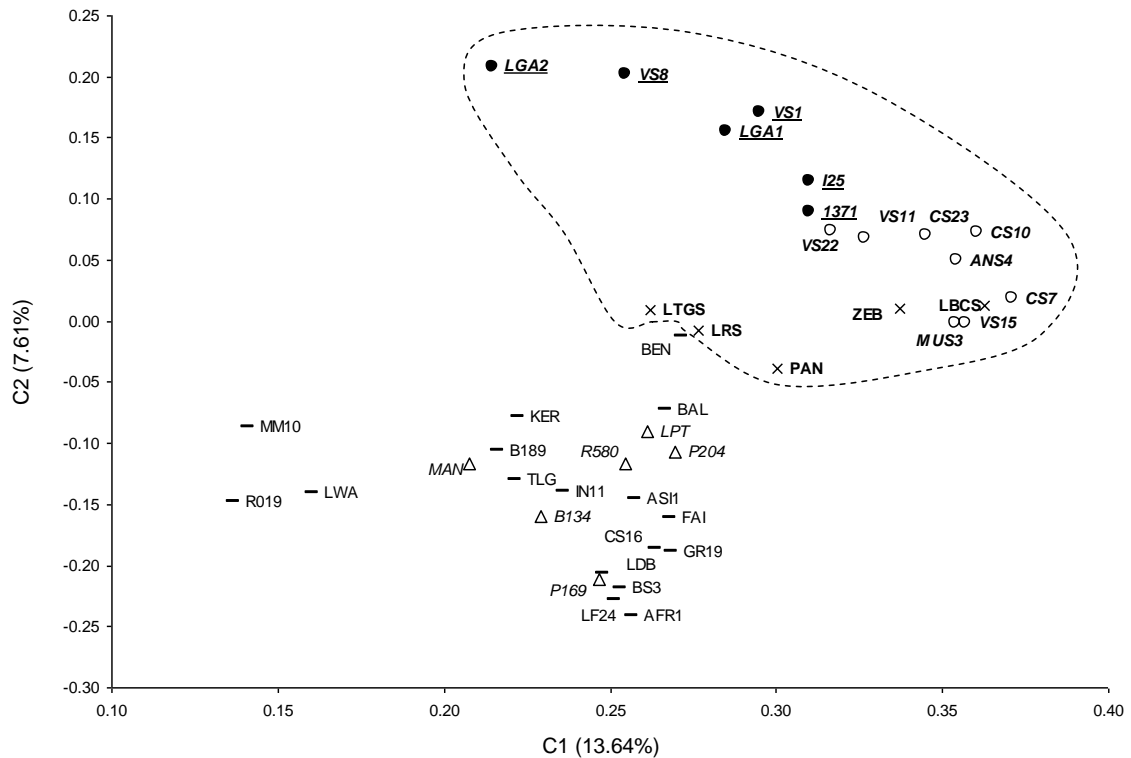
30
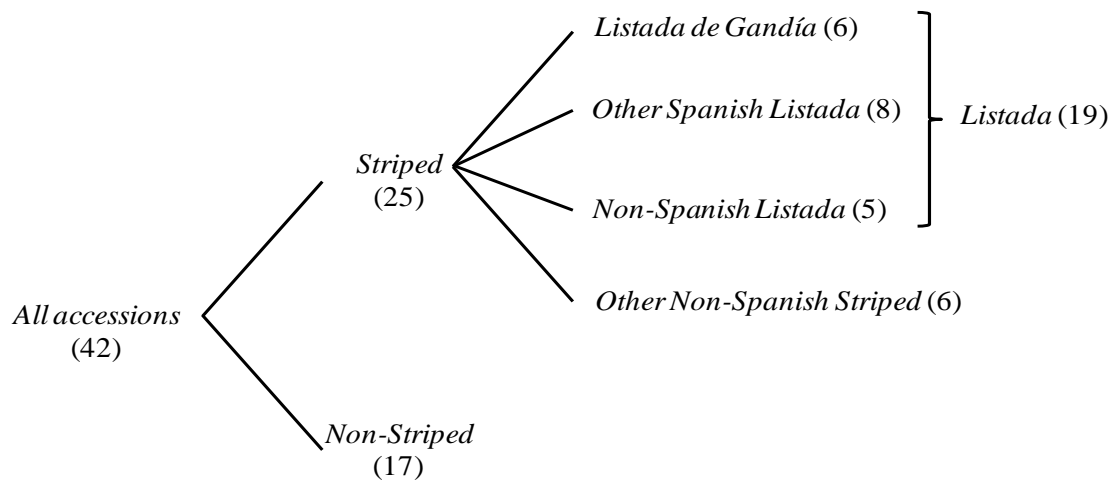
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538 **Fig. 1.** Diagrammatic representation and nomenclature used for the groups of eggplant

539 accessions at the different levels considered.

540

Fig. 2. Relationships between all the eggplant groups studied (42 accessions) based on
principal coordinates analysis (13.64% and 7.61% of the total variation explained by the
first and second component, C1 and C2, respectively) using SSR-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). The three *Listada*
groups are enclosed by a dashed line.

551
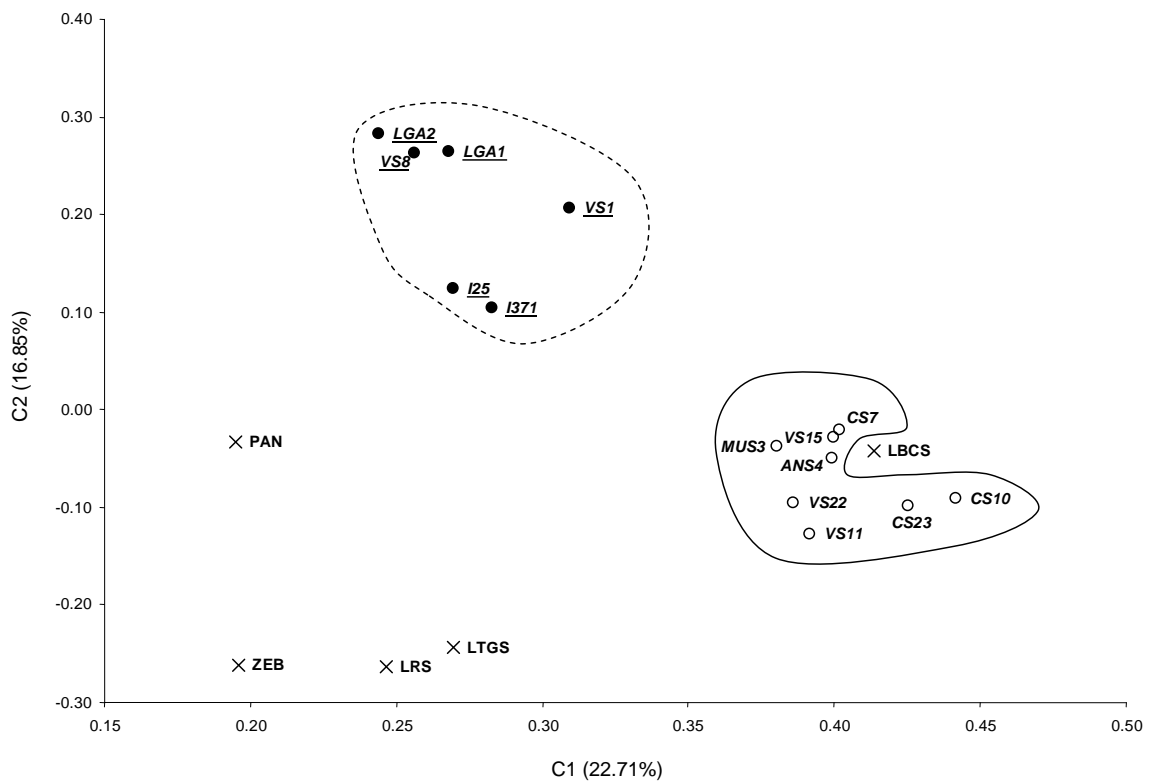
Fig. 3. Relationships between the *Listada* groups studied (25 accessions) based on

principal coordinates analysis (22.71% and 16.85% of the total variation explained by

the first and second component, C1 and C2, respectively) using SSR-based genetic

similarities. ●=*Listada de Gandía* (in bold, italics, underlined font); ○=*Other Spanish*

Listada (in bold, italics font); ×=*Non-Spanish Listada* (in bold font). The *Listada de*

Gandía accessions are enclosed by a dashed line, while the *Other Spanish Listada* are

enclosed by a continuous line.

559

560
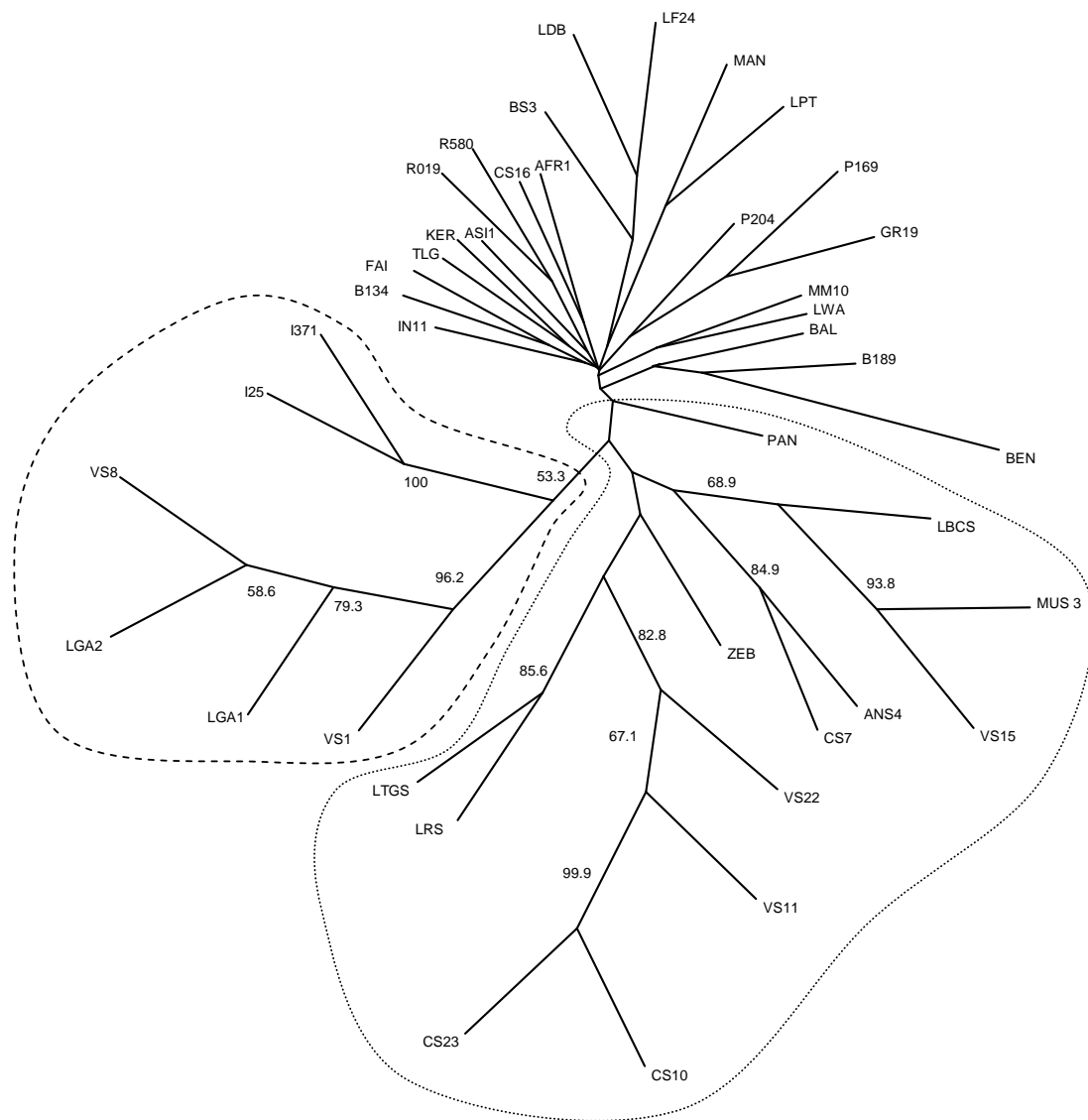
Fig. 4. Unrooted-UPGMA phenogram of 42 accesions of eggplant based on SSR

markers. Phenetic relationships were derived from Dice (Sorensen) pairwise genetic

distances. Bootstrap values (%) (1000 replications) are indicated at each node. Only

nodes with a bootstrap value >50% have been represented. The *Listada de Gandía*

accessions are enclosed by a dashed line, while the accessions of the *Other Spanish*

Listada and *Non-Spanish Listada* groups are enclosed by a dotted line.