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Additional Information

1 **Morphological and molecular characterization of local varieties, modern cultivars**  
2 **and wild relatives of an emerging vegetable crop, the pepino (*Solanum muricatum*),**  
3 **provides insight into its diversity, relationships and breeding history**

4

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13

14

15 **Abstract**

16 Availability of standardized morphological and molecular characterization data is  
17 essential for the efficient development of breeding programmes in emerging crops.  
18 Pepino (*Solanum muricatum*) is an increasingly important vegetatively propagated  
19 vegetable crop for which concurrent data on morphological descriptors and molecular  
20 markers are not available. We evaluated 58 morphological traits, using a collection of  
21 14 accessions of pepinos (including local Andean varieties and modern cultivars) and 8  
22 of wild relatives, using the IPGRI and COMAV descriptors lists coupled with 20 EST-  
23 SSRs from tomato. High morphological diversity was found in both cultivated and wild  
24 accessions; all morphological traits except three were variable. Cultivated pepino and  
25 wild relatives were significantly different for 26 traits. Also, local varieties and modern  
26 cultivars of pepino were different from each other for 13 morphological traits and were  
27 clearly separated in a principal components analysis (PCA). Fourteen of the 20 tomato  
28 EST-SSRs were polymorphic, with an average number of alleles per locus of 4.07 and a  
29 polymorphic information content (*PIC*) value of 0.4132. This revealed a high degree of  
30 transferability from tomato to pepino and wide molecular diversity in the collection.  
31 Cultivated materials manifest high levels of observed heterozygosity, suggesting that it  
32 is related to heterosis for yield associated with heterozygosity. SSR data clearly  
33 differentiated cultivated and wild materials. Furthermore, for pepinos, the modern  
34 varieties were genetically much less diverse than the traditional local varieties.  
35 However, both groups of cultivated material expressed a low degree of genetic  
36 differentiation. A strong correlation ( $r=0.673$ ) between morphological and molecular  
37 distances was found. Our results provide foundational information for programmes of  
38 germplasm conservation, and that can be used to enhance breeding for this emerging  
39 crop.

40 **Keywords:** Breeding · Descriptors · Germplasm · Heterozygosity · *Solanum muricatum*  
41 · SSRs

42

### 43 **Introduction**

44

45 Modern breeding programmes in emerging crops are often limited by scanty or non-  
46 existent phenotypic and genetic information, and by small germplasm collections (FAO  
47 2010; Mayes et al. 2012). Complementary studies of morphological and molecular  
48 diversity provide relevant information for identifying sources of variation in breeding  
49 programmes, for establishing relationships among plant materials, as well as a  
50 foundation for promoting breeding and for germplasm conservation (Rao and Hodgkin  
51 2002; Khoury et al. 2010).

52         The pepino (*Solanum muricatum* Aiton) is an emerging usually vegetatively  
53 propagated vegetable crop native to the Andean region (Anderson et al. 1996). This crop  
54 is phylogenetically close to tomato (*S. lycopersicum* L.) and potato (*S. tuberosum* L.)  
55 (Spooner et al. 1993; Särkinen et al. 2013). The pepino is cultivated for its juicy and  
56 aromatic fruits. Although the pepino is locally important in the Andean region since  
57 long ago (Prohens et al. 1996), in recent decades the increasing interest in exotic fruit  
58 markets has promoted increasing interest in pepino cultivation in several countries  
59 including New Zealand, Australia, Spain, Turkey, Israel and China (Levy et al. 2006;  
60 Yalçın 2010; Rodríguez-Burruezo et al. 2011; Abouelnasr et al. 2014). Nutritionally,  
61 pepino fruits contain high levels of potassium and vitamin C, and it is low in calories.  
62 Furthermore, it offers some properties of medicinal interest, such as antidiabetic,  
63 antidiuretic and antihypotensive activities (Hsu et al. 2011; Rodríguez-Burruezo et al.  
64 2011; Sudha et al. 2012).

65 Most of the plant material cultivated in the Andean region consists of local  
66 varieties that have not been subjected to formal breeding and are adapted to local  
67 climatic conditions and preferences for flavour, size and fruit shape and colour  
68 (Anderson et al. 1996; Prohens et al. 1996). Local varieties of the pepino are commonly  
69 cultivated outdoors in their native range, and they usually have a poor performance  
70 when introduced in other regions (where the pepino is cultivated either outdoors or in  
71 greenhouses: Prohens et al. 1996; Rodríguez-Burruezo et al. 2011). As a consequence of  
72 the usually poor performance, several improved cultivars adapted to non-Andean  
73 climates and to protected cultivation have been developed in New Zealand, Spain, and  
74 Israel (Dawes and Pringle 1984; Simms et al. 1996; Ruiz et al. 1997; Prohens et al.  
75 2002; Rodríguez-Burruezo et al. 2004a, 2004b; Levy et al. 2006). These materials have  
76 been developed using conventional approaches including generating genetically variable  
77 populations by means of seed propagation of collections from the Andean region or by  
78 hybridization between different vegetatively propagated clones in order to exploit  
79 heterosis (Rodríguez-Burruezo et al. 2011).

80 Wild pepino relatives which, like the domesticated pepino, are included in the  
81 section *Basarthurum* of genus *Solanum* (Anderson 1975, 1979) represent a genetic  
82 resource of interest for pepino breeding (Rodríguez-Burruezo et al., 2003a). Among the  
83 wild relatives, the highly variable *S. caripense* Humb. and Bonpl. ex Dun., as well as *S.*  
84 *tabanoense* Correll, form part of the primary genepool of pepino. Fully fertile  
85 interspecific hybrids and backcross generations to pepino have been obtained among  
86 these species (Anderson 1979; Rodríguez-Burruezo et al. 2003a, 2011). Other species of  
87 interest for pepino breeding include *S. trachycarpum* Bitter and Sodiro, which grows in  
88 dry areas (Anderson 1979), and *S. catilliflorum* G.J. Anderson, Martine, Prohens and  
89 Nuez and *S. perlongistylum* G.J. Anderson, Martine, Prohens and Nuez, which are

90 among the most recent species discovered and described for this section (Anderson et al.  
91 2006) and that remain to be studied as potential genetic resources for pepino breeding.

92         Given the interests in crop diversity and enhancement, the precise and  
93 standardized morphological and molecular characterization of the pepino would be of  
94 great utility for breeding programmes, for germplasm conservation and for comparison  
95 of experimental data of different trials and plant materials (Rao and Hodgkin 2002;  
96 Khoury et al. 2010). Fortunately, an internationally accepted list of morphological  
97 descriptors for the extensive characterization of vegetative, inflorescence and flower,  
98 fruit and seed traits of pepino is available (IPGRI and COMAV 2004). However, no  
99 reports are known to us on the utilization of this list of descriptors for the morphological  
100 characterization of pepino collections. Although several studies have been made on  
101 phenotypic diversity of pepino, including wild relatives of interest for breeding, they  
102 have mostly dealt with specific traits of agronomic interest (Rodríguez-Burruezo et al.  
103 2003a, 2011; Muñoz et al. 2014)

104         Similarly, few studies have been done on the molecular diversity of collections  
105 of cultivated pepino and wild relatives (Anderson et al. 1996; Blanca et al. 2007). The  
106 evaluation of the cpDNA-RFLPs polymorphism in the pepino and wild relatives of  
107 *Solanum* section *Basarthurum* revealed that the cultivated pepino was closely related to  
108 *S. caripense* and *S. tabanoense* (Anderson et al. 1996). A subsequent study using AFLP  
109 markers and the sequence variation in the DNA sequence of the nuclear gene 3-  
110 methylcrotonyl-CoA carboxylase revealed that cultivated pepino is highly diverse and  
111 showed that this cultigen was genetically differentiated from wild relatives (Blanca et  
112 al. 2007). AFLP markers have also been used to evaluate the genetic distances among  
113 four pepino cultivars as a predictor for heterosis for yield traits (Rodríguez-Burruezo et  
114 al. 2003b). However, no studies have been performed with other molecular markers in

115 pepino. Unlike AFLPs, which are dominant (Meudt and Clarke 2007), SSRs are co-  
116 dominant and particularly valuable because they allow the precise assignment of allelic  
117 states and evaluation of the level of heterozygosity of individual pepino clones.  
118 Furthermore, SSRs (1) have a high reproducibility and therefore are ideal for  
119 comparison among different experiments and laboratories, (2) are multiallelic, (3) have  
120 locus specificity, (4) are abundant and (5) are randomly distributed throughout the  
121 genome (Kalia et al. 2011). For species like the pepino in which no genomic libraries or  
122 expressed sequence tags (EST) sequences are available, SSRs may be transferred from  
123 close relatives, like tomato, in which there has been an abundance of SSRs developed  
124 (Frary et al. 2005; Suresh et al. 2014). In this respect, EST-SSRs usually offer a greater  
125 degree of transferability among species, as transcribed regions have a greater degree of  
126 conservation than non-transcribed regions (Kalia et al. 2011).

127         The simultaneous study of morphological and molecular diversity of the pepino  
128 and wild relatives also provides information on the morphological and molecular  
129 variation and relationships of the crop to wild relatives, as well as on the association  
130 between morphological and molecular variation. Here, we evaluate the morphological  
131 and molecular diversity using standardized descriptors and highly repeatable SSR  
132 markers in a collection of local varieties and modern cultivars of pepino, as well as in a  
133 set of accessions from wild relatives of interest for breeding. The information obtained  
134 will be of interest for breeders and germplasm managers, as well as for understanding  
135 the evolution of the crop.

136

## 137 **Material and methods**

138

139 Plant material

140

141 We studied a total of 22 accessions, of which six corresponded to local pepino varieties  
142 from the Andean region, eight to improved pepino cultivars, and eight to wild relatives  
143 (different species) (Table 1). Local varieties originated in Colombia (1), Chile (2),  
144 Ecuador (2) and Peru (1). Modern varieties were developed in New Zealand (2), Spain  
145 (5) and the United Kingdom (1) as a result of selection and breeding programmes  
146 (Dawes and Pringle 1984; Simms et al. 1996; Ruiz et al. 1997; Prohens et al. 2002;  
147 Rodríguez-Burruezo et al. 2004a, 2004b). Wild relatives were represented by accessions  
148 of *S. caripense* (4), *S. catilliflorum* (1), *S. perlongistylum* (1), *S. tabanoense* (1) and *S.*  
149 *trachycarpum* (1). The material is part of the germplasm collection of the Instituto de  
150 Conservación y Mejora de la Agrodiversidad valenciana (Valencia, Spain).

151 Five clonal replicates obtained by *in vitro* micropropagation (Cavusoglu and  
152 Sulusoglu 2013) were used for each of the 22 accessions. Clonal replicates were grown  
153 in a glasshouse in Valencia (GPS coordinates: lat. 39° 29' 01'' N, long. 0° 20' 27'' W)  
154 using a completely randomized design. Rooted plantlets were transplanted to benches  
155 filled with quartz sand in January 2014. Plants were spaced 55 cm in the bench, with  
156 115 cm between bench centers. Plants were drip irrigated every 4 h for 5 min.  
157 Fertilization was applied through the drip irrigation system during the growing cycle. A  
158 combination of different fertilizers was used to achieve a final concentration of main  
159 ions and cations in the irrigation solution of 11.47 mM NO<sub>3</sub><sup>-</sup>, 1.00 mM NH<sub>4</sub><sup>+</sup>, 1.50 mM  
160 H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, 6.75 mM K<sup>+</sup>, 3.25 mM Ca<sup>2+</sup>, 2.50 mM Mg<sup>2+</sup> and 2.82 mM SO<sub>4</sub><sup>2-</sup>.  
161 Microminerals were supplied by adding the following salts to the irrigation water: 50  
162 μM H<sub>3</sub>BO<sub>3</sub>, 10 μM FeEDTA, 4.5 μM MnCl<sub>2</sub>, 3.8 μM ZnSO<sub>4</sub>, 0.3 μM CuSO<sub>4</sub> and 0.1  
163 μM (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>. Flowers were vibrated mechanically (to approximate the natural bee  
164 pollination syndrome of vibratile pollination; Anderson and Symon 1988) twice a week

165 to stimulate fruit set. For the self-incompatible wild species *S. caripense*, *S.*  
166 *perlongistylum* and *S. tabanoense* (Mione and Anderson 1992; Anderson et al. 1996),  
167 manual pollination using pollen from other plants from each of the species was used in  
168 order to ensure fruit set. Phytosanitary treatments against spider mites (*Tetranychus*  
169 *urticae* Koch.) and whiteflies (*Bemisia tabaci* Gennadius) were performed when  
170 necessary.

171

#### 172 Morphological and agronomic characterization

173

174 Individual plants were characterized using 58 primary descriptors (IPGRI and COMAV  
175 2004). These descriptors include two plant (P code), seven stem (St code), 12 leaf (L  
176 code), three inflorescence (I code), six flower (Fl code), 24 fruit (Fr code), and four seed  
177 (Se code) traits. Eighteen traits corresponding to these primary descriptors are  
178 quantitative, seven are meristic (traits in which the parts or components are counted)  
179 and the other 33 traits are measured in a scale with predetermined values (Table 2).

180

#### 181 Molecular characterization

182

183 Genomic DNA was extracted from young leaves of each clone according to the CTAB  
184 procedure (Doyle and Doyle, 1987). DNA quality was evaluated on 0.8% agarose gels,  
185 dyed with GelRed Nucleic Acid Stain (Biotium, Hayward, CA, USA) and the DNA  
186 concentrations estimated using a Nanodrop ND-1000 (Nanodrop Technologies,  
187 Wilmington, Delaware, USA) spectrophotometer. Extracted DNA was diluted to a  
188 concentration of 20 ng/ $\mu$ L.

189 We used 20 simple sequence repeat (SSR) markers that proved to be  
190 polymorphic in tomato (Table 3) and that are distributed throughout the tomato genome  
191 (Frery et al. 2005). SSRs were amplified following the M13-tail method described by  
192 Schuelke (2000) to facilitate the incorporation of a dye label during PCR.  
193 Amplifications were performed in a total volume of 10 ng DNA, 1 mM MgCl<sub>2</sub>, 0.05 μM  
194 of forward primer, 0.25 μM of reverse primer, 0.2 μM of fluorescent-labelled M-13  
195 primer, 0.2 mM of dNTPs and 1 unit of *Taq* polymerase in 1X PCR buffer. PCR  
196 amplifications were performed in a Mastercycler ep gradient S thermocycler  
197 (Eppendorf, Hamburg, Germany) using the following programme: 1 cycle for 2 min at  
198 94 °C, 35 cycles of 15 s at 94°C, 30 s at annealing temperature (Table 3), 45 s at 72 °C,  
199 followed by 10 min extensive at 72 °C. SSR alleles were resolved on an ABI PRISM  
200 3100 DNA (Applied Biosystems, Carlsbad, California, USA) genetic analyzer using  
201 GeneScan 3.7 (Applied Biosystems) software and precisely sized using GeneScan 500  
202 LIZ molecular size standards with genotyper 3.7 (Applied Biosystems) software.

203

204 Data analysis

205

206 Range and mean values for the morphological descriptors for the 14 accessions of  
207 cultivated pepino and for the eight accessions of its wild relatives, as well as for the six  
208 local varieties and eight modern cultivars of cultivated pepino, were calculated using  
209 average values for each accession. Significance of differences among groups (cultivated  
210 pepino vs. wild species, and local varieties vs. modern cultivars) was tested using  
211 Student's *t* tests. A principal components analysis (PCA) was performed for  
212 standardized morphological data using pairwise Euclidean distances among accessions.  
213 Monomorphic traits were excluded from the PCA analysis.

214 For the molecular (SSR) data, the number of alleles and of private alleles for  
215 each of the groups considered (all accessions, all cultivated accessions, local varieties,  
216 modern cultivars, and wild relatives) were calculated. The polymorphism information  
217 content (*PIC*) for each SSR marker was calculated as indicated Botstein et al. (1980).  
218 Observed heterozygosity (*H<sub>o</sub>*) was calculated for each accession. Pairwise genetic  
219 similarities among accessions were calculated using the codominant genetic distance  
220 (Smouse and Peakall 1999). In this context, for a single-locus with four different alleles  
221 (*i, j, k* and *l*) a set of squared distances are defined as  $d^2(ii, ii)=0$ ,  $d^2(ij, ij)=0$ ,  $d^2(ii, ij)=1$ ,  
222  $d^2(ij, ik)=1$ ,  $d^2(ij, kl)=2$ ,  $d^2(ii, jk)=3$ , and  $d^2(ii, jj)=4$ . In order to obtain the genetic  
223 distance between two accessions, genetic distances are summed across loci under the  
224 assumption of independence (Smouse and Peakall 1999). A principal coordinates  
225 analysis (PCoA) was performed using pairwise genetic similarities. Total genetic  
226 diversity (*H<sub>T</sub>*), among groups genetic diversity (*D<sub>ST</sub>*), within groups genetic diversity  
227 (*H<sub>S</sub>*), relative magnitude of genetic differentiation (*G<sub>ST</sub>*) and standardized *G<sub>ST</sub>* (*G'<sub>ST</sub>*)  
228 were calculated according to Nei (1973). Correlations between morphological and  
229 molecular distances were investigated with a Mantel (1967) test.

230

## 231 **Results**

232

### 233 Morphological characterization

234

235 A wide morphological diversity was found in the collection (Figure 1). Fifty-five out of  
236 the 58 morphological descriptors evaluated were variable in the collections studied. The  
237 three morphological traits which were not variable were Fr-Stripes (all clones bore fruits  
238 with stripes), Fr-Locules (all clones bore fruits with two locules), and Se-Type (all

239 clones had seeds with no wings). Furthermore, when considering only the cultivated  
240 materials, Fl-CorollaShape was also monomorphic (all clones had rotate a corolla).

241

242 *Differences between cultivated and wild clones*

243

244           Significant differences were found between the cultivated pepino and wild  
245 relatives for 26 traits (Table 4). On average, the cultivated pepino is less tall than the  
246 wild relatives, with significantly lower values for traits related to plant size (P-Size, St-  
247 LengthInfl1, St-InternLength or I-LeavesInfl1). The cultivated pepino plants are  
248 characterized by: more root protuberances at the stem nodes (St-Protuberances), less  
249 pubescence (St-Pubescence), fewer divided leaves (L-Type) (i.e., fewer compound, and  
250 more simple leaves) and more bifurcated (I-Type) inflorescences than the wild relatives  
251 (Table 4). Regarding sexual reproduction traits, the cultivated pepino has less style  
252 exertion (Fl-StyleExsertion), lower pollen production (Fl-PollenProd) and fewer seeds  
253 per fruit (Se-SeedsFruit) than wild relatives. Many differences are found for fruit traits;  
254 in particular cultivated pepinos are not surprisingly larger (Fr-Length, Fr-Width, Fr-  
255 PlacentLength, Fr-PlacentBreadth), have more luminous (Fr-L\*), yellow (Fr-b\*) and  
256 glossy (Fr-Glossiness) skin, and more yellow (Fr-FleshColour), and better tasting (Fr-  
257 Flavour and Fr-OffFlavour) flesh, although with less soluble solids content (Fr-Soluble  
258 Solids), than the wild relatives (Table 4). However, the range of variation within  
259 cultivated pepinos and related wild species was generally large and overlapped for all  
260 but six traits, of which three were related to fruit size (Fr-Length, Fr-Width, Fr-  
261 PlacentLength), two to fruit taste (Fr-Flavour and Fr-SolubleSolids), and the remaining  
262 one to the number of seeds per fruit (Se-SeedsFruit) (Table 4).

263

264 *Differences between local varieties and modern cultivars*

265

266           Local pepino varieties differed significantly from modern cultivars for 13 traits  
267 (Table 5). However, despite the significance of differences in the averages of the two  
268 categories of cultivated pepinos for these traits, the range of variation for all traits of  
269 local cultivars and modern varieties overlapped. Local varieties, on average, had more  
270 pigmented stem and leaves (St-Colour and L-AnthVeins) and shorter internode length  
271 (St-InternLength) than modern varieties. Most modern varieties had simple leaves,  
272 while local varieties mostly had compound and flat leaves, which resulted in differences  
273 among both groups for several leaf shape and type traits (L-LaminaWidth, L.LWRatio,  
274 L-Type, L-Leaflets, L-Surface) (Table 5). Modern varieties had, on average, greater  
275 pollen production (Fl-PollenProd) and a larger number of seeds (Se-SeedsFruit) than  
276 local varieties. Also, fruits of modern varieties were, on average larger and more  
277 elongated (Fr-Length and Fr-LW Ratio), and had a higher intensity of green colour (Fr-  
278 a\*) than local varieties.

279

280 *Principal components analysis*

281

282 The first and second components of the PCA performed with all accessions accounted,  
283 respectively, for 29.7% and 11.8%, of the total variation among accession means. The  
284 first component was positively correlated with plant size vigour and growth traits (P-  
285 Size, St-LengthInfl1, St-InternLength, I-LeavesInfl1), high pollen and seed production  
286 (Fl-PollenProd and Se-SeedsFruit), and with fruits having off-flavour (Fr-OffFlavour)  
287 and high soluble solids content (Fr-SolubleSolids), and negatively with the density of  
288 root protuberances in the stem nodes (St-Protuberances), convex leaf surface (L-

289 Surface), multiparous inflorescences (I-Type), fruit size traits (Fr-Length, Fr-Width, Fr-  
290 PlacentLength, and Fr-PlacentBreadth), fruit glossiness (Fr-Glossiness), fruit flesh with  
291 no chlorophyll (Fr-FleshColour), and sweet flavour (Fr-Flavour) (Table 6). The second  
292 principal component was positively correlated with anthocyanin pigmentation of plant  
293 parts (St-Anthocyanins, St-Colour, L-PetioleColour, and L-AnthVeins), compound  
294 leaves (L-LaminaWidth, L-Type and L-Leaflets), greater number of flowers per  
295 inflorescence (I-NFlowers), more luminous (Fr-L\*), less green (Fr-a\*), mottled (Fr-  
296 Mottling), and fasciated (Fr-Fasciation) fruits, and negatively with dropping (L-  
297 Attitude), elongated (L-LWRatio) and convex (L-Surface) leaves, pigmented flowers  
298 (Fl-CorollaColour) and obovoid fruits (Fr-WidestPart) (Table 6).

299         The projection of the accessions on a two-dimensional PCA plot showed that the  
300 first component clearly separates wild accessions in the right part (i.e., positive values)  
301 and cultivated pepino in the left part (i.e., negative values) of the graph (Figure 2). No  
302 overlap was found for the first component values between cultivated pepino and wild  
303 relatives. The second component clearly separates local varieties and modern cultivars  
304 of cultivated pepino, so that the former plot in the upper part (i.e., positive values) of the  
305 graph, while the latter plot in the lower part (i.e., negative values) (Figure 2). This  
306 second component also separates the different wild species from each other. The highest  
307 values belong to *S. caripense*, followed by the group of the morphologically similar *S.*  
308 *perlongistylum* and *S. catilliflorum*, then by *S. tabanoense*, and finally by *S.*  
309 *trachycarpum* (Figure 2). The PCA plot also shows that the groups of local varieties of  
310 pepino and modern varieties show a considerable degree of dispersion in the PCA  
311 graph. Although the four accessions of the wild *S. caripense* plot in the same section of  
312 the PCA graph, they are distinct for the second component (Figure 2). Interestingly, the

313 local varieties originating in Chile (CH and OV) and Colombia (Co) plot close to most  
314 of the modern varieties developed in Spain (SL, SR, Tu and Va) (Figure 2).

315

316 Molecular characterization

317

318 Out of the 20 tomato SSRs tested, 14 were found to be polymorphic. The six other SSRs  
319 either did not amplify (SSR13, SSR51 and SSR136) or were monomorphic (SSR38,  
320 SSR150 and SSR248).

321

322 *SSR characterization*

323

324 The 14 polymorphic SSRs amplified 57 alleles, with an average of 4.07  
325 alleles/locus and a range between 2 and 8 in the collection (Table 7). When considering  
326 cultivated accessions only, two of the SSRs (SSR14 and SSR66) were monomorphic,  
327 and the average number of alleles per locus was 2.5, with a range between 1 and 6. The  
328 number of alleles for each SSR locus for the local varieties of cultivated pepino was  
329 identical to that found for all pepino accessions, except for locus SSR20, in which five  
330 alleles were found instead of six (Table 7). As a result, the average number of alleles per  
331 locus was very similar to that obtained for all the cultivated accessions. Modern  
332 varieties have many fewer alleles per locus, with an average of 1.29, and polymorphism  
333 was only found for four SSR loci, in which only two alleles were detected (Table 7).  
334 For wild relatives, all SSR loci were polymorphic, except locus SSR578. The average  
335 number of alleles per locus was 3.0, with up to 5 alleles being detected for loci SSR45  
336 and SSR306 (Table 7). No SSR was found to be specific and universal to cultivated or  
337 wild accessions. The average value for the *PIC* parameter of the 14 polymorphic SSRs

338 was of 0.4132, with a range for individual SSR loci between 0.0499 (SSR66) and  
339 0.7021 (SSR306) (Table 7).

340 The mean value for observed heterozygosity ( $H_o$ ) was 0.149, with a range  
341 between 0 and 0.333 (Table 8). All the alleles were homozygous for the accessions of  
342 the modern pepino cultivar, Sweet Round. Similarly, the wild accessions P-80 (*S.*  
343 *catilliflorum*), P-62 (*S. perlongistylum*) and E-257 (*S. tabanoense*) were homozygous.  
344 When considering average values, local varieties of cultivated pepino had the highest  $H_o$   
345 value (0.193), while the wild relatives had the lowest (0.117).

346

#### 347 *Principal coordinates analysis*

348

349 The first and second principal coordinates of the PCoA analysis performed with  
350 SSR data account for 26.0% and 10.6% of the total variation, respectively. The first  
351 principal coordinate clearly separated cultivated (right part of the graph) and wild (left  
352 part of the graph) accessions (Figure 3). As occurred with the PCA for morphological  
353 data, no overlap was found for the first coordinate values between cultivated pepino and  
354 wild relatives. With the exception of accession 37A, which showed highly negative  
355 values for the second principal coordinate, all cultivated pepino accessions had positive  
356 or moderately negative values for the second component (Figure 3). Regarding wild  
357 relatives, the second principal coordinate clearly separated two groups of wild relatives,  
358 one formed by *S. caripense* and *S. tabanoense*, with positive values for the second  
359 coordinate, and another one formed by *S. catilliflorum*, *S. perlongistylum* and *S.*  
360 *trachycarpum*, with negative values. All modern varieties clustered together in the same  
361 area of the PCoA plot, while local varieties were more dispersed (Figure 3).

362

363 *Genetic differentiation*

364

365 Total diversity ( $H_T$ ) of the collection had a value of  $H_T=0.458$ , with the cultivated  
366 pepino having a  $H_T=0.237$  and wild relatives a  $H_T=0.458$  (Table 9). The among-groups  
367 diversity ( $D_{ST}$ ) between cultivated pepino and wild relatives had a value of  $D_{ST}=0.107$ ,  
368 resulting in a relative magnitude of genetic differentiation ( $G_{ST}$ ) value of  $G_{ST}=0.274$  and  
369 a standardized  $G_{ST}$  value ( $G'_{ST}$ ) of  $G'_{ST}=0.430$  (Table 9). When comparing the local  
370 varieties and modern cultivars of pepino, the total diversity of local varieties was much  
371 higher ( $H_T=0.336$ ) than that of modern varieties ( $H_T=0.096$ ), with the among groups  
372 diversity being relatively very low ( $D_{ST}=0.021$ ), resulting in low values of  $G_{ST}$  (0.047)  
373 and  $G'_{ST}$  (0.089) (Table 9).

374

375 *Correlation between morphological and genetic distances*

376

377 Correlations obtained from the Mantel test between the matrices of morphological and  
378 genetic distances were high ( $r=0.673$ ). The graphical representation of the relationships  
379 between morphological and genetic distances shows that for both distances the values  
380 between local varieties are generally higher than those of modern varieties (Figure 4).  
381 For the wild species, there was a wide range of morphological and genetic distances,  
382 with the lowest values for both distances being between *S. caripense* accessions. When  
383 comparing accessions of local varieties and modern cultivars of the pepino, it became  
384 evident that some local accessions (Chilean accessions) are morphologically and  
385 molecularly similar to most of the modern varieties, while others are as different as  
386 local varieties among themselves (Figure 4). Values for both morphological and genetic

387 distances between cultivated (local varieties and modern cultivars) and wild accessions  
388 were high (Figure 4).

389

## 390 **Discussion**

391

392 A combination of morphological and molecular data provides relevant complementary  
393 and synergistic information of great interest for plant breeders and for germplasm  
394 curators, in particular for those working with emerging crops (Rao and Hodgkin 2002;  
395 Khoury et al. 2010; Rodríguez-Burruezo et al. 2011; Yildiz 2014). In the case of the  
396 pepino, a standardized morphological descriptors list is available (IPGRI and COMAV  
397 2004), but the descriptors previously have not been validated or used for the  
398 characterization of a diverse germplasm collection of pepino. We have demonstrated  
399 that most of the IPGRI and COMAV (2004) descriptors used are variable (95% for the  
400 whole collection and 93% for cultivated pepino). This allows the acquisition of multiple  
401 characterization (i.e., phenomics) data of agronomic interest in the pepino and wild  
402 relatives for a precise morphological description. Among the few non-variable traits,  
403 some are of relevance for the taxonomic discrimination, like the type of seed (Se-Type),  
404 which is specific for discrimination between the species used here and other wild  
405 relatives of *Solanum* section *Basarhtrum* (Anderson 1979), or in the case of the  
406 cultivated pepino, the corolla shape (Fl-CorollaShape) which is rotate, while in the wild  
407 *S. tabanoense* is stellate (Anderson 1975).

408         Regarding molecular data, SSR markers are preferred to other molecular  
409 markers for the standardized characterization of germplasm (Ghislain et al. 2009;  
410 Vilanova et al., 2014) as, among other properties, they are highly repeatable, co-  
411 dominant, and allow an adequate discrimination among closely related materials (Kalia

412 2011). Because there are no SSR markers available for the pepino, we tested tomato  
413 EST-SSRs for transferability, given that the pepino and tomato are phylogenetically  
414 close relatives (Spooner et al. 1993; Särkinen et al. 2013), indicated conclusion  
415 supported as well by the viable somatic hybrids between the two species that have  
416 produced flowers and fruits (Sakomoto and Taguchi 1991). Our results show that a  
417 large proportion (70%) of tomato EST-SSRs are transferrable and polymorphic in the  
418 pepino collection studied. Furthermore, considerable SSR variation has been detected in  
419 the collections of pepino and wild relatives studied, with an average number of alleles  
420 and *PIC* values almost as high as the values obtained for a highly variable tomato  
421 germplasm collection that included wild relatives (Frary et al. 2005). This indicates that  
422 the large set of SSRs available in tomato (Frary et al. 2005; Suresh et al. 2014)  
423 represents a genomic tool of interest for pepino characterization and breeding, as well as  
424 for mapping and synteny studies.

425         The morphological characterization results reveal that the pepino and its close  
426 wild relatives are notably variable but clearly distinct, with significant differences for  
427 average values for almost one half of the descriptors evaluated and a clear separation in  
428 the PCA analysis. The domestication syndrome in the case of the pepino includes larger  
429 fruits and very variable for fruit shape (i.e., the organ for which it is cultivated –  
430 illustrating one of Darwin’s conclusions about domesticates: the greatest variation in  
431 cultivated plants will be in that feature for which they are cultivated) that are more  
432 luminous, glossy and yellow and more compact plants (Anderson et al. 1996; Prohens et  
433 al. 1996). However, we have also found important changes in reproductive traits, like an  
434 increased number of root protuberances at the nodes (that facilitate vegetative  
435 reproduction), shorter styles (that facilitate selfing), a reduction in pollen production  
436 (that may accompany the selfing syndrome, or vegetative reproduction) and fewer seeds

437 per fruit. The fact that pepino is vegetatively propagated probably favoured the selection  
438 of parthenocarpic materials (Prohens et al. 1998), which means that traits that promote  
439 effective sexual reproduction are released from selection. Cultivated pepinos also offer  
440 a better perceived flavour, probably resulting for a selection for lower acidity and lack  
441 of off-flavour (Prohens et al. 2005). But, pepino cultigens also have a lower content in  
442 soluble solids content (Rodríguez-Burruezo et al. 2003a), which is undesirable for  
443 producing sweet tasting fruits, obviously highly desirable in the marketplace  
444 (Rodríguez-Burruezo et al. 2011). As in other crops, selection for yield may have  
445 brought a reduction in the concentration of sugars due to the “dilution effect” associated  
446 to high yields (Davis 2009). However, it has been demonstrated that it is possible to  
447 obtain backcrosses resembling the cultivated pepino with interspecific hybrids derived  
448 from *S. caripense* and *S. tabanoense*. Such hybrids have high yield and soluble solids  
449 content levels higher than those of the cultivated recurrent parent, suggesting that these  
450 wild species contain genes not present in the cultivated species that can be useful for  
451 improving the soluble solids content of pepino (Rodríguez-Burruezo et al. 2003a, 2011).

452         The local varieties and modern cultivars of pepinos also differ by a number of  
453 significant morphological differences, and, as a consequence, they cluster in different  
454 areas of the PCA diagram. Breeding for higher yield and fruit typologies adapted to  
455 markets has resulted in modern varieties with larger and more elongated fruits. The  
456 elongated fruits may be constitute a selection for shipping: they pack better in layers in  
457 boxes, which may result in fewer bruises than in round fruits. Also, modern varieties  
458 have a higher production of pollen and higher number of seeds per fruit, probably as a  
459 result of selection for higher yield under conditions that may not favour expression of  
460 parthenocarpy. Oddly, and surprisingly, although markets favor golden yellow fruits  
461 (Rodríguez-Burruezo et al. 2011), modern varieties have a greener ( $a^*$  parameter) skin

462 colouration than local varieties. In tomato, enhancing chloroplast development in the  
463 fruit increases sugar contents in fruit (Cocaliadis et al. 2014), and if the same occurs in  
464 pepino this might be the underlying reason for which breeders have unconsciously  
465 selected for fruits with a greener skin. However, this hypothesis remains to be tested.

466         The high morphological diversity observed in the collections studied is matched  
467 by high levels of molecular diversity. A high level of molecular diversity was already  
468 observed for AFLP and DNA sequence of a nuclear gene (Blanca et al. 2007). The EST-  
469 SSR markers evaluated are scattered over the genome of tomato and may constitute a  
470 good representation of different regions of the genome of pepinos as well, if the high  
471 degree of synteny exists between the two closely related crops (Peters et al. 2012). The  
472 results reveal that cultivated pepino clones manifest a considerable heterozygosity, which  
473 is expected as a high degree of heterozygosity is associated with heterosis for yield  
474 (Rodríguez-Burruezo et al. 2003b). Heterozygosity for DNA sequence data had already  
475 been observed by Blanca et al. (2007) in some pepino clones and wild relatives. In the  
476 case of modern varieties, despite the lower heterozygosity compared to local varieties,  
477 the level of observed heterozygosity has been similar to that of local varieties. This may  
478 be taken as evidence that breeders have selected for highly heterozygous individuals in  
479 the modern breeding programs. The Sweet Round variety, which has been the only  
480 modern cultivar homozygous for the 14 loci scored must be heterozygous for other loci  
481 as it does not breed true (Ruiz et al. 1997). With the exception of *S. caripense*, wild  
482 relatives present low levels of observed heterozygosity. This is probably caused by the  
483 fact that many populations of wild species of *Basarthurum* other than the widespread *S.*  
484 *caripense* are composed of few individuals (Anderson 1975, 1979), which favours  
485 fixation of alleles, even despite the self-incompatibility of some of these species, like *S.*  
486 *perlongistylum* and *S. tabanoense* (Mione and Anderson 1992; Anderson et al. 1996).

487 Wild relatives show greater molecular diversity than the cultivated pepinos  
488 (Blanca et al. 2007). In addition the genetic differentiation between the cultivated and  
489 wild materials was quite high ( $G_{ST}=0.274$  and  $G'_{ST}=0.430$ ), indicating that wild relatives  
490 contain a large diversity that is not represented in the genetic background of the  
491 cultivated pepino. This suggests that wild relatives constitute an important source of  
492 variation for pepino breeding (Rodríguez-Burruezo et al. 2003a; Blanca et al. 2007).  
493 Local varieties of pepino show much greater genetic diversity than modern varieties, but  
494 their differentiation was very low ( $G_{ST}=0.047$  and  $G'_{ST}=0.089$ ), indicating that the  
495 genetic diversity of the modern varieties is mostly present in the local varieties. This is  
496 expected as modern varieties have been derived by selection of segregating generations  
497 derived from local varieties (Dawes and Pringle 1984; Simms et al. 1996; Ruiz et al.  
498 1997; Prohens et al. 2002; Rodríguez-Burruezo et al. 2004a, 2004b; Levy et al. 2006).  
499 Also, in contrast to tomato (Lin et al. 2014), no modern pepino cultivars have been  
500 released incorporating artificially introgressed traits from wild relatives, which increases  
501 genetic diversity of modern cultivars. The low diversity present in the modern varieties  
502 indicates that, as occurred in many crops (Cooper et al. 2001), a genetic bottleneck has  
503 taken place during the selection and hybridization programmes performed by breeders.  
504 Our data confirm the information provided by breeders (Dawes and Pringle 1984;  
505 Simms et al. 1996; Ruiz et al. 1997; Prohens et al. 2002; Rodríguez-Burruezo et al.  
506 2004a, 2004b; Levy et al. 2006) indicating that they have mostly used local varieties  
507 from the peripheral southern (Chile) range of distribution of pepino, where the diversity  
508 is much lower than in the center of diversity of the crop in Ecuador, southern Colombia  
509 and northern Peru (Anderson et al. 1996; Blanca et al. 2007). In fact in the PCoA  
510 analysis, the local varieties closest to the modern varieties cluster are those from Chile.

511 Thus, different results might be expected with different selections of pepino cultivars  
512 and (particularly) with different *S. caripense* wild collections.

513

## 514 **Conclusions**

515

516 The characterization using the IPGRI and COMAV (2004) morphological descriptors  
517 list and tomato SSRs molecular markers (Frary et al. 2005) has revealed a large  
518 variation in the collection studied. These characterization tools will allow the  
519 identification of new sources of morphological and genetic variation in pepino and wild  
520 relatives, the study of diversity and establishment of the relationships in pepino and  
521 wild relatives. Cultivated pepino and wild relatives display high morphological and  
522 molecular diversity, but the two groups are clearly differentiated from each other.  
523 Modern cultivars are notably morphological different from local varieties, and are much  
524 less variable at the molecular level indicating the existence of a genetic bottleneck  
525 during the modern breeding history of this crop. All of these data are of relevance for  
526 modern and efficient pepino breeding based on phenotypic and molecular marker  
527 selection as well as for the management and conservation of pepino germplasm  
528 collections.

529

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531

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668 **Table 1** Plant materials used for the study of morphological and molecular (SSR)  
669 variation in a germplasm collection of local varieties and modern cultivars of cultivated  
670 pepino (*S. muricatum*) and wild relatives (other species of *Solanum* section  
671 *Basarthrum*).

Accession	Code	Species	Origin <sup>a</sup>
Pepino local varieties			
37-A	37	<i>S. muricatum</i>	Ecuador (Azuay)
Col-1	Co	<i>S. muricatum</i>	Colombia
CH2-22	CH	<i>S. muricatum</i>	Chile
OV-8	OV	<i>S. muricatum</i>	Chile (Limarí)
PT-154	PT	<i>S. muricatum</i>	Peru
RP-1	RP	<i>S. muricatum</i>	Ecuador
Pepino modern cultivars			
El Camino	EC	<i>S. muricatum</i>	New Zealand
Kawi	Ka	<i>S. muricatum</i>	New Zealand
Puzol	Pu	<i>S. muricatum</i>	Spain
Quito	Qu	<i>S. muricatum</i>	United Kingdom
Sweet Long	SL	<i>S. muricatum</i>	Spain
Sweet Round	SR	<i>S. muricatum</i>	Spain
Turia	Tu	<i>S. muricatum</i>	Spain
Valencia	Va	<i>S. muricatum</i>	Spain
Wild relatives			
BIRM/S 1034	c1	<i>S. caripense</i>	Ecuador
E-7	c2	<i>S. caripense</i>	Ecuador (Pichincha)
EC-40	c3	<i>S. caripense</i>	Ecuador (Loja)
QL-013	c4	<i>S. caripense</i>	Ecuador (Cayambe)
P-80	ct	<i>S. catilliflorum</i>	Peru (Abancay)
P-62	pe	<i>S. perlongistylum</i>	Peru (La Mar)
E-257	ta	<i>S. tabanoense</i>	Ecuador (Loja)
E-34	tr	<i>S. trachycarpum</i>	Ecuador (Cotopaxi)

672 <sup>a</sup>Origin refers to the country and province (when known) of the collection in the case of  
673 wild relatives and local varieties of pepinos, and to the country where the modern  
674 cultivar of the pepino was developed.

675 **Table 2** Morphological and agronomic descriptors used for the characterization of  
 676 cultivated pepino (*S. muricatum*) and wild relatives. Full details on each descriptor can  
 677 be consulted elsewhere (IPGRI and COMAV 2004).

Descriptor	Code	Range (scale) / units
Plant descriptors (P)		
Plant size	P-Size	1-9 (3=small; 7=large)
Vigour of the plant	P-Vigour	1-9 (3=weak; 7=strong)
Stem descriptors (St)		
Stem length at first inflorescence	St-LengthInfl1	cm
Degree of ramification	St-Ramification	1-9 (3=low; 7=high)
Intensity of anthocyanin of shoot tip	St-Anthocyanin	0-9 (0=absent; 7=strong)
Root protuberances at the node	St-Protuberances	0-9 (0=absent; 7=many)
Stem pubescence density	St-Pubescence	0-9 (0=glabrous; 7=dense)
Stem colour	St-Colour	1-5 (1=green; 5=dark purple)
Internode length	St-InternLength	cm
Leaf descriptors (L)		
Petiole length	L-PetioleLength	mm
Petiole colour	L-PetioleColour	1-5 (1=green; 5=dark purple)
Foliage density	L-Density	1-9 (3=sparse; 7=dense)
Leaf attitude	L-Attitude	1-3 (1=semi-erect; 3=dropping)
Leaf lamina length	L-LaminaLength	cm
Leaf lamina width	L-LaminaWidth	cm
Leaf blade length/width ratio	L-LWRatio	---
Type of leaves	L-Type	1-2 (1=simple; 2=compound)
Number of leaflets	L-Leaflets	---
Leaf colour	L-Colour	1-5 (1=light green; 5=purple)
Anthocyanin coloration of leaf veins	L-AnthVeins	1-9 (3=green; 7=purple)
Leaf surface attitude	L-Surface	1-9 (3=flat; 7=very convex)
Inflorescence descriptors (I)		
Number of leaves from ground to first inflorescence	I-LeavesInfl1	---
Inflorescence type	I-Type	1-3 (1=generally uniparous; 3=generally multiparous)
Number of flowers per inflorescence	I-NFlowers	---
Flower descriptors (Fl)		
Corolla shape	Fl-CorollaShape	1-3 (1=stellate, 3=rotate)

Corolla colour	Fl-CorollaColour	1-6 (1=white; 6=purple)
Sepal length	Fl-SepalLength	mm
Stamen length	Fl-StamenLength	mm
Style exertion beyond anther cone	Fl-StyleExsertion	mm
Pollen production	Fl-PollenProd	0-9 (0=none; 7=high)
Fruit descriptors (Fr)		
Number of fruits per infructescence	Fr-FruitInfruct	---
Number of fruits per plant	Fr-FruitPlant	---
Fruit size uniformity	Fr-Uniformity	1-9 (3=low; 7=high)
Fruit length	Fr-Length	cm
Fruit width	Fr-Width	cm
Position of the widest part of the fruit	Fr-WidestPart	1-9 (3=less than ¼ way from base to tip; 7=more than ½ way from base to tip)
Fruit length/width ratio	Fr-LWRatio	---
Fruit primary colour L* parameter	Fr-L*	---
Fruit primary colour a* parameter	Fr-a*	---
Fruit primary colour b* parameter	Fr-b*	---
Fruit stripes	Fr-Stripes	0-1 (0=absent; 1=present)
Fruit mottling	Fr-Mottling	0-1 (0=absent; 1=present)
Fruit surface covered by additional colour	Fr-AddColour	1-3 (1=less than 10%; 3=between 30 and 50%)
Fruit epidermis glossiness	Fr-Glossiness	3-7 (3=dull; 7=bright)
Number of locules per fruit	Fr-Locules	---
Inner placental area length	Fr-PlacentLength	cm
Inner placental area breadth	Fr-PlacentBreadth	cm
Inner placental length/breadth ratio	Fr-PlacentLBRatio	---
Fruit flesh colour	Fr-FleshColour	1-8 (1=dark green; 8=salmon)
Fruit flavour	Fr-Flavour	1-9 (3=acidic; 9=sweet)
Presence of bitter off-flavour	Fr-OffFlavour	0-9 (0=absent; 7=strong)
Fruit cracking	Fr-Cracking	0-9 (0=absent; 9=severe)
Fruit fasciation	Fr-Fasciation	0-9 (0=absent; 9=severe)
Fruit soluble solids content	Fr-SolubleSolids	%
Seed descriptors (Se)		
Number of seeds per fruit	Se-SeedsFruit	---
Seed colour	Se-Colour	1-7 (1=white; 7=black)
Seed diameter	Se-Diameter	1-3 (1=small (<1.5 mm); 3=large (>2.5 mm))
Type of seed	Se-Type	1-3 (1=not winged; 3=winged)

679 **Table 3** EST-SSR tomato markers used in the present study along with their repeat  
680 motif, annealing temperature, expected size, and linkage group in which they map in the  
681 tomato genetic map (Frary et al. 2005).

SSR locus	Repeat motif	Annealing temperature	Expected size	Linkage group
SSR13	(AAG) <sub>6</sub>	50	102	5
SSR14	(ATA) <sub>9</sub>	55	166	3
SSR20	(GAA) <sub>8</sub>	50	157	12
SSR38	(TCT) <sub>8</sub>	55	237	8
SSR43	(TAC) <sub>7</sub>	55	237	4
SSR45	(AAT) <sub>14</sub>	50	246	7
SSR51	(ACAA) <sub>6</sub>	50	148	1
SSR52	(AAC) <sub>9</sub>	50	202	7
SSR66	(ATA) <sub>8</sub>	50	185	2
SSR80	(TTCAA) <sub>2</sub> (GTACAA) <sub>2</sub> (CAA) <sub>7</sub>	50	186	11
SSR111	(TC) <sub>6</sub> (TCTG) <sub>6</sub>	50	188	3
SSR128	(CAG) <sub>6</sub> (CAA) <sub>3</sub> (CAG) <sub>7</sub>	50	123	6
SSR136	(CAG) <sub>7</sub>	50	149	11
SSR150	(CTT) <sub>7</sub>	50	217	1
SSR248	(TA) <sub>21</sub>	55	251	10
SSR285	(TTAT) <sub>2</sub> (AT) <sub>6</sub>	55	276	7
SSR306	(ATT) <sub>7</sub>	55	258	4
SSR578	(AAC) <sub>6</sub> (ATC) <sub>5</sub>	55	294	6
SSR590	(TC) <sub>6</sub> (AC) <sub>4</sub>	55	161	5
SSR593	(TAC) <sub>7</sub>	55	295	4

682

683 **Table 4** Mean and range for the morphological descriptors for which significant  
684 differences were found between accessions of the cultivated pepino (*S. muricatum*) and  
685 its wild relatives.

Descriptor <sup>a</sup>	Cultivated species		Wild relatives		Prob. t
	Mean	Range	Mean	Range	
N		14		8	
P-Size	4.9	3.4-6.6	6.5	5.0-7.0	0.0014
St-LengthInfl1	51.9	43-71	101.8	63-144	<0.0001
St-Protuberances	4.6	3.0-7.0	2.3	0.0-3.0	0.0002
St-Pubescence	2.7	0.0-3.0	4.7	0.0-7.0	0.0067
St-InternLength	5.3	4.2-6.0	7.5	4.3-9.3	0.0001
L-LaminaLength	31.7	25-37	26.9	20-34	0.0180
L-LWRatio	1.8	1.0-3.0	1.2	0.8-2.2	0.0469
L-Type	1.4	1.0-2.0	1.9	1.0-2.0	0.0077
L-Surface	4.7	3.0-7.0	3.4	3.0-5.0	0.0026
I-LeavesInfl1	11.6	8-17	16.8	13-19	0.0001
I-Type	2.6	1.0-3.0	1.4	1.0-3.0	0.0008
Fl-StyleExsertion	2.8	1.4-3.9	3.9	1.3-5.2	0.0223
Fl-PollenProd	3.4	0.0-5.4	5.7	5.0-7.0	0.0007
Fr-Uniformity	5.0	3.0-6.2	5.9	5.0-7.0	0.0249
Fr-Length	9.1	4.8-15.4	2.9	1.7-4.6	<0.0001
Fr-Width	7.2	4.1-11.1	2.7	1.8-3.6	<0.0001
Fr-L*	60.3	51-65	54.7	40-63	0.0495
Fr-b*	23.6	17-29	18.7	8-24	0.0241
Fr-Glossiness	4.5	3.0-5.7	3.3	3.0-5.0	0.0039
Fr-PlacentLength	5.1	2.2-9.7	1.4	0.6-2.1	0.0012
Fr-PlacentBreadth	0.68	0.2-1.8	0.15	0.1-0.2	0.0020
Fr-FleshColour	5.2	3.0-6.7	2.8	2.0-4.0	0.0001
Fr-Flavour	5.8	5.0-7.0	2.0	1.0-3.0	<0.0001
Fr-OffFlavour	0.66	0.0-3.0	2.75	0.0-5.0	0.0037
Fr-SolubleSolids	6.6	4.9-7.7	9.7	7.8-11.4	<0.0001
Se-SeedsFruit	0.26	0.0-0.7	3.08	1.0-4.0	<0.0001

686 <sup>a</sup>See Table 2 for a full definition of the descriptors.

687 **Table 5** Mean and range for the morphological descriptors for which significant  
 688 differences were found between local varieties and modern cultivars of cultivated  
 689 pepino (*S. muricatum*).

Descriptor <sup>a</sup>	Local varieties		Modern cultivars		Prob. t
	Mean	Range	Mean	Range	
N	6		8		
St-Colour	3.1	2.0-4.0	2.2	2.0-3.4	0.0195
St-InternLength	4.8	4.2-5.4	5.6	5.1-6.0	0.0024
L-LaminaWidth	24.6	16-34	16.5	11-31	0.0354
L-LWRatio	1.3	1.0-1.7	2.3	1.1-3.0	0.0082
L-Type	1.7	1.3-2.0	1.2	1.0-1.5	0.0057
L-Leaflets	3.0	1.0-5.0	1.6	1.0-3.0	0.0269
L-AnthVeins	4.3	3.0-5.0	3.3	3.0-3.8	0.0196
L-Surface	4.1	3.0-5.4	5.2	4.6-7.0	0.0478
Fl-PollenProd	2.5	0.0-4.6	4.1	3.0-5.4	0.0402
Fr-Length	6.8	4.8-7.9	10.8	6.6-15.5	0.0185
Fr-LWRatio	1.0	0.7-1.8	1.6	0.9-2.2	0.0382
Fr-a*	-3.3	-6.1--1.3	-6.4	-11.7--3.1	0.0393
Se-SeedsFruit	0.10	0.0-0.4	0.38	0.0-0.7	0.0223

690 <sup>a</sup>See Table 2 for a full definition of the descriptors.

691

692

693 **Table 6** Correlation coefficients between morphological descriptors and the two first  
694 components (29.7% and 11.8% of the total variance explained by the first and second  
695 principal components, respectively) for accessions evaluated of the cultivated pepino  
696 and wild relatives. Only those correlations with absolute values  $\geq 0.15$  have been listed.

Descriptor <sup>a</sup>	First principal component	Second principal component
P-Size	0.172	
St-LengthInfl1	0.225	
St-Anthocyanins		0.181
St-Protuberances	-0.178	
St-Colour		0.227
St-InternLength	0.188	
L-PetioleColour		0.280
L-Attitude		-0.190
L-LaminaWidth		0.270
L-LWRatio		-0.235
L-Type		0.201
L-Leaflets		0.249
L-AnthVeins		0.155
L-Surface	-0.178	-0.180
I-LeavesInfl1	0.199	
I-Type	-0.159	
I-NFlowers		0.217
Fl-CorollaShape	0.198	
Fl-CorollaColour		-0.185
Fl-PollenProd	0.163	
Fr-Length	-0.196	
Fr-Width	-0.220	
Fr-WidestPart		-0.160
Fr-L*		0.152
Fr-a*		0.259
Fr-Mottling		0.164
Fr-Glossiness	-0.183	
Fr-PlacentLength	-0.178	
Fr-PlacentBreadth	-0.172	
Fr-FleshColour	-0.186	
Fr-Flavour	-0.224	
Fr-OffFlavour	0.159	
Fr-Fasciation		0.247
Fr-SolubleSolids	0.184	
Se-SeedsFruit	0.211	

697 <sup>a</sup>See Table 2 for a full definition of the descriptors.

698 **Table 7** SSR markers successfully amplified and polymorphic in the collection of  
 699 cultivated pepino and wild relatives evaluated, number of alleles per SSR locus of each  
 700 of the groups considered and PIC value.

SSR locus	Number of alleles					PIC
	All accessions (n=22)	All cultivated accessions (n=14)	Cultivated local varieties (n=6)	Cultivated modern cultivars (n=8)	Wild relatives (n=8)	
SSR14	3	1	1	1	3	0.3360
SSR20	8	6	5	2	3	0.6134
SSR43	4	3	3	1	2	0.2604
SSR45	6	2	2	1	5	0.3665
SSR52	3	2	2	1	2	0.4156
SSR66	2	1	1	1	2	0.0499
SSR80	2	2	2	2	2	0.3715
SSR111	4	2	2	1	4	0.4297
SSR128	5	2	2	1	4	0.3079
SSR285	4	3	3	1	3	0.5188
SSR306	6	4	4	1	5	0.7021
SSR578	2	2	2	2	1	0.3693
SSR590	4	3	3	2	2	0.5774
SSR593	4	2	2	1	4	0.4669
Mean	4.07	2.50	2.43	1.29	3.00	0.4132

701

702

703 **Table 8** Observed heterozygosity ( $H_o$ ) for the polymorphic SSR loci in each of the  
 704 accessions of cultivated pepino (*S. muricatum*) and wild relatives evaluated, and mean  
 705 values ( $\pm$ SE) for the cultivated pepino local varieties, modern cultivars and for wild  
 706 relatives.

Accession	$H_o$
Pepino local varieties	
37-A	0.154
Col-1	0.231
CH2-22	0.214
OV-8	0.167
PT-154	0.091
RP-1	0.300
Mean local varieties	0.193 $\pm$ 0.029
Pepino improved cultivars	
El Camino	0.154
Kawi	0.333
Puzol	0.154
Quito	0.143
Sweet Long	0.154
Sweet Round	0
Turia	0.077
Valencia	0.167
Mean improved cultivars	0.148 $\pm$ 0.033
Wild relatives	
BIRM/S 1034 ( <i>S. caripense</i> )	0.154
E-7 ( <i>S. caripense</i> )	0.250
EC-40 ( <i>S. caripense</i> )	0.154
QL-013 ( <i>S. caripense</i> )	0.286
P-80 ( <i>S. catilliflorum</i> )	0
P-62 ( <i>S. perlongistylum</i> )	0
E-257 ( <i>S. tabanoense</i> )	0
E-34 ( <i>S. trachycarpum</i> )	0.091
Mean wild relatives	0.117 $\pm$ 0.040

707

708 **Table 9** Total genetic diversity ( $H_T$ ), among groups genetic diversity ( $D_{ST}$ ), within  
 709 groups genetic diversity ( $H_S$ ), relative magnitude of genetic differentiation ( $G_{ST}$ ) and  
 710 standardized  $G_{ST}$  ( $G'_{ST}$ ) (Nei, 1973) estimated from data for the cultivated pepino (*S.*  
 711 *muricatum*) and wild relatives accessions.

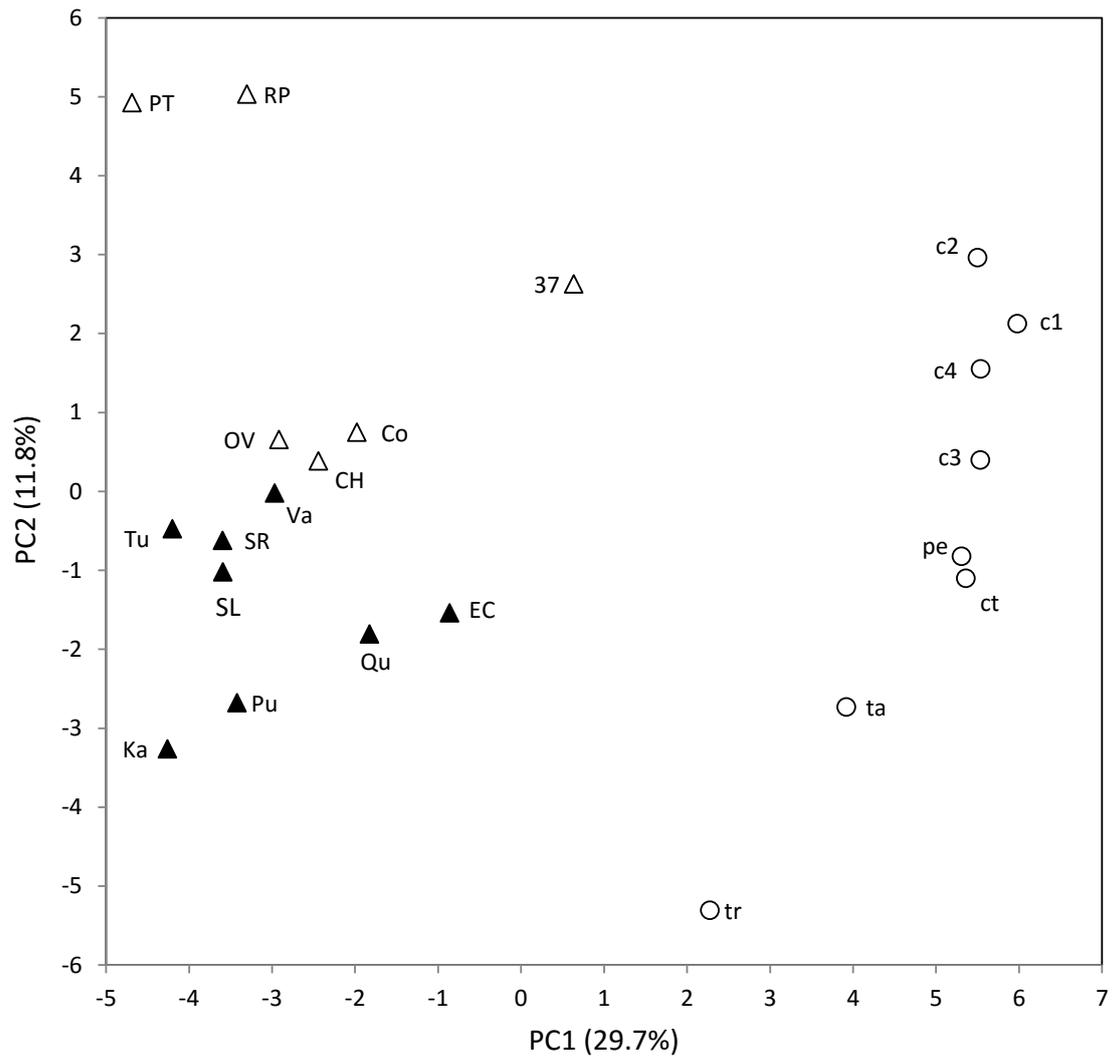
Group	Sample size	$H_T$	$D_{ST}$	$H_S$	$G_{ST}$	$G'_{ST}$
All	22	0.458	0.107	0.350	0.274	0.430
Cultivated pepino	14	0.237				
Wild relatives	8	0.401				
Cultivated pepino	14	0.237	0.021	0.216	0.047	0.089
Local varieties	6	0.336				
Modern cultivars	8	0.096				

712

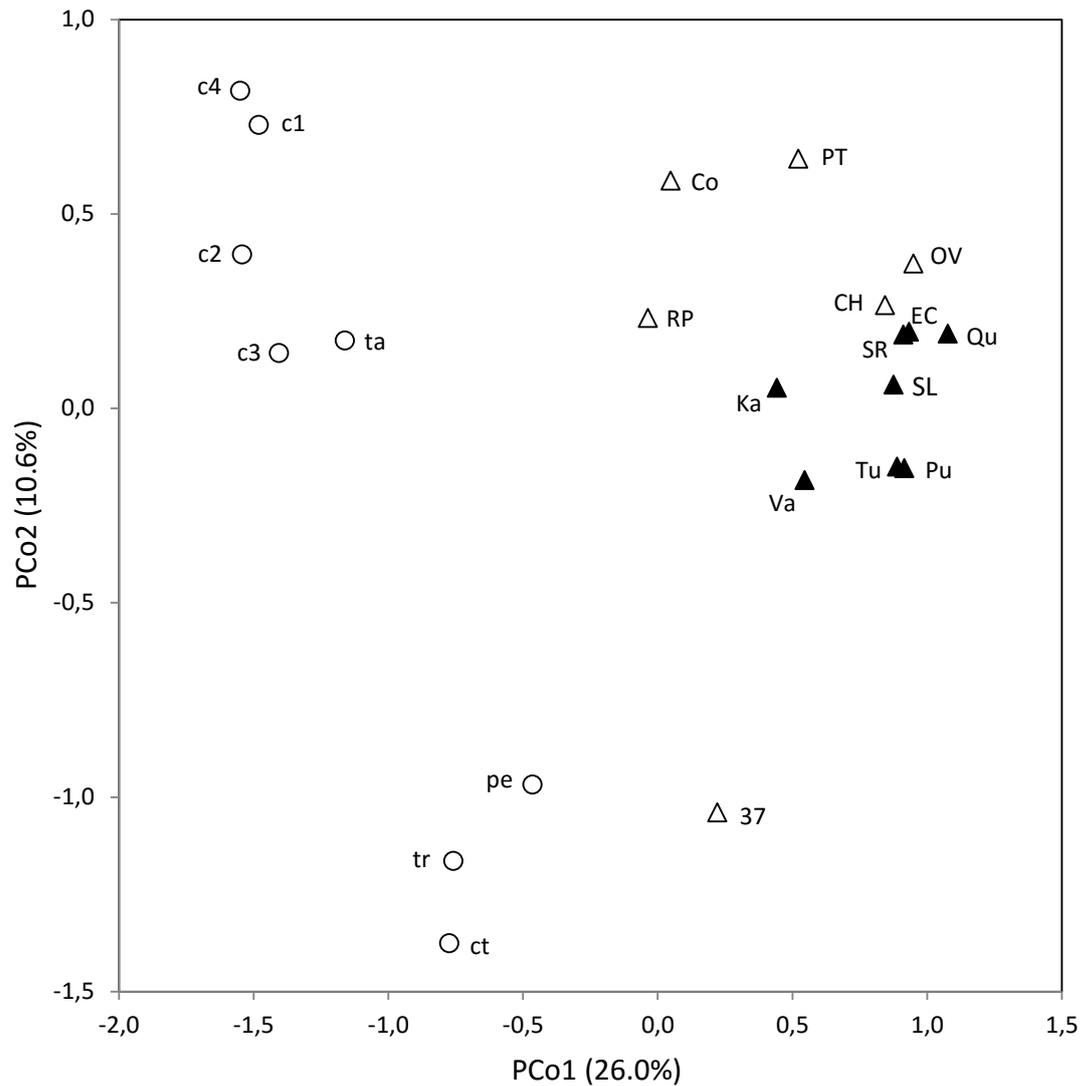


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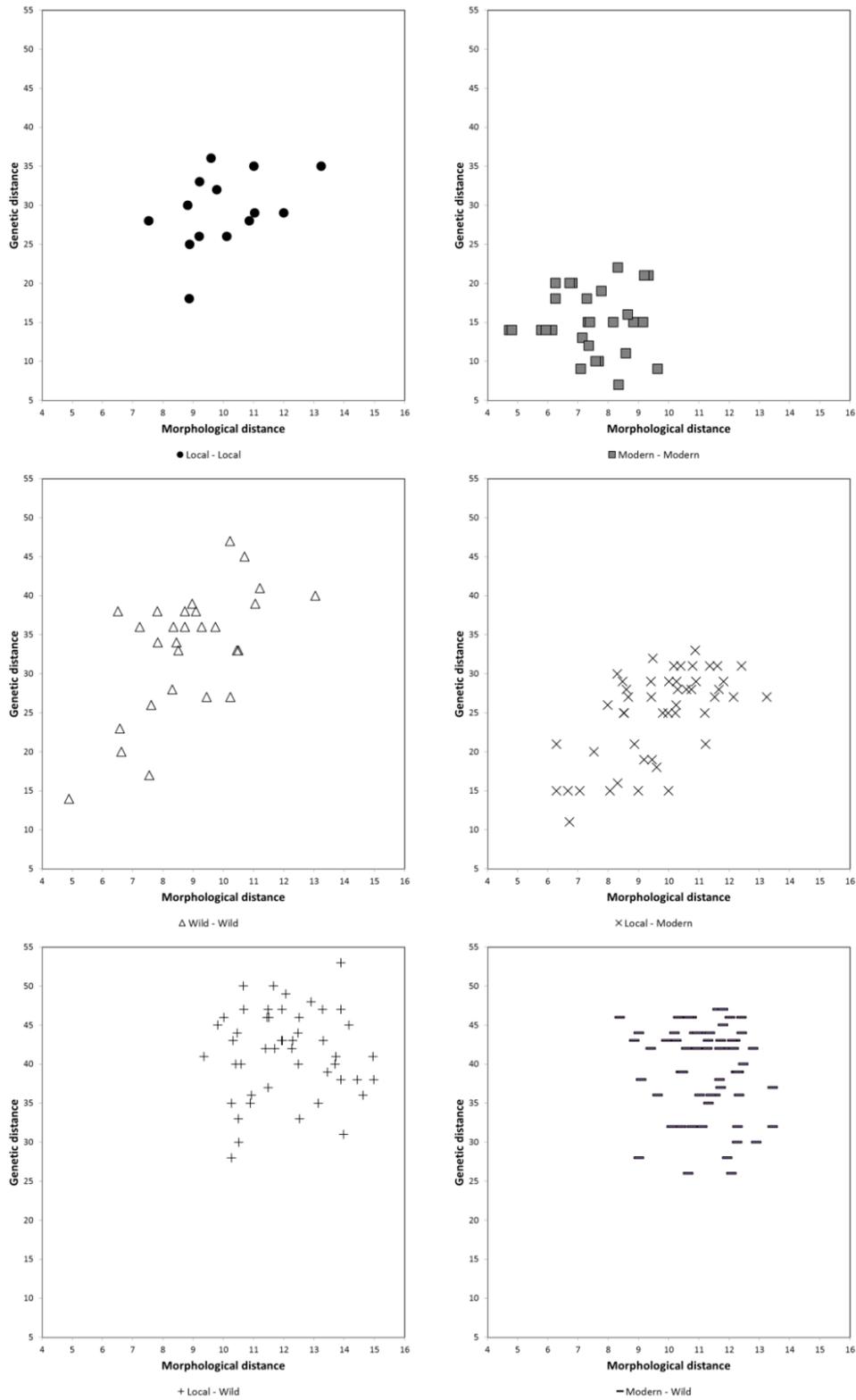
714 **Fig. 1** Diversity in fruit size, shape and colour in the cultivated pepino and wild  
715 relatives collection studied. Fruits of wild species are indicated by white arrows.



716 **Fig. 2** Principal components analysis (PCA) similarities based on 55 variable  
717 morphological descriptors among 22 accessions of local varieties (open triangle),  
718 modern cultivars (solid triangle) of cultivated pepino and wild relatives (open circle).  
719 First (PC1) and second (PC2) principal components account for 29.7% and 11.8% of the  
720 total variation, respectively.  
721  
722



723  
724 **Fig. 3** Principal coordinates analysis (PCoA) similarities based on 14 polymorphic EST-  
725 SSRs among 22 accessions of local varieties (open triangle) and modern cultivars (solid  
726 triangle) of cultivated pepino and wild relatives (open circle). First (PC1) and second  
727 (PC2) principal coordinates account for 26.0% and 10.6% of the total variation,  
728 respectively.



729

730 **Fig. 4** Relationships between morphological and molecular distances among pairs of  
 731 accessions of pepino and wild relatives. Distances between pairs of accessions are  
 732 represented for each combination of groups: Local and local (solid circle; above left);  
 733 modern and modern (grey square; above right); wild and wild (white triangle; center  
 734 left); local and modern (× cross; center right); local and wild (+ cross; below left); and,  
 735 modern and wild (horizontal dash; below right).