1 *Phenotypic and genetic diversity of Spanish tomato landraces.*

2 Jaime Cebolla-Cornejo[1,3], Salvador Roselló[2], Fernando Nuez[1]

3 [1] Instituto Universitario de Conservación y Mejora de la Agrodiversidad Valenciana

4 (COMAV). Universitat Politècnica de València. Cno. de Vera, s.n. 46022. València.

5 Spain.

6 [2] Departamento de Ciencias Agrarias y del Medio Natural, Universitat Jaume I, Campus

7 de Riu Sec, 12071 Castellón, Spain.

8 [3]Corresponding author: +34-963879423; +34-963879422; jaicecor@btc.upv.es

9 ABSTRACT

10 The structure of Spanish landraces of tomato (*Solanun lycopersicum* L.) has been analysed. This
11 diversity has been evaluated using agro-morphological characteristics (43 descriptors), quality
12 parameters (solid soluble contents and individual sugars and organic acids) and DNA markers
13 (Amplified Fragment Length Polymorphisms, AFLP). A wide range of variation was found for
14 all traits but in the DNA marker level. Certain common characteristics could be identified in
15 populations of the same landrace in several of the dimensions analysed, but generally, an
16 overlap of the spectrum of variation of different landraces was found. The results indicate that in
17 each landrace the populations are strongly selected using very basic morphological
18 characteristics such as fruit shape, colour or ribbing, while other traits vary depending on each
19 farmer preferences. Seed mixing and pollen contamination might introduce variation which
20 would be purged by farmers at the morphological level, but would be maintained in quality and
21 yield traits. Despite the introduction of spurious variation it would be still possible to identify
22 certain relations between quality attributes and the morphological traits defining specific
23 landraces. The existence of a wide level of variation in plant yield and quality profiles enables
24 the development of selection programmes targeted to provide farmers with materials with
25 economically viable yield and excellent organoleptic quality. The results also highlight the
26 necessity to stress the efforts in morpho-agronomical and quality characterization over
27 molecular characterization in the ex situ management of these resources, as well as not to
28 underestimate the importance of intra-varietal variability.

29

30 KEYWORDS

31 Germplasm; genetic resources; *Solanum lycopersicum*; quality; traditional variety; amplified
32 fragment length polymorphism

33

34

INTRODUCTION

It is commonly accepted that the tomato (*Solanum lycopersicum* L.) was domesticated from *S. lycopersicon* var. *cerasiforme* in México (Bai & Lindhout, 2007). With the arrival of the Spaniards in America, the tomato participated in the exchange of crops between the New and the Old World. And it reached Europe though Spain probably in the first half of the 16th century, though the exact date remains unknown. From Spain it spread to the Viceroyalty of Naples and to the rest of Italy (Dondarini, 2010). Considering that Spain played a major role in the spread of tomato and the fact that Spain and Italy were the first countries cultivating this crop in Europe, it seems logical that both countries would represent an important secondary centre of diversity.

Over these five centuries of cultivation, numerous ecotypes adapted to different agroclimatic conditions have been developed. It was the farmers themselves who contributed to the diversification of this crop, by carrying out distinct selections in different cultivation areas. Consequently, in the early 20th century a great diversity of tomato landraces existed in the main horticultural areas of Spain.

The term landrace has received numerous definitions and several synonyms refer to the same concept, including local variety, local population, traditional cultivar, farmer variety and farmer population (Zeven, 1998) or traditional variety and primitive variety (Negri et al. (2009). Harlan (1975) described them as follows: "Landraces have a certain genetic integrity. They are recognizable morphologically; farmers have names for them and different landraces are understood to differ in adaptation to soil type, time of seeding, date of maturity, height, nutritive value, use and other properties. Most important, they are genetically diverse." In the same text Harlan stated that landraces "consist of mixtures of genotypes or genetic lines". Louette (2000) in the context of maize cultivation defined a local variety or landrace as the set of farmers' seed lots that bear the same name and are considered as a homogeneous set, and seed lots as the set of kernels of a specific variety selected by one farmer. Again the idea of a landrace or local

61 variety as composed of different selections appears. The different selections of the same

62 landrace made by farmers can be considered as populations of the landrace or as subpopulations

63 being in this case the landrace the population). Considering that usually during germplasm

64 collections the term population is usually used to define the sample obtained at a specific site

65 (Brown and Marshall, 1995; Hawkes et al., 2000), it could be proposed that a landrace maybe

66 formed by different populations that despite sharing common characteristics typical of the

67 landrace to which they belong have suffered different selections by different farmers and have

68 evolved in different environments.

69 In Spain several different tomato landraces can still be found with different colours (red, orange,

70 yellow, pink), shapes (heart-shaped, flattened, rounded and intermediate shapes, cylindrical,

71 pyriform, ellipsoid and elongated) and sizes (up to 1kg). Their origins remain unclear, as in the

72 case of other crops it is difficult to find varietal designations, other than the name of the crop,

73 until the first half of the 20th century. Nowadays it is still difficult to differentiate in some cases

74 between real landraces, selected by farmers, and old obsolete commercial varieties selected by

75 breeders, as only their designations and not their origins are conserved in the spoken tradition.

76 In this context, the evaluation of Spanish landraces seems to be a good model in order to

77 analyse the structure of variation in tomato landraces. Several studies regarding Spanish

78 landraces of tomato have been previously published, but usually they include data on a specific

79 group of characteristics (morphological or quality traits or DNA) and usually including a very

80 limited set of landraces and accessions per landrace (Garcia-Martinez et al., 2006, 2013; Casals

81 et al., 2011a, 2011b; Cebolla-Cornejo et al., 2011).

82 These landraces constitute the main source of variation in the cultivated species and usually

83 show outstanding organoleptic quality. In fact, this last reason has enabled the development of

84 quality markets where consumers are eager to pay a differential of 4.7 over the price of

85 commercial modern varieties (Cebolla-Cornejo *et al*., 2007). The information obtained in the

86 analysis of wide collections of landraces would be of great interest in the management of *ex situ*

87 collections, for their utilization in breeding programmes or for their direct use in quality

88   markets, as the cultivation of these materials could represent a 'true pearl' as defined by

89   Meerburg *et al*. (2009): the one that satisfies societal demands while providing a reasonable

90   income to the farmer.

91

92   In this context, this work analyses agronomical and morphological traits, chemical composition

93   related to organoleptic quality and DNA variation in a wide collection of Spanish landraces,

94   including a wide representation of farmers' selections or populations of several key landraces.

95   To which point are farmers' selections of the same landrace similar? Are different landraces

96   really differentiable? Is this variation clearly structure is separated groups? Landraces are

97   usually distinguished morphologically, but do they have a clear chemical profile defining a

98   characteristic taste? Several authors have analysed a discrete set of Spanish landraces using

99   DNA markers, but are the results consistent when a wide collection of landraces and farmer's

100   selections are considered? These are the questions that this work tries to answer..

101

102   **MATERIALS AND METHODS**

103   A collection of several accessions or populations of different traditional varieties was analysed

104   considering different traits: morpho-agronomical traits, quality-related traits and DNA. The

105   variation in fruit weight and yield (accumulated fruit weights) variation was analysed in depth

106   considering the importance of these traits. The number of accessions evaluated was reduced for

107   plant yield, quality and DNA variation, considering the costs of each characterization. In each

108   case, the populations were selected depending on the socio-economic importance of each

109   variety.

110

111   *Analysis of morpho-agronomical variation*.

112   For the analysis of morpho-agronomical variation 75 populations of 29 landraces were included

113   (Table 1). Although several varieties were included in this study, it was centred in the analysis

114   of four especially important landraces or traditional varieties: 'Valenciano', a heart shape

115   tomato, 'Muchamiel', a flat and ribbed tomato, 'Pimiento' a long variety resembling an Italian

116   pepper and 'Penjar' a small fruited variety with long shelf-life. All the accessions were provided

117   by seedbank of the Instituto Universitario de Conservación y Mejora de la Agrodiversidad

118   Valenciana, COMAV (Valencia, Spain). These populations were evaluated using morphological

119   and agronomical descriptors.

120   A selection of IPGRI (1997) descriptors (marked I-) was used with some additions (marked A-),

121   including 21 qualitative morphological descriptors, 4 qualitative agronomical descriptors, 17

122   morphological quantitative descriptors and 5 agronomical quantitative descriptors. Some

123   agronomical descriptors can also be considered as morphological. Nevertheless, they have been

124   studied together as morpho-agronomical variation.

125   Qualitative descriptors were classified in scales from 1 to 9, generally 1 corresponding to

126   extremely low intensity and 9 to extremely high intensity. Morphological descriptors included

127   were: I-unripe external fruit colour, I-green stripes, I-green shoulder intensity, I-fruit

128   pubescence, I-fruit shape, I-fruit size, I-fruit size homogeneity, I-external ripe fruit colour, I-

129   intensity of ripe external fruit colour, I-secondary fruit shape, I-intensity of fruit ribbing, I-

130   easiness of fruit to detach from pedicel, I-easiness of fruit wall (skin) to be peeled, I-skin colour

131   of ripe fruit, I-flesh colour of pericarp, I-flesh colour intensity, A-core colour, I-intensity of core

132   colour, I-fruit cross-sectional shape, I-shape of pistil scar, I-fruit blossom end shape and I-

133   blossom end scar condition. Qualitative agronomical descriptors were: I-sensorial fruit firmness,

134   I-radial cracking, I-concentric cracking and A-seed yield. Quantitative morphological

135   descriptors and the corresponding units used in the evaluation were: I-fruit length (mm), I-fruit

136   width (mm), A-fruit width /fruit length ratio, I-pedicel length (mm), I-pedicel length from

137   abscission layer (mm), I-width of pedicel scar (mm), I-size of corky area around pedicel scar

138   (mm), I-thickness of pericarp (mm), A-fruit section length (mm), I-size of core (mm), A-

139   minimum number of locules, A-maximum number of locules, I-mean number of locules, A-

140   mean locule size (mm), A-size of hollow area between pericarp and core (mm), A-fruit firmness

141   (measured with a Bertoluzzi FT327 penetrometer with a 8mm probe, kg/mm), A-size of the

142   internal fibrous area associated to pedicel scar (mm). Agronomical quantitative descriptors

143   included: I-mean fruit weight (g), A-mean plant yield (g/plant), A-minimum plant yield

144   (g/plant), A-maximum plant yield (g/plant) and A-percentage of commercial fruits.

145   Cultivation was carried out in the open air in Turis (39º 20' 54''N, 0º, 43' 19''W), in an area

146   with low populations of tomato virus vectors, during one growing cycle. Four blocks were

147   utilized with three plants per accession randomly distributed in each block. Plants of the hybrid

148   'Royesta' were used as borders in order to provide similar growing conditions in the

149   experiment. All the varieties had the same indeterminate growing habit and similar vegetation.

150   Thus, neighbour effects were considered to affect uniformly to all the plants. Plants were staked

151   with a separation of 0.4m between plants and 1.2m between rows. A basal dressing of 30,000

152   kg/ha of manure and 1,500 kg/ha of 15/15/15 NPK was applied. A total top dressing of 2,500

153   kg/ha of ammonium nitrate, 1.500 kg/ha of mono-ammonium phosphate, 3.500 kg/ha of kalium

154   sulphate and 500 kg/ha of magnesium sulphate was applied gradually using drip irrigation.

155   Plants were pruned on a weekly basis.

156   The variation was analysed statistically using multivariate tests. A principal component

157   analysis (PCA) was carried out using the means of the whole set of variables. Qualitative

158   variables were included as they were scored in a 1 to 9 scale. In order to increase the level of

159   variance explained, a second PCA was performed with a selection of descriptors, most of them

160   quantitative, related with varietal recognition by farmers. This set of variables included: fruit

161   weight, length, width, width to length ratio, mean locule number, width of pedicel scar, size of

162   corky area around pedicel scar, thickness of pericarp, size of core, mean locule size, size of

163   hollow are between pericarp and core, fruit firmness and fruit ribbing. In order to determine the

164   number of principal components selected, the eigenvalues were represented in a graph against

165   their indices (scree plot). The first few eigenvalues showed a sharp decline, followed by a much

166   more gradual slope. Those dimensions corresponding to the flat portion of the graph may

167   represent non-differentiable 'noise' components of the system. Therefore the number of

168   components selected depended on the position at which the 'elbow' of the scree plot appeared.

169   This criterion is defined in Krzanowski (2000). With all quantitative and qualitative traits, a

170   cluster analysis was performed. In this case, two sets of variables suffered different pre-

171   treatments. Quantitative variables and those qualitative variables representing a value of

172   intensity were scaled to 0-1 using a range transformation: $(x_i\text{-min}(x))/((\text{max}(x)\text{-min}(x))$. On the

173   other hand, qualitative variables not indicating a degree of intensity, such as fruit shape or

174   colour, were decomposed in dummy variables. For example in the case of the fruit shape

175   descriptor, 9 new variables were created such as "heart-shaped fruit" or "pyriform fruit" each

176   one with a binary notation (present/absent : 1/0). As each initial variable was converted in a

177   different number of new dummy variables it was necessary to avoid that those decomposed in a

178   higher number of dummy variables would have an extra weight in the analysis. Therefore,

179   considering that this matrix would be used to calculate Euclidean distances, for each dummy

180   variable instead of using the common 1/0 annotation, the value of the squared root of the

181   number of new dummy variables of the descriptor minus 1 was used instead of 1. Following this

182   procedure, when the Euclidean distance is calculated, in cases of maximum difference the sum

183   of distances for all the dummy variables arising from the same descriptor would sum 1. This

184   transformation ensures that a single descriptor decomposed into x dummy variables will have

185   the same statistical weight in the analysis as a descriptor decomposed into y dummy variables.

186   This approach was adapted from the theoretical foundations described in Kiers (1989). After the

187   pre-treatment both sets of variables were combined in a single matrix and Euclidean distance

188   was calculated after bootstrapping (with 1000 repetitions and 0.3 substitutions). Dendrograms

189   were obtained using the unweighted pair group method with arithmetic means (UPGMA).

190   Stable clusters were identified using stability of nodes obtained with the bootstrap analysis. As

191   statistical software S-PLUS-8 (Insightful Corp., Seattle, USA), Phylip (Felsenstein, 1989) and

192   Phyltools (Buntjer, 2001) were used.

193   *Analysis of fruit weight and plant yield variation*.

194   A total of 39 populations belonging to the traditional varieties 'Valenciano' (heart

195   shaped), 'Muchamiel' (flat and ribbed), 'Pimiento' (long, resembling Italian pepper) and

196 'Penjar' (small sized, long-term conservation) were selected to evaluate the level of variation in

197 fruit weight and yield in different scales (Table 1). This analysis was not extended to all the

198 populations characterized, due to the difficulty of weighing individual fruits. Therefore the

199 populations of the most important socio-economic varieties were prioritized, selecting random

200 populations in each variety. The hybrid ''Royesta'' with high acceptance in Mediterranean areas

201 (FAO, 2002) was used as a reference. The growing conditions and experimental field design

202 were the same described previously.

203　　　　Fruit weight was measured in a per plant basis, and all the fruits up to the fifth truss

204 were weighed individually. Mean fruit weight and plant yield were calculated. The objective of

205 this work was not to detect significant differences in fruit weight or yield but to provide a

206 description of the level of variation. Intra population fruit weight CV was calculated as the

207 coefficient of variation between plant means for fruit weight. The homoscedasticity of plant

208 fruit weight variation was analysed per population using Bartlett's test. Logarithmic and square

209 root transformations were applied to transform the data in order to homogenise variances. Plant

210 yields were calculated as the sum of plant fruit weights. Mean, maximum and minimum yields

211 were determined and the level of variation expressed as a standard coefficient of variation.

212

213　　　　*Analysis of quality-related variation*

214 Samples were obtained from a selection of 52 of the 75 populations characterized morpho-

215 agronomically (Table 1). Populations were selected considering the socio-economic importance

216 of the variety and the ripening conditions of the fruits, as in some populations it was difficult to

217 obtain a minimum number of fruits in the precise ripening stage required. It was also prioritized

218 the analysis of inter-varietal diversity rather than intra-varietal diversity. Four fruits representing

219 the predominant fruit shape and size were collected from each of the 12 plants at the mature-red

220 stage (only from the first three trusses), avoiding the unusual fruits (deformations, big size, etc.)

221 that usually develop in different proportions in the first and second trusses of several of these

222 traditional varieties. Longitudinal wedges were obtained from the fruits and ground at low

223 temperature, and a bulked sample was obtained from each block (3 plants per block). One

224 aliquot was used for the determination of basic parameters and the rest were kept frozen at -80

225 ºC until analysis of individual components. Each sample was analysed three times.

226 Basic quality traits included the determination of total soluble solids content (SSC), measured

227 with an Pr-1 refractometer (Atago Co Ltd., Tokyo, Japan) and expressed as g/100g sucrose, and

228 total titratable acidity measured with three volumetric determinations and expressed as g citric

229 acid/ 100g.

230 The sugars fructose, glucose and sucrose and the organic acids oxalic, malic and citric were

231 quantified following the method described by Roselló *et al*. (2002). Capillary electrophoresis

232 was performed with a P/ACE MDQ (Beckman Instruments Inc., Fullerton, CA, USA),

233 controlled by the software 32 Karat™ V.5.

234 *Analysis of DNA variation*.

235 A selection of 35 accessions was used to analyse the DNA variation between populations (Table

236 1). Populations were selected prioritizing the analysis of inter-varietal diversity rather than

237 intra-varietal diversity. Tomato breeding lines RDD and UPV-1 and accession PE-45 from

238 *Solanum pennellii* Correll were included as controls and outgroup.

239 Genomic DNA was extracted (Doyle & Doyle, 1990) from the first true leaf of 6 plants per

240 accession. After quantification the DNA of the 6 plants were pooled together in the same

241 proportion. AFLP analysis (Vos *et al*., 1995) was performed with the commercial kit Invitrogen

242 AFLP® Core reagent Nº cat.: 40482-016 (Invitrogen®, Carlsbad, CA, EE.UU.). EcoRI and

243 MSEI were selected as restriction enzymes and the experimental procedure reproduced the

244 indications of the kit.

245 The adapters used in the analysis were:

246 *Eco R1Adapter*:   5'-CTCGTAGACTGCGTACC
                           CATCTGACGCATGGTTAA-5'

247

248 *Mse I Adapter*:   5'-GACGATGAGTCCTGAG
                           TACTCAGGACTCAT-5'

249

250   Pre-amplification primers were complementary: Eco R1-A and Mse I-C and amplification

251   primer combinations included Eco RI-ACA / Mse I-CAC, Eco RI-AGC / Mse I-CAA, Eco RI-

252   AAC / Mse I-CAC, Eco RI-ACT / Mse I-CAA and Eco RI-AGC / Mse I-CAC, marked with

253   either 6FAM, NED, HEX and JOE flurofors. AFLP products were separated in an automated

254   DNA sequencer ABI/PRISM® 310 (PE Biosystems, Foster City, CA, EE.UU.). The software

255   GeneScan v. 3.1.2 (Applied Biosystems, Foster City, CA, EE.UU.) and Genographer v. 1.6.0.

256   (Montana State University, Montana, MO, EE.UU.), were used to obtain the binary matrix

257   corresponding to presence/absence of amplification. Phylip (Felsenstein, 1989) and Phyltools

258   (Buntjer, 2001) were used for the cluster analysis using Nei and Jaccard distances and UPGMA

259   with a bootstrap of 1000 repetitions and 0.3 substitution. Stable clusters were identified using

260   stability of nodes obtained with the bootstrap analysis.

261   *Analysis of relationships between sets of variables.*

262   In order to analyse the correlation among sets of variables two approaches were followed:

263   canonical correlation analysis and distance matrix correlation analysis. The canonical

264   correlation analysis (CCA) was applied between the morpho-agronomical and quality data sets

265   in order to identify common patterns between both sets of variables avoiding the influence of

266   within-set correlation. The CCA transforms the p morpho-agronomical variables and the q

267   quality variables to s pairs of new variates $(u_1, v_1), \ldots(u_s, v_s)$ being the s canonical correlations

268   the pure expression of association between the sets of morpho-agronomical and quality

269   variables.. This analysis was not carried out between these data sets and the AFLP marker data

270   due to its binary structure.

271   The CCA was performed using the GenStat V.12 software (VSN International Ltd., Hemel

272   Hempstead, UK). The number of canonical variates (CaV) to be included in the analysis of the

273   results was determined using the Bartlett's statistic described by Krzanowski (2000). Following

274   this same guidelines, for the interpretation of the results the canonical variates were expressed in

275   terms of standardized original variables.

276   For the distance matrix correlation analysis, following the methodology already described in

277   previous sections, new distance matrices and cluster dendrograms were calculated for morpho-

278   agronomical (Euclidean distance, UPGMA), quality (Euclidean distance, UPGMA) and AFLP

279   data (Nei distance, UPGMA) considering only the 27 populations used in the three analysis. The

280   cophenetic coefficients and correlations between pairs of distance matrices were calculated

281   using NTSYSpc v.2.02 software package (Applied Biostatistics Inc., Setautek, NY, EE.UU.)

282   and for the estimation of the significance of the correlations, Mantel tests with 1000

283   permutations were performed.

284   In order to further analyze the possible correlation between AFLP marker data and geographical

285   distance between collection sites, a spatial autocorrelation analysis was performed (Smouse &

286   Peakall, 1999).

287   *Access to data generated in this work*.

288   Raw data for main quantitative descriptors and data related to organoleptic quality is provided

289   in supplementary tables 1 and 2. The rest of the data can be consulted in the COMAV seedbank.

290   **RESULTS**

291   *Analysis of morpho-agronomical variation*.

292   A principal component analysis (PCA) was performed with the whole set of variables in order

293   to obtain a general overview of the structure of variation within and between traditional

294   varieties. The first two components explained 0.332 of the variation, a low value probably due

295   to the high number of variables considered and the presence of qualitative traits. In order to

296   increase the percentage of variation explained by the analysis, the number of variables was

297   reduced trying to maximize the variance explained by the model. In the new PCA the first two

298   principal components now explained 0.366 and 0.146 of the variation respectively, and were

299   selected for the interpretation of the results. The first component was mainly related to traits

300   regarding fruit size and the second with traits related to fruit shape. The graphical representation

301   of the PCA showed a broad dispersion of the populations. Despite the high number of varieties

302   and populations analysed, the populations of 'Valenciano', 'Muchamiel', 'Pimiento', 'Penjar'

and 'De la pera' were grouped together in a higher or lower degree (Fig. 1). Nevertheless, it was possible to identify some populations placed outside the main area of distribution of each variety. For example, this was the case of the populations BGV5709 (Fig. code 2.2) of 'Muchamiel' and BGV5461 (Fig. code 3.4) of 'Pimiento'. In these cases though, there was no reason to discard these populations as errors of varietal adscription, once the characterization and passport data were individually reviewed. Nevertheless, especially in the overrepresented varieties such as 'Valenciano' or 'Muchamiel' the gradient of variation was quite wide and their area of distribution overlapped with other varieties.

A more precise view of the wide level of variation present among the populations of each variety was observed in the cluster analysis (Fig. 2), where all the morpho-agronomical variables were included. A high cophenetic coefficient (0.86) was obtained (Mantel test p=0.02 with 100 permutations) but low bootstrap values were obtained in most nodes, indicating a lack of robustness of the clustering. In fact, the populations of the same variety appeared in different nodes in several cases.

Analysis of fruit weight and plant yield variation.

In order to examine in detail the variation in agronomical key traits fruit weight and plant yield were selected from the pool of morpho-agronomic variables. A wide range of mean fruit weight could be observed, especially in the varieties 'Valenciano' and 'Muchamiel', both having a high number of populations represented. In the case of 'Valenciano', it ranged from populations with small fruits of 113.7g to populations with big fruits of 302.9g (Table 2). In this variety, the most stable characteristic was the heart shape of its fruits, which was identifiable in all the populations. Nevertheless, a certain level of variation in the width to length ratio could be detected. Something similar happened in 'Muchamiel'. In this case fruit weight ranged from populations with a mean of 198.6g to populations with 356.4g. In this case, all the populations showed flat and heavily ribbed fruits in variable degrees. In the rest of varieties the number of populations assayed was too small to obtain general conclusions. In this sense, though

329 'Pimiento' showed medium size, long fruits, with a low number of seeds and 'Penjar' showed

330 uniformly small fruits with rounded or ovoid shapes.

331 Intra-population coefficient of variation for fruit weight ranged from 0.07 and 0.34 in

332 'Valenciano', though the lower value was obtained in a population with low fitness. In

333 'Muchamiel' the coefficient of variation ranged between 0.18 and 0.37, in 'Penjar' from 0.25

334 and 0.26 and in 'Pimiento' from 0.26 and 0.31 (Table 2). The level of variation among plants in

335 each population was examined using the Bartlett's test. Most part of the populations showed a

336 lack of homoscedasticity (Table 2). The logarithmic and especially the square root

337 transformations improved the uniformity of variances, but still a lack of homoscedasticity was

338 detected. Consequently only the results using untransformed data were included.

339 Plant yield was also extremely variable (Table 2). The mean coefficient of variation of yield in

340 the traditional populations was 0.54, 3.4 times higher than the detected in the commercial

341 reference (0.16). The high amount of variation in yield detected in the traditional populations

342 was mainly related to the lack of fitness of some of the plants of the same population.

343 Accordingly, minimum and maximum yields were usually very different (Table 2).

344 Nevertheless, in each population was possible to identify plants with acceptable productions. It

345 was also possible to identify in each variety populations with either an extreme performance

346 (maximum yield) or homogeneity in yield (low coefficient of variation) or both characteristics.

347 *Analysis of organoleptic quality related variation*.

348 Regarding the variability observed in basic parameters related to fruit organoleptic quality a

349 wide distribution was observed in the populations and varieties evaluated (Fig. 3). This

350 variability was especially evident in the overrepresented varieties 'Valenciano' and

351 'Muchamiel'. In both of them a wide gradient, in both total soluble solids content and total

352 titratable acidity, was found. Nevertheless, a common general pattern could be identified. In this

353 sense, 'Muchamiel' tended to show low values of both variables, while 'Valenciano' showed

354 intermediate values (Fig. 3). The same would apply to variety 'De la pera', with intermediate

355 values of SSC and low acidity, or 'Pimiento' that in general showed both high SSC and acidity.

356 The range of variation in each variety enabled the identification of accessions with values in this

357 variables corresponding to better organoleptic quality (both high SSC and titratable acidity).

358 In order to get a better idea of the variation in the variables affecting organoleptic quality

359 including both basic parameters and individual compounds, a PCA was carried out. The first

360 component explained 0.333 of the variation and was positively and highly correlated with

361 glucose, fructose and citric and total soluble solids content, positively and moderately correlated

362 with total titratable acidity and moderately and negatively correlated with pH and malic acid

363 content. The second component explained 0.248 of the variation and was positively correlated

364 with pH, glucose and fructose content and negatively correlated with total titratable acidity.

365 That would mean that higher values in the first component would be related to higher flavour

366 intensity and the second component would mainly represent the acidic note.

367 The analysis of the dispersion of populations in the first two components showed that in each of

368 the overrepresented varieties there was a wide range of variation (Fig. 4). In fact, the level of

369 variation among populations of the same variety was similar or higher than the variation among

370 different varieties (Fig. 4). In that sense, the populations belonging to 'Valenciano' were

371 scattered covering almost the whole variation spectrum, and the same applied to the varieties

372 'Penjar' and 'Morado'. Nevertheless, as it happened with the basic parameters, it was possible

373 to appreciate some general trends for specific varieties. For example, it could be said, that

374 despite the wide variation detected in the variety 'Muchamiel', it usually showed low levels of

375 single compounds and a rather acidic note. Likewise, the populations of 'Pimiento' were

376 characterized by high individual compound contents and a slight acidic note.

377 *DNA marker variation*

378 AFLPs markers were used to characterize some of the landraces evaluated. DNA from 6 plants

379 of each landrace was pooled for this purpose. With the five primer combinations 253 bands

380 were amplified, with a mean of 51 bands per amplification. Thirty three of the bands appeared

381 exclusively in the outgroup of *S. pennellii*. Globally, the percentage of polymorphic bands

382 (frequency lower than 0.95) was 0.253. In the case of cultivated tomato 220 bands were

383 observed, and 0.258 were polymorphic. The mean frequency of band presence was 0.592,

384 though the real distribution was biased towards very frequent or very infrequent alleles.

385 Among the populations belonging to the variety 'Valenciano' the level of detected

386 polymorphism was 0.092. In the case of 'Muchamiel' a higher level, 0.18, was detected. In both

387 cases, 195 bands were observed. The mean genetic diversity was 0.23 for 'Valenciano'

388 populations, 0.08 for 'Muchamiel' populations and 0.14 for the whole set of accessions

389 analysed. The mean genetic distance using Nei's coefficient was 0.062±0.001 though the pair

390 grouped distances were distributed asymmetrically with a preponderance of low coefficients.

391 In a first cluster analysis using Nei's index, the outgroup of *S. pennellii* was clearly

392 differentiated from *S. lycopersicum* populations (Fig. 5). Once checked the validity of the

393 analysis, the outgroup was removed to analyse the diversity in the cultivated species. A high

394 cophenetic coefficient of 0.98 (Mantel test p=0.99, 100 permutations) was obtained in the

395 cluster analysis using Nei's index. Nevertheless, the bootstrap analysis showed that the nodes

396 obtained were not stable, as most of them obtained frequencies lower than 0.50. The same

397 analysis using the Jaccard index showed a similar topology (data not showed). As it happened in

398 the analysis of morpho-agronomical variables, the distribution of the populations of each variety

399 was nearly random, as they appeared mixed in different nodes.

400 *Correlation analysis between sets of variables*.

401 The first five canonical variates (CaV) obtained in the analysis were selected, representing a

402 cumulated correlation of 0.783 (Table 3). For the first CaV, length to width ratio and fibrous

403 area associated to pedicel scar and fructose and titratable acidity showed the highest loadings

404 respectively in each set of variables, meaning that these variables bear a higher level of

405 association between them. For the second CaV, fruit length to width ratio, fruit section length,

406 fruit ribbing and size of core and citric, malic and titratable acidity showed the highest loadings.

407 For the third CaV, the highest loadings were obtained with fruit length, mean number of locules,

408 size of hollow area between pericarp and core and minimum plant yield and citric acid, glucose

409 and SSC (ºBrix). The variables with the highest loadings with the fourth CaV were L/W ratio,

fruit section length and size of core and SSC. For the fifth CaV the highest loadings were obtained with the size of fibrous area associated to pedicel scar and malic acid. From this analysis, it seems then that variables related to fruit shape and structure, usually linked to variety recognition, bear some level of association with quality parameters. This may lead to the general trends in quality parameters associated to certain varieties observed in the study.

In order to obtain a different perspective of the relations between the different data sets new distance matrices were obtained for each standardized data set, only considering the accessions with representation in the three analyses. The correlation between the distance (Euclidean) matrices of the standardized morpho-agronomical data and standardized quality data was significant and moderate: R=0.40 (Mantel test, p=0.002 with 1000 permutations). The correlation between the distance (Euclidean) matrices of the standardized morpho-agronomical data and the distance (transformed Nei's coefficient) matrix of the AFLP marker data was not significant (r=0.07, Mantel test, p=0.36 with 1000 permutations). And finally, the correlation between the distance matrix of the standardized quality data and the distance matrix of the APLP marker data was significant (Mantel test, p=0.0.02 with 1000 permutations) but reduced (r=0.25).

The cluster analysis of the three distance matrices (Fig. 6) showed no consistent clustering of the same accessions, despite showing high cophenetic coefficients (AFLP: r=0.84; Quality: r=0.71; Morpho-agronomical: r=0.87). It seems therefore that, again, although there is some relation between morpho-agronomical characteristics and quality and between quality and AFLP data, these relations are not consistent enough to provide a clear identification of different varieties. This seems quite clear when analysing the clustering behaviour of "'Muchamiel'" varieties in the dendrograms.

Finally, in order to analyse if there was an underlying geographic structure in the genetic structure of the populations analysed, the distances between collection sites were calculated. The correspondence analysis between the genetic distance (transformed Nei's coefficient) and the geographic distance between collection sites showed no correlation (r=-0,003; p=0.48). In

437   the same sense, the spatial autocorrelation analysis, showed no significant genetic structures in

438   20km scales (data not shown).

439   **DISCUSION**

440   The heterogeneity present in a landrace or traditional variety is an inherent characteristic of

441   these materials. Zeven (1998) reviewed the definitions given to landraces by several authors and

442   in most of them the genetic diversity played an important role. In this study the diversity present

443   in a set of traditional varieties of tomato has been analysed considering different traits. The

444   analysis of fruit weight and yield was perhaps one of the most enlightening, as it gave an idea of

445   the variability present in a certain population, in a variety or varietal type and among different

446   traditional varieties. The evaluation was only performed during one year, and thus important

447   information such as environmental effects or population x environment interactions could not be

448   evaluated. Nevertheless, the results obtained can still be valuable, as all the plants were grown

449   in the same environment and our interest was focused on genotypic effects. The levels of

450   variation found between plants in fruit mean weight were variable, though the lack of

451   homoscedasticity prevented the comparison between populations and with the hybrid control. In

452   each variety a wide range of variation in mean fruit weight among different populations was

453   identified. Terzopoulos & Bebeli (2010) also obtained a wide range of phenotypic variation in

454   fruit weight among Greek landrace populations between 0 and 0.61 with a mean value of 0.36,

455   and Mazzucato *et al*. (2010) have also found considerable level of variation in fruit weight in

456   their analysis of the Italian landrace collection Abruzzese, ranging from 190g to 366g. At least

457   in our case, it seems that this parameter might not be especially important in the recognition of

458   the variety and might oscillate depending on farmer's preference. In fact, lower variation was

459   found in characters related to fruit shape such as the length to width ratio of the degree of fruit

460   ribbing, which seem more important in varietal recognition than fruit size.

461   In the varieties 'Penjar' and 'Pimiento' with lower number of populations the range of variation

462   of mean fruit weight was low. In the case of the variety 'Penjar', the main characteristic of the

463   variety is its long shelf life, recently associated with the presence of the *alc* mutation where

464   additionally, an extended shelf life has been related to small fruit size (Casals *et al*., 2011a).

465   Therefore, it would be reasonable that a strong selection would have been made for small fruits,

466   then justifying the lower range of variation in mean fruit weight among populations detected in

467   this study.

468   More important than the variation in fruit weight was the high variation in plant yield. Usually

469   in most populations low and high producing plants could be identified, causing a high

470   coefficient of variation in plant yield. Consequently, the mean level of variation in the

471   traditional populations (0.54) represented more than three times (3.37) the variation of the

472   commercial hybrid. This enormous variation led to especially low mean yields in the traditional

473   varieties as plant with low fitness reduced drastically the mean value, thus considerably

474   lowering their competitiveness. Nevertheless, the existence of this level of variation also enables

475   the development of intra-population and intra-varietal selections to improve yield in this

476   cultivars. Terzopoulos *et al*. (2009) also found high levels of variation in Greek traditional

477   varieties of tomato, with coefficients of variation ranging from 0.31 to 0.51, values only slightly

478   lower than those reported here. It should be noted that in our case the estimates of variation in

479   fruit weight and yield were obtained using a relatively low number of plants, 12, but the

480   estimates have enough accuracy to obtain the conclusions explained.

481   It should be noted that the farmers that usually cultivate these traditional materials hold the idea

482   of seed "degeneration", where a variety loses its characteristics or its fitness during successive

483   generations. This idea of "degeneration" and the results obtained may be related to the observed

484   high variation in plant yield. It has been previously considered that this seed "degeneration"

485   referred by farmers could be related to the continuous interchange and eventual mix of seeds

486   from different populations of the same variety or by the pollen contamination with other

487   populations (Zeven, 1999; Cebolla-Cornejo, *et al*., 2007).

488   When both agronomical and morphological variation were analysed jointly it could be

489   recognized that the different populations that constitute a single traditional variety represent a

490   wide gradient of variation that eventually overlaps the range of variation of different varieties.

491   In fact, the evaluation of variation has shown that sometimes there are more differences in

492   morphological traits or in the chemical profile between two populations of the same landrace

493   than between two populations of different landraces. This wide range would be logical if it is

494   assumed that each farmer would have selected the next generation considering his own

495   priorities. In that case, the recognition of the variety would rely on very few and basic

496   morphological characteristics such as fruit shape, colour and ribbing, or shelf life in the case of

497   the 'Penjar' variety. Strong selection would have been applied by farmers for these traits,

498   reducing its variation and discarding off-types arising from pollen contamination, while the rest

499   would greatly vary attending to farmer preferences. This would explain that some general trends

500   in quality parameters could be identified in certain landraces. In fact these trends would also be

501   the basis of the relations found between morpho-agronomical and quality data in the canonical

502   correlation analysis or the correlation between the distance matrices for these traits. But again,

503   despite the existence of a general trend, no consistent clustering patterns were obtained.

504   In other landraces it has been highlighted that a variation in fruit shape might not be so

505   important. In this sense, Terzopoulos & Bebeli (2010) identified three main fruit shapes in the

506   variety Santorini, depending on the use given by farmers and Mazzucato *et al*. (2010) also

507   observed variation in this trait in the landrace A pera Abruzzese. In the present study the

508   varieties analysed showed a reasonably uniform fruit shape and that level of variation was only

509   found in 'Penjar' tomatoes, where the distinctiveness of the landrace is defined by the long

510   shelf-life trait and shape might very depending on the genetic background where the *alc*

511   mutation has been naturally introgressed (Casals *et al*., 2011a). The landrace 'Penjar' satisfies

512   all the requirements set by Camacho-Villa et al. (2005) to be considered as a landrace: its origin

513   is lost in time, it has only be selected by farmers, it has some level of local adaptation, it's used

514   in traditional farming systems (though it is also grown in industrialized systems as well), it is

515   obviously genetically diverse and it has a distinct identity. It should be considered, though, that

516   distinctness is restricted to one single trait, long shelf-life, controlled by a single gene. .

517 Regarding quality traits, usually landraces are associated with better organoleptic quality and

518 this has led to the development of quality niche markets. Nevertheless, the results obtained

519 showed high variation in objective parameters related to flavour perception. In the case of SSC

520 and TA, which are the most basis variables related with consumer preference (Stevens, 1972), a

521 high gradient was found among the populations of the same landrace. In a more complex

522 analysis, a similar variation was obtained when single compounds were analysed. The specific

523 content of individual sugars and organic acids has recently been correlated with consumer

524 acceptance or preference and received a further analysis (Baldwin *et al*., 1998; Fulton *et al*.,

525 2002, Cebolla-Cornejo *et al*., 2011). Nonetheless, in both cases general trends could be

526 identified associated to certain landraces.

527 This high level of variation and the overlap in landrace distribution would again coincide with

528 the results on plant yield and morphological characteristics. Again seed mixing and pollen

529 contamination might be the explanation for this wide level of variation. Nevertheless, in this

530 case it should be added that the purge of a contaminated population might be easy considering

531 directly perceived morphological characteristics (leading to simpler variety recognition), but

532 very complicated when sensory quality are to be considered.

533 It is obvious that the high organoleptic quality of landraces exists, as there are consumers

534 willing to pay higher prices for these materials, but our results also show that the landraces

535 might "degenerate" in quality characteristics. This would be a problem as it may risk the

536 existence of niche markets and therefore should be controlled (Casals *et al*., 2011b).

537 Fortunately, again the existence of a wide range of variation also enables the selection of the

538 best populations that might help to consolidate these niche markets.

539 The variation present in morphological, agronomical and quality traits represents quite a

540 problem in the context of promoting on-farm conservation. In agreement with definition given

541 by Maxted et al. (1997) this type of conservation should be sustainable. In the case of the

542 Spanish traditional varieties studied here, it depends on their economic viability, as old farmer's

543 that still prefer them are not being replaced by the next generations (Cebolla-Cornejo et al.,

544   2007). This viability depends on the existence of an added value such as a recognized

545   organoleptic quality and the existence of niche markets. But, the existence of 'too much'

546   variability in these materials hinder this possibility. The expected organoleptic quality is not

547   always present in all the farmer's selections of a landrace, the variation present in

548   morphological traits interferes consumer recognition and the variation present in yield per plant

549   reduces drastically potential benefits. In this case, as it has been stated some level of selection

550   would aid to develop conservation alternatives. Some degree of selection targeted to develop

551   several lines of a landrace, offering higher morphological uniformity (and thus facilitating

552   recognition by non trained consumers), the best organoleptic quality present in the landrace

553   (satisfying consumer demands) and with higher yields (improving farmer income) will facilitate

554   the maintenance of these materials. It seems reasonable that this alternative should be led by

555   public institutions with the participation of farmers in the process. Nevertheless, it should be

556   considered that if after some level of selection these materials would still be landraces, but also

557   if without that selection those materials would completely disappear.

558   As DNA data analysis is regarded, the genetic diversity present in traditional varieties of tomato

559   is highly limited. AFLP markers have been used to develop unique fingerprints of tomato

560   varieties (Park et al., 2004), but its use in the fingerprinting of traditional varieties seems quite

561   difficult. The introgression of wild genetic background from the 50s might improve the

562   identification of unique profiles, but this is much more difficult in traditional not formally bred

563   materials. In fact, in our study accessions with a high level of genetic similarity showed clear

564   morphological differences.

565   The limited variability of cultivated tomato has been previously described using RAPD and

566   RFLP markers (Williams & St. Clair, 1993; Archak et al., 2002). SSR markers have also been

567   employed, though mainly in genetic fingerprinting or diversity studies using only modern

568   cultivars with a different genetic structure (Bredemeijer et al., 2002) or a mixture of tomato

569   cultivars and wild relatives (Alvarez et al., 2001; He et al., 2003) that cannot be compared with

570   the results of traditional varieties. Anyway, the low genetic diversity in tomato, especially in

571 secondary centres of diversity has been explained by a founder effect, selfing and natural and

572 artificial selection (Rick, 1958; Rick & Fobes, 1975).

573 In this study a relatively low level of diversity has been found, with an irregular distribution,

574 similar to that described by Villand *et al*. (1998) using RAPD markers, with a preponderance of

575 bands with very high or very low frequencies. This situation led in or study to low paired

576 genetic distances probably resulting in low stability nodes in the cluster analysis with population

577 of different varieties being mixed. This lack of stability with low bootstrap values was also

578 observed by Garcia-Martinez *et al*. (2006) using also AFLP and Spanish landraces, though in

579 that case 'Muchamiel' populations were grouped together and in this case they appeared

580 scattered in different nodes. Recent analysis by the same group using (GATA)$_4$ probes have

581 proved to be more efficient in the discrimination between and with accessions, though even then

582 a similar cluster analysis compared to AFLPs was obtained at least in the case of 'De la Pera'

583 landrace (García-Martínez et al., 2013).

584 The lack of relation between molecular and morpho-agronomical data, was somehow expected.

585 Terzopoulos & Bebeli (2008), also observed no correlation between those sets of information in

586 Greek landraces of tomato, and in the Italian Abruzzese variety collection analysed by

587 Mazzucato *et al*. (2010). The lack of correlation among geographic collection distance and

588 genetic distance can also be considered normal. As it has been suggested in traditional landraces

589 of corn in Mexico, landrace differentiation at regional or local level might be prevented by a

590 high level of seed exchange among farmers (Pressoir & Berthaud, 2003). In our opinion the

591 same would be applicable in our case considering previous collection information (Cebolla-

592 Cornejo *et al*., 2007). Although a low correlation between AFLP and quality data has been

593 found, and the absence of consistent clustering patterns, again reinforces the idea that there is no

594 clear relation between AFLP data and the phenotype nor geographic origin of the populations.

595 During the last decades several studies have confirmed that very few QTL are responsible for

596 most part of the variation in fruit size and shape (Grandillo *et al*., 1999). The loci *fw1.1*, *fw2.2*,

597 *fw3.1* and *fw4.1* affect only fruit size, the loci *fasciated* and *locule number*, affecting fruit size

22

and shape via carpel number, and the loci *ovate*, *sun* and *fs8.1* affect fruit shape (Tanksley, 2004). In order to obtain the characteristics of a certain variety a combination of alleles of these few loci would be enough. In this sense the variety Giant heirloom, that morphologically resembles some of the big size tomato analysed here, owes its big size to the combined effect of the loci *fw1.1*, *fw2.2*, *fw3.1*, *locule number* and *fasciated* (Lippman & Tanksley, 2001) and the variety Long John with long fruits resembling variety 'Pimiento', shows the combined effect of loci *ovate* and *sun* (van der Knaap *et al.*, 2002).

Therefore, it seems that the few exclusive traits defining a traditional variety might be determined by a few genes and therefore most part of the genome might be common for most varieties. Genetic differences between accessions might be the results of spurious variation and would not affect morphological or quality traits. Consequently, when applying molecular characterization, for example to identify duplicates in seedbanks, a high level of probability of including spurious information should be taken into account. The morphological, agronomical and quality characterization should be prioritized in this case in the management of tomato germplasm.

Other practical considerations rise as a result of the structure of traditional populations. For example the degree of variation present in landraces, or simply the existence of different morphotypes in a landrace as in the case of 'Valenciano', is almost incompatible with the degree of variation allowed in the technical examinations carried out for the registration of a material as a conservation variety under the European regulations. Similarly, when selecting accessions to be included in core collections or in special collections, such as the AEGIS (*A European Genebank Integrated System*), a special emphasis should be made on phenotypic characteristics over molecular data. In this sense it should also be consider that selecting only one representative population of a single landrace might exclude a significant amount of variation. Old questions might arise again, as how many populations of a single landrace should be conserved in a genebank? Our results seem to highlight that the correct answer would be as much as possible, as they might represent different variation with a possible future use. In a

625 context of climate change and increasing food demands, the main sources of food are more

626 genetically vulnerable than ever before, and it is an imperative to fully exploit the variation

627 present in traditional varieties either *per se* or as sources of variation in breeding programs. The

628 variation present in local or traditional varieties of different crops should not be neglected as it

629 will be a valuable resource to develop new cultivars whilst reducing genetic vulnerability.

633 **REFERENCES**

634 Archak, S., Karihaloo, J.L., Jain, A. 2002. RAPD markers reveal narrowing genetic base of
635 Indian tomato cultivars. Curr. Sci. 82, 1139–1143.

636 Alvarez, A.E., Van De Wiel, C.C.M., Smulders, M.J.M., Vosman, B. 2001. Use of
637 microsatellites to evaluate genetic diversity and species relationships in the genus *Lycopersicon*.
638 *TAG* Theor. App. Genet. 103, 1283-1292.

639 Bai, Y., Lindhout, P. 2007. Domestication and breeding of tomatoes, What have we gained and
640 what can we gain? Ann. Bot. 100, 1085-1094.

641 Baldwin, E.A., Scott, J.W., Einstein, M.A., Malundo, T.M.M., Carr, B.T., Shewfelt, R.L.,
642 Tandon, K.S. 1998. Relationship between sensory and instrumental analysis for tomato flavor.
643 J. Am. Soc. Hort. Sci. 123, 906-915.

644 Bredemeijer, G.M.M., Cooke, R.J., Ganal, M.W., Peeters, R., Isaac, P., Noordijk, Y., Rendell,
645 S., Jackson, J., Röder, M.S., Wendehake, K., Dijcks, M., Amelaine, M., Wickaert, V., Bertrand,
646 L., Vosman, B. 2002. Construction and testing of a microsatellite database containing more than
647 500 tomato varieties. TAG Theor. App. Genet. 105, 1019-1026.

648 Brown, A.H.D., Marshall, D.R. A. 2000. Basic sampling strategy: theory and practice, in:
649 Guarino, L., Rao, V.R., Reid, R. (Eds.) Collecting plant genetic diversity. CAB International,
650 Wallingford, pp. 75-91

651 Buntjer, J.B. 2001. PhylTools; Phylogenetic Computer Tools V.1.32. Wageningen University
652 and Research Centre, The Netherlands.
653 http://www.plantbreeding.wur.nl/UK/software_PhylTools.html. Accessed June 2011.

654 Casals, J., Pascual, L., Cañizares, J., Cebolla-Cornejo, J., Casañas, F., Nuez, F. 2011a. Genetic
655 basis of long shelf life and variability into Penjar tomato. Genet. Res. Crop. Evol. 59, 219-229.

656 Casals, J., Pascual, L., Cañizares, J., Cebolla-Cornejo, J., Casañas, F., Nuez, F. 2011b. The risks
657 of success in quality vegetable markets, Possible genetic erosion in Marmande tomatoes
658 (*Solanum lycopersicum* L.) and consumer dissatisfaction. Sci. Hort. 130, 78-84.

659   Cebolla-Cornejo, J., Soler, S., Nuez, F. 2007. Genetic erosion of traditional varieties of
660   vegetable crops in Europe, tomato cultivation in Valencia (Spain) as a case Study. Int. J. Plant
661   Prod. 1, 113-128.

662   Cebolla-Cornejo, J., Roselló, S., Valcárcel, M., Serrano, E., Beltrán, J., Nuez, F. 2011.
663   Evaluation of Genotype and Environment Effects on Taste and Aroma Flavor Components of
664   Spanish Fresh Tomato Varieties. J. Agric. Food Chem. 59, 2440-2450.

665   Dondarini, R. 2010. Storia e arte, Aspetti storici, in: Angelini, R. (Ed.) Il pomodoro. Art Servizi
666   Editoriali, Bologna, pp. 19-45.

667   Doyle, J.J., Doyle, J.L. 1990. Isolation of plants DNA from fresh tissue. Focus 12, 13-15.

668   FAO, Food And Agriculture Organization Of The United Nations. 2002. El Cultivo protegido
669   en clima mediterráneo. Estudio FAO producción y protección vegetal, 90. FAO, Rome.

670   Felsenstein, J. 1989. PHYLIP - Phylogeny Inference Package (Version 3.2). Cladistics 5, 164-
671   166.

672   Fulton, T.M., Bucheli, P., Voirol, E., López, J., Pétiard, V., Tanksley, S.D. 2002. Quantitative
673   trait loci (QTL) affecting sugars, organic acids and other biochemical properties possibly
674   contributing to flavor, identified in four advanced backcross populations of tomato. Euphytica
675   127, 163-177.

676   Garcia-Martinez, S., Andreani, L., Garcia-Gusano, M., Geuna, F., Ruiz, J.J. 2006. Evaluation of
677   amplified fragment length polymorphism and simple sequence repeats for tomato germplasm
678   fingerprinting, utility for grouping closely related traditional cultivars. Genome 49, 648-656.

679   Garcia-Martinez, S., Corrado, G., Ruiz, J.J., Rao, R. 2013. Diversity and structure of a sample
680   of traditional Italian and Spanish tomato accessions. Genet. Resour. Crop Evol. 60, 789-798.

681   Grandillo, S., Ku, H.M., Tanksley, S.D. 1999. Identifying the loci responsible for natural
682   variation in fruit size and shape in tomato. TAG Theor. App. Genet. 99, 978-987.

683   Harlan, J.R. 1975. Our vanishing genetic resources. Science 188, 618–621.

684   Hawkes, J.G., Maxted, N., Ford-Lloyd, B.V. 2000. The ex situ conservation of plant genetic
685   resources. Kluwer Academic Publishers, Dordrecht.

686   He, C., Poysa, V., Yu, K. 2003. Development and characterization of simple sequence repeat
687   (SSR) markers and their use in determining relationships among *Lycopersicon esculentum*
688   cultivars. TAG Theor. App. Genet. 106, 363-373.

689   Hector, A., Von Felten, S., Schmid, B. 2010. Analysis of variance with unbalanced data, an
690   update for ecology & evolution. J. Anim. Ecol. 79, 308-316.

691   IPGRI, International Plant Genetic Resources Institute. 1997. Descriptors for tomato
692   (*Lycopersicon* spp.). IPGRI (Bioversity Int.), Rome.

693   Kiers, H.A.L. 1989. Three-way methods for the analysis of qualitative and quantitative two-way
694   data. DWSO Press, Leiden.

695   Krzanowski, W.J. 2000. Principles of multivariate analysis, a user's perspective. Oxford
696   University Press, Oxford.

697   Louette, D. 2000. Traditional management of seed and genetic diversity: what is a landrace? in:
698   Brush, S.B. (Ed.) Genes in the field: on-farm conservation of crop diversity. Lewis Publishers,
699   Florida, pp. 109-142.

700   Lippman, Z., Tanksley, S.D. 2001. Dissecting the Genetic Pathway to Extreme Fruit Size in
701   Tomato Using a Cross Between the Small-Fruited Wild Species *Lycopersicon pimpinellifolium*
702   and *L. esculentum* var. Giant Heirloom. Genetics 158, 413-422.

703   Maxted, N., Ford-Lloyd, B.V., Hawkes, J.G. 1997. Complementary conservation strategies, in:
704   Maxted, N., Ford-Lloyd, B.V., Hawkes, J.G. (Eds), Plant genetic conservation: the in situ
705   approach. Chapman and Hall, London, pp. 5-39.

706   Mazzucato, A., Ficcadenti, N., Caioni, M., Mosconi, P., Piccinini, E., Reddy Sanampudi, V.R.,
707   Sestili, S., Ferrari, V. 2010. Genetic diversity and distinctiveness in tomato (*Solanum
708   lycopersicum* L.) landraces, The Italian case study of 'A pera Abruzzese'. Sci. Hortic. 125, 55-
709   62.

710   Meerburg, B.G., Korevaar, H., Haubenhofer, D.K., Blom-Zandstra, M., Van Keulen, H. 2009.
711   The changing role of agriculture in Dutch society. J. Agric. Sci. 147, 511.

712   Negri, V., Maxted, N., Veteläinen, M. 2009. European landrace conservation: an introduction,
713   in: Veteläinen, M., Negri, V., Maxted, N. (Eds.). European landraces: on-farm conservation
714   management and use, Biodiversity Technical Bulletin 15. Bioversity International, Macaresse,
715   pp. 1-22.

716   Park, Y.H., West, M.A.L., St. Clair, D.A. 2004. Evaluation of AFLPs for germplasm
717   fingerprinting and assessment of genetic diversity in cultivars of tomato (*Lycopersicon
718   esculentum* L.). Genome 47, 510-518.

719   Pressoir, G., Berthaud, J. 2003. Patterns of population structure in maize landraces from the
720   Central Valleys of Oaxaca in Mexico. Heredity 92, 88-94.

721   Rick, C.M. 1958. The role of natural hybridization in the derivation of cultivated tomatoes of
722   western South America. Econ. Bot. 12, 346–367.

723   Rick, C.M., Fobes, J.F. 1975. Allozyme Variation in the Cultivated Tomato and Closely Related
724   Species. Bull. Torrey Bot. Club 102, 376-384.

725   Roselló, S., Galiana-Balaguer, L., Herrero-Martínez, J.M., Maquieira, A., Nuez, F. 2002.
726   Simultaneous quantification of the main organic acids and carbohydrates involved in tomato
727   flavour using capillary zone electrophoresis. J. Sci. Food Agric. 82, 1101-1106.

728   Smouse, P.E., Peakall, R. 1999. Spatial autocorrelation analysis of individual multiallele and
729   multilocus genetic structure. Heredity 82, 561-573.

730   Stevens, M.A. 1972. Relationships between components contributing to quality variation
731   among tomato lines. J Am Soc Hortic Sci 97, 70-73.

732   Tanksley, S.D. 2004. The Genetic, Developmental, and Molecular Bases of Fruit Size and
733   Shape Variation in Tomato. Plant Cell Online 16, S181-S189.

734   Terzopoulos, P.J., Bebeli, P.J. 2008. DNA and morphological diversity of selected Greek
735   tomato (*Solanum lycopersicum* L.) landraces. Sci. Hortic. 116, 354-361.

736   Terzopoulos, P.J., Walters, S.A., Bebeli, P.J. 2009. Evaluation of Greek Tomato Landrace
737   Populations for Heterogeneity of Horticultural Traits. Eur. J. Hortic. Sci. 74, 24–29,

738   Terzopoulos, P.J., Bebeli, P.J. 2010. Phenotypic diversity in Greek tomato (*Solanum
739   lycopersicum* L.) landraces. Sci. Hortic. 126, 138-144.

740   Van Der Knaap, E., Lippman, Z.B., Tanksley, S.D. 2002. Extremely elongated tomato fruit
741   controlled by four quantitative trait loci with epistatic interactions. TAG Theor. App. Genet.
742   104, 241-247.

743   Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van De Lee, T., Hornes, M., Frijters, A., Pot, J.,
744   Pelman, J., Kuiper, M., Zabeau, M. 1995. AFLP, A new technique for DNA fingerprinting.
745   Nucleic Acids Res. 23, 4407-4414.

746   Villand, J. Skroch, P.W., Lai, T., Hanson, P., Kuo, C.G., Nienhuis, J. 1998. Genetic Variation
747   among Tomato Accessions from Primary and Secondary Centers of Diversity. Crop Sci. 38,
748   1339-1347.

749   Williams, C.E., St. Clair, D.A. 1993. Phenetic relationships and levels of variability detected by
750   restriction fragment length polymorphism and random amplified polymorphic DNA analysis of
751   cultivated and wild accessions of *Lycopersicon esculentum*. Genome 36, 619-630.

752   Zeven, A.C. 1998. Landraces, a review of definitions and classifications. Euphytica 104, 117-
753   139.

754   Zeven, A.C. 1999. The traditional inexplicable replacement of seed and seed ware of landraces
755   and cultivars, A review. Euphytica 110, 181-191.

756

Table 1. Origin and description of the populations analysed.

| | | | Origin | | Assays | | | | |
Accession code	Local name	Basic description	Town	Province	Weight&Yield	Morpho-agronomical	Quality	DNA	Figure code
BGV5654	`Valenciano`	Heart shape	Cullera	Valencia	x				
BGV5524	`Valenciano`	Heart shape	Segorbe	Castellón	x				
BGV5421	`Valenciano`	Heart shape	Siete Aguas	Valencia	x				
BGV5530	`Valenciano`	Heart shape	Liria	Valencia	x				
BGV5422	`Valenciano`	Heart shape	Siete Aguas	Valencia	x				
BGV5561	`Valenciano`	Heart shape	Casas Altas	Valencia	x				
BGV5577B	`Valenciano`	Heart shape	Alboraya	Valencia	x				
BGV5587	`Valenciano`	Heart shape	Canyada	Alicante	x				
BGV5594	`Valenciano`	Heart shape	Villena	Alicante	x				
BGV5595	`Valenciano`	Heart shape	Villena	Alicante	x				
BGV5616	`Valenciano`	Heart shape	Turís	Valencia	x				
BGV5642	`Valenciano`	Heart shape	Valencia	Valencia	x				
BGV5653	`Valenciano`	Heart shape	Foios	Valencia	x				
BGV5656	`Valenciano`	Heart shape	Monacada	Valencia	x				
BGV5437	`Valenciano`	Heart shape	Algar	Valencia	x				
BGV5412	`Valenciano`	Heart shape	La Punta	Valencia	x				
BGV5458	`Valenciano`	Heart shape	Picassent	Valencia	x				
BGV14992	`Valenciano`	Heart shape	Chelva	Valencia	x				
BGV5688	`Valenciano`	Heart shape	Alboraya	Valencia		x	x		1.1
BGVJ323	`Valenciano`	Heart shape	Alboraya	Valencia		x	x		1.2
BGVJ324	`Valenciano`	Heart shape	El Puig	Valencia		x	x	x	1.3
BGV5520	`Beninova`	Heart shape	Valencia	Valencia		x		x	1.4
BGV5530	`Valenciano`	Heart shape	Líria	Valencia		x	x		1.5
BGV5577A	`Valenciano`	Heart shape	Alboraya	Valencia	x	x	x		1.6
BGV5652	`Valenciano`	Heart shape	El Perelló	Valencia	x	x		x	1.7
BGV5655	`Valenciano`	Heart shape	Vinalesa	Valencia	x	x		x	1.8
BGV5657	`Valenciano`	Heart shape	Moncada	Valencia	x	x	x	x	1.9
BGV5670	`Valenciano`	Heart shape	Paterna	Valencia		x	x		1.10
BGV5673	`Valenciano`	Heart shape	L'Alcudia	Valencia		x		x	1.11
BGVJ321	`Valenciano`	Heart shape	Turís	Valencia		x			1.12
BGVJ322	`Valenciano`	Heart shape	Turís	Valencia		x			1.13
BGV5716	`Muchamiel`	Flat, strong ribbing	Novelda	Alicante		x		x	2.1
BGV1027	`Muchamiel`	Flat, strong ribbing	Laujar de Andarax	Almería	x				
BGV978	`Muchamiel`	Flat, strong ribbing	Alhama	Granada	x				
BGV1569	`Muchamiel`	Flat, strong ribbing	Porreres	Baleares	x				
BGV3877	`Muchamiel`	Flat, strong ribbing	La Gineta	Albacete	x				
BGV3912	`Muchamiel`	Flat, strong ribbing	San Clemente	Cuenca	x				
BGV4397A	`Muchamiel`	Flat, strong ribbing	Lorca	Murcia	x				
BGV4397B	`Muchamiel`	Flat, strong ribbing	Lorca	Murcia	x				
BGV5650	`Muchamiel`	Flat, strong ribbing	Alboraya	Valencia	x				
BGV5648	`Muchamiel`	Flat, strong ribbing	San Juan	Alicante	x				
BGV5709	`Muchamiel`	Flat, strong ribbing	Torrellano	Alicante		x	x		2.2
BGV5711	`Muchamiel`	Flat, strong ribbing	Muchamiel	Alicante		x	x		2.3
BGV5713	`Anaranjado`	Flat, strong ribbing	Orihuela	Alicante		x	x	x	2.4
BGVJ325	`Muchamiel`	Flat, strong ribbing	Orihuela	Alicante		x	x	x	2.5
BGVJ326	`Muchamiel`	Flat, strong ribbing	Orihuela	Alicante		x	x	x	2.6
BGV4407	`Muchamiel`	Flat, strong ribbing	Lorca	Murcia	x	x	x	x	2.7
BGV5554	`Muchamiel`	Flat, strong ribbing	Campello	Alicante		x	x	x	2.8
BGV5622	`Muchamiel`	Flat, strong ribbing	Muchamiel	Alicante		x		x	2.9
BGV5626	`Muchamiel`	Flat, strong ribbing	Muchamiel	Alicante		x	x		2.10
BGV5627	`Muchamiel`	Flat, strong ribbing	Muchamiel	Alicante		x	x		2.11
BGV5649	`Muchamiel`	Flat, strong ribbing	San Juan	Alicante	x	x	x		2.12
BGV5651	`Muchamiel`	Flat, strong ribbing	San Juan	Alicante	x	x	x	x	2.13
BGV5659	`Pimiento`	Long shape	Moncada	Valencia	x				
BGV5586	`Pimiento`	Long shape	Yátova	Valencia		x	x		3.1
BGV5591	`Pimiento`	Long shape	Cañada	Alicante		x	x		3.2
BGV5658	`Pimiento`	Long shape	Catarroja	Valencia	x	x	x	x	3.3
BGV5461	`Pimiento`	Long shape	Culla	Castellón		x	x		3.4
BGV5478	`Pimiento`	Long shape	Fontanares	Valencia		x	x		3.5
BGV5661	`Penjar`	Small ovoid long cons.	Moncada	Valencia	x				
BGV5426	`Penjar`	Small long conservation	Lliber	Alicante		x	x		4.1
BGV5592	`Penjar`	Small long conservation	Cañada	Alicante		x		x	4.2
BGV5660	`Penjar`	Small round long cons.	Serra	Valencia	x	x	x		4.3
BGV5663	`Penjar`	Small ovoid long cons.	Benicarló	Castellón	x	x	x	x	4.4
BGV5413	`Penjar`	Small long conservation	Chelva	Valencia		x	x	x	4.5
BGV5460	`Penjar`	Small long conservation	Borriol	Castellón		x	x		4.6
BGV5715	`De la pera`	Indeterminate pear shape	El Saladar	Alicante		x			5.1
BGV5717	`Elche`	Indeterminate pear shape	Novelda	Alicante		x			5.2
BGV5712	`De la pera`	Indeterminate pear shape	Almoradí	Alicante		x			5.3
BGV5714	`De la pera`	Indeterminate pear shape	Orihuela	Alicante		x			5.4

Table 1. Origin and description of the populations analysed (continuation).

Accession code	Local name	Basic description	Origin		Assays				Figure code
			Town	Province	Weight&Yield	Morpho-agronomical	Quality	DNA	
BGV5547	`De pera gruesa`	Indeterminate pear shape	Crevillente	Alicante		x	x		5.5
BGV5548	`Elchero`	Rounded angular section	Elche	Alicante		x	x	x	6.1
BGV5623	`Elchero`	Rounded angular section	Muchamiel	Alicante		x	x		6.2
BGV5536	`Morado`	Big slightly flat pink	Aras del Alpuente	Valencia		x	x	x	7.1
BGV5582	`Morado`	Big slightly flat pink	Yátova	Valencia		x			7.2
BGV5459A	`Morado`	Small slightly flat pink	Albocaser	Castellón		x		x	7.3
BGV5459B	`Morado`	Small slightly flat red	Albocaser	Castellón		x			7.4
BGV5477	`Morado`	Medium size slightly flat pink	Fontanares	Valencia		x	x		7.5
BGV5708	`Aperado`	Determinate pear shape	Torrellano	Alicante		x			8.1
BGV5581	`De pruna`	Determinate pear shape	Yátova	Valencia		x	x		8.2
BGV5545	`De San Juan`	Slightly flat, slight ribbing	S. Fulgencio	Alicante		x	x		9.1
BGV5552	`De San Juan`	Slightly flat, slight ribbing	San Juan	Alicante		x	x	x	9.2
BGV5423	`Cuarenteno `	Slightly flat, slight ribbing	Aldaya	Valencia		x			10.1
BGV5416	`Cuarenteno`	Slightly flat, slight ribbing	Chelva	Valencia		x	x	x	10.2
BGV5512	`Bombillero`	small pear shaped pink	Fanzara	Castellón		x		x	11
BGV5482	`De penjar`	Very small rounded red	Onda	Castellón		x	x	x	12
BGV5429	`Petroblanco`	Red rounded	Novelda	Alicante		x	x	x	13
BGV5466	`Ademuz`	Red rounded	Ademuz.	Valencia		x	x		14
BGV5450	`De la zona`	Big flat red	Viver	Castellón		x	x	x	15
BGV5486	`Francés`	Flat ribbed pink	La Foya	Castellón		x	x	x	16
BGV5441	`Tomate`	Red rounded	Alcoleja	Alicante		x	x		17
BGV5515	`Del terreno`	Small rounded red	Argelita	Castellón		x	x	x	18
BGV5533	`Primerenco`	Small rounded	Aras del Alpuente	Valencia		x	x	x	19
BGV5551	`De Elda`	Flat, strong ribbing	Elda	Alicante		x	x	x	20
BGV5579	`Gordo`	Big slightly flat red	Buñol	Valencia		x	x	x	21
BGV5608A	`De Castellón`	Big slightly flat pink	Castalla	Alicante		x	x	x	22.1
BGV5608B	`De Castellón`	Big slightly flat red	Castalla	Alicante		x	x	x	22.2
BGV5522	`Catalana`	Small rounded red	Vinaroz	Castellón		x	x	x	23
BGV5523	`Palo de santo`	Red rounded	Vinaroz	Castellón		x	x		24
BGV5455A	`Catalán`	Small rounded red	Jérica	Castellón		x			25.1
BGV5455B	`Catalán`	Small rounded pink	Jérica	Castellón		x			25.2
BGV5550	`Del País`	Big slightly flat red	Novelda	Alicante		x			26
BGV5565	`Bombillero`	Long shape	Sta Cruz Moya	Valencia		x			27
BGV5710	`Redondo`	Red rounded	Muchamiel	Alicante		x	x		28
Royesta	Comercial hybrid	Flat slight ribbing	-	-	x	x	x		40
RDD	Breeding line	Red rounded				x		x	41
BGV12406	Breeding line	Red rounded					x		42
UPV-1	Breeding line	Red rounded				x	x	x	43
BGV7972	*S. pennellii*							x	45

Table 2. Results of the analysis of fruit weight and plant yield variation. Varieties: 1: 'Valenciano', 2: 'Muchamiel', 3: 'Penjar', 4: 'Pimiento'. (CV: coefficients of variation).

Variety	Population	Mean Fruit Weight (g)	Fruit weight intra population variation		Mean Yield (g)	Yield CV	Min. yield (g)	Max.yield (g)
			Bartlett test (p value)	Fruit weight CV				
1	BGV5421	207.9	0,06	0,30	1899.6	0.50	475.2	3074.4
1	BGV5530	228.6	0,96	0,34	2252.2	0.59	21.7	4732.6
1	BGV5422	198.1	0,51	0,28	2114.1	0.73	416.1	5263.9
1	BGV5577A	254.5	0,16	0,28	2645.9	0.34	1518.4	4144.7
1	BGV5577B	152.4	0,04	0,21	2272.0	0.49	658.6	4621.9
1	BGV5587	194.9	0.06	0.18	908.4	0.77	135.9	2051.4
1	BGV5594	266.7	0,01	0,26	1358.8	0.92	154.7	3486.9
1	BGV5595	113.7	0,00	0,21	2361.9	0.32	1040.5	3649.2
1	BGV5616	201.2	0,17	0,19	1323.8	0.61	335.8	2675.1
1	BGV5642	233.8	0,03	0,18	656.8	0.57	247.7	1464.2
1	BGV5652	289.6	0,05	0,29	2908.8	0.28	1855.6	4451
1	BGV5653	257.2	0.03	0,31	987.3	1.35	258.5	2982.6
1	BGV5654	198.4	0.02	0,07	563.3	0.08	531.4	595.2
1	BGV5655	208.2	0,33	0,22	2126.9	0.46	930.2	3122.3
1	BGV5656	302.9	0,18	0,08	1211.4	0.41	861.2	1561.6
1	BGV5657	200.7	0,02	0,34	1753.3	0.50	798.1	3832.8
1	BGV5437	184.8	0,00	0,24	1336.0	0.72	412	3642.7
1	BGV5412	266.2	0,20	0,27	1769.2	0.57	269.4	3403.1
1	BGV5458	240.0	0,01	0,26	1798.3	0.57	605.6	3183.8
1	BGV14992	211.5	0,02	0,33	1979.0	0.38	903.2	3038.8
2	BGV1027	286.7	0,02	0,28	4820.0	0.35	2998.3	8621.9
2	BGV978	356.4	0,12	0,26	2460.1	0.38	1559.6	4249.8
2	BGV1569	233.0	0,14	0,30	3843.3	0.54	1637.3	8713
2	BGV3877	253.6	0,00	0,37	3847.8	0.64	2354.5	6710.7
2	BGV3912	202.9	0,00	0,29	3526.8	0.51	922	6012.1
2	BGV4397A	268.8	0,15	0,31	3978.4	0.48	1314	6847.2
2	BGV4397B	260.3	0,37	0,26	3572.6	0.44	907.1	6105.9
2	BGV4407	237.5	0,00	0,29	4491.5	0.45	1998.8	7417.8
2	BGV5524	198.6	0,01	0,28	3168.4	0.27	1419.1	4159.4
2	BGV5561	233.2	0,02	0,22	1776.9	0.96	446	4413.8
2	BGV5648	223.5	0,10	0,23	2393.6	0.51	245.6	4741.1
2	BGV5649	272.3	0,01	0,28	4882.3	0.60	1437.5	9044.2
2	BGV5650	251.8	0,14	0,23	2876.1	0.35	1777.1	4392
2	BGV5651	254.1	0,34	0,18	1473.9	0.49	713.3	2971.7
3	BGV5658	217.2	0,17	0,26	2710.4	0.82	305.7	6498.2
3	BGV5659	183.4	0,02	0,25	2367.1	0.51	394.7	4511.1
4	BGV5660	145.7	0,50	0,28	1484.2	0.58	384.8	2648.8
4	BGV5661	131.4	0,14	0,31	2506.9	0.36	1287.3	3940.9
4	BGV5663	127.9	0,08	0,26	2432.2	0.39	1271.2	4061
40	ROYESTA	189.6	0,02	0,18	5570.0	0.16	3964.8	7221.2

Table 3. Transformed loadings obtained in the canonical correspondence analysis (5 variates selected) for each initial set of variables (morpho-agronomical and quality). Only loadings contributing more than 20% of global loading sum are shown.

Canonical variate	1	2	3	4	5
Correlation	0.178	0.166	0.158	0.146	0.135

Canonical variate	1	2	3	4	5
			Loadings		
Weight			0.05		0.16
Length			0.17		
Width	0.24				
L/W ratio	0.70	0.13		0.63	
Width of pedicel scar			0.00	0.09	
Size of corky area in pedicel scar			0.02		
thickness of pericarp		0.04	0.05		
Fruit section length		0.17		0.22	
size of core		0.17	-0.08	0.17	
Mean number of locules	0.17		0.15		0.18
Maximum number of locules			-0.07		
Size of hollow area between pericarp and core			0.14		
Size of fibrous area associated to ped. scar	0.43		0.07		0.46
Maximum fruit firmness		0.04			
Minimum fruit firmness		0.09	0.08		
Green shoulder intensity			-0.06		
Fruit size homogeneity		0.03	-0.02		
Intensity of ripe external fruit colour		0.05	-0.01		
Sensorial fruit firmness		0.03	-0.06		
Fruit ribbing		0.12			
Radial cracking			0.05		
Concentric cracking			-0.08		
Seed yield			-0.05		
Mean plant yield		0.03	-0.06	0.16	
Maximum plant yield			-0.05		
Minimum plant yield			0.13		

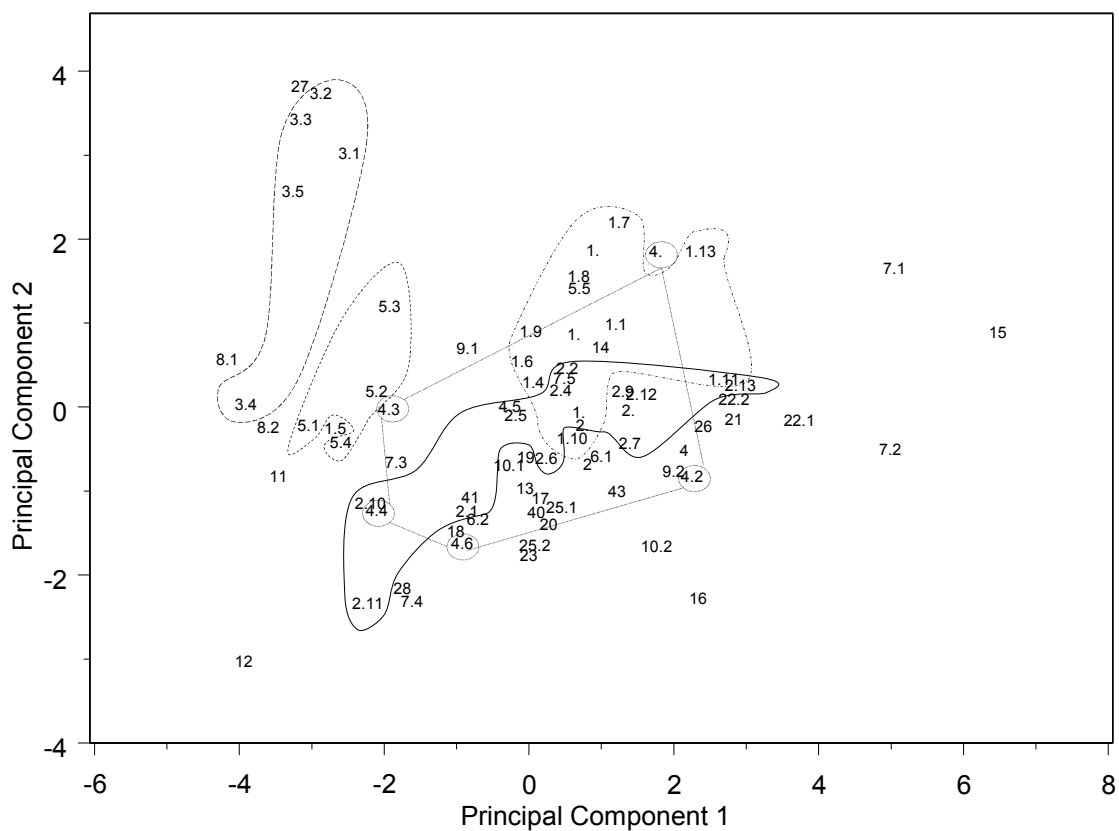| Canonical variate	1	2	3	4	5
Malic acid		0.03	-0.01		0.13
Citric acid		0.03	-0.04	0.04	0.06
Fructose	0.18				0.07
Glucose			0.08		
Titratable acidity	0.08	0.05			
SSC (g/100g sucrose)		0.00		0.04	0.16
pH		-0.01			

Fig. 1. Representation of the populations of traditional varieties in the first (0.366 of variance) and second (0.146 of variance) principal components obtained in the analysis of the morpho-agronomical variables. The first figure indicates variety or varietal type: 1: 'Valenciano', 2: 'Muchamiel', 3: 'Forma pimiento', 4: 'De penjar', 5: 'De la pera', 6: 'Elchero', 7: 'Morada', 8: 'De pera', 9: 'De San Juan', 10: 'Cuarenteno', 11-28: other types, 40-43: Controls. See accession codes in table 1. Lines identify the populations belonging the landraces 1 to 5.
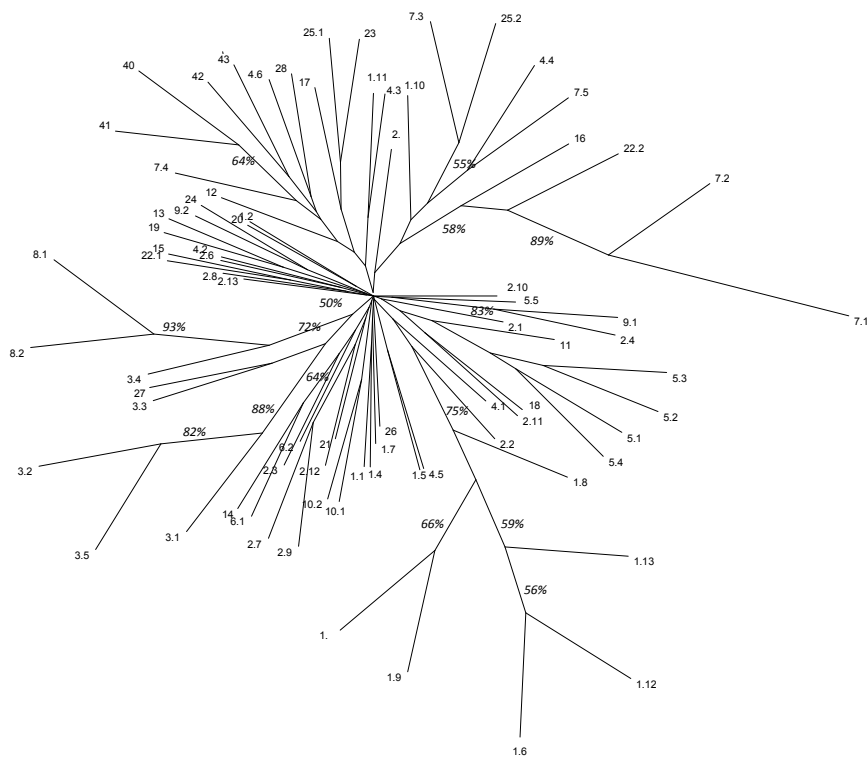
Fig. 2. Dendrogram obtained in the cluster analysis using the morpho-agronomical (quantitative and qualitative) variables. The first figure indicates variety or varietal type: 1: 'Valenciano', 2: 'Muchamiel', 3: 'Forma pimiento', 4: 'De penjar', 5: 'De la pera', 6: 'Elchero', 7: 'Morada', 8: 'De pera', 9: 'De San Juan', 10: 'Cuarenteno', 11-28: other types, 40-43: Controls. Percentages (only >50% shown) indicate the stability of nodes in the bootstrap analysis (1000 repetitions, 30% substitution). See accession codes in table 1.
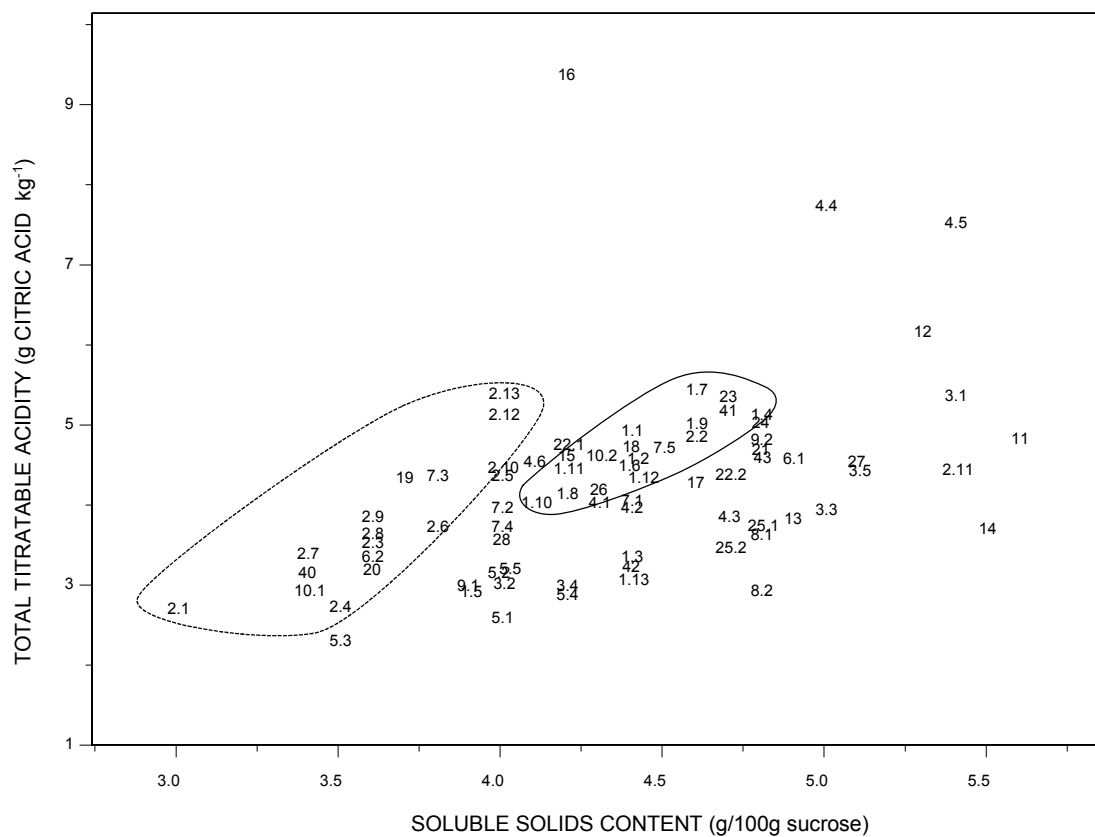
Fig. 3. Basic quality parameters. The lines delimit general patterns in the varieties 'Valenciano' (continuous line) and 'Muchamiel' (dotted line). The first figure indicates variety or varietal type: 1: 'Valenciano', 2: 'Muchamiel', 3: 'Forma pimiento', 4: 'De penjar', 5: 'De la pera', 6: 'Elchero', 7: 'Morada', 8: 'De pera', 9: 'De San Juan', 10: 'Cuarenteno', 11-28: other types, 40-43: Controls. See accessions codes in table 1.
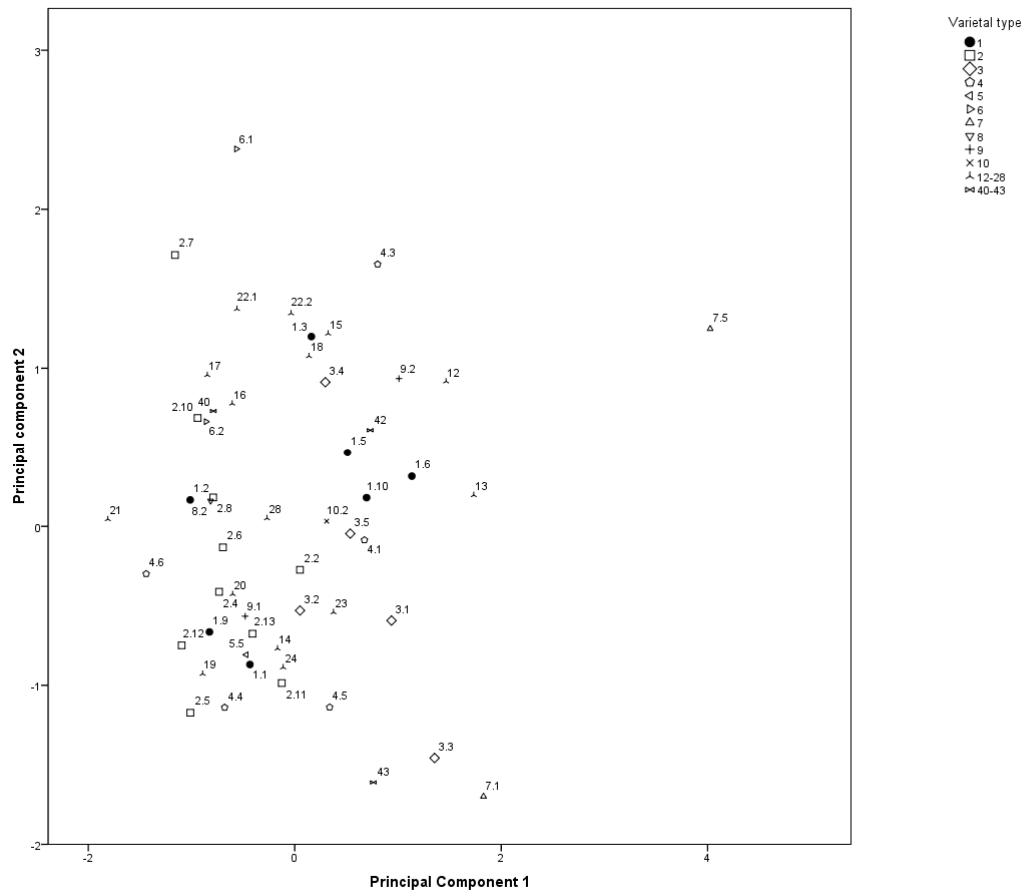
Fig. 4. Representation of the populations of traditional varieties in the first (0.333 of variation) and second (0.248 of variation) principal components obtained in the PCA of basic parameters and the content in individual sugars and organic acids related to organoleptic quality. The first figure indicates variety or varietal type: 1: 'Valenciano', 2: 'Muchamiel', 3: 'Forma pimiento', 4: 'De penjar', 5: 'De la pera', 6: 'Elchero', 7: 'Morada', 8: 'De pera', 9: 'De San Juan', 10: 'Cuarenteno', 11-28: other types, 40-43: Controls. See accession codes in table 1.
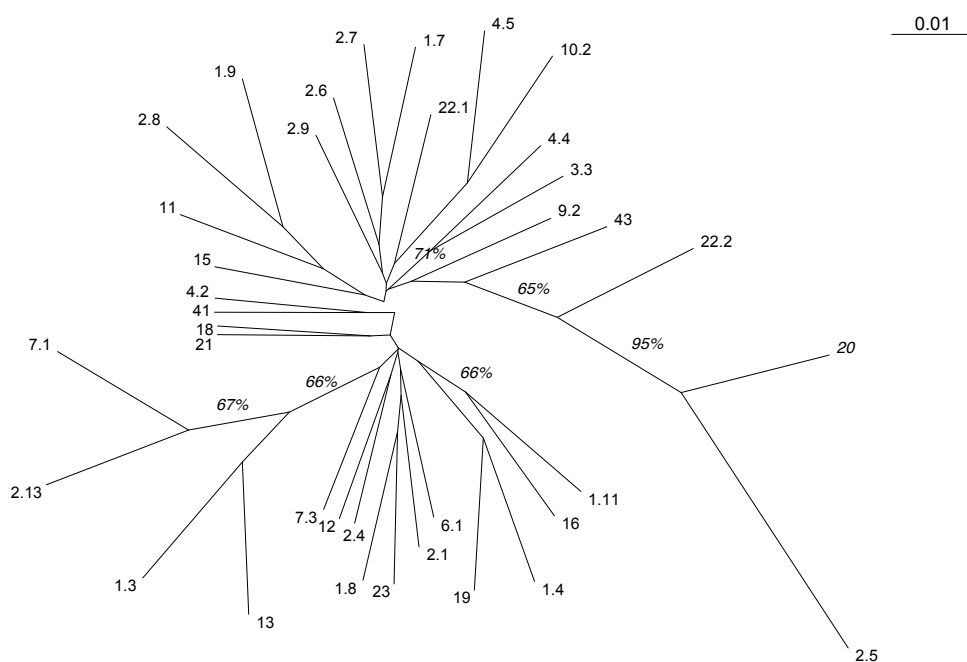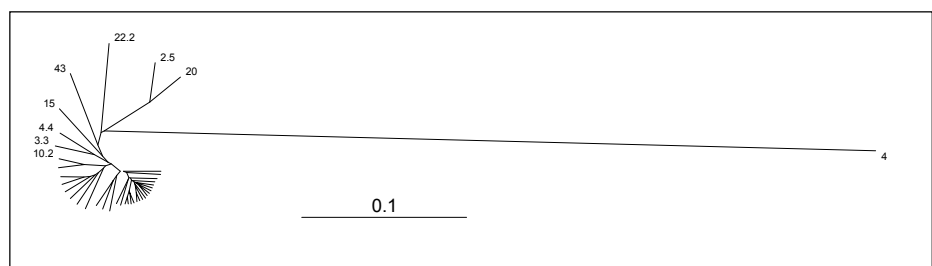
Fig. 5. Dendrograms obtained from the cluster analysis of AFLP data using Nei's distance, bootstrapping and UPGMA. Upper diagram represents the results including the outgroup control from *Solanum pennellii* Correll. The first figure indicates variety or varietal type: 1: 'Valenciano', 2: 'Muchamiel', 3: 'Forma pimiento', 4: 'De penjar', 6: 'Elchero', 7: 'Morada', 9: 'De San Juan', 10: 'Cuarenteno', 11-28: other types, 40-43: Controls. Percentages (only >50% shown) indicate the stability of nodes in the bootstrap analysis (1000 repetitions, 30% substitution). See accession codes in table 1.
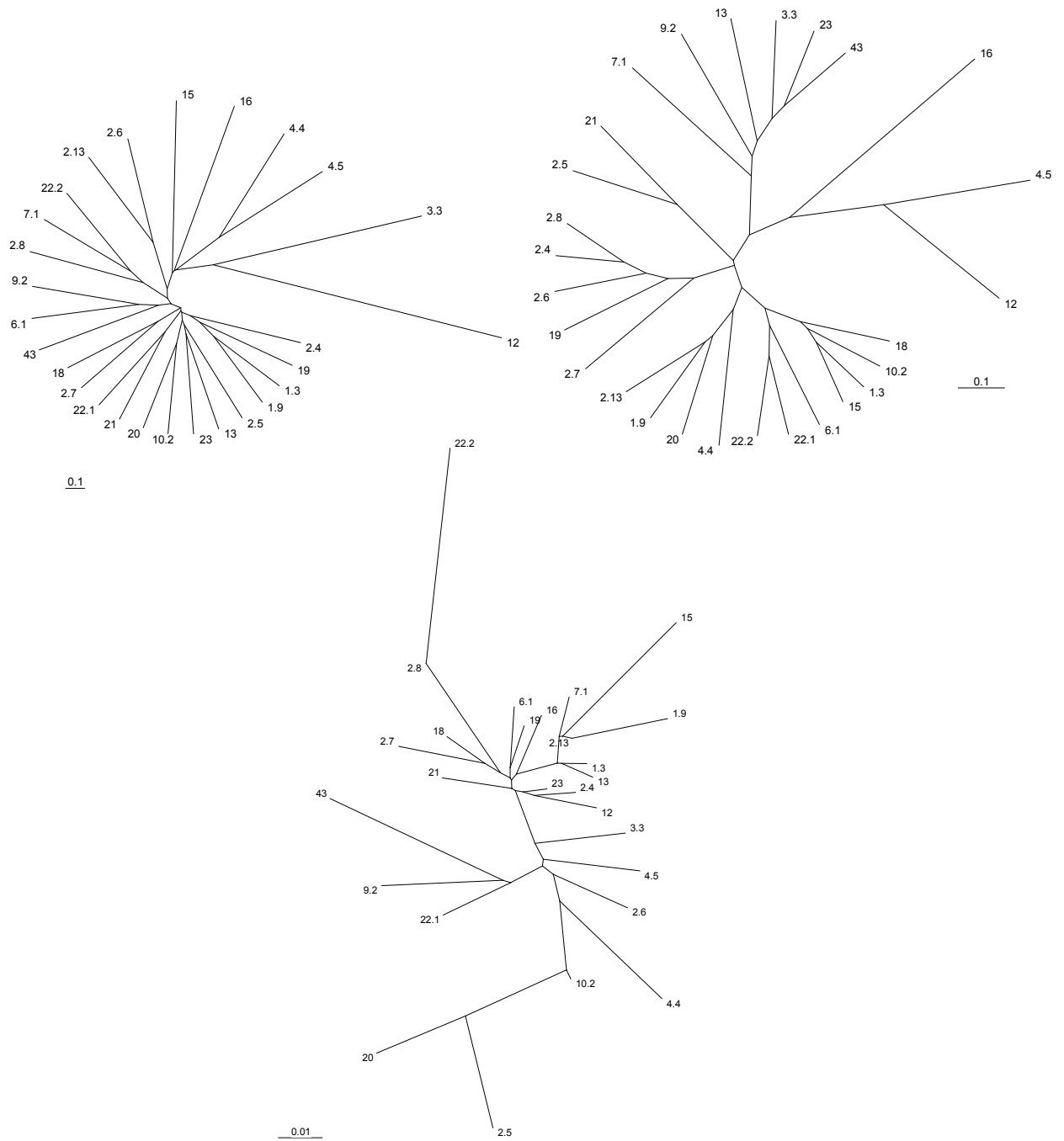
Fig. 6. Cluster analysis of the distance matrices obtained for standardized morpho-agronomical data (Euclidean distance, upper left corner), standardized quality data (Euclidean distance, upper right corner) and AFLP marker data (Nei's coefficient, center).