Phenotypic and genetic diversity of Spanish tomato landraces.

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ABSTRACT

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

KEYWORDS

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

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

markets, as the cultivation of these materials could represent a 'true pearl' as defined by Meerburg *et al*. (2009): the one that satisfies societal demands while providing a reasonable income to the farmer.

In this context, this work analyses agronomical and morphological traits, chemical composition related to organoleptic quality and DNA variation in a wide collection of Spanish landraces, including a wide representation of farmers' selections or populations of several key landraces. To which point are farmers' selections of the same landrace similar? Are different landraces really differentiable? Is this variation clearly structure is separated groups? Landraces are usually distinguished morphologically, but do they have a clear chemical profile defining a characteristic taste? Several authors have analysed a discrete set of Spanish landraces using DNA markers, but are the results consistent when a wide collection of landraces and farmer's selections are considered? These are the questions that this work tries to answer..

MATERIALS AND METHODS

A collection of several accessions or populations of different traditional varieties was analysed considering different traits: morpho-agronomical traits, quality-related traits and DNA. The variation in fruit weight and yield (accumulated fruit weights) variation was analysed in depth considering the importance of these traits. The number of accessions evaluated was reduced for plant yield, quality and DNA variation, considering the costs of each characterization. In each case, the populations were selected depending on the socio-economic importance of each variety.

Analysis of morpho-agronomical variation.

For the analysis of morpho-agronomical variation 75 populations of 29 landraces were included (Table 1). Although several varieties were included in this study, it was centred in the analysis 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: $(x_i\text{-min}(x))/((\max(x)\text{-min}(x))$. 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), ….(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.

For the distance matrix correlation analysis, following the methodology already described in previous sections, new distance matrices and cluster dendrograms were calculated for morpho-agronomical (Euclidean distance, UPGMA), quality (Euclidean distance, UPGMA) and AFLP data (Nei distance, UPGMA) considering only the 27 populations used in the three analysis. The cophenetic coefficients and correlations between pairs of distance matrices were calculated using NTSYSpc v.2.02 software package (Applied Biostatistics Inc., Setautek, NY, EE.UU.) and for the estimation of the significance of the correlations, Mantel tests with 1000 permutations were performed.

In order to further analyze the possible correlation between AFLP marker data and geographical distance between collection sites, a spatial autocorrelation analysis was performed (Smouse & Peakall, 1999).

Access to data generated in this work.

Raw data for main quantitative descriptors and data related to organoleptic quality is provided in supplementary tables 1 and 2. The rest of the data can be consulted in the COMAV seedbank.

RESULTS

Analysis of morpho-agronomical variation.

A principal component analysis (PCA) was performed with the whole set of variables in order to obtain a general overview of the structure of variation within and between traditional varieties. The first two components explained 0.332 of the variation, a low value probably due to the high number of variables considered and the presence of qualitative traits. In order to increase the percentage of variation explained by the analysis, the number of variables was reduced trying to maximize the variance explained by the model. In the new PCA the first two principal components now explained 0.366 and 0.146 of the variation respectively, and were selected for the interpretation of the results. The first component was mainly related to traits regarding fruit size and the second with traits related to fruit shape. The graphical representation of the PCA showed a broad dispersion of the populations. Despite the high number of varieties and populations analysed, the populations of 'Valenciano', 'Muchamiel', 'Pimiento', 'Penjar'

303 and ‘De la pera’ were grouped together in a higher or lower degree (Fig. 1). Nevertheless, it was
304 possible to identify some populations placed outside the main area of distribution of each
305 variety. For example, this was the case of the populations BGV5709 (Fig. code 2.2) of
306 ‘Muchamiel’ and BGV5461 (Fig. code 3.4) of ‘Pimiento’. In these cases though, there was no
307 reason to discard these populations as errors of varietal adscription, once the characterization
308 and passport data were individually reviewed. Nevertheless, especially in the overrepresented
309 varieties such as ‘Valenciano’ or ‘Muchamiel’ the gradient of variation was quite wide and their
310 area of distribution overlapped with other varieties.
311 A more precise view of the wide level of variation present among the populations of each
312 variety was observed in the cluster analysis (Fig. 2), where all the morpho-agronomical
313 variables were included. A high cophenetic coefficient (0.86) was obtained (Mantel test p=0.02
314 with 100 permutations) but low bootstrap values were obtained in most nodes, indicating a lack
315 of robustness of the clustering. In fact, the populations of the same variety appeared in different
316 nodes in several cases.
317 *Analysis of fruit weight and plant yield variation*.
318 In order to examine in detail the variation in agronomical key traits fruit weight and plant yield
319 were selected from the pool of morpho-agronomic variables. A wide range of mean fruit weight
320 could be observed, especially in the varieties ‘Valenciano’ and ‘Muchamiel’, both having a high
321 number of populations represented. In the case of ‘Valenciano’, it ranged from populations with
322 small fruits of 113.7g to populations with big fruits of 302.9g (Table 2). In this variety, the most
323 stable characteristic was the heart shape of its fruits, which was identifiable in all the
324 populations. Nevertheless, a certain level of variation in the width to length ratio could be
325 detected. Something similar happened in ‘Muchamiel’. In this case fruit weight ranged from
326 populations with a mean of 198.6g to populations with 356.4g. In this case, all the populations
327 showed flat and heavily ribbed fruits in variable degrees. In the rest of varieties the number of
328 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

observed, and 0.258 were polymorphic. The mean frequency of band presence was 0.592, though the real distribution was biased towards very frequent or very infrequent alleles.

Among the populations belonging to the variety 'Valenciano' the level of detected polymorphism was 0.092. In the case of 'Muchamiel' a higher level, 0.18, was detected. In both cases, 195 bands were observed. The mean genetic diversity was 0.23 for 'Valenciano' populations, 0.08 for 'Muchamiel' populations and 0.14 for the whole set of accessions analysed. The mean genetic distance using Nei's coefficient was 0.062±0.001 though the pair grouped distances were distributed asymmetrically with a preponderance of low coefficients.

In a first cluster analysis using Nei's index, the outgroup of *S. pennellii* was clearly differentiated from *S. lycopersicum* populations (Fig. 5). Once checked the validity of the analysis, the outgroup was removed to analyse the diversity in the cultivated species. A high cophenetic coefficient of 0.98 (Mantel test p=0.99, 100 permutations) was obtained in the cluster analysis using Nei's index. Nevertheless, the bootstrap analysis showed that the nodes obtained were not stable, as most of them obtained frequencies lower than 0.50. The same analysis using the Jaccard index showed a similar topology (data not showed). As it happened in the analysis of morpho-agronomical variables, the distribution of the populations of each variety was nearly random, as they appeared mixed in different nodes.

Correlation analysis between sets of variables.

The first five canonical variates (CaV) obtained in the analysis were selected, representing a cumulated correlation of 0.783 (Table 3). For the first CaV, length to width ratio and fibrous area associated to pedicel scar and fructose and titratable acidity showed the highest loadings respectively in each set of variables, meaning that these variables bear a higher level of association between them. For the second CaV, fruit length to width ratio, fruit section length, fruit ribbing and size of core and citric, malic and titratable acidity showed the highest loadings. For the third CaV, the highest loadings were obtained with fruit length, mean number of locules, size of hollow area between pericarp and core and minimum plant yield and citric acid, glucose and SSC (ºBrix). The variables with the highest loadings with the fourth CaV were L/W ratio,

410 fruit section length and size of core and SSC. For the fifth CaV the highest loadings were
411 obtained with the size of fibrous area associated to pedicel scar and malic acid. From this
412 analysis, it seems then that variables related to fruit shape and structure, usually linked to
413 variety recognition, bear some level of association with quality parameters. This may lead to the
414 general trends in quality parameters associated to certain varieties observed in the study.
415 In order to obtain a different perspective of the relations between the different data sets new
416 distance matrices were obtained for each standardized data set, only considering the accessions
417 with representation in the three analyses. The correlation between the distance (Euclidean)
418 matrices of the standardized morpho-agronomical data and standardized quality data was
419 significant and moderate: R=0.40 (Mantel test, p=0.002 with 1000 permutations). The
420 correlation between the distance (Euclidean) matrices of the standardized morpho-agronomical
421 data and the distance (transformed Nei's coefficient) matrix of the AFLP marker data was not
422 significant (r=0.07, Mantel test, p=0.36 with 1000 permutations). And finally, the correlation
423 between the distance matrix of the standardized quality data and the distance matrix of the
424 APLP marker data was significant (Mantel test, p=0.0.02 with 1000 permutations) but reduced
425 (r=0.25).
426 The cluster analysis of the three distance matrices (Fig. 6) showed no consistent clustering of
427 the same accessions, despite showing high cophenetic coefficients (AFLP: r=0.84; Quality:
428 r=0.71; Morpho-agronomical: r=0.87). It seems therefore that, again, although there is some
429 relation between morpho-agronomical characteristics and quality and between quality and
430 AFLP data, these relations are not consistent enough to provide a clear identification of different
431 varieties. This seems quite clear when analysing the clustering behaviour of "'Muchamiel'"
432 varieties in the dendrograms.
433 Finally, in order to analyse if there was an underlying geographic structure in the genetic
434 structure of the populations analysed, the distances between collection sites were calculated.
435 The correspondence analysis between the genetic distance (transformed Nei's coefficient) and
436 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

598 and shape via carpel number, and the loci *ovate*, *sun* and *fs8.1* affect fruit shape (Tanksley,
599 2004). In order to obtain the characteristics of a certain variety a combination of alleles of these
600 few loci would be enough. In this sense the variety Giant heirloom, that morphologically
601 resembles some of the big size tomato analysed here, owes its big size to the combined effect of
602 the loci *fw1.1*, *fw2.2*, *fw3.1*, *locule number* and *fasciated* (Lippman & Tanksley, 2001) and the
603 variety Long John with long fruits resembling variety 'Pimiento', shows the combined effect of
604 loci *ovate* and *sun* (van der Knaap *et al*., 2002).
605 Therefore, it seems that the few exclusive traits defining a traditional variety might be
606 determined by a few genes and therefore most part of the genome might be common for most
607 varieties. Genetic differences between accessions might be the results of spurious variation and
608 would not affect morphological or quality traits. Consequently, when applying molecular
609 characterization, for example to identify duplicates in seedbanks, a high level of probability of
610 including spurious information should be taken into account. The morphological, agronomical
611 and quality characterization should be prioritized in this case in the management of tomato
612 germplasm.
613 Other practical considerations rise as a result of the structure of traditional populations. For
614 example the degree of variation present in landraces, or simply the existence of different
615 morphotypes in a landrace as in the case of 'Valenciano', is almost incompatible with the degree
616 of variation allowed in the technical examinations carried out for the registration of a material as
617 a conservation variety under the European regulations. Similarly, when selecting accessions to
618 be included in core collections or in special collections, such as the AEGIS (*A European*
619 *Genebank Integrated System*), a special emphasis should be made on phenotypic characteristics
620 over molecular data. In this sense it should also be consider that selecting only one
621 representative population of a single landrace might exclude a significant amount of variation.
622 Old questions might arise again, as how many populations of a single landrace should be
623 conserved in a genebank? Our results seem to highlight that the correct answer would be as
624 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.

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756

Table 1. Origin and description of the populations analysed.

Accession code	Local name	Basic description	Origin		Assays				Figure code
			Town	Province	Weight&Yield	Morpho-agronomical	Quality	DNA	
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			

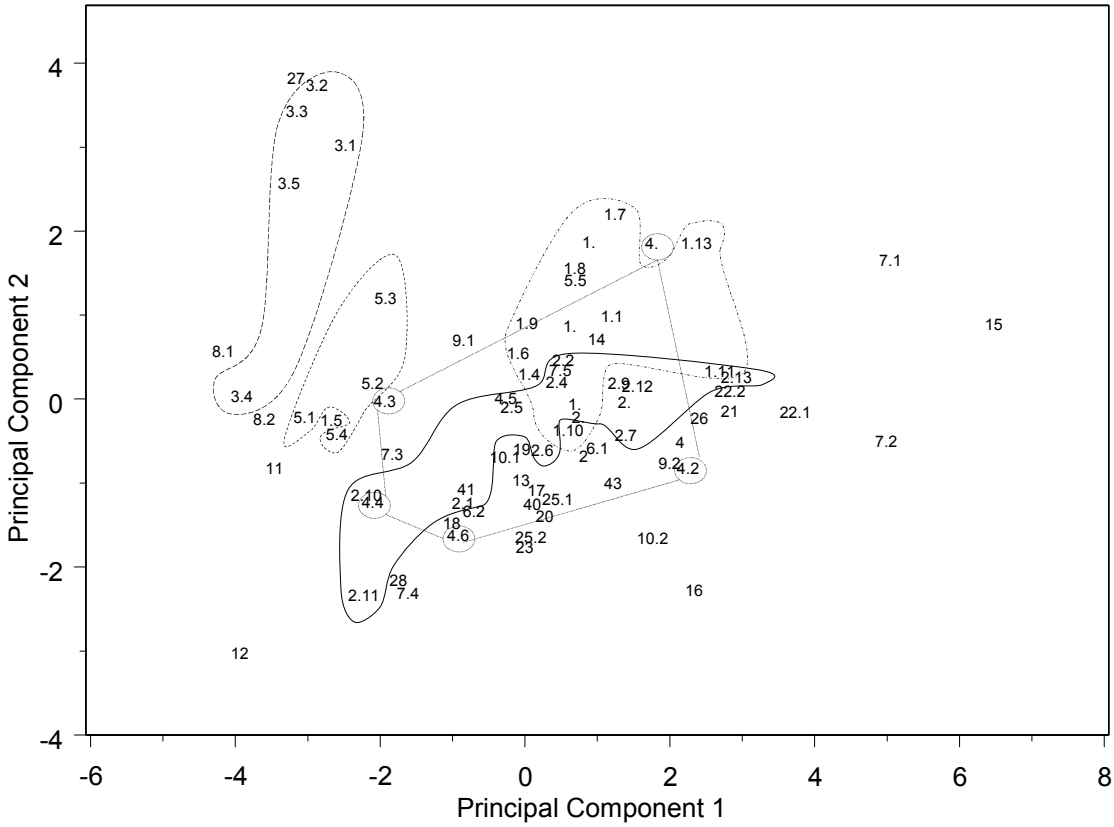

Fig. 1. Representation of the populations of traditional varieties in the first (0.366 of variance) and second (0.146 of variance) principal components obtained in the analysis of the morpho-agronomical variables. The first figure indicates variety or varietal type: 1: 'Valenciano', 2: 'Muchamiel', 3: '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.

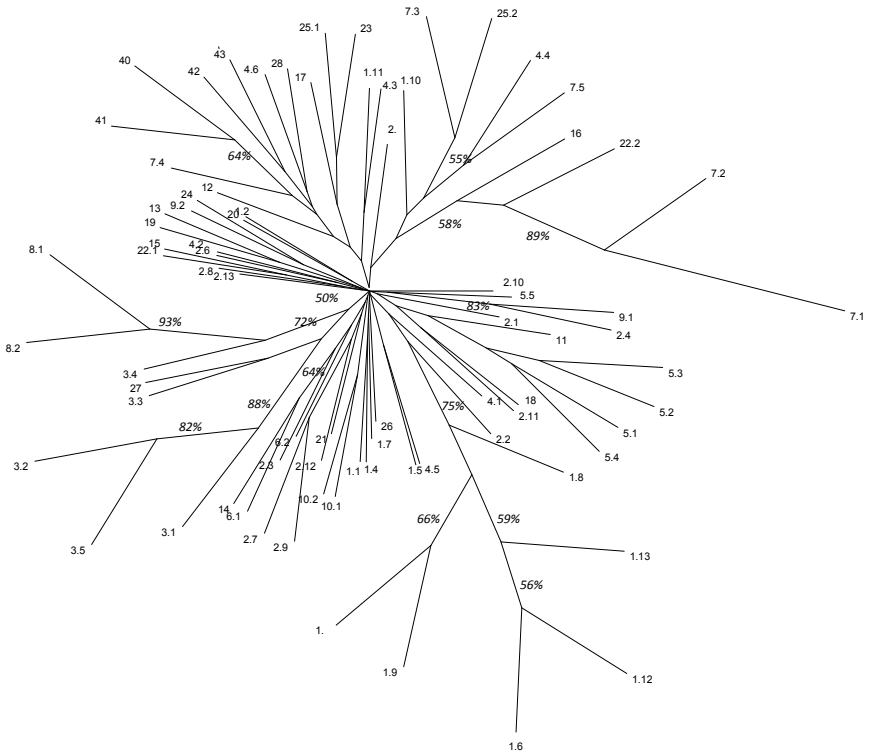

Fig. 2. Dendrogram obtained in the cluster analysis using the morpho-agronomical (quantitative and qualitative) variables. The first figure indicates variety or varietal type: 1: 'Valenciano', 2: 'Muchamiel', 3: 'Forma pimiento', 4: 'De penjar', 5: 'De la pera', 6: 'Elchero', 7: 'Morada', 8: 'De pera', 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.

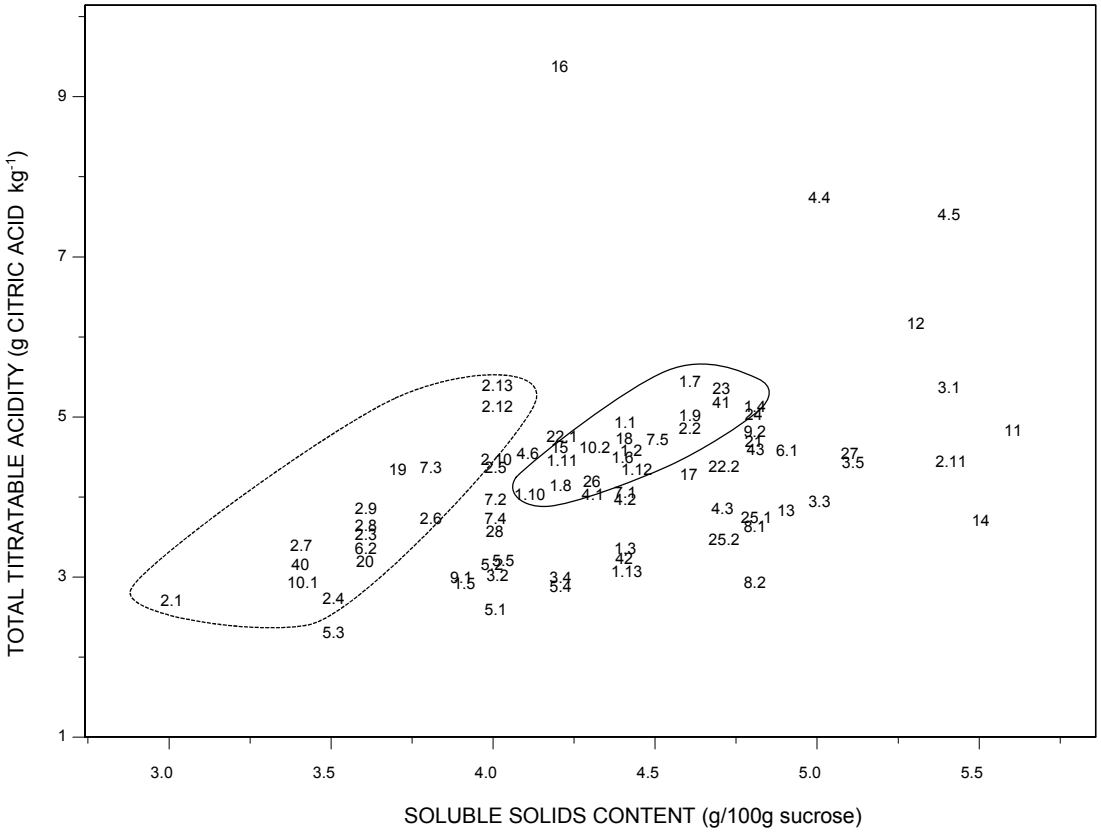

Fig. 3. Basic quality parameters. The lines delimit general patterns in the varieties 'Valenciano' (continuous line) and 'Muchamiel' (dotted line). The first figure indicates variety or varietal type: 1: 'Valenciano', 2: 'Muchamiel', 3: 'Forma pimiento', 4: 'De penjar', 5: 'De la pera', 6: 'Elchero', 7: 'Morada', 8: 'De pera', 9: 'De San Juan', 10: 'Cuarenteno', 11-28: other types, 40-43: Controls. See accessions codes in table 1.

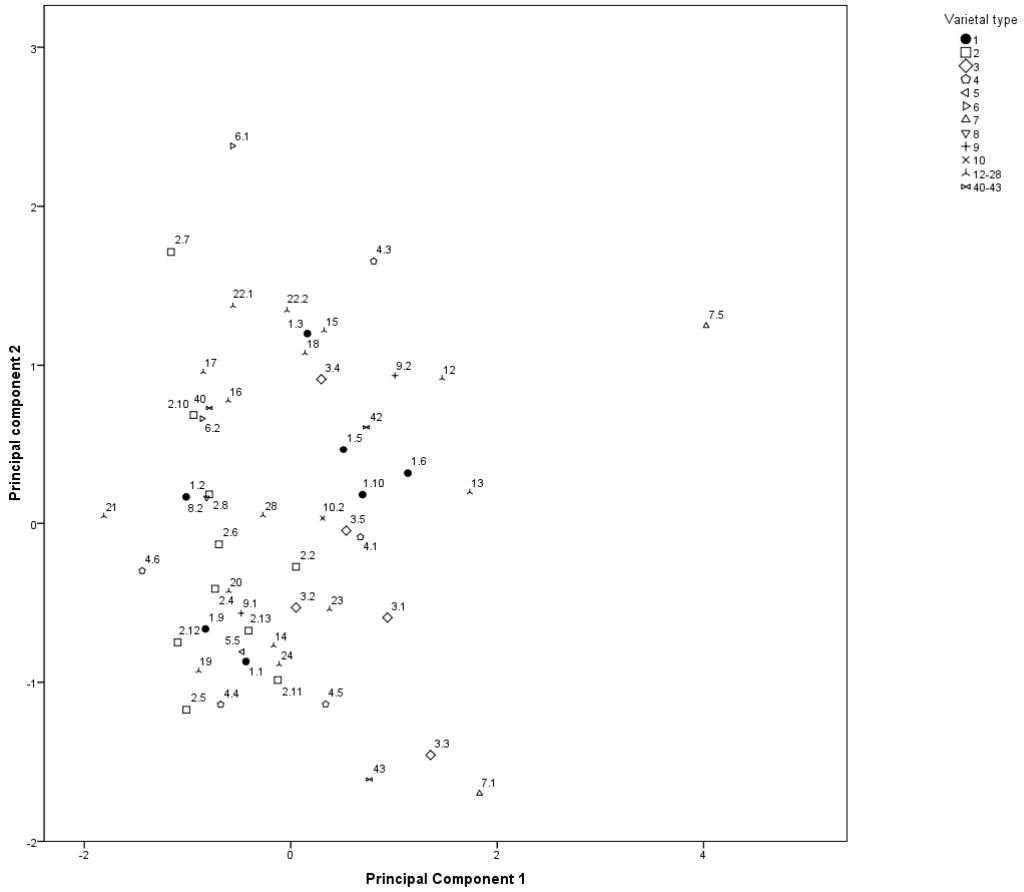

Fig. 4. Representation of the populations of traditional varieties in the first (0.333 of variation) and second (0.248 of variation) principal components obtained in the PCA of basic parameters and the content in individual sugars and organic acids related to organoleptic quality. The first figure indicates 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.

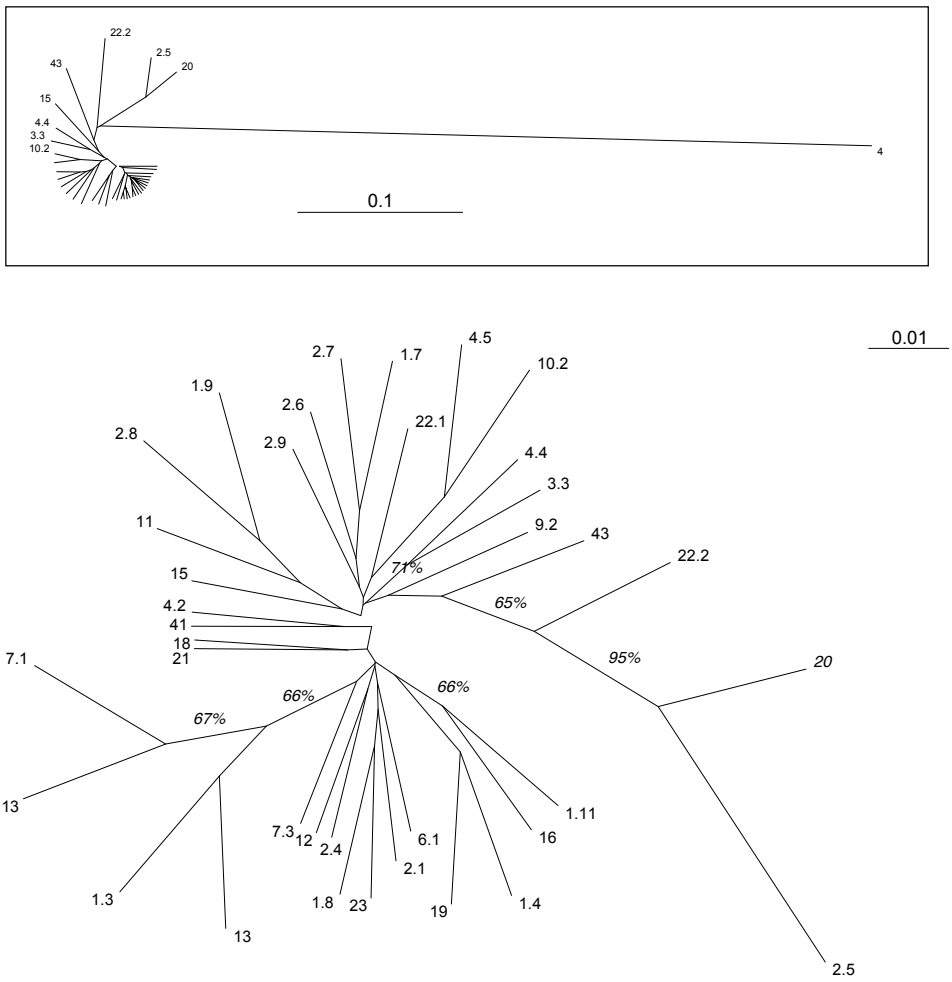

Fig. 5. Dendrograms obtained from the cluster analysis of AFLP data using Nei's distance, bootstrapping and UPGMA. Upper diagram represents the results including the outgroup control from *Solanum pennellii* Correll. The first figure indicates variety or varietal type: 1: 'Valenciano', 2: 'Muchamiel', 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.

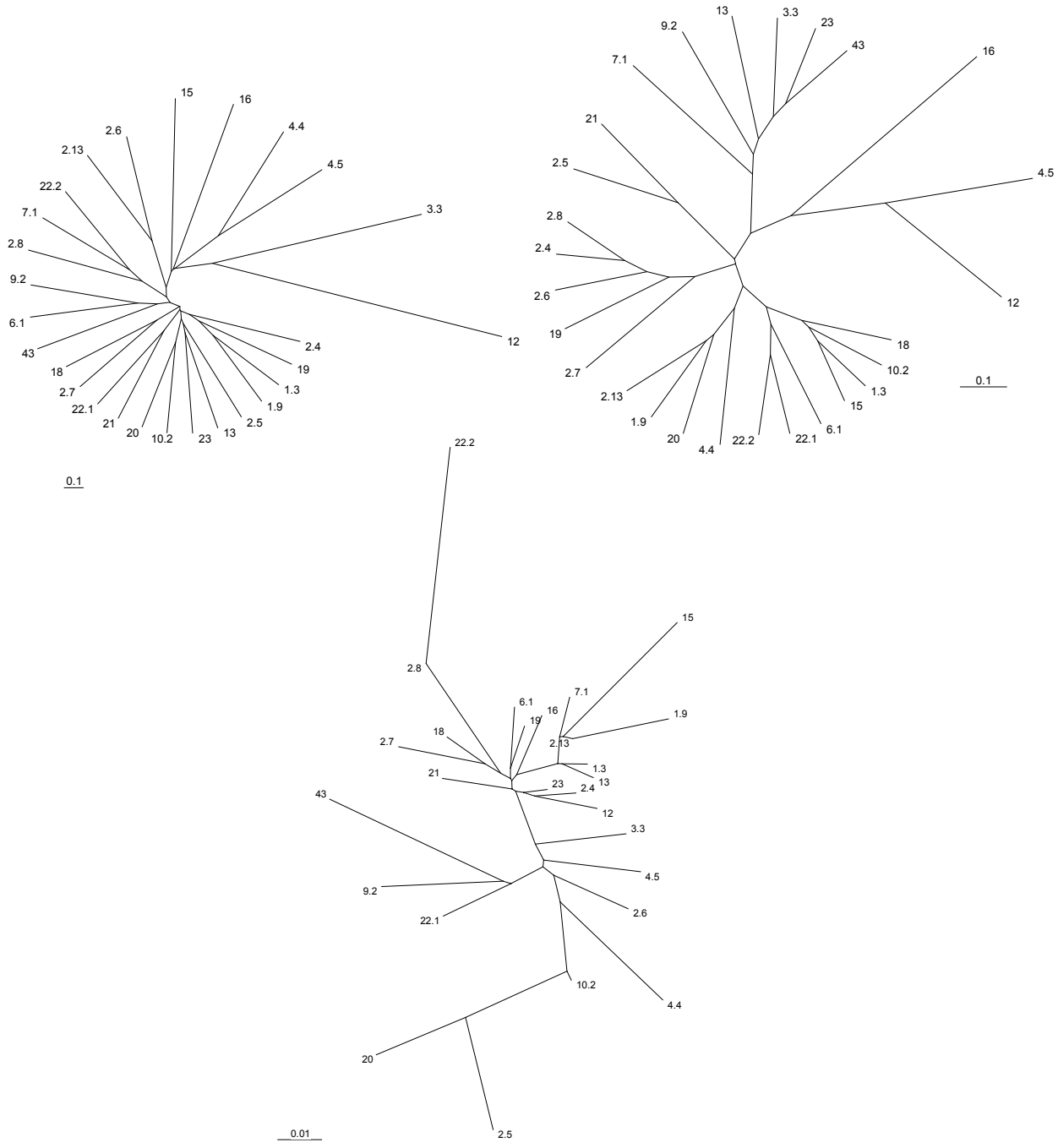

Fig. 6. Cluster analysis of the distance matrices obtained for standardized morpho-agronomical data (Euclidean distance, upper left corner), standardized quality data (Euclidean distance, upper right corner) and AFLP marker data (Nei's coefficient, center).