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Additional Information

1 **Variation of morphological descriptors for the evaluation of tomato germplasm**
2 **and their stability across different growing conditions**

3
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12
13 **ABSTRACT**

14 Germplasm and breeding materials are usually characterized using morphological and
15 agronomic descriptors, which should have a high heritability. Despite the widespread
16 use of tomato (*Solanum lycopersicum*) standardized descriptors, little information exists
17 on environmental effects on descriptor values and their heritability. We have evaluated
18 12 tomato accessions from seven cultivar groups in three different environments (open-
19 field conventional, open-field organic, and greenhouse) and characterized them with 36
20 descriptors. A wide range of variation was found for most descriptors, demonstrating
21 their utility for describing tomato materials and their diversity and relationships. The
22 analysis of descriptors variation reveals that while for some descriptors with a simple
23 genetic control the accession effect accounts for 100% of the variation, for others like
24 yield per plant only 10.83% of the variation observed is due to the accession effect.
25 Although significant differences were found among environments for most descriptors,
26 including a much higher yield in the open-field conventional environment than in the
27 two others, the environmental effect was low for most traits. However, the genotype ×
28 environment effect generally had an important contribution to the structure of variation
29 for many descriptors, and for three traits it had the highest contribution to the
30 percentage of the sum of squares. As a result of the variation structure, the heritability
31 values are high (>0.7) for only 10 descriptors, while for five is low (<0.3). Principal
32 components analysis (PCA) reveals that projections in the PCA graph of a same
33 accession grown in different environments plot together in the same area of the PCA
34 graph. Although cultivar groups are generally clearly separated in the PCA graph,

35 accessions from the same cultivar group in some cases are intermixed. These results
36 have important implications for detecting tomato duplicates and establishing core
37 collections, as well as for analyzing germplasm and breeding results, when using data
38 sets containing data of accessions grown in different environments.

39

40 **Keywords:**

41 Characterization

42 Descriptors

43 Genotype \times environment interaction

44 Germplasm

45 Heritability

46 *Solanum lycopersicum*

47

48

49 **1. Introduction**

50

51 Standardized descriptor lists for the characterization of germplasm collections
52 and breeding stocks constitute an important tool for germplasm banks and breeders as
53 they allow using an internationally agreed format, facilitating comparison of
54 characterization data sets among germplasm banks and trials (Gotor et al., 2008). Up to
55 now, Bioversity International has published descriptors lists for over 100 crops
56 (Bioversity International, 2017). Also, the UPOV has descriptors lists for the
57 characterization of new varieties in distinctness, uniformity and stability (DUS) tests
58 (UPOV, 2017). The characterization and evaluation descriptors lists include
59 morphological and agronomic traits that are of relevance for breeders. Depending on the
60 trait, descriptors are metric, meristic, measured according to an arbitrary quantitative
61 scale, or assigned to qualitative states (Grum and Atieno, 2007). Ideally, standardized
62 descriptors should display a wide variation in the collections of materials characterized,
63 as well as having a high heritability (Ortiz Ríos, 2015), which in turn requires a low
64 environmental influence. Descriptors having these characteristics are highly
65 informative. However many traits that are of interest for breeders, in particular those
66 polygenic, are influenced by the environment (Annicchiarico, 2002). For example, yield
67 is a typical example of an important trait highly affected by the environment (van
68 Bueren et al., 2011; van Ittersum et al., 2013). A way to overcome the influence of the

69 environment is using common controls in the trials, so that an estimate of the
70 environment effect can be obtained allowing its removal in the comparisons of data sets
71 from different environments (Ortíz Ríos, 2015). However, when important genotype ×
72 environment exists, the comparisons of distinct materials grown in different
73 environments are flawed, and this can lead to unreliable results (Annichiarico, 2002).
74 High genotype × environment interaction also represents a challenge for the
75 morphological traits-based detection of duplicates in germplasm banks (Diederichsen,
76 2009).

77 Tomato (*Solanum lycopersicum* L.) is the most important vegetable crop, and
78 over 75,000 accessions being conserved in germplasm banks (Robertson and Labate,
79 2006). Bioversity International standardized descriptors have been available for tomato
80 for over two decades (IPGRI, 1996). Since then, Bioversity International descriptors for
81 tomato have been widely used by germplasm banks and breeders (Mazzucato et al.,
82 2008; Gonçalves et al., 2009; de Castro et al., 2010; Cebolla-Cornejo et al., 2013;
83 Cortés-Olmos et al., 2015; Figàs et al., 2015; Parisi et al., 2016). These reports generally
84 show that IPGRI (1996) descriptors display a large range of variation and are useful to
85 distinguish among accessions and varietal groups. However, amazingly, in most of the
86 cases where germplasm is characterized using IPGRI (1996) descriptors they contain
87 data of a single location and year, and there are few works reporting data of several
88 years or environments. One exception is the work done by Mazzucato et al. (2008),
89 whom used 22 morpho-physiological traits, largely conforming with IPGRI (1996)
90 tomato descriptors, in 61 tomato and wild relatives accessions grown in two locations.
91 In this work, significant genotype × environment interaction was found for 21 out of the
92 22 descriptors, although the authors indicate that this interaction was mostly caused by
93 the performance of a few genotypes for each trait (Mazzucato et al., 2008). In another
94 work, Rao et al. (2006) used UPOV descriptors to evaluate ‘San Marzano’ accessions
95 for three years. These authors found that some homogeneous accessions that matched
96 the ‘San Marzano’ type in one year did not match it in other years. Overall, these data
97 seem to indicate that, while IPGRI (1996) tomato descriptors are appropriate for
98 describing the main morphological characteristics of tomato materials as well as for
99 assessing variation in germplasm and breeding collections, their values and scores may
100 be influenced by the environment and by genotype × environment interaction.

101 The lack of information on the stability of morphological descriptors in tomato
102 in different environments contrasts with the large number of studies evaluating the

103 effects of genotype \times environment in tomato for agronomic and fruit quality traits (Ortiz
104 et al., 2007; Cebolla-Cornejo et al., 2011; Adalid et al., 2012; Panthee et al., 2012,
105 2013). In general, these works reveal that there is a large genotype \times environment
106 interaction for yield and composition traits, and a moderate or low one for fruit shape
107 traits. Given the importance of standardized descriptors, like those of IPGRI (1996), in
108 tomato germplasm management and in breeding, it is necessary to have an assessment
109 of the genotype \times environment interaction of these widely used descriptors, in particular
110 when comparing data from data sets from different environments or years,.

111 In this work we use a set of IPGRI (1996) descriptors to evaluate 12 tomato
112 accessions in three different cultivation conditions (open-field conventional, open-field
113 organic, and greenhouse conventional). The results will provide information on the
114 stability of the different descriptors in different environmental conditions, and on the
115 utility of the utilization of a multiple set of standardized descriptors for providing a
116 characterization profile that allow differentiation among varieties grown in different
117 environments. All this information will be relevant for tomato germplasm
118 characterization and breeding.

119

120 **2. Material and methods**

121

122 *2.1. Plant material*

123

124 Twelve phenotypically diverse local varieties from the region of València
125 (Spain) were used in the present study (Table 1; Figure 1). The accessions belong to
126 seven different cultivar groups of local Valencian varieties (Borseta, Cor, Penjar, Plana,
127 Pruna, Redona, Valenciana) as described in Figàs et al. (2015). Four accessions belong
128 to the Penjar group, which is characterized by the presence of the *alc* mutation, which
129 confers a long shelf-life (Casals et al., 2012), and small or medium-sized fruits, three to
130 the Plana group, characterized by large flattened fruits, and one accession to each of the
131 groups Borseta (pyriform), Cor (slightly heart-shaped), Pruna (cylindrical), Redona
132 (rounded), and Valenciana (heart-shaped) (Table 1).

133

134 *2.2. Cultivation conditions*

135

136 All accessions were grown under three cultivation conditions: i) open field under
137 conventional management (open-field conventional), ii) open field under organic
138 management (open-field organic), and iii) greenhouse under conventional management
139 (greenhouse conventional). Seeds for the conventional cultivation trials were disinfected
140 with a 1:10 w/v solution of dodecahydrate trisodium phosphate ($\text{Na}_3\text{PO}_4 \cdot 12 \text{H}_2\text{O}$) for 3
141 h and rinsed three times with distilled water for 15 min; subsequently, the seeds were
142 subjected to an additional round of disinfection with commercial bleach (40 g/l of
143 NaOCl) at 30% for 7 min and rinsed three times with distilled water for 7 min. After
144 that, the seeds were left to dry for several weeks on filter paper and then subjected to
145 thermotherapy at 80°C for 24 h. The seeds for the organic cultivation conditions were
146 subjected to the same treatments except that the trisodium phosphate disinfection was
147 not performed. Seedling trays for 96 plants filled with Humin-substrat N3 (Klasmann-
148 Deilmann, Germany) substrate for the conventional cultivation conditions or Natur Pots
149 Premium (Projar) organic substrate for organic conditions were used for sowing the
150 seeds. Seedling trays were kept in a climatic chamber with a 14 h light / 10 h dark
151 photoperiod and a 25°C (light) / 18 °C (dark) temperature regime. For the open-field
152 conventional and organic trials, five plants per accession were grown, while for the
153 greenhouse trial six plants per accession were grown. In all trials plants were
154 transplanted on the 23rd of March of 2014, spaced 1.25 m among rows and 0.33 m
155 within the row and distributed according to a completely randomized design.

156 The open-field conventional trial was located in La Pobla de Vallbona
157 (Valencia, Spain; geographical coordinates: 39°34'33" N, 0°33'13" W, 90 m.a.s.l.). A
158 background fertilization of 0.15 kg/m² of fertilizer containing 15% N, 15% P₂O₅, and
159 15% K₂O (NPK(S) 15-15-15 (20), Fertiberia, Madrid, Spain) was applied before
160 transplant. An additional top-dressing fertilization at a dose of 0.05 kg/m² of the same
161 fertilizer was applied three months after transplant. Flood irrigation was used for
162 watering the plants, which were stacked with canes. Weeds were removed manually.
163 Phytosanitary treatments against spider mites, aphids, caterpillars, and tomato leaf
164 miner were performed using spinosad, emamectin, imidacloprid, and dimethoate. A
165 total of six treatments were performed throughout the crop.

166 The open-field organic trial was also located in La Pobla de Vallbona (Valencia,
167 Spain; 39°34'34" N, 0°33'16" W, 91 m.a.s.l.) in an organic certified farm with a organic
168 cultivation history of 5 years. Fertilization consisted in the background application of
169 0.6 kg/m² of horse manure. Irrigation, plant conduction and weeding were performed as

170 for the conventional cultivation system. A single phytosanitary treatment using the
171 insecticide spinosad, which is authorized for organic agriculture, was performed.

172 The greenhouse conventional trial was located in the campus of Universitat
173 Politècnica de València (Valencia, Spain; GPS coordinates: 39°28'56" N, 0° 20' 16" W,
174 5 m.a.s.l.). Plants were transplanted to 15 L pots filled with coconut peat (Horticoco,
175 Valimex, Valencia, Spain) and fertilized and watered using a drip irrigation system
176 using pressure compensating emitters. The final concentration of the main anions and
177 cations in the irrigation water was the following: 11.47 mM NO₃⁻, 1.00 mM NH₄⁺, 1.50
178 mM H₂PO₄⁻, 6.75 mM K⁺, 3.25 mM Ca²⁺, 2.50 mM Mg²⁺ and 2.82 mM SO₄²⁻.
179 Microminerals were supplied by adding the following salts to the irrigation water: 50
180 μM H₃BO₃, 10 μM FeEDTA, 4.5 μM MnCl₂, 3.8 μM ZnSO₄, 0.3 μM CuSO₄ and 0.1
181 μM (NH₄)₆Mo₇O₂₄. Excess water was applied in order to avoid salt build up in the pots.
182 Plants were trellised using vertical strings. Phytosanitary treatments against spider mites
183 and whiteflies were performed using the following pesticides: spinosad, emamectin,
184 imidacloprid, and dimethoate. A total of six treatments were performed throughout the
185 crop.

186

187 2.3. Characterization

188

189 Individual plants were characterized using 36 morphological and agronomic
190 descriptors, of which 31 were IPGRI (1996) descriptors (Table 2), which are commonly
191 used in tomato germplasm characterization trials (Mazzucato et al., 2008; Gonçalves et
192 al., 2009; de Castro et al., 2010; Cebolla-Cornejo et al., 2013; Cortés-Olmos et al.,
193 2015; Figàs et al., 2015; Parisi et al., 2016). All characterizations were performed
194 jointly by the same characterization team (MF, SS and CC). The five other descriptors
195 corresponded to yield, fruit firmness, and CIELAB fruit colour parameters L*, h*, and
196 c*, which are traits of relevance for tomato breeding (Rodríguez-Burruezo et al., 2005;
197 Figàs et al., 2005a). Twelve traits were quantitative, four meristic, 17 measured in a
198 quantitative scale, and three dichotomous. IPGRI descriptors data were taken according
199 to the instructions and recommendations given in IPGRI (1996). All fruits of each plant
200 were used to determine yield. Fruit firmness and fruit colour were determined in three
201 fruits (with three measures in three different areas of the mid-part of the fruit separated
202 by 120°) per plant when available using, respectively, a Fruit Pressure Tester FT327
203 (Effegi, Alfonsine, Italy) with a 8-mm tip probe, and a CR-300 (Minolta, Osaka, Japan)

204 chromameter. Each plant was considered as a replicate; so multiple measurements from
205 a single plant were used to obtain an average of each plant.

206

207 *2.4. Data analyses*

208

209 Individual plant data were used to calculate the mean value for each accession in
210 each of the environments. Average data of accessions in each environment were used to
211 calculate the global mean and range for each of the descriptors. Individual plant data
212 were subjected to a two factorial analysis of variance (ANOVA) to test the effects of
213 accession and cultivation environment, their interaction and the residual effects (Little
214 and Hills, 1978). The total sums of squares was partitioned in the sums of squares for
215 accession, environment, accession \times environment, and residual effects, and expressed in
216 percentage over the total sums of squares. Broad sense heritability (H^2) was calculated
217 according to Wricke and Weber (1986) using the formula $H^2 = \sigma_G^2 / [\sigma_G^2 + \sigma_{GE}^2/e$
218 $+ \sigma_E^2/(r \times e)]$, where σ_G^2 is the genetic variance, σ_{GE}^2 is the genotype \times environment
219 interaction, σ_E^2 is the residual (error) variance, e is the number of environments (e=3),
220 and r is the average number of replicates per environment (r=[5+5+6]/3) (Wricke and
221 Weber, 1986). Differences among environments for each descriptor were assessed using
222 a Student-Newman-Keuls multiple range test at a significance level of P<0.05. Pairwise
223 Euclidean distances among the three cultivation environments were calculated for each
224 accession using standardized data ($\mu=0$; $\sigma=1$) for each descriptor. These pairwise
225 Euclidean distances were also used for a multivariate principal components analysis
226 (PCA).

227

228 **3. Results**

229

230 *3.1. Descriptors range of variation*

231

232 None of the 36 descriptors assessed was uniform in the data from 12 accessions
233 evaluated in three environments (Table 2). In general, for quantitative and meristic
234 traits, wide ranges of variation were found. For example, differences of over 11-fold
235 were found for Size of corky area around pedicel scar, of almost 10-fold for Number of
236 locules, and of around seven-fold for Yield per plant, Number of flowers per

237 inflorescence, and Fruit weight (Table 2). In other traits, the differences were smaller in
238 relative terms, but also reflected a wide variation in the collection of accessions grown
239 in three environments. For traits measured in a quantitative scale, in some cases like
240 Inflorescence type, Intensity of greenback (shoulder), Fruit size homogeneity, Fruit
241 shoulder shape, Fruit cross-sectional shape, Fruit blossom end shape, Radial cracking,
242 and Fruit fasciation, the full scale range as given in the IPGRI (1996) descriptors for
243 tomato was represented (Table 2). However, in other cases, the range variation observed
244 for quantitative scale traits was reduced, like for Leaf attitude (only values of 6 and 7
245 for a scale from 3 to 7), or for Exterior colour of immature fruit (with values from 1 to 5
246 for a scale from 1 to 9). For the three dichotomous traits, variation was found in the
247 collection, although most of the accessions presented one of the descriptor states (e.g.
248 “standard” for Leaf type, “present” for Presence of green (shoulder) trips on the fruit,
249 and “yellow” for the skin colour of ripe fruit) (Table 2).

250

251 3.2. *Structure of descriptors variation*

252

253 The decomposition of the sums of squares from the ANOVA analyses revealed
254 that the accession effect was highly significant ($P < 0.001$) for the 36 descriptors
255 evaluated (Table 3). The percentage of sums of squares for accession varied between
256 10.83% for Yield per plant and 100.00% for Leaf type and Skin colour for ripe fruit.
257 The accession effect was the largest contributor to the sums of squares for 31 out of the
258 36 traits evaluated, the exception being for Leaf attitude, Leaf type, Yield per plant, and
259 Number of inflorescences. For 26 traits, the contribution of accession to the sum of
260 squares was higher than 50% (Table 3). The environment effect was low for most traits,
261 with 27 traits out of the 36 evaluated with a contribution below 10% to the total sums of
262 squares. The largest values were observed for Yield per plant (68.34%), which was the
263 only trait for which the environment effect was the largest contributor to the sums of
264 squares, and for Concentric cracking (27.71%) (Table 3). The contribution of the
265 accession \times environment interaction to the total sums of squares was generally higher
266 than that of environment. The only traits for which the environment effect had a larger
267 contribution than accession \times environment interaction to the total sums of squares were
268 Yield per plant and Number of flowers per inflorescence. The accession \times environment
269 interaction effect was the greatest contributor to the total sums of squares for three traits
270 (Leaf attitude, Number of inflorescences, and Flesh colour intensity) (Table 3). The

271 residual effect contribution to the sums of squares was variable, ranging from 0.00% for
272 Leaf attitude, Leaf type, Presence of green (shoulder) trips on the fruit, Skin colour of
273 ripe fruit, and Puffiness appearance, to 39.47% for Number of leaves under 1st
274 inflorescence. The residual effect was not the greatest contributor to the total sums of
275 squares for any of the descriptors (Table 3).

276 Heritability (H^2) values ranged between 0.14 for Leaf attitude to 1.00 for Leaf
277 type and Skin colour of ripe fruit (Table 3). For 10 traits out of 36, the H^2 values were
278 higher than 0.7, for 21 descriptors the H^2 values ranged between 0.3 and 0.7, and for
279 five descriptors the H^2 values were below 0.3. The traits with larger H^2 values included
280 Leaf type, some fruit size and shape descriptors (Fruit weight, Fruit length, Fruit width,
281 Size of corky area around pedicel scar, Size of core, Number of locules, and Fruit
282 fasciation), and Skin colour of ripe fruit. The lowest values for H^2 were for Leaf
283 attitude, Yield per plant, Number of inflorescences, Exterior colour of immature fruit,
284 and Puffiness appearance (Table 3).

285

286 *3.3. Differences among environments*

287

288 Significant differences ($P < 0.05$) among average values in each environment
289 were observed for all descriptors, except for Leaf type, Skin colour of ripe fruit, and
290 Number of locules (Table 4). Plants from the open-field conventional environment were
291 characterized by higher yield and number of flowers per inflorescence, greater fruit size
292 homogeneity, larger values for several descriptors related to fruit size, more intensely
293 coloured fruits, and fruits with higher degree of fasciation and puffiness, as well as less
294 bifurcated inflorescences and less firm fruits than those of the open field-organic or
295 greenhouse environments (Table 4). Those from the open-field organic environment had
296 plants with smaller size, more erect leaves, less number of flowers per inflorescence,
297 with a greater proportion and intensity of green shoulder, less fruit size homogeneity,
298 thinner pericarp, less fruit colour intensity, more pointed fruits, and with higher
299 cracking incidence than those of open-field organic or greenhouse cultivation. Finally,
300 plants from the greenhouse environment had a higher vegetative development with
301 dropping leaves, immature fruits less green, and with less intensity of greenback in the
302 shoulder, smaller fruits, with less cracking and fasciation, and with lower fruit colour
303 lightness than those of open-field cultivation (Table 4).

304 When considering all descriptors together, the three environments were almost
305 equidistant, with pairwise Euclidean differences averaged over the 12 varieties among
306 them not being significantly different for the three possible comparisons (Table 5).
307 However, the accessions evaluated had a different performance, with highest values for
308 accession MA2 (Pruna type) with an average value of 8.01 for Euclidean distances
309 among environments, while for one accession of the Cor type (DA2) and two Penjar
310 accessions (VI1 and VS1) the average Euclidean distances among environments were
311 much smaller, below 5. When considering specific pairwise comparisons in individual
312 accessions, the range of Euclidean distances among environments varies between 3.56
313 for VS1 for the open-field conventional vs. greenhouse environments comparison,
314 and 8.81 for MA2 for the open-field conventional and open field-organic environments
315 comparison. Also, for five accessions (AX2, DA2, MA2, RE2, and XA1) the largest
316 environmental distances were found between open-field conventional and open-field
317 organic environments, for two accessions (PI1 and VS1) were between open-field
318 conventional and greenhouse environments, while for six accessions were between
319 open-field organic and greenhouse environments (Table 5).

320

321 *3.4. Principal components analysis*

322

323 The first and second components of the PCA made with 36 descriptors evaluated
324 accounted for 30.21% and 14.06%, respectively, of the total variance (Table 6). The
325 first principal component was positively correlated to descriptors related to divided
326 inflorescences (Inflorescence type), multiple fruit size and weight descriptors, large
327 pedicel scars and corky area around them, intensely coloured fruits, irregular cross
328 section (Fruit cross-sectional shape, Fasciation and Number of locules), Size of core and
329 Radial cracking, and negatively to Fruit size homogeneity, Thickness of pericarp and
330 Fruit firmness (Table 6). The second principal component was positively correlated to
331 Foliage density, dropping (Leaf attitude) and standard (Leaf type) leaves, number of
332 inflorescences, Fruit size and Fruit length, yellow colour of ripe fruit (Skin colour of
333 ripe fruit), and Exterior fruit chroma value (c^*), and negatively with indeterminate
334 growth (Plant growth type), Number of leaves under 1st inflorescence, Number of
335 flowers per inflorescence, Presence of green (shoulder) trips on the fruit, and Exterior
336 fruit colour lightness (L^*) and hue (h^*).

337 The projection of accessions grown in each of the environments in the PCA plot
338 reveals that the different cultivar groups plot in separated areas of the graph, with the
339 exception of Cor and Redona groups, which cluster together (Figure 2). The first
340 component separates accessions of the Plana (large and fasciated fruits) and
341 Valenciana type (large fruits), which generally present positive values for this first
342 component from those of the Pruna, Penjar and Borseta types, which have negative
343 values. The second component separates the Pruna, Cor, Redona, Valenciana and
344 Borseta types, which have positive values from the Penjar type, which displays negative
345 values (Figure 2). Accessions grown in the three different environments group together,
346 and generally are separated from the other accessions, with the exception of accessions
347 DA2 (Cor type) and XA1 (Redona type) on one side, and accessions AX1, VI1, and
348 VS1 (Penjar type) on the other, which are intermingled (Figure 2). Accessions of the
349 Plana type are separated in the first component, with FU1, RE2 and OR3 having
350 highest, intermediate and lower values, respectively. The AX2 (Penjar) accession is
351 characterized by having a combination of lower values for the first component and
352 higher ones for the second component than the rest of Penjar accessions (Figure 2).

353

354 **4. Discussion**

355

356 Our work is the first, to our knowledge, to evaluate the stability of tomato
357 descriptors in characterization trials from different cultivation environments.
358 Amazingly, despite the importance of characterization data for breeding and germplasm
359 management (Engels and Visser, 2002; Ortiz Ríos, 2015), there is little information on
360 the stability of tomato descriptors and the influence this may have in comparison of data
361 sets from different trials and environments.

362 We have found that the morphological and agronomic descriptors used, most of
363 them corresponding to the Bioversity International descriptors list for tomato (IPGRI,
364 1996), are useful to describe the variation existing in a collection of tomato local
365 varieties, with wide ranges of variation having being observed, as occurred in other
366 works (Mazzucato et al., 2008; Gonçalves et al., 2009; de Castro et al., 2010; Cebolla-
367 Cornejo et al., 2013; Cortés-Olmos et al., 2015; Figàs et al., 2015; Parisi et al., 2016).
368 This again confirms the utility of these descriptors for providing a description for
369 relevant traits in tomato, as well as for comparing varieties and cultivar groups from a
370 single trial. In our case, we have compared data from three different cultivation

371 environments in three different sites. In order to evaluate the descriptors in different
372 environments, we included open-field conventional, open-field organic and greenhouse
373 cultivation conditions.

374 Ideally, descriptors should have high heritability and, in consequence, the
375 cultivation environment and genotype \times environment interaction should have a low
376 influence in the expression of the trait scored (Ortiz Ríos, 2015). This would allow
377 direct comparisons among characterization data sets. However, the comparison of the
378 scores of the same descriptors in three characterization trials revealed that wide
379 differences exist among descriptors for the effects of environment and genotype \times
380 environment. This is in agreement with some previous evidence (Rao et al., 2006;
381 Mazzucato et al., 2008). These latter authors reported significant genotype \times
382 environment interaction for morphological descriptors in the characterization of specific
383 sets of local tomato varieties in different environments. However, these studies do not
384 provide information on the relative contribution of the environment or genotype \times
385 environment interaction to the variation of descriptors (Rao et al., 2006; Mazzucato et
386 al., 2008). In our work, we have found large differences among individual descriptors in
387 the contribution of the accession, environment, or genotype \times environment interaction
388 effects. In this respect, descriptors for some monogenic traits, like Leaf type, controlled
389 by gene *C* (Busch et al., 2011), or Skin colour, controlled by gene *Y* (Ballester et al.,
390 2010), are not influenced by the environment and display no genotype \times environment
391 interaction and therefore have a heritability of 1. Other traits that had relatively high
392 values for heritability were those related to fruit shape. In tomato, it is known that the
393 expression of fruit shape traits has a high degree of genetic determination (Gonzalo and
394 van der Knaap, 2008, Rodríguez et al., 2011). On the other side, other descriptors, for
395 important traits, like yield per plant, had high environmental influence and genotype \times
396 environment interaction, as well as differences among plants within a trial, and therefore
397 have a low heritability. This is in agreement with other works (Avdikos et al., 2011; El-
398 Gabry et al., 2014), that reveal that yield, being a complex trait affected by multiple
399 genetic factors affected by the environment generally has a low heritability.
400 Remarkably, few descriptors had high heritability values, with only 10 out of the 36
401 descriptors having a heritability value above 0.7. This is in contrast with the
402 recommendation that descriptors for germplasm characterization should have high
403 heritability (Ortiz Ríos, 2015). It also suggests that comparisons of data sets
404 corresponding to different trials, in particular, when environmental conditions are very

405 dissimilar should be made with caution, unless only descriptors with high heritability or
406 low genotype \times environment contribution to the observed variation are used. In this
407 respect, the fact that for most descriptors the contribution of the genotype \times
408 environment effect is larger than that of the environment indicates that the use of
409 controls or environmental indexes to remove the environmental effect may only
410 partially contribute to make data comparable (Wricke and Weber, 1986; Becker and
411 León, 1988).

412 The three cultivation systems that we have evaluated presented many differences
413 in the crop management (Martínez-Blanco et al., 2011; van Bueren et al., 2011; de Ponti
414 et al., 2012). This has resulted in many significant differences among environments for
415 the traits evaluated. In particular, average yield has been much higher in the open-field
416 conventional environment than in the open-field organic or greenhouse environments,
417 probably reflecting the reduced input of fertilizers in the organic field and the poor
418 performance of many local tomato varieties in greenhouse (Bettiol et al., 2004; Jones,
419 2007; Kläring and Krumbein, 2013). The higher incidence of fruit cracking in the open-
420 field organic than in the open-field conventional conditions suggests that there has been
421 higher fluctuation of soil water content in the soil and/or deficiencies of calcium or
422 boron that, apart from increasing cracking may have contributed to reduce yield
423 (Pascual et al., 2000; Liebisch et al., 2009). The differences among environments for
424 each accession have also been studied using pairwise Euclidean distances among the
425 standardized values for the 36 descriptors. While the three environments were
426 approximately equidistant when considering the average values across all varieties,
427 considerable differences have been observed among varieties, both in the average value
428 and the environmental distances among pairs of environments. In this respect, some
429 varieties were more stable than others, and consequently had lower differences for
430 characterization data among environments. Others, on the contrary displayed much
431 higher differences among environments, reflecting a lower stability. Important
432 differences in stability for several traits have been observed in tomato (Ortiz and
433 Izquierdo, 1994). This has implications when choosing controls for comparison of
434 environments, as depending on the variety or varieties chosen, the estimation of the
435 environmental effect may be variable (Annicchiarico, 2002).

436 Despite wide differences among individual descriptors for the contribution of the
437 environment and genotype \times environment effects to their variation, the multivariate
438 principal component analysis (PCA) shows that observations of a single accession

439 grown in different environments plot in the same area of the PCA graph. This indicates
440 that IPGRI (1996) descriptors, despite the fact that some of them have low heritability,
441 when analyzed together provide a reliable characterization. As found in other works
442 (Cebolla-Cornejo et al., 2013; Figàs et al., 2015), the IPGRI (1996) morphological
443 descriptors allow a clear separation among tomato cultivar groups. However, the PCA
444 analysis also reveals that for accessions of the same cultivar group, some of the
445 accessions are intermingled, and therefore the comparison of characterization data of
446 different accessions of the same cultivar group evaluated in different environments
447 could lead to misleading results about the relationships among them. This has important
448 implications for detecting duplicates and creating nuclear collections in germplasm
449 banks (Dwivedi et al., 2005; Diederichsen, 2009), as a genetically uniform accession
450 evaluated in one environment when compared to itself and other accessions of the same
451 cultivar group grown in another environment may plot closer to other accessions.
452 Similarly, different accessions evaluated in different environment can plot together in a
453 PCA analysis.

454

455 **5. Conclusions**

456 In conclusion, our work confirms that morphological and agronomic descriptors
457 commonly used for characterization of tomato, like those of IPGRI (1996), are suited
458 for providing a detailed description of germplasm accessions or other plant materials.
459 This makes them of great utility for evaluating diversity, to study relationships among
460 accessions, and to assign them to cultivar groups. However, many descriptors, when
461 compared over dissimilar environments, have low or moderate heritability. This,
462 coupled with large genotype \times environment interaction effects indicates that comparison
463 of tomato characterization data sets that include accessions that have not been grown in
464 the same trial should be made with caution, even when controls are used to remove the
465 environmental effect. Nonetheless, multivariate principal components analysis using
466 data coming from different environments have proved useful for a reliable separation of
467 accessions according to cultivar group. All this information has important implications
468 for tomato germplasm conservation and management as well as for breeding.

469

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471

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614 **Table 1**

615 Local tomato varieties used for the present study including the varietal type (according
 616 to Figàs et al., 2015), and origin (municipality and province) within the Valencian
 617 Region (Spain).

Accession code	Varietal type	Predominant fruit shape	Origin	
			Municipality	Province
AX1	Penjar	Flattened	Alcalà de Xivert	Castelló
AX2	Penjar	Rounded	Alcalà de Xivert	Castelló
DA2	Cor	Slightly heart-shaped	Dos Aigües	València
FU1	Plana	Flattened	Fuenterrobles	València
MA2	Pruna	Cylindrical	Massalfassar	València
OR1	Borseta	Pyriiform	Oriola	Alacant
OR3	Plana	Flattened	Oriola	Alacant
PI1	Valenciana	Heart-shaped	Picanya	València
RE2	Plana	Flattened	Requena	València
VII	Penjar	Slightly flattened	Vinaròs	Castelló
VS1	Penjar	Flattened	Vistabella	Castelló
XA1	Redona	Rounded	Xàtiva	València

618

619

620

621 **Table 2**

622 Descriptors used and the global mean and range of varietal means observed in the three environments evaluated in the 12 local tomato varieties
 623 studied. Full details of the Bioversity International descriptors can be consulted elsewhere (IPGRI, 1996).

Descriptors	IPGRI descriptor code	Units/scale	Mean	Range
<i>Plant descriptors</i>				
Plant growth type	7.1.2.1	1=Dwarf; 4=Indeterminate	3.73	2.00-4.00
Foliage density	7.1.2.6	3=Sparse; 7=Dense	5.23	4.00-7.00
Number of leaves under 1st inflorescence	7.1.2.7	---	7.84	5.00-12.50
Leaf attitude	7.1.2.8	3=Semi-erect; 7=Drooping	6.86	6.00-7.00
Leaf type ^a	7.1.2.9	2=Potato leaf; 3=Standard	2.92	2.00-3.00
Yield per plant	---	g	2776	883-6167
<i>Inflorescence descriptors</i>				
Inflorescence type	7.2.1.1.	1=Generally uniparous; 3=Generally multiparous	2.03	1.00-3.00
Number of inflorescences	8.1.4	---	7.70	3.50-24.60
Number of flowers per inflorescence	8.1.5	---	7.22	4.48-11.44
<i>Fruit descriptors</i>				
Exterior colour of immature fruit	7.2.2.1	1=Greenish-white; 9=Very dark green	2.29	1.00-5.00

Presence of green (shoulder) trips on the fruit	7.2.2.2	0=Absent; 1=Present	0.94	0.00-1.00
Intensity of greenback (shoulder)	7.2.2.3	3=Slight; 7=Strong	4.51	0.00-7.00
Fruit size	7.2.2.6	1=Very small; 5=Very large	3.43	2.00-5.00
Fruit size homogeneity	7.2.2.7	3=Low; 7=High	5.21	2.00-7.00
Fruit weight	7.2.2.8	g	165.8	62.2-446.9
Fruit length	7.2.2.9	mm	60.8	40.7-94.6
Fruit width	7.2.2.10	mm	72.0	46.0-116.4
Fruit shoulder shape	7.2.2.16	1=Flat; 7=Strongly depressed	3.39	1.00-7.00
Width of pedicel scar	7.2.2.20	mm	10.7	5.7-19.6
Size of corky area around pedicel scar	7.2.2.21	mm	3.60	0.88-10.10
Skin colour of ripe fruit	7.2.2.23	1=Colourless; 2=Yellow	1.75	1.00-2.00
Thickness of pericarp	7.2.2.25	mm	7.42	5.10-9.90
Flesh colour intensity	7.2.2.27	3=Light; 7=Dark	5.78	4.00-7.00
Colour (intensity) of core	7.2.2.28	1=Green; 7=Dark	4.74	2.00-7.00
Fruit cross-sectional shape	7.2.2.29	1=Round; 3=Irregular	1.62	1.00-3.00
Size of core	7.2.2.30	cm	3.62	1.43-7.31
Number of locules	7.2.2.31	---	6.54	2.00-19.60
Fruit blossom end shape	7.2.2.33	1=Indented; 3=Pointed	1.63	1.00-3.00
Radial cracking	8.2.3	1=Corky lines; 7=Severe	2.83	0.00-7.00
Concentric cracking	8.2.4	1=Corky lines; 7=Severe	1.24	0.00-5.00

Fruit fasciation	8.2.5	3=Slight; 7=Severe	2.31	0.00-7.00
Puffiness appearance	8.2.9	3=Slight; 7=Severe	1.19	0.00-6.00
Fruit firmness	---	kg/cm ²	1.90	1.29-2.84
Exterior fruit colour lightness (L*)	---	0=black; 100=white	36.3	29.5-46.9
Exterior fruit colour hue (h*)	---	0°=red; 90°=yellow; 180°=green; 270°=blue	36.7	25.7-56.0
Exterior fruit colour chroma (c*)	---	0=completely unsaturated; 100=fully saturated	26.0	16.1-35.4

624 ^aQualitative descriptor potentially polytomous, but which has been found to be dichotomous in the collection

625 **Table 3**

626 Percentage of the total sums of squares for the effects of accessions, environment, interaction between accession and environment and residuals
 627 for the descriptors evaluated in 12 tomato accessions grown in three environments.

Descriptors	Sums of squares (%) ^a				Heritability (H^2)
	Accession	Environment	Accession × Environment	Residual	
Plant growth type	64.19 ^{***}	1.97 ^{***}	12.94 ^{***}	15.15	0.57
Foliage density	59.69 ^{***}	17.79 ^{***}	19.98 ^{***}	2.53	0.61
Number of leaves under 1st inflorescence	48.43 ^{***}	5.22 ^{***}	6.87 ^{ns}	39.47	0.49
Leaf attitude	35.33 ^{***}	17.60 ^{***}	47.07 ^{***}	0.00	0.14
Leaf type	100.00 ^{***}	0.00 ^{ns}	0.00 ^{ns}	0.00	1.00
Yield per plant	10.83 ^{***}	68.34 ^{***}	6.45 ^{***}	14.38	0.25
Inflorescence type	67.78 ^{***}	2.44 ^{***}	10.62 ^{***}	19.17	0.65
Number of inflorescences	36.50 ^{***}	4.61 ^{***}	40.79 ^{***}	18.09	0.17
Number of flowers per inflorescence	50.16 ^{***}	14.63 ^{***}	7.92 ^{**}	27.29	0.56
Exterior colour of immature fruit	49.96 ^{***}	0.41 ^{***}	48.74 ^{***}	0.88	0.26
Presence of green (shoulder) trips on the fruit	65.31 ^{***}	2.89 ^{***}	31.80 ^{***}	0.00	0.51
Intensity of greenback (shoulder)	56.89 ^{***}	17.98 ^{***}	24.86 ^{***}	0.27	0.54
Fruit size	76.90 ^{***}	3.25 ^{***}	18.64 ^{***}	1.21	0.70
Fruit size homogeneity	66.27 ^{***}	5.72 ^{***}	27.58 ^{***}	0.44	0.56

Fruit weight	71.37***	10.80***	12.38***	5.45	0.73
Fruit length	80.89***	4.42***	7.37***	7.32	0.81
Fruit width	83.56***	1.64***	5.66***	9.14	0.83
Fruit shoulder shape	85.03***	2.08***	12.38***	0.51	0.81
Width of pedicel scar	54.25***	5.68***	9.04**	31.02	0.54
Size of corky area around pedicel scar	76.83***	1.47***	9.53***	12.18	0.74
Skin colour of ripe fruit	100.00***	0.00 ^{ns}	0.00 ^{ns}	0.00	1.00
Thickness of pericarp	52.97***	6.36***	14.80***	25.88	0.50
Flesh colour intensity	31.42***	5.26***	63.32***	0.00	0.00
Colour (intensity) of core	66.36***	1.40***	31.71***	0.53	0.51
Fruit cross-sectional shape	78.55***	0.24***	19.82***	1.38	0.69
Size of core	82.47***	1.02***	9.17***	7.33	0.79
Number of locules	92.68***	0.08 ^{ns}	1.74***	5.50	0.92
Fruit blossom end shape	74.59***	1.33***	15.18***	8.91	0.68
Radial cracking	51.27***	10.99***	37.38***	0.36	0.37
Concentric cracking	38.41***	27.71***	33.51***	0.36	0.30
Fruit fasciation	88.49***	1.31***	10.13***	0.07	0.85
Puffiness appearance	50.22***	0.04***	49.73***	0.00	0.24
Fruit firmness	46.69***	2.75**	19.33***	31.23	0.39
Exterior fruit colour lightness (L*)	44.35***	10.21***	23.26***	22.17	0.37

Exterior fruit colour hue (h*)	65.69***	7.21***	10.69***	16.42	0.66
Exterior fruit colour chroma (c*)	59.12***	3.68***	12.93***	24.27	0.56

628 ^a ns, *, ***, and *** indicate non-significant, or significant at P<0.05, >0.01, and <0.001, respectively.

629 **Table 4**

630 Values averaged over 12 tomato accessions for each descriptor in the three
 631 environments evaluated and significance of differences.

Descriptors	Open field conventional	Open field organic	Greenhouse
Plant growth type ^a	3.75 b	3.61 a	3.82 b
Foliage density	5.33 b	4.83 a	5.53 c
Number of leaves under 1st inflorescence	7.25 a	7.73 a	8.53 b
Leaf attitude	6.92 b	6.67 a	7.00 c
Leaf type ^a	2.92 a	2.92 a	2.92 a
Yield per plant	4717 b	1890 a	1721 a
Inflorescence type	1.87 a	2.15 b	2.08 b
Number of inflorescences	8.73 b	7.42 a	6.96 a
Number of flowers per inflorescence	7.67 b	6.04 a	7.95 b
Exterior colour of immature fruit	2.33 b	2.33 b	2.21 a
Presence of green (shoulder) trips on the fruit	0.92 a	1.00 b	0.92 a
Intensity of greenback (shoulder)	4.25 b	5.50 c	3.79 a
Fruit size	3.50 b	3.53 c	3.25 a
Fruit size homogeneity	5.58 c	4.87 a	5.17 b
Fruit weight	188.9 b	187.0 b	121.5 a
Fruit length	6.48 c	5.96 b	5.81 a
Fruit width	7.53 b	7.08 a	6.98 a
Fruit shoulder shape	3.75 c	3.17 a	3.25 b
Width of pedicel scar	1.16 b	1.08 b	0.95 a
Size of corky area around pedicel scar	0.40 c	0.32 a	0.36 b
Skin colour of ripe fruit	1.75 a	1.75 a	1.75 a
Thickness of pericarp	0.77 b	0.69 a	0.76 b

Flesh colour intensity	5.50 a	5.92 b	5.92 b
Colour (intensity) of core	5.00 c	4.47 a	4.75 b
Fruit cross-sectional shape	1.58 a	1.67 c	1.62 b
Size of core	3.79 c	3.64 b	3.45 a
Number of locules	6.56 a	6.69 a	6.36 a
Fruit blossom end shape	1.58 a	1.75 b	1.56 a
Radial cracking	2.75 b	3.80 c	1.94 a
Concentric cracking	1.43 b	2.20 c	0.08 a
Fruit fasciation	2.58 c	2.42 b	1.93 a
Puffiness appearance	1.25 b	1.17 a	1.17 a
Fruit firmness	1.80 a	1.93 b	1.96 b
Exterior fruit colour lightness (L*)	37.64 c	36.21 b	34.92 a
Exterior fruit colour hue (h*)	40.08 b	34.74 a	35.35 a
Exterior fruit colour chroma (c*)	27.40 b	25.39 a	25.22 a

632 ^aMeans within rows separated by different letters are significantly different at $P < 0.05$,
633 according to the Student-Newman-Keuls test.
634

635 **Table 5**
 636 Pairwise Euclidean distances among three cultivation environments (open field
 637 conventional, open field organic, and greenhouse) for 12 tomato accessions based on
 638 normalized data of 36 characterization descriptors.

Accessions	Open field conventional vs. Open field organic	Open field conventional vs. Greenhouse	Open field organic vs. Greenhouse	Average
AX1	4.61	4.90	6.04	5.18
AX2	6.70	4.62	5.33	5.55
DA2	5.06	4.52	4.00	4.53
FU1	4.98	6.10	6.47	5.85
MA2	8.81	7.57	7.65	8.01
OR1	4.96	6.19	6.37	5.84
OR2	5.80	5.80	6.14	5.91
PI1	5.30	5.67	4.87	5.28
RE2	6.05	5.30	4.60	5.32
VI1	4.82	4.77	5.29	4.96
VS1	5.52	3.56	5.67	4.92
XA1	7.09	5.53	4.96	5.86
Average±SE	5.81±0.34	5.38±0.28	5.62±0.27	5.60±0.24

639

640

641 **Table 6**
642 Correlation coefficients between tomato descriptors for plant, inflorescence and fruit of
643 12 accessions grown in three environments and the two first principal components of a
644 multivariate principal components analysis. Correlations with absolute values above
645 0.150 are represented in bold font.

Descriptor	First principal component	Second principal component
Plant growth type	0.089	-0.335
Foliage density	-0.039	0.193
Number of leaves under 1st inflorescence	-0.023	-0.271
Leaf attitude	0.116	-0.157
Leaf type ^a	0.060	0.155
Yield per plant	0.083	0.054
Inflorescence type	0.218	-0.082
Number of inflorescences	-0.122	0.213
Number of flowers per inflorescence	0.014	-0.217
Exterior colour of immature fruit	-0.076	-0.056
Presence of green (shoulder) trips on the fruit	0.116	-0.251
Intensity of greenback (shoulder)	0.104	-0.147
Fruit size	0.224	0.183
Fruit size homogeneity	-0.178	-0.081
Fruit weight	0.270	0.094
Fruit length	0.018	0.372
Fruit width	0.292	0.008
Fruit shoulder shape	0.272	-0.019
Width of pedicel scar	0.264	0.001
Size of corky area around pedicel scar	0.267	0.075
Skin colour of ripe fruit	-0.050	0.188
Thickness of pericarp	-0.171	-0.040
Flesh colour intensity	0.091	0.145
Colour (intensity) of core	0.181	0.141
Fruit cross-sectional shape	0.191	-0.121
Size of core	0.275	-0.014

Number of locules	0.288	0.077
Fruit blossom end shape	-0.070	0.146
Radial cracking	0.164	-0.044
Concentric cracking	-0.010	-0.104
Fruit fasciation	0.212	-0.103
Puffiness appearance	-0.078	-0.018
Fruit firmness	-0.203	0.059
Exterior fruit colour lightness (L*)	-0.104	-0.168
Exterior fruit colour hue (h*)	-0.115	-0.265
Exterior fruit colour chroma (c*)	-0.040	0.317
Eigenvalue	10.88	5.06
Variance explained (%)	30.21	14.06

646

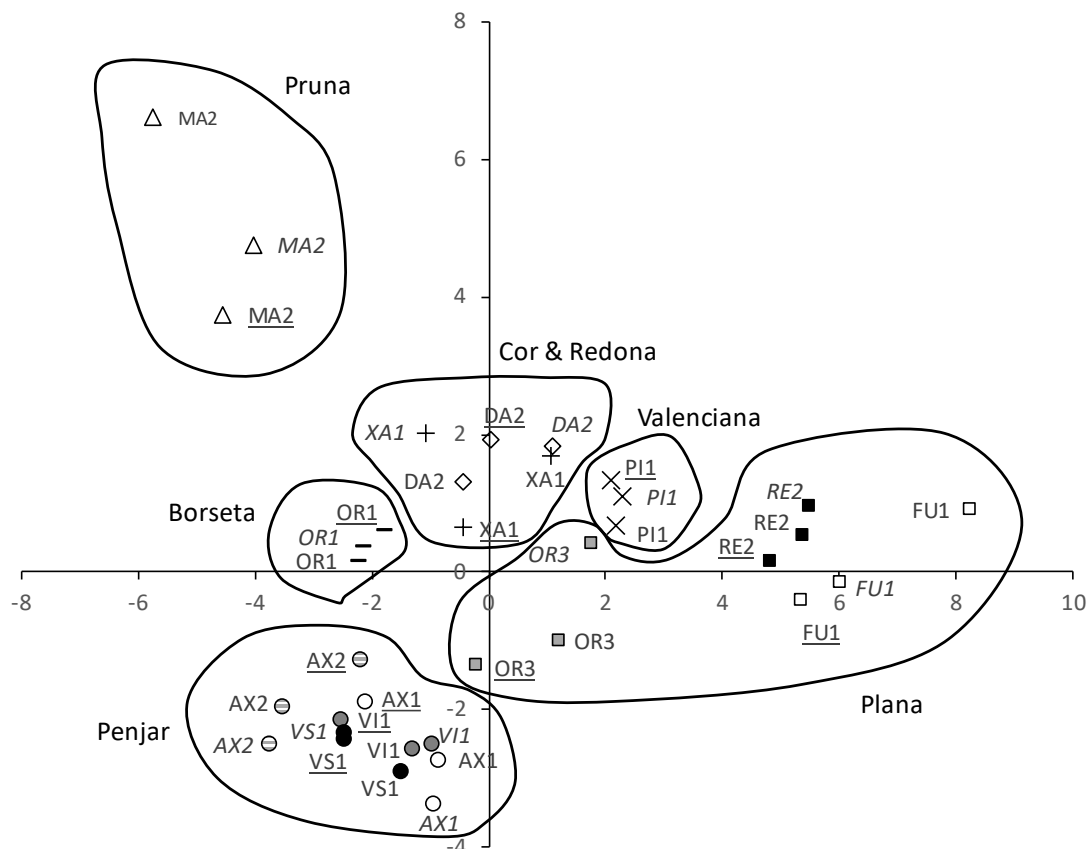


647

648 **Figure 1**

649 Fruits of the 12 local tomato varieties (AX1 to XA1) from the Valencian Region (Spain)
 650 used in the morphological and agronomic characterizations. The varietal type of each of
 651 them can be consulted in Table 1. Fruits are not depicted at the same scale; the divisions
 652 in the ruler correspond to 1 cm.

653



654

655 **Figure 2**

656 First (x-axis) and second (y axis) principal components scatterplot, based on 36
 657 morphological and agronomic descriptors, of 12 tomato accessions grown under three
 658 environments. The first and second principal components account for 30.21% and
 659 14.06% of the total variation. Each variety is indicated by its code (AX1 to XA1) and
 660 by the varietal type: Borseta (horizontal lines), Cor (diamonds), Penjar (circles), Plana
 661 (squares), Pruna (triangles), Redona (plus signs), and Valenciana (multiplication signs).
 662 The three cultivation conditions are indicated by the font type: open-field conventional
 663 (normal font), open-field organic (italic font), or greenhouse (underlined) cultivation
 664 conditions. Lines encompass the accessions included in each cultivar group.

665