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Additional Information

1 **Diversity for chemical composition in a collection of different varietal types of tree**
2 **tomato (*Solanum betaceum* Cav.), an Andean exotic fruit**

3

4 Running title: Diversity for chemical composition in tree tomato

5

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19

20

21

22 **Abstract**

23

24 We evaluated 23 tree tomato (*Solanum betaceum*) accessions from five cultivar groups
25 and one wild relative (*S. cajanumense*) for 26 composition traits. For all traits we found
26 highly significant differences ($P < 0.001$) among the materials studied. The high diversity
27 found within *S. betaceum* for composition traits was matched by a high diversity within
28 each of the cultivar groups. We found that sucrose and citric acid were the most
29 important soluble sugar and organic acid, respectively, in tree tomato. Fruit in the
30 anthocyanin pigmented (purple) group had a carotenoid content similar to that in the
31 yellow-orange cultivar groups. Total phenolic content was significantly correlated
32 ($r = 0.8607$) with antioxidant activity. Analyses of mineral content showed that tree
33 tomato is a good source of K, Mg, and Cu. Multivariate principal components analysis
34 (PCA) confirmed that an important diversity exists within each cultivar group. The
35 results we have obtained indicate that the high diversity found within the tree tomato
36 could be exploited for selection and breeding for developing the tree tomato as a
37 commercial crop.

38

39 **Keywords:** antioxidants, minerals, organic acids, proximate composition, soluble
40 sugars, tree tomato

41

42

43 **1. Introduction**

44

45 The so-called exotic fruits represent an important part of the human diet in their
46 regions of origin and are increasingly present in specialty markets in developed
47 countries. Many exotic fruits have beneficial properties for human health due to their
48 high levels of biologically active metabolites (Dembitsky et al., 2011), which may
49 further enhance demand and consumption. The Andean region is the native home of
50 many neglected exotic fruits with a high potential for developing as new high value
51 export crops (National Research Council, 1989).

52

53 One of the Andean fruit crops to have attracted increasing interest in the last few
54 years is the tree tomato (*Solanum betaceum* Cav., syn. *Cyphomandra betacea* (Cav.),
55 Sendtn.), also known as tamarillo. The tree tomato is a neglected, fast-growing, small
fruit tree (Prohens & Nuez, 2000). It is cultivated for its edible, fleshy, juicy, and

56 flavourful (fresh) fruits, which have a characteristic acidic taste (Bohs, 1989). Tree
57 tomato has been extensively studied from the taxonomy and systematics point of view
58 (Bohs, 1991, 1994, 1995, Bohs, 2007). However, until recently, few studies have
59 studied the diversity of the domesticated tree tomato with the aim of plant breeding and
60 increased intake. Acosta-Quezada, Martínez-Laborde, & Prohens (2011) differentiated
61 five cultivar groups based on fruit colour and shape: orange, orange pointed, red, red
62 conical and purple, and found considerable morphological diversity within each of the
63 cultivar groups. The low differentiation among cultivar groups from the morphological
64 point of view was confirmed with molecular data (Acosta-Quezada, Vilanova,
65 Martínez-Laborde, & Prohens, 2012). As a result of these studies, strategies for the
66 conservation of germplasm, as well as for the breeding and development of new
67 cultivars, were proposed.

68 The development of a successful tree tomato-breeding programme would benefit
69 from improvement of fruit quality, in particular those constituents impacting taste (e.g.,
70 sugars and acids) and compounds with functional activity or nutritional relevance (El-
71 Zeftawi et al., 1988). A number of studies have been devoted to the study of the
72 chemical composition and bioactive constituents of tree tomato, including general
73 composition (Dawes & Callaghan, 1970; Heatherbell, Reid, & Wrolstad, 1982; El-
74 Zeftawi et al., 1988; Romero-Rodriguez, Vazquez-Oderiz, Lopez-Hernandez, & Simal-
75 Lozano, 1994), sugars and organic acids (Boyes & Strübi, 1997; Ordóñez, Vattuone, &
76 Isla, 2005; Vasco, Avila, Ruales, Svanberg, & Kamal-Eldin, 2009), carotenoids
77 (Rodríguez-Amaya, Bobbio, & Bobbio, 1983; Mertz, Brat, Caris-Veyrat, & Gunata,
78 2010), phenolics (Hurtado, Morales, González-Miret, Escudero-Gilete, & Heredia,
79 2009; Osorio et al., 2012), or minerals (Clark, Smith, & Gravett, 1989). These studies
80 show that tree tomato contain relatively high levels of soluble sugars and organic acids
81 and remarkable levels of carotenoids and phenolics. Tree tomato also contain high
82 levels of K, with values similar to those of banana (Sulaiman et al., 2011).

83 Although the studies performed up to now have provided considerable
84 information about the composition and properties of tree tomato fruit, they have been
85 performed with only one or a few (with a maximum of three) varieties. These studies
86 have provided evidence of difference in composition among the varieties (Dawes &
87 Callaghan, 1970; Romero-Rodriguez, Vazquez-Oderiz, Lopez-Hernandez, & Simal-
88 Lozano, 1994; Boyes & Strübi, 1997; Vasco, Avila, Ruales, Svanberg, & Kamal-Eldin,
89 2009). However, the diversity of fruit composition within the species as well as within

90 and among cultivar groups has not been explored. This information is essential for the
91 development of selection and breeding programmes for improving the composition of
92 tree tomato, which is seen as key for the success of new varieties (El-Zeftawi et al.,
93 1988).

94 This work aimed to study the chemical composition, including proximate
95 composition traits (moisture, soluble solids content, titratable acidity, and protein),
96 soluble sugars (glucose, fructose, and sucrose), organic acids (malic and citric acid),
97 chlorophylls, total carotenoids, total phenolics, antioxidant activity, and minerals, in a
98 collection of tree tomato varieties from five cultivar groups (Acosta-Quezada, Martínez-
99 Laborde, & Prohens, 2011). The objective was to obtain information for the selection
100 and breeding of tree tomato varieties with improved taste and nutritional quality.

101

102 **2. Material and methods**

103

104 *2.1. Plant material and cultivation conditions*

105

106 A total of 24 tree tomato accessions, 23 of which correspond to the cultivated *S.*
107 *betaceum* and one to the wild relative *S. cajanumense*, were used for the analyses of
108 composition. The cultivated accessions belonged to five cultivar groups as defined by
109 Acosta-Quezada, Martínez-Laborde, & Prohens (2011): orange, orange pointed, red, red
110 conical, and purple (Table 1). Most of these accessions were previously
111 morphologically and molecularly characterized (Acosta-Quezada, Martínez-Laborde, &
112 Prohens, 2011; Acosta-Quezada, Vilanova, Martínez-Laborde, & Prohens, 2012). Plant
113 materials used were provided by the germplasm banks of the Universitat Politècnica de
114 València (UPV; Spain) and the Universidad Técnica Particular de Loja (UTPL;
115 Ecuador).

116 Plants from which the fruits were harvested were grown in an experimental field
117 plot of the UTPL in Loja (Ecuador; GPS: 4° 0' 1.59" S and 79° 10' 48.46" W) at 2160
118 m of altitude. The plantation was established in October 2007.

119

120 *2.2. Preparation of samples*

121

122 Commercially ripe fruits, i.e., in which the final size had been attained and with fully
123 coloured skin, were harvested between July and August 2011. A total of six samples of

124 fruit were taken for each accession. Each sample consisted of approximately 0.5 kg,
125 except for four accessions (A18, A27, A21, A40) in which the number of fruits
126 available was limited and the sample was around 250 g. Fruits were washed, peeled, cut
127 longitudinally and the seeds removed. Each sample was divided in two parts; one (fresh
128 fruit) was analysed at UTPL while the other (lyophilised) was analysed at UPV. For the
129 four accessions with reduced numbers of fruit, all fruits were lyophilised and no
130 analysis of fresh fruits was performed.

131 Fresh fruit samples were squeezed using a domestic juice extractor and
132 immediately analysed. Samples were frozen in liquid N₂ and stored at -80°C prior to
133 lyophilisation. One accession (A26) was lost due to a technical problem. Lyophilised
134 tissue from remaining samples was shipped to UPV using a courier service. Fruit
135 moisture, soluble solids content (SSC), and acidity (TA) were measured in fresh fruit
136 samples only, while other analyses were performed on the lyophilised samples.

137

138 *2.3. Analytical methods*

139

140 Moisture was determined as $(100 \times (\text{fresh weight} - \text{dry weight})/\text{fresh weight})$
141 and expressed as g/100 g. Soluble solids content (SSC; %) was measured with a hand
142 held refractometer. Titratable acidity (TA) was determined by titrating diluted juice with
143 0.1 mol/L NaOH to the phenolphthalein-end point and expressed as g/100 g of
144 anhydrous citric acid. The SSC:TA ratio was calculated from the SSC and TA values.
145 Protein concentration was estimated using the Kjeldahl method described by Raigón,
146 Prohens, Muñoz-Falcón, & Nuez (2008).

147 Glucose, fructose and sucrose contents were determined using a commercial kit
148 (Sucrose, D-fructose and D-glucose kit, Megazyme International Ltd., Wicklow,
149 Ireland). Analyses of malic and citric acids were also performed with commercial
150 enzymatic kits (MA9906 kit for malic acid and CI9920 kit for citric acid, BEN S.r.l.,
151 Milano, Italy). For both soluble sugars and organic acids, determinations were
152 performed following the manufacturers' instructions.

153 Chlorophylls *a* and *b*, total chlorophylls, and total carotenoids were measured
154 spectrophotometrically after extraction with a mixture of acetone and water (80:20 v/v)
155 using the method described by Wellburn (1994). Total phenolics were determined
156 according to the Folin-Ciocalteu method using the procedure indicated in Raigón,
157 Prohens, Muñoz-Falcón, & Nuez (2008). Results were expressed as chlorogenic acid

158 equivalents. Antioxidant activity was estimated using the colourimetric DPPH (1,1-
159 diphenyl-2-picrylhydrazyl) assay described in Sánchez-Moreno, Larrauri, & Saura-
160 Calixto (1998). Antioxidant capacity results were presented as Trolox equivalents (TE).

161 The analyses of minerals were performed as described in Raigón, Prohens,
162 Muñoz-Falcón, & Nuez (2008). Two g of lyophilised sample were calcined in a furnace
163 at 450 °C for 2 h and ashes were dissolved in 2 mL HCl. The mixture was heated until
164 the first vapours appeared and immediately 2 mL distilled water were added.
165 Subsequently, the mixture was filtered and brought to 100 mL with distilled water. P
166 was analysed by spectrophotometry using the molibdovanadate method, K by flame
167 photometry using a Jenway PF7 flame photometer (Jenway, Essex, UK), and Ca, Mg,
168 Fe, Cu, and Zn by atomic absorption spectrophotometry with a Thermo Elemental
169 (SOLAAR AA Spectrometers, Cambridge, UK) spectrometer.

170

171 *2.4. Statistical analyses*

172

173 Data were subjected to factorial analyses of variance (ANOVA) using a fixed-
174 effects model for variety. For each trait measured the least significant difference (LSD)
175 was calculated from the corresponding ANOVA. For each cultivar group the average
176 and standard error (SE) were calculated. Spearman rank correlations were calculated
177 between pairs of composition traits and significance of correlations were evaluated with
178 the Bonferroni test (Hochberg, 1988). The Spearman rank correlation was chosen
179 instead of the commonly used Pearson correlation in order to avoid spurious
180 correlations arising from extreme values (Little & Hills, 1978). The Bonferroni test,
181 which is very conservative, was chosen in order to minimize the risk of false positives
182 (Hochberg, 1988). Principal components analysis (PCA) was performed for
183 standardized composition data using pairwise Euclidean distances among accessions.
184 Only accessions with data for all the composition traits were included in the PCA
185 analysis.

186

187 **3. Results**

188

189 *3.1. Proximate composition*

190

191 Highly significant differences ($P < 0.001$) among accessions were found for all
192 proximate composition traits (Table 2). Moisture content in the *S. betaceum* accessions
193 ranged between 86.1% and 87.7%, while for the wild *S. cajanumense* was 93.2% (Table
194 2). SSC varied between 10.9% and 12.5% with an average value of 11.3%. No
195 differences were found among the cultivar groups, although the wild accession A15 had
196 values higher than those of the cultivated materials. TA varied widely, with wild *S.*
197 *cajanumense* values much lower than that of cultivated accessions (Table 2). Also,
198 considerable differences for TA were found within and among the different cultivar
199 groups. Cultivar groups red conical, red and orange pointed had average TA values
200 lower than those of the orange group. This wide variation for TA also resulted in very
201 variable values for SSC:TA ratio (Table 2). On average the orange accessions had lower
202 SSC:TA ratio than the other cultivar groups. Protein content ranged between 4.89 g/100
203 g in *S. cajanumense* and 9.58 g/100 g in the *S. betaceum* accession A41 (red conical
204 group) (Table 2).

205

206 3.2. Soluble sugars and organic acids

207

208 Differences among accessions for all soluble sugars and organic acids were
209 highly significant ($P < 0.001$). Glucose and fructose values were similar (average values
210 of 9.1 and 9.3 g/100 g, respectively), and lower than those of sucrose (average value of
211 23.5 g/100 g) (Table 3). Glucose contents were very variable ranging from 2.0 g/100 g
212 in the *S. cajanumense* accession to 12.4 g/100 g in *S. betaceum* accession A17 (Table
213 3). Differences within the cultivated species were less; A23 had the lowest value at 6.3
214 g glucose/100 g. Few differences were found for average values among the cultivar
215 groups, the most significant being the low value for the red conical group compared
216 with the orange and orange pointed groups. For fructose contents, the results were
217 comparable to those obtained for glucose, with the lowest values being found in *S.*
218 *cajanumense* (1.1 g/100 g), and the highest in accession A19 (13.2 g/100 g) (Table 3).
219 Sucrose contents were less variable than those for glucose or fructose. Although
220 important differences were found within each cultivar group, average values in orange
221 cultivar group and the wild *S. cajanumense* were highest (Table 3). Average total sugars
222 was 41.9 g/100 g, and ranged between 28.1 g/100 g and 52.0 g/100 g in accessions A23
223 and A17 (both of the orange pointed group). As with individual sugars, the orange

224 group had higher total sugars than the other cultivar groups, which in turn were higher
225 the wild *S. cajanumense* (Table 3).

226 Average malic acid content (0.70 g/100 g) was much lower than that of citric
227 acid (6.07 g/100 g). Malic acid content was highest in *S. cajanumense* (2.11 g/100 g);
228 the highest and lowest contents in *S. betaceum* were found in accessions A24 (0.99
229 g/100 g) and A17 (0.34 g/100 g) (Table 3). For citric acid, the highest and lowest values
230 were found within *S. betaceum*, ranging from 4.01 g/100 g (A18) to 7.54 g/100 g (A16).
231 For both malic and citric acids wide differences were found within cultivar groups but
232 not among groups. For total acids content, the results were similar to those of the
233 predominant citric acid (Table 3). The average value for the sugars:acids ratio was 6.43,
234 and the values varied between 4.38 (A39) and 9.37 (A18). On average, the cultivated *S.*
235 *betaceum* groups have higher values for the sugars:acids ratio than those of the wild *S.*
236 *cajanumense* (Table 3).

237

238 3.3. Pigments and antioxidants

239

240 All the pigment and antioxidant traits measured presented highly significant
241 ($P < 0.001$) differences among accessions. Chlorophyll *b* content was higher than
242 chlorophyll *a* content with mean values of 1.53 and 0.66 mg/100 g, respectively (Table
243 4). The only exception was *S. cajanumense* which had a chlorophyll *b* content higher
244 than that of chlorophyll *a*. Chlorophyll *a* content was much higher in the wild *S.*
245 *cajanumense* (4.50 mg/100 g) than in the cultivated *S. betaceum*, which had values
246 ranging from 0.19 mg/100 g (A34) to 0.97 mg/100 g (A21 and A40) (Table 4). Despite
247 wide differences within cultivar group, the purple, red and red conical groups had
248 higher average values than those of the orange and orange pointed groups. For
249 chlorophyll *b* the content ranged between 0.42 mg/100 g (A31) and 6.57 mg/100 g
250 (A40). Wide variation for chlorophyll *b* content was found within cultivar groups (Table
251 4). Total chlorophyll content was maximal in *S. cajanumense* (8.64 mg/100 g) and
252 minimal in *S. betaceum* A31 (0.67 mg/100 g). *Solanum betaceum* A40 had much higher
253 total chlorophyll content (7.54 mg/100 g) than the rest of cultivated accessions. Purple,
254 red conical and red groups had higher contents of total chlorophylls than the orange and
255 orange red groups.

256 The content in carotenoids varied between 2.60 mg/100 g (A24) and 11.20
257 mg/100 g (A40) (Table 4). Again there were important differences within cultivar

258 groups; the red conical and purple groups had higher average values than the rest of
259 groups. The total phenolic content was much higher than that of the chlorophylls and
260 carotenoids, with an average value of 3.78 g/100 g (Table 4). Individual accession
261 values ranged from 2.82 g/100 g (A35) to 6.18 g/100 g (A19) (Table 4). As with
262 chlorophylls and carotenoids wide differences were found within groups, but the
263 differences among groups were not significant. The antioxidant activity varied
264 considerably among accessions with values ranging from 12.2 $\mu\text{mol TE/g}$ (A23) to 48.7
265 $\mu\text{mol TE/g}$ (A40) (Table 4). Also, wide differences were found within groups, but not
266 among groups.

267

268 3.4. Minerals

269

270 As with the other traits studied, highly significant differences ($P < 0.001$) were
271 found among accessions for the concentration of all minerals studied. By far, the
272 mineral with highest concentration was K, with an average value of 2511 mg/100 g,
273 followed by Mg (141 mg/100 g), P (124 mg/100 g), Ca (60 mg/100 g), Fe (1.39 mg/100
274 g), Zn (0.97 mg/100 g) and Cu (0.57 mg/100 g) (Table 5). For all minerals, a wide range
275 of variation was found. In the case of P, the red conical accession A41 had much higher
276 values (226 mg/100 g) than the rest of accessions, and differences among the rest of
277 cultivar groups were not significant (Table 5). Conversely to what occurred for P,
278 accession A41 had the lowest content in K, with values considerably lower than those of
279 the rest of cultivar groups. For Ca the wild *S. cajanumense* had much higher values (117
280 mg/100 g) than the cultivated accessions (Table 5). In the case of Mg, accession A41
281 had the lowest value (48 mg/100 g); also, some of the purple accessions had low Mg
282 values (Table 5). For microelements Fe, Cu, and Zn, very important differences were
283 found among the accessions, in particular for Cu (Table 5). However, the differences
284 among cultivar groups were non-significant in most cases.

285

286 3.5. Correlations between traits

287

288 Ten out of 378 Spearman rank correlations between traits were significant
289 according to the Bonferroni significance test at $P \leq 0.05$. A negative correlation was
290 found between TA and the SSC:TA ratio ($r = -0.9304$); the rest of significant correlations
291 had positive values. Out of the nine significant positive correlations, four involve

292 content in sugars (glucose and fructose, $r=0.9745$; glucose and total sugars, $r=0.9607$;
293 fructose and total sugars, $r=0.9565$; sucrose and total sugars, $r=0.8238$), one content in
294 acids (citric acid and total acids, $r=0.9338$), three the content in chlorophylls
295 (chlorophyll *a* and chlorophyll *b*, $r=0.8310$; chlorophyll *a* and total chlorophylls,
296 $r=0.9042$; chlorophyll *b* and total chlorophylls, $r=0.9792$), and the last one involves total
297 phenolic content and antioxidant activity ($r=0.8607$).

298

299 3.6. *Multivariate analysis*

300

301 The first and second components of the PCA performed with all the accessions
302 accounted for 37.1 and 15.8% of the total variation, respectively. This PCA analysis
303 (not shown) basically separates the wild *S. cajanumense* accession A15 from the
304 cultivated tree tomato accessions. Therefore, we performed another PCA including only
305 cultivated accessions. In this PCA the first and second components accounted,
306 respectively, for 26.1% and 19.2% of the total variation. The first principal component
307 was positively correlated to moisture content, acidity (TA, malic acid, citric acid, and
308 total acids), and chlorophylls (chlorophyll *a*, chlorophyll *b*, and total chlorophylls), and
309 negatively correlated to sugars content (glucose, sucrose, fructose, and total sugars) and
310 ratio sugars:acids (Figure 1). The second component was positively correlated to Fe and
311 negatively correlated to sugars (glucose, sucrose, fructose, and total sugars), total
312 phenolic content, antioxidant activity and minerals P, Ca, and Zn (Figure 1). The
313 projection of individual accessions in the PCA plot shows that accessions of cultivar
314 groups are intermingled (Figure 1). However, most of the accessions of the orange
315 pointed group are characterized by low values of both the first and second components
316 (i.e., associated to high content in sugars and sugars:acids ratio). In this respect, no
317 accessions from any of the other groups had negative values for both the first and
318 second principal components (Figure 1). Also, no accessions of the orange pointed
319 group are found in the PCA graph quadrant having positive values for the first
320 component and negative values for the second one (i.e., associated to high content in
321 acids, chlorophylls, carotenoids, and minerals P, K, Ca, and Zn).

322

323 4. Discussion

324

325 Tree tomato is an emerging exotic fruit crop (Acosta-Quezada, Martínez-
326 Laborde, & Prohens, 2011). The successful build-up as a commercially important crop
327 requires the development of improved cultivars with good fruit quality. In this respect,
328 selection and breeding of new cultivars requires the identification of sources of variation
329 for the traits of interest. Although the composition of tree tomato fruit had been already
330 studied (Dawes & Callaghan, 1970; Heatherbell, Reid, & Wrolstad, 1982; Rodríguez-
331 Amaya, Bobbio, & Bobbio, 1983; El-Zeftawi et al., 1988; Clark, Smith, & Gravett,
332 1989; Romero-Rodriguez, Vazquez-Oderiz, Lopez-Hernandez, & Simal-Lozano, 1994;
333 Boyes & Strübi, 1997; Ordóñez, Vattuone, & Isla, 2005; Hurtado, Morales, González-
334 Miret, Escudero-Gilete, & Heredia, 2009; Vasco, Avila, Ruales, Svanberg, & Kamal-
335 Eldin, 2009; Osorio et al., 2012; Mertz, Brat, Caris-Veyrat, & Gunata, 2010), the results
336 obtained here are the first to examine the diversity of chemical composition in sufficient
337 tree tomatoes, including different cultivar groups and one wild relative (*S.*
338 *cajanumense*).

339 We have shown that cultivated tree tomato and wild *S. cajanumense* have
340 different composition profiles. For example, *S. cajanumense* fruits have greater
341 moisture content, lower TA and soluble sugars, higher SSC:TA ratio, malic acid,
342 chlorophylls, and Ca than those of cultivated *S. betaceum*. These differences in
343 composition profile can be exploited for the introgression of favourable traits from the
344 wild species into the genetic background of the cultivated species. The lower TA of *S.*
345 *cajanumense* could be a trait of great interest for the improvement of cultivated tree
346 tomato, as tree tomato fruit are generally perceived as acidic (Bohs, 1989; Boyes &
347 Strübi, 1997; Vasco, Avil, Ruales, Svanberg, & Kamal-Eldin, 2009).

348 Tree tomato fruit display statistically significant differences for all the
349 composition traits studied. Although for moisture content and SSC the range of
350 variation was quite narrow, for all the other traits measured a wide range of variation
351 was found. Our study is in agreement with previous works that used a limited genetic
352 diversity (two or at the most three accessions) in this crop, in which differences among
353 varieties for composition traits were reported (Dawes & Callaghan, 1970; Romero-
354 Rodriguez, Vazquez-Oderiz, Lopez-Hernandez, & Simal-Lozano, 1994; Boyes &
355 Strübi, 1997; Vasco, Avila, Ruales, Svanberg, & Kamal-Eldin, 2009). In particular, for
356 the microminerals studied (Fe, Cu, Zn) differences of several-fold were found among
357 the varieties having the lowest and the highest content. The wide differences for
358 composition traits affecting flavour (sugars and acids), colour (chlorophylls,

359 carotenoids, phenolics), and functional quality (carotenoids, phenolics, antioxidant
360 activity) reveal improvement of tree tomato fruit quality can be achieved through
361 selection and breeding.

362 As occurs with morphological and molecular diversity (Acosta-Quezada,
363 Martínez-Laborde, & Prohens, 2011; Acosta-Quezada, Vilanova, Martínez-Laborde, &
364 Prohens, 2012), we have found a wide diversity for composition traits within each of the
365 cultivar groups. Also, differences have been observed among cultivar groups for some
366 relevant composition traits. For example, the orange group had higher TA and lower
367 SSC:TA ratio than the rest of cultivar groups. This is in agreement with the results
368 obtained by Boyes & Strübi (1997) who, in a study with two red and one yellow-orange
369 cultivars found the red cultivars had a sweeter taste than the yellow-orange one. These
370 results suggest the orange cultivar group varieties will have a more acidic taste than the
371 rest of cultivars.

372 When considering soluble sugars, we found that the most abundant sugar was
373 sucrose. Glucose and fructose were present at similar levels. For organic acids, citric
374 acid content was much higher than malic acid. These results are in agreement with
375 previous results (Heatherbell, Reid, & Wrolstad, 1982; Romero-Rodríguez, Vazquez-
376 Oderiz, Lopez-Hernandez, & Simal-Lozano, 1984; Boyes & Strübi, 1997; Vasco, Avila,
377 Ruales, Svanberg, & Kamal-Eldin, 2009), but differ from those obtained by Dawes and
378 Callaghan (1970) and by Ordóñez, Vattuone, & Isla (2005), who found higher levels of
379 fructose and glucose than of sucrose. The wide variation among varieties for the
380 sugars:acids ratio, with differences up to two-fold among accessions, indicates that
381 selection for a sweeter taste could be achieved (El-Zeftawi et al., 1988; Mwithiga,
382 Mukolwe, Shitanda, & Karanja, 2007). Correlations among the three soluble sugars
383 studied, on one hand, and between the two organic acids, on the other, were high,
384 indicating variation in the composition profile of both sugars and acids was low.

385 The flesh colour of tree tomato depends on the content in chlorophylls,
386 carotenoids, and anthocyanins. The presence of chlorophyll in the fruit flesh is a
387 negative trait for fruits of the orange or red fleshed cultivars, as it reduces the chroma
388 and luminosity of the fruit flesh colour (McGhie & Ainge, 2002). However, for the
389 purple-fleshed cultivars, the presence of chlorophyll may be favourable, as chlorophylls
390 may contribute to a darker flesh colour. In this respect, we have found that the purple
391 group, on average, had higher content in chlorophylls than the rest of accessions.
392 Carotenoids, which are responsible of the yellow or orange colour of the flesh of the

393 orange, orange pointed, red, and red conical cultivars (Mertz, Brat, Caris-Veyrat, &
394 Gunata, 2010), display wide variation in the collection tested. However, on average,
395 yellow- or orange-fleshed cultivars did not have more carotenoid than purple cultivars
396 (Mertz et al., 2009; Vasco, Avila, Ruales, Svanberg, & Kamal-Eldin, 2009). This
397 indicates that anthocyanins in the purple-fleshed cultivars mask the yellow or orange
398 colour conferred by carotenoids.

399 Phenolic content was much greater (several hundred-fold) than carotenoids
400 (Mertz et al., 2009). The high correlation value ($r=0.8607$) observed by us between
401 phenolics content and antioxidant activity indicates that, as in other crops (Plazas et al.,
402 2013), phenolics are the major antioxidants in the fruit flesh of tree tomato. Although
403 the tree tomato also contains appreciable quantities of ascorbic acid (Romero-
404 Rodriguez, Vazquez-Oderiz, Lopez-Hernandez, & Simal-Lozano, 1994; Vasco, Avila,
405 Ruales, Svanberg, & Kamal-Eldin, 2009), the levels found are much lower than those of
406 phenolics. Furthermore, given that tree tomato is mostly consumed in juices, ascorbic
407 acid is quickly oxidized and lost (Mertz, Brat, Caris-Veyrat, & Gunata, 2010), and in
408 practice will not represent a significant contribution to the antioxidant intake.

409 As with other *Solanum* fruit crops (Raigón, Prohens, Muñoz-Falcón, & Nuez,
410 2008; Giuffrida, Martorna, & Leonardi, 2009), the tree tomato has only moderate levels
411 of minerals. Nonetheless, the important variation observed indicates that selection and
412 breeding could result in improvements in the content of nutritionally important
413 minerals. In this respect, one serving (100 g) of tree tomato would provide relevant
414 amounts of the minerals K (up to 8.8% of the recommended dietary allowance; RDA),
415 Mg (up to 7.6% of the RDA), and Cu (up to 50% of the RDA) (Institute of Medicine,
416 2000, 2001; Vasco, Avila, Ruales, Svanberg, & Kamal-Eldin, 2009).

417 The multivariate PCA analysis reflected the wide diversity existing within
418 cultivar groups in composition profile, and allowed varieties that are more or less
419 similar in composition to be identified. It also showed that the accessions belonging to
420 different cultivar groups intermingle, suggesting that chemometric classification using
421 PCA may not be a reliable tool to assign accessions to cultivar groups. However, the
422 PCA analysis showed some orange-pointed accessions had similar composition profile,
423 characterised by high contents of sugars and ratio sugars:acids, and were different from
424 the rest of cultivars. These accessions could be considered of interest for improving the
425 flavour of this cultivar group, and also could be sources of variation for improving these

426 traits in other cultivar groups (El-Zeftawi et al., 1988; Mwithiga, Mukolwe, Shitanda, &
427 Karanja, 2007).

428 Overall, our results indicate that wide variation exists among tree tomato
429 accessions for composition traits. But, there are good prospects for the selection of tree
430 tomato with better organoleptic, nutritional, and functional quality. This may help in
431 developing the tree tomato as a commercially important crop.

432

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434

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437

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- 543

544 **Table 1**

545 Tree tomato accessions used for the present study and information on the cultivar group

546 they belong to and origin, including province or department and country.

Accession	Germplasm bank	Accession code in germplasm bank	Origin
<i>Orange</i>			
A16	UPV	ECU-1134	Morona Santiago (Ecuador)
A22	UTPL	ECUt-002	Azuay (Ecuador)
A29	UTPL	ECUt-008	Cotopaxi (Ecuador)
<i>Orange pointed</i>			
A17	UPV	ECU-1221	Azuay (Ecuador)
A19	UPV	ECU-1295	Carchi (Ecuador)
A23	UTPL	ECUt-003	Azuay (Ecuador)
A31	UPV	UNT-08	Lima (Peru)
A32	UPV	PT-087	Chachapoyas (Peru)
A33	UPV	PT-221	Cajamarca (Peru)
A34	UPV	PT242	Cajamarca (Peru)
A35	UPV	BOL-14	Cochabamba (Bolivia)
A36	UPV	BOL-116	Santa Cruz (Bolivia)
<i>Red</i>			
A18	UPV	ECU-1248	Tungurahua (Ecuador)
A24	UTPL	ECUt-004	Azuay (Ecuador)
A26	UTPL	ECUt-006	Tungurahua (Ecuador)
A27	UTPL	ECUt-007	Tungurahua (Ecuador)
<i>Red conical</i>			
A41	UTPL	ECUt-009	Saraguro (Ecuador)
<i>Purple</i>			
A21	UTPL	ECUt-001	Loja (Ecuador)
A25	UTPL	ECUt-005	Azuay (Ecuador)
A30	UPV	QB-54	Boyacá (Colombia)
A37	UPV	EUR-CY-1	Lisboa (Portugal)
A39	UPV	NZ-1	New Zealand
A40	UPV	NZ-2	New Zealand
<i>Wild (S. cajanumense)</i>			
A15	UTPL	ECUts-100	Loja (Ecuador)

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548

549

550 **Table 2**

551 Mean values, probability of the *F*-test for differences among accessions and least
 552 significant difference (LSD; *P*=0.05) for proximate composition traits measured in 24
 553 tree tomato accessions, and average values±SE for each of the four cultivar groups
 554 containing two or more accessions.

Cultivar group and accession	Moisture (g/100 g f.w.)	SSC (%)	TA (g/100 g f.w.)	Ratio SSC:TA	Protein (g/100 g d.m.)
<i>Orange</i>					
A16	86.1	11.2	1.50	7.48	7.13
A22	87.1	12.1	1.68	7.22	4.41
A29	86.2	11.2	1.52	7.40	6.88
Average	86.46±0.32	11.5±0.3	1.56±0.06	7.37±0.08	6.14±0.87
<i>Orange pointed</i>					
A17	86.2	11.0	1.24	8.88	7.41
A19	86.1	11.0	1.47	7.49	7.21
A23	86.2	11.7	1.54	7.64	6.82
A31	86.3	10.9	1.43	7.64	7.43
A32	86.2	12.0	1.31	9.15	6.23
A33	86.4	11.2	1.49	7.50	6.81
A34	86.1	11.1	1.40	7.92	5.60
A35	86.1	11.1	1.45	7.65	4.92
A36	86.3	11.0	1.41	7.84	6.13
Average	86.21±0.03	11.2±0.1	1.41±0.03	7.97±0.20	6.51±0.29
<i>Red</i>					
A18	---	---	---	---	6.31
A24	87.0	11.0	1.37	8.03	5.05
A26	86.9	11.1	1.39	8.01	---
A27	---	---	---	---	7.12
Average	86.95±0.03	11.1±0.1	1.38±0.01	8.02±0.01	6.16±0.60
<i>Red conical</i>					
A41	87.2	11.3	1.33	8.55	9.58
<i>Purple</i>					
A21	---	---	---	---	6.70
A25	87.1	11.1	1.29	8.61	6.53
A30	87.1	11.1	1.24	8.96	6.34
A37	87.4	11.9	1.55	7.73	7.26
A39	87.7	11.3	1.75	6.45	6.13
A40	---	---	---	---	7.15
Average	87.34±0.13	11.3±0.2	1.46±0.12	7.94±0.56	6.69±0.18
<i>Wild (S. cajanumense)</i>					
A15	93.2	12.5	0.70	18.78	4.89
Global mean	86.95±0.35	11.3±0.1	1.40±0.05	8.45±0.56	6.52±0.23
Prob. <i>F</i> -test	<0.001	<0.001	<0.001	<0.001	<0.001
LSD (<i>P</i> =0.05)	0.4	0.2	0.07	0.48	1.25

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 556

557 **Table 3**

558 Mean values, probability of the *F*-test for differences among accessions and least
 559 significant difference (LSD; P=0.05) for soluble sugars and organic acids measured in
 560 23 tree tomato accessions, and average values±SE for each of the four cultivar groups
 561 containing two or more accessions.

Cultivar group and accession	Soluble sugars				Organic acids			Ratio sugars: acids
	Glucose (g/100 g d.m.)	Fructose (g/100 g d.m.)	Sucrose (g/100 g d.m.)	Total (g/100 g d.m.)	Malic acid (g/100 g d.m.)	Citric acid (g/100 g d.m.)	Total (g/100 g d.m.)	
<i>Orange</i>								
A16	10.9	11.7	27.1	49.7	0.53	7.54	8.06	6.18
A22	10.9	10.9	27.0	48.8	0.74	7.28	8.02	6.20
A29	8.8	8.8	24.3	41.8	0.58	5.80	6.39	6.56
Average	10.1±0.7	10.4±0.9	26.1±0.9	46.8±2.5	0.61±0.06	6.87±0.54	7.49±0.55	6.31±0.12
<i>Orange pointed</i>								
A17	12.4	13.0	26.7	52.0	0.34	5.42	5.76	9.10
A19	11.1	13.2	26.9	51.2	0.78	5.88	6.65	8.23
A23	6.3	6.3	15.5	28.1	0.46	6.13	6.59	4.86
A31	9.1	8.9	21.2	39.2	0.59	4.92	5.51	7.16
A32	9.9	9.8	25.1	44.9	0.68	6.53	7.21	6.31
A33	8.9	8.8	19.8	37.5	0.72	6.91	7.63	5.07
A34	11.0	11.0	23.6	45.5	0.53	5.51	6.03	7.85
A35	9.7	9.8	25.6	45.1	0.43	6.54	6.97	6.54
A36	10.9	10.9	26.4	48.1	0.48	6.22	6.70	7.19
Average	9.9±0.6	10.2±0.7	23.4±1.3	43.5±2.5	0.56±0.04	6.01±0.21	6.56±0.23	6.92±0.47
<i>Red</i>								
A18	8.9	8.8	24.1	41.8	0.46	4.01	4.46	9.37
A24	10.2	9.9	24.0	44.2	0.99	5.98	6.97	6.43
A27	8.0	8.4	20.8	37.2	0.69	5.97	6.66	5.67
Average	9.0±0.6	9.0±0.5	23.0±1.1	41.1±2.0	0.71±0.15	5.32±0.66	6.03±0.79	7.16±1.13
<i>Red conical</i>								
A41	8.4	8.8	22.2	39.4	0.59	6.39	6.97	5.72
<i>Purple</i>								
A21	8.3	8.4	19.8	36.5	0.76	6.91	7.67	4.99
A25	9.4	10.1	23.1	42.6	0.74	5.50	6.24	6.90
A30	9.6	9.5	23.5	42.6	0.55	6.03	6.59	6.64
A37	9.0	9.0	23.6	41.6	0.88	6.38	7.25	5.74
A39	7.4	7.4	18.3	33.1	0.88	6.64	7.53	4.38
A40	9.5	9.6	25.8	44.8	0.53	6.56	7.10	6.32
Average	8.9±0.3	9.0±0.4	22.4±1.1	40.2±1.8	0.73±0.06	6.34±0.21	7.06±0.22	5.83±0.40
<i>Wild (S. cajanumense)</i>								
A15	2.0	1.1	25.6	28.6	2.11	4.51	6.62	4.52
Global mean	9.1±0.4	9.3±0.5	23.5±0.6	41.9±1.3	0.70±0.07	6.07±0.18	6.76±0.17	6.43±0.28
Prob. F-test	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD (P=0.05)	2.1	2.0	4.8	8.3	0.23	0.88	0.95	1.66

563 **Table 4**
564 Mean values, probability of the *F*-test for differences among accessions and least
565 significant difference (LSD; *P*=0.05) for pigments and antioxidants measured in 23 tree
566 tomato accessions, and average values±SE for each of the four cultivar groups
567 containing two or more accessions.

Cultivar group and accession	Chlorophylls			Total carotenoids (mg/100 g d.m.)	Total phenolics (g/100 g d.m.)	Antioxidant activity (μmol TE/g d.m.)
	Chlorophyll <i>a</i> (mg/100 g d.m.)	Chlorophyll <i>b</i> (mg/100 g d.m.)	Total (mg/100 g d.m.)			
<i>Orange</i>						
A16	0.40	0.78	1.18	4.11	5.26	34.2
A22	0.51	0.98	1.49	4.05	2.98	16.4
A29	0.25	0.87	1.12	3.68	4.24	28.2
Average	0.39±0.07	0.87±0.06	1.26±0.11	3.95±0.14	4.16±0.66	26.3±5.2
<i>Orange pointed</i>						
A17	0.52	1.25	1.77	4.60	3.69	15.1
A19	0.37	1.36	1.73	6.21	6.18	37.6
A23	0.68	1.36	2.04	3.30	2.84	12.2
A31	0.25	0.42	0.67	4.16	3.55	16.6
A32	0.26	0.66	0.92	4.48	4.15	25.3
A33	0.47	1.09	1.57	6.59	4.19	21.5
A34	0.19	0.71	0.90	3.08	2.98	17.6
A35	0.27	1.03	1.29	4.75	2.43	14.7
A36	0.38	1.08	1.47	5.16	2.54	13.2
Average	0.38±0.05	1.00±0.11	1.37±0.15	4.70±0.39	3.62±0.39	19.3±2.7
<i>Red</i>						
A18	0.56	1.32	1.88	4.66	4.39	23.4
A24	0.67	1.52	2.19	2.60	2.94	17.7
A27	0.46	0.93	1.38	4.53	3.91	20.9
Average	0.56±0.06	1.26±0.17	1.82±0.23	3.93±0.67	3.75±0.43	20.6±1.7
<i>Red conical</i>						
A41	0.56	1.84	2.40	7.35	3.65	23.6
<i>Purple</i>						
A21	0.97	2.27	3.24	4.80	3.66	17.7
A25	0.46	1.23	1.68	4.97	4.67	31.2
A30	0.54	1.00	1.54	3.84	2.82	15.4
A37	0.30	1.27	1.57	4.68	2.58	12.5
A39	0.59	1.63	2.22	6.14	4.05	29.8
A40	0.97	6.57	7.54	11.20	6.12	48.7
Average	0.64±0.11	2.33±0.87	2.97±0.95	5.94±1.09	3.98±0.53	25.9±5.5
<i>Wild (S. cajanumense)</i>						
A15	4.50	3.94	8.64	4.00	3.19	28.8
Global mean	0.66±0.18	1.53±0.27	2.19±0.41	4.91±0.37	3.78±0.22	22.7±1.9
Prob. <i>F</i> -test	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD (<i>P</i> =0.05)	0.38	0.59	0.98	1.43	0.78	6.1

568 **Table 5**

569 Mean values, probability of the *F*-test for differences among accessions and least
 570 significant difference (LSD; *P*=0.05) minerals traits measured in 23 tree tomato
 571 accessions, and average values±SE for each of the four cultivar groups containing two
 572 or more accessions.

Cultivar group and accession	P (mg/100 g d.m.)	K (mg/100 g d.m.)	Ca (mg/100 g d.m.)	Mg (mg/100 g d.m.)	Fe (mg/100 g d.m.)	Cu (mg/100 g d.m.)	Zn (mg/100 g d.m.)
<i>Orange</i>							
A16	173	2848	70	230	0.68	0.57	1.13
A22	106	2430	48	145	1.00	0.23	0.94
A29	93	2397	35	103	1.95	0.07	0.64
Average	124±25	2558±145	51±10	159±37	1.21±0.38	0.29±0.15	0.90±0.14
<i>Orange pointed</i>							
A17	125	2003	66	195	1.47	1.13	1.07
A19	160	2466	66	182	1.21	0.93	1.24
A23	108	2264	57	158	1.15	0.26	1.44
A31	57	1958	34	87	2.32	0.11	0.21
A32	118	2195	54	155	1.08	0.15	0.82
A33	115	2596	66	184	1.71	0.32	1.00
A34	130	2477	60	187	1.30	0.60	1.11
A35	142	2467	62	160	2.01	3.25	1.25
A36	112	2939	80	58	1.01	0.34	0.87
Average	118±9	2374±102	61±4	152±16	1.47±0.15	0.79±0.33	1.00±0.12
<i>Red</i>							
A18	133	2005	66	221	1.12	0.82	1.83
A24	118	2814	70	197	1.23	0.36	1.06
A27	70	2489	42	113	1.54	0.06	0.46
Average	107±19	2436±235	60±9	177±33	1.30±0.12	0.41±0.22	1.12±0.40
<i>Red conical</i>							
A41	226	1868	63	48	1.64	0.59	1.31
<i>Purple</i>							
A21	124	2203	53	214	1.18	0.63	0.71
A25	130	3211	78	149	1.00	0.28	1.11
A30	56	2231	16	124	2.43	0.07	0.35
A37	130	2975	73	59	1.13	0.70	0.91
A39	159	3114	65	60	1.36	0.52	1.04
A40	172	2998	44	54	1.75	0.54	1.13
Average	129±16	2789±184	55±9	110±26	1.47±0.22	0.46±0.10	0.88±0.12
<i>Wild (S. cajanumense)</i>							
A15	104	2805	117	161	0.75	0.58	0.64
Global mean	124±8	2511±82	60±4	141±12	1.39±0.10	0.57±0.14	0.97±0.08
Prob. F-test	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD (<i>P</i> =0.05)	33	384	25	51	0.76	0.37	0.47

573 Figure 1. Diagram showing the relationships among the 26 composition traits (above)
574 and among 18 accessions of cultivated tree tomato (*S. betaceum*) (below) based on the
575 two first principal components of PCA (26.1 and 19.2% of the total variation,
576 respectively). The different cultivar groups are represented in the accessions plot
577 (below) by different symbols: orange (open circle), orange pointed (filled circle), red
578 (open triangle), red conical (filled triangle), and purple (open square). Results are based
579 on the data obtained from 18 accessions of the cultivated tree tomato (*S. betaceum*) for
580 which data were available for all composition traits studied.
581