

1	Phenotypic diversity among local Spanish and foreign peach and
2	nectarine [Prunus persica (L.) Batsch] accessions
3	
4	Carolina Font i Forcada ^a , Thomas M. Gradziel ^b , Yolanda Gogorcena ^a , María
5	Angeles Moreno ^a *
6	^a Departamento de Pomología, Estación Experimental de Aula Dei (CSIC). Apdo.
7	13034, 50080 Zaragoza, Spain
8	^b Department of Plant Sciences, University of California, Davis, CA 95616, USA
9	*Corresponding author, E-mail: mmoreno@eead.csic.es, Tel: +34 976 71 61 36. Fax:
10	+34 976 71 61 45.
11	
12	

13 ABSTRACT

1	Δ
	_

15 Phenotypic data for tree and fruit characteristics was collected over three consecutive years from a 16 germplasm collection of 94 peach and nectarine accessions representing both traditional Spanish as well 17 as foreign cultivars with widespread global plantings. All accessions were grown at the Experimental 18 Station of Aula Dei (CSIC) located in the Ebro Valley (northern Spain, Zaragoza) under a Mediterranean 19 climate. Tree traits evaluated included bloom and harvest date, vigor, yield, yield efficiency and flower 20 and leaf characteristics. Fruit traits included fresh weight, firmness, soluble solids, titratable acidity, 21 levels of individual soluble sugars (sucrose, glucose, fructose and sorbitol), vitamin C, total phenolics, 22 flavonoids, anthocyanins, relative antioxidant capacity and ripening index. Extensive variability was 23 observed for most qualitative and quantitative traits with significant correlations identified between many 24 traits. While the traditional Spanish accessions demonstrated good adaptability to the northern Spain 25 evaluation site, opportunities for continued improvement in tree and fruit quality traits were demonstrated 26 by an extensive phenotypic variability within the germplasm collection.

- 27
- 28 Keywords: yield, fruit quality, sugars, antioxidant activity, vitamin C, trait correlations
- 29

30 Introduction

31

Peach [*Prunus persica* (L.) Batsch] is one of the most important tree fruit in global commerce. Within the economically important *Rosaceae*, it ranks behind only apples and pears. Peach is also the fruit species with the largest number of commercial cultivars, representing a diverse international germplasm. In recent years, peach production has doubled as a result of the introduction of improved cultivars and rootstocks along with improved cultural techniques. World production has increased from 11.4 million tons in 1995 to more than 20.2 million tons in 2010 (FAOSTAT 2012). The largest producer is China, followed by Italy, Spain, and United States.

39

40 Many peach breeding programs are currently pursuing improved fruit quality and productivity within 41 locally adapted germplasm (Monet and Bassi 2008; Byrne et al. 2012). Initial breeding goals include 42 improved external fruit quality, postharvest life, and disease/pest resistance, as well as a greater range of 43 fruit maturities and types (Byrne 2005). More recently, improved fruit eating quality including nutritional 44 composition, has also been targeted. Early results indicate that important tree and fruit quality parameters 45 may not be independent of each other (Cantín et al. 2010; Abidi et al. 2011; Font i Forcada et al. 2012) as 46 might be anticipated owing to their complex genetic and physiological control. Genetic control of traits 47 affecting plant growth and architecture, yield, blooming and harvesting time are usually quantitative 48 (Dirlewanger et al. 1999). Fruit size is reported to be a polygenic trait with a low to moderate heritability 49 (Souza et al. 1998) and so largely affected by environmental conditions, plant nutrition, and cultural 50 practices. Fruit texture (melting vs. non-melting) is largely determined by the multi-allelic F locus (Lester 51 et al. 1996). Both color and acidity levels in peach fruit are reportedly controlled by qualitative genes 52 (Souza et al. 1998). Total soluble solids concentration (SSC) reportedly has a moderate heritability, which 53 may be sufficient to allow steady improvement of fruit sugar levels in spite of the variations caused by 54 environmental, maturity and production differences among regions and years (Cantín et al. 2009a).

55

More recently, the biochemical components of peach as well as several other fruits have received greater attention because of their potential health benefits (Prior and Cao 2000). The major soluble sugars in peach are sucrose followed by glucose and fructose, with lower levels of sorbitol (Brooks et al. 1993). In ripe fruit, these sugars comprise about 60% of the SSC (Cantín et al. 2009a). Glucose and fructose 60 concentrations show a continuous increase during fruit development, while sucrose accumulates primarily 61 during maturation (Hancock 1999). Both sucrose and fructose have been shown to have beneficial effects 62 on gastrointestinal health (Muir et al. 2009) while sorbitol can be used as a glucose substitute for diabetics 63 (Forni et al. 1992). Fructose is perceived to be between 1.75 and 1.8 times sweeter than sucrose (Doty 64 1976) while glucose is reported to be perceived as less sweet than sucrose (Yamaguchi et al. 1970). 65 Because most previous breeding efforts targeted improved yield and vigor (Byrne et al. 2012), the fruit 66 nutrient composition as well as variability among cultivars remains poorly understood.

67

68 Peach fruits are also a rich source for antioxidant compounds (Tomás-Barberán and Robins 1997). 69 Phenolic compounds are a major source of antioxidants (Gil et al. 2002) and appear to be under strong 70 genetic control (Gil et al. 2002; Cevallos-Casals et al. 2006). Phenolics have also been found to be natural 71 antimicrobial agents for increasing the shelf life of fresh fruit while inhibiting the growth of pathogenic 72 microorganisms (Bowles and Juneja 1998). Flavonoids and anthocyanins also show strong antioxidant 73 capacity (Wang et al. 1997). Antioxidant capacity to neutralize free radicals appears important for 74 protection against certain diseases, such as heart or vascular diseases and cancer. While peach has a lower 75 antioxidant capacity compared with other fruit types such as strawberry, kiwifruit, orange or apple, it is 76 one of the few tree fruits available during spring and summer and so becomes an important contributor to 77 human diets during this period (Besco et al. 2007).

78

The Spanish peach industry has traditionally been based on non-melting, clingstone and yellow flesh peach cultivars. Recently, the supplementation of traditional Spanish peaches with cultivars developed in other countries has introduced the melting- freestone and nectarine types (Badenes et al. 1998). Unfortunately, peach genetic diversity has been shown to be relatively low within the foreign cultivars since most share a common and recent ancestry (Aranzana et al. 2003).

84

85 In the present work, a diverse peach germplasm is evaluated, including traditional Spanish accessions as 86 well as cultivars with extensive commercial plantings in other growing regions. Tree and fruit quality for 87 these 94 peach and nectarine accessions have been characterized and associations between traits 88 evaluated.

90 Materials and methods

91

92 Plant material and field trials

93

94 A total of 94 peach and nectarine accessions from the peach germplasm collection at the 'Experimental 95 Station of Aula Dei' (CSIC) were evaluated (Table 1). This set includes 43 native local Spanish 96 accessions and 51 foreign ones mostly from U.S. programs, but also from France, Italy, New Zealand and 97 South Africa (Font i Forcada et al. 2013). All accessions were budded onto the 'Pollizo' plum rootstock 98 'Adesoto' (Moreno et al. 1995) and established in an experimental orchard (three trees per genotype) in 99 the winter of 2004-2005. Most accessions are non-melting, clingstone and yellow flesh peaches. Among 100 them, only 7 out of 94 accessions were nectarines, 4 had white flesh, 10 had melting flesh and 5 were 101 freestone.

102

103 The germplasm collection was located in the Ebro Valley (Northeast Spain, Zaragoza), and grown under a 104 Mediterranean climate, on a heavy and calcareous soil with 27% total calcium carbonate, 8% active lime, 105 water pH 8.3, and a clay-loam texture. Standard commercial practices were used for fertilization, 106 irrigation, pest and disease control, spring thinning and winter pruning. Trees were hand-thinned at 45-50 107 days after full bloom (DAFB) leaving approximately 20 cm between fruits. Open vase trees were pruned 108 to strengthen existing scaffold branches and eliminate vigorous shoots inside and outside the vase that 109 would compete with selected scaffolds or shade fruiting wood. The plot was level-basin irrigated every 12 110 days during the summer. Most vegetative and fruit quality traits have been evaluated over four 111 consecutive years (2008-2011).

112

113 Tree and fruit quality characterization

114

Blooming date was recorded for each accession according to Baggiolini (1952). The average date for
bloom beginning (E stage), full bloom (F stage) and bloom end (G stage) was scored for each accession.
The mean harvest date was also calculated for each accession. Harvest date ranged from late-June to lateOctober.

Agronomic traits including trunk cross-sectional area (TCSA), yield, annual yield efficiency and fruit weight were evaluated. Trunk girths were measured during the dormant season at 20 cm above the graft union, and TCSA was calculated. At harvest, all fruits from each tree (three single-tree replications for each accession) were counted and weighed to determine total yield per tree (Kg/tree) and mean fruit weight. For the final two years (2010-2011), data was recorded for cumulative yield per tree and annual yield efficiency (cumulative yield in kilograms per final tree TCSA).

126

Other traits including leaf gland type (reniform/globose) and flower type (showy/non-showy) were measured directly in the field, while fruit type (peach/nectarine), flesh color (yellow/white), flesh type (melting/non-melting), and stone type (clingstone/freestone) were determined in the laboratory immediately after harvest.

131

132 From 2008 to 2011, twenty mature fruits per accession and per year were harvested at commercial 133 maturity. Fruits were harvested when ground green color turned to yellow. Fruit samples were randomly 134 harvested by a single person to maintain a consistent maturity standard. Basic quality traits such as flesh 135 firmness (FF), soluble solids content (SSC), titratable acidity (TA) and ripening index (RI) were then 136 evaluated. Flesh values of L* (brightness or lightness), a^* (- a^* = greenness, + a^* = redness), b^* (- b^* = 137 blueness, $+b^* =$ yellowness), C* (chroma) and H (lightness's angle) were measured using a colorimeter 138 (Chroma Meter, CR-400 Konica Minolta, Japan). Flesh firmness was measured using a penetrometer 139 (Model FT-327) on both cheek-sides of each fruit after removing 1 mm thick disk of skin, with an 8 mm 140 diameter probe. SSC was measured with a digital refractometer (Atago PR-101, Tokyo, Japan). TA and 141 pH were determined using an automatic titration system (Metrohm Ion analysis, 807 Dosing Unit, 142 Switzerland) with NaOH titrated to pH end-point of 8.1. RI was calculated based on SSC/TA ratio.

143

Sugars, total phenolics, flavonoids, anthocyanins, relative antioxidant capacity (RAC) and vitamin C were evaluated for the three final years (2009-2011). Fruits were peeled and cut longitudinally into two halves and a portion of the mesocarp was removed from each half and cut into small pieces. For each analysis, a composite sample of 5 g was obtained by mixing all pieces from the selected fruits. This sample was frozen in liquid nitrogen and kept at -20°C until analyzed.

150 For analysis of sugars content, samples were homogenized with 10 mL of extraction solution consisting 151 of 800 mL/L ethanol/Milli-Q water, using an Ultra-Turrax homogenizer (IKA Works, Inc., Wilmington). 152 A sample of 250 µL of the homogenized extract was incubated at 80°C for 20 min in 200 µL of 800 mL/L 153 ethanol/water, with 5 g/L manitol added as an internal standard. Samples were purified using ion 154 exchange resins (Bio-Rad Barcelona, Spain) as reported by Moing et al. (1992). Samples were then 155 vacuum concentrated and then re-suspended to 1 mL of Milli-Q water, before High Performance Liquid 156 Chromatography (HPLC) analysis. Then, sucrose, glucose, fructose and sorbitol were analyzed by HPLC 157 (Aminex HPX-87C column, 300 mm x 7.8 mm; Bio-Rad, Barcelona, Spain) with a refractive index 158 detector (Waters 2410) as previously reported (Cantín et al. 2009a). PC Millenium 3.2 software (Waters) 159 was used to perform sugar quantification. Standard calibration curves were used to quantify each different 160 sugar and the concentrations were expressed as g per kg of fresh weight (FW).

161

162 Samples for vitamin C determination were kept at -20°C in metaphosphoric solution (5% HPO₃) until 163 analysis for preservation of oxidation. For analysis of antioxidant compounds, samples were 164 homogenized with 10 mL of extraction solution consisting of 0.5 N HCl in methanol/Mili-Q water (80% 165 v/v). Vitamin C and antioxidant compounds were analyzed using a spectrophotometer photodiode array 166 detector DU 800 (Beckman Coulter, Inc., Fullerton, CA) as described by Cantín et al. (2009b). 167 Absorbance for vitamin C was determined at 525 nm and the results were expressed as mg of ascorbic 168 acid (AsA) per 100 g of FW. The Folin-Ciocalteau reagent at 0.25 N was used to determine the total 169 phenolics content, and the absorbance was measured at 725 nm and the results were expressed as mg of 170 Gallic acid equivalents (GAE) per 100 g FW. The flavonoid content absorbance was measured at 510 nm 171 and the results were expressed as mg of catechin equivalents per 100 g of FW. For determining 172 anthocyanin content, spectrophotometric readings at 535 nm were taken subtracting absorbance at 700 nm 173 (due to turbidity) and the results were expressed as mg of cyanidin 3-glucoside equivalents (C3GE) per 174 kg of FW (using a molecular weight of 494 and a molar extinction absorptivity coefficient $\varepsilon = 25,965/cm$ 175 M). The relative antioxidant capacity (RAC) was determined using the 1,1-diphenyl-2-picrylhydrazyl 176 (DPPH) and the absorbance was measured at 515 nm and the results were expressed as µg of Trolox 177 equivalents per g of FW.

178

179 Statistical analysis

180

181 All statistical analyses were performed with the program SPSS 19.0 (SPSS, Inc, Chicago, USA). When 182 comparing two different fruit types (yellow and white flesh; peach vs. nectarine, non-melting vs. melting 183 flesh, clingstone vs. freestone flesh, reniform vs. globose leaf, showy vs. non-showy flower) or accessions 184 origin (local Spanish vs. foreign) a t test ($P \le 0.05$) was used. Correlations using Pearson correlation 185 coefficient at $P \le 0.05$ were calculated between traits to reveal possible associations among data based on 186 the average of three trees per accession and year over the three years. Principal components analysis 187 (PCA) was used to study associations among traits. A 2D PCA plot was designed using combined data 188 from three years of the study using the program Unscrambler version 9.6 program package (CamoASA, 189 2001). 190

191 Results

192

193 Accession influence and phenotypic evaluation

194

Extensive phenotypic variation was found for all parameters studied. Ranges and means for tree and fruit
quality traits are shown in Table 2. ANOVA results showed that accessions influenced SSC, TA, RI,
glucose, fructose, sorbitol and RAC.

198

In this study, full bloom date was mainly recorded in the second half of March (from 79 to 87 Julian days). The earliest accessions to bloom were the nectarines 'Big Top' and 'Fantasia', and the peach accessions 'Shasta' and 'Stanford' (approximately 80 JD). The latest accessions to reach full bloom included 'Amarillo Calanda 131 AD' and 'Oropel' (approximately 87 JD). The earliest accessions to be harvested (181 JD, late June) were 'Maria Serena' and 'Super Crimson Gold'. In contrast, the traditional Spanish accessions from the Ebro Valley (Northeast Spain) 'Alcañiz 1', 'Bonet V' and 'Calanda Tardío' were harvested in late October (approximately 272-274 JD).

206

Vigor of trees was estimated based on TCSA (cm²). Among accessions, 'Bonet III' and 'Paloro B' had the highest values (approximately 280 cm²), while 'Fortuna' (48 ± 9.2 cm²) and 'Shasta' (44 ± 12 cm²) showed the lowest values. The mean value for yield among all accessions was 14.2 kg/tree, but a high 210 variability was also found. 'Lovell' (47±3.2)', 'Sudanell GF 2804 AD' (43±5.6) and 'GF3' (32±3.5) 211 showed the highest yields. Mean value for annual yield efficiency was 0.30 kg/cm², with 'Lovell' having 212 the highest values (1.31±0.08), and 'Sudanell GF 2804 AD' (0.69±0.02) and 'Suncling' (0.66±0.03) 213 showing intermediate values. Fruit weight varied greatly among accessions with a range of 64 to 315 g. 214 Among them, 'Alejandro Dumas' (315±15) and 'Baby Gold 6' (312±18.5) showed the higher fruit weight 215 values, 'Klamt' (233±15) and 'Lovell' (223±15) had intermediate values, while 'Benasque' (64±15), 216 'Diamante Amarillo' (102±12), 'Nectar del Jalón (114±10) and 'Super Crimson Gold' (129±13) 217 presented lower ones.

218

219 Firmness, SSC, TA and RI, greatly varied among accessions. A range of 9 to 61 N was found for 220 firmness. SSC varied from 12 to 18 °Brix, TA ranged from 0.4 to 0.9 g malic acid/ 100 g FW while 221 SSC/TA ranged from 15 to 67. The non-melting native Spanish peaches 'Borracho de Jarque' (61±1.5N), 222 'Amarillo Calanda 131 AD' (58±0.5N), 'Bonet III' (56±3.2N), 'Calanda Tardío' (55±2.5N) and 'Sudanell 223 1' ($52\pm4.3N$), as well as the commercial accessions 'Keimoes' ($54\pm1.2N$), 'Lovell' ($52\pm1.3N$) and 224 'Vivian' (52±1.3N) presented the highest fruit firmness. In contrast, the white flesh peach 'Benasque' 225 $(17\pm1.5N)$ (a seedling peach rootstock), and the nectarines 'Fantasia' ($9\pm1.2N$) and 'Super Crimson Gold' 226 $(17\pm1N)$ showed the lowest levels of firmness.

227

228 For SSC, the native non-melting peaches 'Bonet I', 'Bonet III', 'Borracho de Jarque', 'Rojo del Rito', and 229 'Sudanell 1' demonstrated the highest °Brix (~18), along with the non-native accessions 'Nuevo' (~18), 230 'Golden Queen', 'Halford', 'Paloro A', 'Oropel' and 'Vivian' (~17 °Brix). In contrast, the melting 231 nectarine 'Queen Giant' and the melting peach 'Redhaven' showed the lowest values (~12 °Brix). For 232 fruit acidity, 'Maria Serena' and 'Tebana' showed the lowest acidity (~0.4 g malic acid/ 100 g FW) based 233 on TA, followed by the native non-melting clingstone Spanish peaches 'Alcañiz 2', 'Borracho de Jarque', 234 'Calabacero', 'Calanda San Miguel', 'Fraga', 'Goiri', 'Jerónimo de Alfaro', and 'Zaragozano Rojo', and 235 the commercial yellow peaches 'Andross', 'Babygold 6', 'Babygold 9', 'Carson', 'Dixon', 'Stanford', 236 and 'Suncling' (~0.5). 'Andora', 'Calanda Tardío' and 'Paloro B' presented the highest content of TA 237 (~0.9). Among accessions, the native local Spanish accession 'Borracho de Jarque' showed the highest RI 238 value at 67±2.3. Intermediate RI values were observed for 'Maria Serena' (~36), 'Alcañiz 2', 'Nuevo'

and 'Tebana' (~33), 'Dixon' (~32), and 'Andross', 'Bonet I' and 'Rojo del Rito' (~31). 'Andora'
(15±0.5) and 'Queen Giant' (17±0.8) showed the lowest RI values.

241

242 Sucrose was the major sugar present in peach fruit. Total sugars varied from 63 to 136 g/kg FW. The 243 local Spanish accessions 'Bonet III' (136±5.6), 'Calabacero' and 'Calanda San Miguel' (~134) showed 244 the higher contents, while the nectarines 'Super Crimson Gold' (80.9±10.9) and 'Venus' (71.5±12.5), as 245 well as the peaches 'Alcañiz 1' (75.5±10.2) and 'Amarillo Calanda 131 AD' (63±15.3) showed the lowest 246 levels. Sucrose content values varied from 35 to 98 g/kg FW with 'Calabacero' (98±9.1), 'Jungerman' 247 (93 ± 5.3) and 'Diamante Amarillo' (90 ± 2.4) showing the highest contents. Glucose values varied from 4 248 to 15 g/kg FW, with 'Babygold 9' and 'Bonet IV' (~15) and 'Calabacero' and 'Fantasia' (~14) presenting 249 the higher contents. Fructose varied from 2 to 14 g/kg FW, with 'Amarillo Calanda 2400 AD', 'Babygold 250 9', 'Bonet IV', 'Calabacero', 'Fantasia', 'Infanta Isabel', and 'Venus' showing the higher fructose 251 contents (~14). Finally, sorbitol varied from 2 to 35 g/kg FW and two native accessions, 'Bonet III' 252 (35 ± 5.3) and 'Rojo del Rito' (31 ± 4.8) , followed by the commercial accession 'Vivian' (27.4 ± 2.5) showed 253 the highest levels.

254

255 Phytochemical compounds also showed a wide variability (Table 2). Vitamin C ranged from 3 to 28 mg 256 ASA/100 g FW, with 'Shasta' showing the highest value (27.8±1.9), followed by native Spanish peaches 257 'Alcañiz 2' (~20) and 'Goiri' (~19). Total phenolics, as determined by the Folin-Ciocalteau assay, varied 258 among accessions from 18 to 62 mg of GAE /100 g of FW, with the native peach 'Alcañiz 1' having the 259 highest values for phenolic contents (62±2.8), followed by other Spanish peaches including 'Amarillo 260 Calanda 131 AD', 'Calanda San Miguel' and 'Miraflores' (~52) and the non-native accessions 'Golden 261 Queen', 'Nuevo', 'Paloro B' and 'Vivian' (~49). Flavonoid content ranged from 3 to 63 mg of CE per 262 100 g of FW. Among accessions, 'Nuevo' (63±5.6), 'Alcañiz 2' (60±2.5), 'Amarillo Calanda 131 AD' 263 (57±2.5) and 'Zaragozano Amarillo' (56±1.6) showed the highest values. Total anthocyanins varied 264 among accessions (0.7 to 12 mg of cyaniding 3-glucoside equivalents (C3GE) per kg of FW) depending 265 on the percentage of red pigmentation of the flesh. Accessions with red mesocarp flesh, such as 'Flavortop' (12±0.9), 'Rojo del Rito' (10±4), 'Amarillo de Gallur', 'Brasileño' and 'Vivian' (~8), and 266 267 'Borracho de Jarque' and 'Fantasia' (~7) had higher anthocyanins content than accessions with pure 268 yellow flesh, such as 'Andora' (0.7±0.06), 'Goiri' (0.8±0.01) and 'Maria Serena' (0.9±0.01). Relative antioxidant capacity (RAC) varied from 186 to 1184 µg TE/g FW with the native Spanish accessions
'Alcañiz 2', 'Amarillo Calanda 131 AD', 'Bonet III' and 'Zaragozano Amarillo' showing the highest
values (between 1130 and 1184 µg TE/g FW), followed by other native peaches 'Amarillo de Gallur',
'Benasque', 'Bonet IV', 'Calanda Tardío', 'Fraga', 'Sudanell 1', 'Sudanell Blanco', 'Tipo Campiel' and
the non-native commercial accessions 'Golden Queen', 'Gomes', 'Halford', 'Kakamas', 'Nuevo', 'Paloro
A', 'Paloro B' and 'Vivian' (between 1000 and 1130). In contrast, 'Big Top', 'Maria Serena' and 'Venus'
showed lower content on RAC (between 180 and 400 µg TE/g FW).

276

277 Influence of tree and fruit traits on several fruit quality traits

278

279 Significant fruit quality and phytochemical differences were found among accessions with different tree280 and fruit characteristics (Tables 3, 4, 5).

281

282 Local Spanish and yellow flesh accessions had higher fruit weights than foreign and white flesh 283 accessions, respectively (Table 3). Firmness was lower for melting flesh accessions compared to the non-284 melting ones, as well as for white flesh compared to yellow flesh. The SSC was higher for foreign 285 peaches when compared to local Spanish peaches as well as nectarines. Significantly higher TA was 286 observed for nectarine, white and melting flesh and freestone fruits. On the other hand, peaches showed 287 higher RI than nectarines due to their reported higher SSC. Foreign and peach-type cultivars had higher 288 L*, b*, C* (chroma) and H (lightness's angle) values than local Spanish and nectarine accessions, 289 respectively (Table 4). In general, peach and foreign accessions had significantly higher content on 290 sorbitol, total sugars, vitamin C, phenolics, flavonoids and RAC when compared to nectarines and local 291 Spanish accessions, respectively (Table 5). Also, clingstone accessions generally had higher sucrose and 292 total sugars than freestone accessions.

293

294 Correlations between traits

295

The Pearson's correlation coefficients between pairs of traits are shown in Table 6. Harvesting date showed significant and positive correlations with bloom date, fruit weight, SSC, sucrose, fructose, sorbitol, total sugars, phenolics, flavonoids and RAC contents. When fruits are harvested later, they are, in general, larger and with higher SSC. In contrast, harvest date was negatively correlated with flesh firmness. Annual yield efficiency was also positively correlated with fruit weight but negatively correlated with fructose.

302

303 Significant positive correlations were also found between fruit weight and SSC, TA, glucose, fructose and 304 total sugars, between fruit weight and phenolics, flavonoids and RAC, and between SSC and phenolics, 305 flavonoids and RAC. A significant negative correlation was found between flesh firmness and ripening 306 index, sucrose, glucose, fructose, sorbitol, total sugars, phenolics and flavonoids. However, a significant 307 positive correlation was found between flesh firmness and TA and SSC. High and significant correlations 308 were found between individual and total sugars, and between total sugars and phytochemical compounds. 309 Other important positive and significant correlations were found between vitamin C and RAC, between 310 phenolics and both flavonoids and RAC, as well as between flavonoids and RAC.

311

312 Principal components analysis

313

314 The principal components analysis (PCA) can help to determine the accessions with better quality 315 performance. The results for the 21 tree and fruit traits are presented in Figure 1 and Table 7. PCA 316 analysis showed that more than 54% of the observed variance could be explained by the first two 317 components. The PC1 and PC2 axes explained 35.2% and 19.1% of total variability, respectively. This 318 biplot showed a clear separation between fruit quality traits, agronomic traits, and antioxidant 319 compounds. PC1 represents mainly harvest date, SSC, FF, sucrose, sorbitol, total sugars, vitamin C, 320 phenolics, flavonoids, RAC and C* (chroma color). PC2 explains mainly yield efficiency, SSC, TA, 321 glucose, fructose, anthocyanins and the rest of the components of the color. Accessions on the PC2 322 loadings suggested that separation on this component was mainly due to some basic fruit parameters such 323 as TA, SSC, as well as total and individual (glucose, fructose, sorbitol) sugars content and anthocyanins.

324

An examination of PC1 loadings suggested that accessions in the positive side had in general higher value on yield efficiency and were, in general, less acid, less firmness, and accumulated less sugars than those on the negative side. Accessions on the PC2 loadings suggested that separation on this component was mainly due to some basic fruit parameters such as TA, flesh firmness, SSC, as well as total and individual
 sugars content and anthocyanins, phenolics and flavonoids.

330

331 Discussion

332

333 Extensive phenotypic variation was found for all parameters studied. It had previously been demonstrated 334 that levels of some quality traits in peach fruit differ among rootstocks or cultivars (Colaric et al. 2005; 335 Orazem et al. 2011). In this study, full bloom date was mainly recorded in the second half of March and 336 the latest bloom dates were recorded at approximately 87 JD. The earliest accessions to be harvested were 337 recorded in late June, and in contrast, the latest accessions were harvested in late October. Early blooming 338 is a desirable character in Mediterranean areas in order to obtain the earliest possible yields (George and 339 Nissen 1992) though it increases the risk that spring frosts may reduce production in some years. Bloom 340 and harvest traits have been established as quantitatively inherited in peach and other Prunus species 341 (Dirlewanger et al. 1999). The peach fruit development period is highly dependent on cultivar (Mounzer 342 et al. 2008). Nevertheless, bloom and harvest date may change every year depending on the 343 environmental conditions, especially temperature (Mounzer et al. 2008) making it particularly vulnerable 344 to climate change. The values of vigor, yield, annual yield efficiency and fruit weight varied among 345 accessions. Yield depends on the genetic background of the cultivar (density of flower buds and flowers, 346 fruit set, fruit size) and on agronomic and environmental factors (Milatović et al. 2010). Yield and fruit 347 weight are also known to be quantitatively inherited (Dirlewanger et al. 1999). Also, the fruit quality 348 parameters, such as firmness, SSC, TA and RI, greatly varied among accessions. A range of 9 to 61 N 349 was found for firmness, showing that the maximum level of fruit firmness for marketing fresh peaches 350 and nectarines is 63.7 N (Commission Regulation EC, No.1861/2004 of 28 October 2004). SSC varied 351 from 12 to 18 °Brix with a minimum value of SSC for consumer acceptance reported to be 10 °Brix 352 (Kader 1999). The values of TA and SSC/TA were within the range reported by other peach studies 353 (Cantín et al. 2010; Abidi et al. 2011). RI is a major organoleptic quality trait of mature fruit in peaches 354 (Bassi and Selli 1990) which depends on the SSC/TA ratio.

355

Regarding individual and total sugars, a large and significant range of values were reported. For example, the content of total sugars varied from 63 to 136 g/kg FW, for sucrose the content values varied from 35 358 to 98 g/kg FW, and for sorbitol the content values varied from 2 to 35 g/kg FW. Sucrose, glucose, 359 fructose and sorbitol play an important role in peach flavor quality (Robertson et al. 1988). Also, sorbitol 360 showed the highest association with peach aroma and taste among carbohydrates and organic acids 361 (Colaric et al. 2005). Values for sucrose, glucose, fructose, sorbitol, and total sugars are within the range 362 reported by other authors (Yoshida 1970; Cantín et al. 2009a; Abidi et al. 2011). Likewise, phytochemical 363 compounds showed a wide variability among accessions. The contents of vitamin C, phenolics, 364 flavonoids, anthocyanins and relative antioxidant capacity were evaluated. Results support peach fruit as 365 a good source of vitamin C, emphasizing its importance in the evaluation of commercial peach 366 accessions. Others studies showed similar values for vitamin C (Gil et al. 2002). The range recorded for 367 total phenolics (from 18 to 62 mg of GAE /100 g of FW) were within the range reported in the literature 368 by other authors (Tavarini et al. 2008; Cantín et al. 2009b; Abidi et al. 2011). Similarly, the content of 369 flavonoids, anthocyanins and RAC found in our study agrees with others studies in peach (Tomás-370 Barberán et al. 2001; Gil et al. 2002; Cevallos-Casals et al. 2006; Abidi et al. 2011). The health value of 371 high fruit flavonoids has been summarized by Vauzour et al. (2008).

372

373 Concerning the influence of tree traits on several fruit quality traits, foreign peach and nectarine 374 accessions showed lower average yield and yield efficiency than native peaches as would be expected 375 because of extensive local Spanish selection for natives. Several non-native accessions, however, showed 376 very high yield and yield efficiencies, indicating substantial opportunities for the continued genetic 377 improvement of local Spanish peaches (for example: 'Baby Gold 5', 'GF3' or 'Lovell'). Different results 378 were obtained concerning flesh color and firmness. Firmness was lower for melting flesh accessions 379 compared to the non-melting ones, as well as for white flesh compared to yellow flesh, in agreement with 380 Crisosto et al. (2001) and Cantín et al. (2010). In general, peach-type fruit and foreign accessions had 381 significantly higher content on phytochemical compounds than nectarine-typess and local Spanish 382 accessions, and clingstone accessions generally had higher sucrose and total sugars than freestone 383 accessions. In contrast, Cantín et al. (2010) reported that nectarine-white flesh fruits and freestone 384 genotypes had higher contents of sucrose, glucose and fructose than yellow fleshed peaches. These 385 differences were probably due to the smaller number of nectarine and white flesh accessions in this study. 386 Fructose content and yellow/white flesh reportedly are co-localized to the same QTL in LG1, which 387 might explain the linked segregation of these two traits (Bliss et al. 2002; Quilot et al. 2004). In addition,

388

control of sucrose and glucose content has been reported on LG4, near the F-locus controlling fruit

389 texture (melting vs. non-melting) and stone adhesion (clingstone vs. freestone) (Quilot et al. 2004).

390

391 On the other hand, the significant and positive correlations found between some agronomic and fruit 392 quality parameters such as harvesting date, fruit weight and SSC, are in agreement with previous reports 393 (Dirlewanger et al. 1999; Cantín et al. 2010). These results showed that when fruits have later harvests, 394 they are, in general, larger and with higher SSC. The significant positive or negative correlations between 395 yield, annual yield efficiency, fruit weight, TA, fructose, sorbitol and fructose suggest that yield increases 396 with fruit weight but several sugars decreases as consequence of higher crop loads inducing lower fruit 397 total sugar content, possibly a result of sink competition among fruits as reported by Morandi (2008). As 398 expected, significant positive correlations between fruit weight and SSC, TA, glucose, fructose and total 399 sugars were found, since the amount of translocated carbohydrates determines fruit growth rate (Morandi 400 2008). The co-location of QTLs for sucrose, fructose and sorbitol (Dirlewanger et al. 1999) along with 401 possible pleiotropic effects, could partly explain these results. Also, significant positive correlations were 402 found between fruit weight and phenolics, flavonoids and RAC, and between SSC and phenolics, 403 flavonoids and RAC in agreement with different studies in peach (Cantín et al. 2009b; Abidi et al. 2011) 404 and in other species including plums (Díaz-Mula et al. 2008), apricots (Bureau et al. 2009) and sweet 405 cherries (Serrano et al. 2005). In addition, DeJong (1999) has shown that sufficient accumulation of 406 sugars in or near the fruit is essential for subsequent phenolics compounds synthesis. The different 407 positive or negative significant correlations found between flesh firmness, ripening index, individual and 408 total sugars, phenolics, flavonoids, TA and SSC suggests that softer fruit is associated with lower acidity 409 in agreement with Byrne et al. (1991) and Cantín et al. (2010). The positive relationship between firmness 410 and SSC has also been reported in sweet cherry (Jiménez et al. 2004) suggesting that, at the same level of 411 ripening, firmer fruits show a tendency to have higher SSC. The significant and positive correlations 412 found between individual and total sugars have been reported (Dirlewanger et al. 1999; Cantín et al. 413 2009a; Abidi et al. 2011). Among individual sugars, the highest correlation was found between glucose 414 and fructose as previously reported (Dirlewanger et al. 1999; Cantín et al. 2009a). Moreover, the 415 significant and positive correlations between individual and total sugars with phytochemical compounds 416 agree with the study reported in cherries by Pirie and Mullins (1977), due to the role of sugars in the 417 regulation of phenolic biosynthesis. Finally, the significant and positive correlations found between the

418 phytochemical traits demonstrate the importance of these bioactive compounds for antioxidant activity in

```
419 peaches, in agreement with the findings of Cantín et al. (2009a; 2009b) and Abidi et al. (2011).
```

420

421 A set of accessions with specific fruit quality values could be identified through PCA analysis. These 422 results confirmed the higher contents for vitamin C and RAC (positive side of PC2) for the local Spanish 423 accession 'Rojo del Rito' and the non-native cultivar 'Vivian'. These accessions could be a good source 424 of vitamin C with stronger antioxidant activity. Regarding individuals sugars, 'Keimoes', 'Golden Queen' 425 and 'Walgant' showed high sucrose content, and 'Borracho de Jarque' showed high fructose content. In 426 addition, local accessions such as 'Borracho de Jarque' and 'Calabacero', as well as 'Gomes' and 427 'Kakamas' showed higher SSC, individual and total sugars content. On the other hand, 'Campiel Rojo' 428 and 'Zaragozano' located on the positive side of PC2, and showed high fruit weight. Finally, for 429 agronomical parameters, 'Alcañiz 1', 'Carson' and 'Stanford' accessions showed higher yield efficiency, 430 probably because of their lower vigor and a stronger sink competition of fruit versus vegetative growth, as 431 previously reported by Font i Forcada et al. (2012). In other PCA studies (Cantín et al. 2010; Reig et al. 432 2013), different distribution of traits were found, probably due to different characteristics and fruit types 433 in the plant material studied. In the present work, old local Spanish accessions or others coming from very 434 early peach breeding programs were included in the analysis. In contrast, other studies analyzed 435 genotypes currently under selection or more recently released cultivars from current breeding programs 436 (Cantín et al. 2010; Reig et al. 2013). Ongoing changes in market preferences (and so breeding emphasis) 437 for fruit quality, including nutritional and antioxidant value have been well documented in recent reviews 438 (Bassi and Selli 1990; Byrne 2005; Byrne et al. 2012).

439

440 Conclusion

441

442 Considerable variation was found in sampled peach and nectarine germplasm for all traits studied. The 443 wide variability in tree and fruit traits suggests sufficient genetic opportunities exist for continued 444 breeding progress to satisfy evolving market and consumer demands. Results also demonstrate the value 445 of traditional and well adapted germplasm as a foundation for future tree and fruit quality improvement 446 through genetic recombination.

448 Acknowledgments

450 We thank R. Giménez, E. Sierra, S. Segura and N. Miguel for technica	l assistance and pl	lant management
--	---------------------	-----------------

- 451 in the field. We gratefully acknowledge S. Jiménez and G. Reig for statistical analysis. This study was
- 452 funded by the Spanish Ministry of Science and Innovation (MICINN) grants AGL2005-05533,
- 453 AGL2008-00283 and AGL2011-24576, and RFP 2009-00016 cofunded by FEDER and the Regional
- 454 Government of Aragon (A44). C. Font was supported by a JAE fellowship from Consejo Superior de
- 455 Investigaciones Científicas (CSIC).

- 456 **References**
- 457
- 458 Abidi W, Jiménez S, Moreno MA, Gogorcena Y (2011) Evaluation of antioxidant compounds and total
- 459 sugar content in a nectarine [*Prunus persica* (L.) Batsch] progeny. Int J Mol Sci 12:6919-6935
- 460 Aranzana MJ, Carbó J, Arús P (2003) Microsatellite variability in peach [Prunus persica (L.) Batsch]:
- 461 cultivar identification, marker mutation, pedigree inferences and population structure. Theor Appl Genet
- 462 106:1341-352
- 463 Badenes ML, Werner DJ, Martínez-Calvo J, Lorente M, Llácer G (1998) An overwiew of the peach
 464 industry of Spain. Fruit Var J 52:11-17
- 465 Baggiolini M (1952) Stades repères du pêcher. Revue Romande d'Agriculture, Viticulture et
 466 Arboriculture 4:28-29
- 467 Bassi D, Selli R (1990) Evaluation of fruit quality in peach and apricot. Adv Hort Sci 4:107-112.
- 468 Besco E, Elena B, Vertuani S, Ziosi P, Brazzo F, Bruni R, Sacchetti G, Manfredini S (2007) The use of
- 469 photochemiluminescence for the measurement of the integral antioxidant capacity of baobab products.
- 470 Food Chem 102:1352-1356
- 471 Bliss FA, Arulsekar S, Foolad MR, Becerra AM; Gillen A, Warburton ML, Dandekar AM, Kocsisne GM,
- 472 Mydin KK (2002) An expanded genetic linkage map of *Prunus* based on an interspecific cross between
- 473 almond and peach. Genome 45:520-529
- 474 Bowles BL, Juneja VK (1998) Inhibition of foodborne bacterial pathogens by naturally occurring food
- 475 additives. J Food Safety 18:101-112
- 476 Brooks SJ, Moore JN, Murphy JB (1993) Quantitative and qualitative changes in sugar content of peach
- 477 genotypes [Prunus persica (L.) Batsch]. J Am Soc Hortic Sci 118:97-100
- 478 Bureau S, Renard C, Reich M, Ginies C, Audergon JM (2009) Change in anthocyanin concentrations in
- 479 red apricot fruits during ripening. LWT Food Sci Technol 42:372-377
- 480 Byrne DH (2005) Trends in stone fruit cultivar development. Hort Technol 15:494-500
- 481 Byrne DH, Nikolic AN, Burns EE (1991) Variability in sugars, acids, firmness, and color characteristics
- 482 of 12 peach genotypes. J Am Soc Hortic Sci 116:1004-1006
- 483 Byrne DH, Raseira MC, Bassi D, Piagnani MC, Gasic K, Reighard GL, Moreno MA, Pérez S (2012)
- 484 Peach. In: Fruit Breeding, Handbook of Plant Breeding 8. pp, 505-565. Ed. Springer Science+Business
- 485 Media, LLC 2012. Editors: M.L. Badenes, D.H. Byrne

- 486 CamoASA (2001) The Unscrambler 9.6 User Manual; Camo: Oslo, Norway
- 487 Cantín CM, Gogorcena Y, Moreno MA (2010) Phenotypic diversity and relationships of fruit quality
- 488 traits in peach and nectarine [Prunus persica (L.) Batsch] breeding progenies. Euphytica 171:211-226
- 489 Cantín CM, Gogorcena Y, Moreno MA (2009a) Analysis of phenotypic variation of sugar profile in
- 490 different peach and nectarine [Prunus persica (L.) Batsch] breeding progenies. J Sci Food Agric 89:1909-
- 491 1917
- 492 Cantín CM, Moreno MA, Gogorcena Y (2009b) Evaluation of the antioxidant capacity, phenolic
- 493 compounds, and vitamin C content of different peach and nectarine [Prunus persica (L.) Batsch] breeding
- 494 progenies. J Agric Food Chem 57:4586-4592
- 495 Cevallos-Casals BA, Byrne D, Okie WR, Cisneros-Zevallos L (2006) Selecting new peach and plum
- 496 genotypes rich in phenolics compounds and enhanced functional properties. Food Chem 96:273-280
- 497 Colaric M, Veberic R, Stampar F, Hudina M (2005) Evaluation of peach and nectarine fruit quality and
- 498 correlations between sensory and chemical attributes. J. Sci Food Agric 85:2611-2616
- 499 Crisosto CH, Day KR, Crisosto GM, Garner D (2001) Quality attributes of white flesh peaches and
- 500 nectarines grown under California conditions. J Am Pomol Soc 55:45-51
- 501 DeJong TM (1999) Developmental and environmental control of dry-matter partitioning in peach.
- 502 HortScience 34:1037-1040
- 503 Dirlewanger E, Moing A, Rothan C, Svanella L, Pronier V, Guye A, Plomion C, Monet R (1999)
- 504 Mapping QTLs controlling fruit quality in peach [*P. persica* (L.) Batsch]. Theor Appl Genet 98:18-31
- 505 Díaz-Mula HM, Zapata PJ, Guillén F, Castillo S, Martínez- Romero D, Valero D, Serrano M (2008)
- 506 Changes in physicochemical and nutritive parameters and bioactive compounds during development and
- 507 on-tree ripening of eight plum cultivars: a comparative study. J Sci Food Agric 88:2499-2507
- 508 Doty TE (1976) Fructose sweetness: a new dimension. Cereal Foods World 2:62-63
- 509 FAOSTAT (2012) http://FAOSTAT.fao.org/
- 510 Font i Forcada C, Gogorcena Y, Moreno MA (2012) Agronomical and fruit quality traits of two peach
- 511 cultivars on peach-almond hybrid rootstocks growing on Mediterranean conditions. Sci Hortic 140:157-
- 512 163
- 513 Font i Forcada C, Oraguzie N, Igartua E, Moreno MA, Gogorcena Y (2013) Population structure and
- 514 marker-trait associations for pomological traits in peach and nectarine cultivars. Tree Genet Genomes.
- 515 331-349

- 516 Forni E, Erba ML, Maestrelli A, Polesello A (1992) Sorbitol and free sugar contents in plums. Food
- 517 Chem 44:269-275
- 518 George AP, Nissen RJ (1992) Effects of water stress, nitrogen and paclobutrazol on flowering, yield and
- 519 fruit quality of the low-chill peach cultivar Flordaprince. Sci Hort 49:197-209
- 520 Gil MI, Tomás-Barberán FA, Hess-Pierce B, Kader AA (2002) Antioxidant capacities, phenolic
- 521 compounds, carotenoids and vitamin C contents of nectarine, peach and plum cultivars from California. J
- 522 Agric Food Chem 50:4976-4982
- 523 Hancock JF (1999) Strawberries. Wallingford, United Kingdom: CABI Publishing, pp 77
- 524 Jiménez S, Garín A, Albás ES, Betrán JA, Gogorcena Y, Moreno MA (2004) Effect of several rootstocks
- on fruit quality of 'Sunburst' sweet cherry. Acta Hort 658: 353-358
- 526 Kader AA (1999) Fruit maturity, ripening, and quality relationships. Acta Hort 485:203-208
- 527 Lester DR, Sherman WB, Atwell BJ (1996) Endopolygalacturonase and the melting flesh (M) locus in
- 528 peach. J Amer Soc Hort Sci 121:231-235
- 529 Milatović D, Nikolić D, Đurović D (2010) Variability, heritability and correlations of some factors
- affecting productivity in peach. HortScience 37:79-87
- Moing A, Carbonne F, Rashad MH, Gaudillère JP (1992) Carbon fluxes in mature peach leaves. Plant
 Physiol 100:1878-1884
- 533 Monet R, Bassi D (2008) Classical genetics and breeding. In: D.R. Layne and D. Bassi (eds.), The peach,
- botany, production and uses. CAB International, Wallingford (UK), pp. 61-84
- Morandi B (2008) Carbohydrate availability affects growth and metabolism in peach fruit. Physiol Plant
 133:229-241
- 537 Moreno MA, Tabuenca MC, Cambra R (1995) Adesoto 101, a plum rootstock for peaches and other stone
- 538 fruit. Hortscience 30:1314-1315
- 539 Mounzer OH, Conejero W, Nicolás E, Abrisqueta I, García-Orellana YV, Tapia LM, Vera J, Abrisqueta
- 540 JM, Ruíz-Sánchez MC (2008) Growth pattern and phenological stages of early-maturing peach trees
- 541 under a Mediterranean climate. HortScience 43:1813-1818
- 542 Muir JG, Rose R, Rosella O, Liels K, Barrett JS, Shepherd SJ, Gibson PR (2009) Measurement of short-
- 543 chain carbohydrates in common australian vegetables and fruits by high-performance liquid
- 544 chromatography (HPLC). J Agric Food Chem 57 (2):554-565

- 545 Orazem P, Stampar F, Hudina M (2011) Fruit quality of Redhaven and Royal Glory peach cultivars on
- seven different rootstocks. J Agric Food Chem 59:9394-9401
- 547 Pirie A, Mullins MG (1977) Interrelationships of sugars, anthocyanins, total phenols and dry weight in
 548 the skin of grape berries during ripening. Am J Enol Vitic 28:204-209
- 549 Prior RL, Cao GH (2000) Antioxidant phytochemicals in fruits and vegetables: Diet and health 550 implications. HortScience 35:588-592
- 551 Quilot B, Wu BH, Kervella J, Genard M, Foulongne M, Moreau K (2004) QTL analysis of quality traits
- in an advanced backcross between *Prunus persica* cultivars and the wild relative species *P. davidiana*.
- 553 Theor Appl Genet 109:884-897
- Reig G, Iglesias I, Gatius F, Alegre S (2013) Antioxidant capacity, quality, and anthocyanin and nutrient
- 555 contents of several peach cultivars [*Prunus persica* (L.) Batsch] grown in Spain. J Agric Food Chem 61:
- 6344-6357
- Robertson JA, Meredith, Scorza R (1988) Characteristics of fruit from high- and low-quality peach
 cultivars. HortScience 23:1032-1034
- 559 Serrano M, Guillén F, Martínez-Romero D, Castillo S, Valero D (2005) Chemical constituents and
- antioxidant activity of sweet cherry at different ripening stages. J Agric Food Chem 53:2741-2745
- 561 Souza VAB, Byrne DH, Taylor JF (1998) Heritability, genetic and phenotypic correlations, and predicted
- 562 selection response quantitative traits in peach. II. An analysis of several fruit traits. J Amer Soc Hort Sci
- 563 123:604-611
- Tavarini S, Degl'Innocenti E, Remorini D, Massai R, Guidi L (2008) Preliminary characterisation of
 peach cultivars for their antioxidant capacity. J Food Sci Technol 43:810-815
- 566 Tomás-Barberán FA, Gil MI, Cremin P, Waterhouse AL, Hess-Pierce B, Kader AA (2001) HPLC-DAD-
- 567 ESIMS analysis of phenolic compounds in nectarines, peaches, and plums. J Agric Food Chem 49:4748-568 4760
- 569 Tomás-Barberán FA, Robins RJ (1997) Phytochemistry of fruit and vegetables; Oxford University Press:
- 570 New York, 1997, pp. 398
- 571 Vauzour D, Vafeiadou K, Rodríguez-Mateos A, Rendeiro C, Spencer J (2008) The neuroprotective
- 572 potential of flavonoids: A multiplicity of effects. Genes Nutr 115-26
- Wang H, Cao G, Prior RL (1997) Oxygen radical absorbing capacity of anthocyanins. J Agric Food Chem
 45:304-309

- 575 Yamaguchi S, Yoshikawa T, Ikeda S, Ninomiya T (1970) Studies on the taste of some sweet substances.
- 576 Part I. Measurement of the relative sweetness. Agr Biol Chem 34:181-186
- 577 Yoshida M (1970) Genetical studies on the fruit quality of peach varieties. Bull Hort Res Sta Jpn 9:1-15

Accessions	Accession number	Accession classification	Origin	Harvest date (JD)	Fruit type	Flesh color	Flesh type	Shape type	Stone type	Gland type	Bloom type
(1) Adriatica	3323 AD	Foreign	Italy	188	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Showy
(2) Alcañiz 1	3097 AD	Local Spanish	Teruel, SP	274	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Showy
(3) Alcañiz 2	3098 AD	Local Spanish	Teruel, SP	246	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Showy
(4) Alejandro Dumas	351 AD	Local Spanish	La Rioja, SP	245	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(5) Amarillo Calanda	131 AD	Local Spanish	Huesca, SP	256	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(6) Amarillo Calanda	2400 AD	Local Spanish	Huesca, SP	266	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Showy
(7) Amarillo Gallur	2361 AD	Local Spanish	Zaragoza, SP	244	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Showy
(8) Andora	2273 AD	Foreign	USA	223	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(9) Andross	3253 AD	Foreign	USA	213	Peach	Yellow	Non-melting	Round	Clingstone	Globose	Non-showy
(10) Baby Gold 5	2562 AD	Foreign	USA	205	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(11) Baby Gold 6	2563 AD	Foreign	USA	203	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(12) Baby Gold 7	2564 AD	Foreign	USA	210	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(13) Baby Gold 8	2565 AD	Foreign	USA	205	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(14) Baby Gold 9	2566 AD	Foreign	USA	203	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Showy
(15) Baladin	3209 AD	Foreign	France	188	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(16) Benasque	3135 AD	Local Spanish	Huesca, SP	235	Peach	White	Melting	Ovate	Freestone	Reniform	Showy
(17) Big Top	3656 AD	Foreign	USA	184	Nectarine	Yellow	Melting	Round	Clingstone	Reniform	Showy
(18) Bonet I	2831 AD	Local Spanish	Lérida, SP	231	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Showy
(19) Bonet II	2832 AD	Local Spanish	Lérida, SP	232	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(20) Bonet III	2833 AD	Local Spanish	Lérida, SP	261	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(21) Bonet IV	2834 AD	Local Spanish	Lérida, SP	258	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(22) Bonet V	2835 AD	Local Spanish	Lérida, SP	272	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Showy
(23) Borracho de Jarque	3185 AD	Local Spanish	Zaragoza, SP	255	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Showy
(24) Brasileño	2184 AD	Local Spanish	Murcia, SP	193	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(25) Calabacero	2247 AD	Local Spanish	Murcia, SP	221	Peach	Yellow	Non-melting	Round	Clingstone	Globose	Non-showy
(26) Calanda San Miguel	2383 AD	Local Spanish	Teruel, SP	251	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Showy
(27) Calanda Tardío	1920 AD	Local Spanish	Teruel, SP	273	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Showy
(28) Campiel	3139 AD	Local Spanish	Huesca, SP	242	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(29) Campiel Rojo	3142 AD	Local Spanish	Huesca, SP	231	Peach	Yellow	Non-melting	Round	Clingstone	Globose	Non-showy

Table 1 Accession number, classification, origin and main fruit characteristics of the 94 accessions studied

(30) Carolyn	2274 AD	Foreign	USA	199	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Showy
(31) Carson	2957 AD	Foreign	USA	194	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Showy
(32) Catherina	3137 AD	Foreign	USA	190	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Showy
(33) Del Gorro	2830 AD	Local Spanish	Teruel, SP	245	Peach	Yellow	Non-melting	Round	clingstone	Reniform	Showy
(34) Diamante Amarillo	2581 AD	Local Spanish	Teruel, SP	245	Peach	Yellow	Non-melting	Round	Clingstone	Globose	Non-showy
(35) Dixon	2278 AD	Foreign	USA	231	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(36) Everts	3060 AD	Foreign	USA	210	Peach	Yellow	Non-melting	Ovate	Clingstone	Reniform	Showy
(37) Fantasia	2971 AD	Foreign	USA	237	Nectarine	Yellow	Melting	Round	Freestone	Reniform	Showy
(38) Flamekist	2970 AD	Foreign	USA	202	Nectarine	Yellow	Melting	Ovate	Clingstone	Reniform	Showy
(39) Flavortop	2969 AD	Foreign	USA	196	Nectarine	Yellow	Melting	Round	Freestone	Reniform	Non-showy
(40) Fortuna	2279 AD	Foreign	USA	243	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(41) GF3	3045 AD	Foreign	France	204	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(42) Goiri	3035 AD	Local Spanish	Bilbao, SP	208	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(43) Golden Queen	2282 AD	Foreign	NZL	253	Peach	Yellow	Non-melting	Round	Clingstone	Globose	Showy
(44) Gomes	3063 AD	Foreign	USA	248	Peach	Yellow	Non-melting	Round	Clingstone	Globose	Non-showy
(45) Halford	3059 AD	Foreign	USA	239	Peach	Yellow	Non-melting	Round	Clingstone	Globose	Non-showy
(46) Infanta Isabel	1068 AD	Local Spanish	Castellón, SP	216	Peach	Yellow	Non-melting	Round	Clingstone	Globose	Non-showy
(47) Jerónimo de Alfaro	3010 AD	Local Spanish	Murcia, SP	226	Peach	Yellow	Non-melting	Round	Clingstone	Globose	Showy
(48) Jungerman	2959 AD	Foreign	USA	216	Peach	Yellow	Non-melting	Round	Clingstone	Globose	Showy
(49) Kakamas	2801 AD	Foreign	South Africa	241	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Showy
(50) Keimoes	3245 AD	Foreign	South Africa	235	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Showy
(51) Klamt	3144 AD	Foreign	USA	228	Peach	Yellow	Non-melting	Round	Clingstone	Globose	Non-showy
(52) Loadel	2802 AD	Foreign	USA	197	Peach	Yellow	Non-melting	Round	Clingstone	Globose	Non-showy
(53) Lovell	3046 AD	Foreign	USA	252	Peach	Yellow	Melting	Round	Freestone	Reniform	Non-showy
(54) Maluenda	2375 AD	Local Spanish	Zaragoza, SP	246	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Showy
(55) Maria Serena	3320 AD	Foreign	Italy	181	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Showy
(56) Maruja	2261 AD	Local Spanish	Murcia, SP	196	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(57) Maruja Porvenir	2955 AD	Local Spanish	Murcia, SP	196	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(58) Miraflores	2844 AD	Local Spanish	Zaragoza, SP	250	Peach	Yellow	Non-melting	Round	Clingstone	Globose	Showy
(59) Mountaingold	3254 AD	Foreign	USA	205	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(60) Nectar del Jalón	561 AD	Local Spanish	Aragón, SP	218	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(61) NJC 97	3422 AD	Foreign	USA	183	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Showy
(62) Nuevo	2803 AD	Foreign	USA	235	Peach	Yellow	Non-melting	Round	Clingstone	Globose	Non-showy

(63) Oropel	2582 AD	Local Spanish	Teruel, SP	258	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Showy
(64) Paloro A	3057 AD	Foreign	USA	248	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(65) Paloro B	3058 AD	Foreign	USA	246	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(66) Queen Giant	3639 AD	Foreign	USA	188	Nectarine	White	Melting	Round	Clingstone	Globose	Non-showy
(67) Redhaven	3640 AD	Foreign	USA	186	Peach	Yellow	Melting	Round	Clingstone	Reniform	Non-showy
(68) Rojo del Rito	3189 AD	Local Spanish	Lérida, SP	251	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(69) San Jaime	2355 AD	Local Spanish	Lérida, SP	199	Peach	Yellow	Non-melting	Round	Clingstone	Globose	Non-showy
(70) San Lorenzo	2358 AD	Local Spanish	Huesca, SP	218	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(71) Sarell (Oom)	3246 AD	Foreign	South Africa	207	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Showy
(72) Selma	255 AD	Foreign	USA	220	Peach	Yellow	Non-melting	Round	Clingstone	Globose	Non-showy
(73) Shasta	2286 AD	Foreign	USA	198	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(74) Stanford	2033 AD	Foreign	USA	237	Peach	Yellow	Non-melting	Round	Clingstone	Globose	Non-showy
(75) Starn	3062 AD	Foreign	USA	244	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(76) Sudanell 1	2211 AD	Local Spanish	Lérida, SP	226	Peach	Yellow	Non-melting	Round	Clingstone	Globose	Non-showy
(77) Sudanell 2	2212 AD	Local Spanish	Lérida, SP	231	Peach	Yellow	Non-melting	Round	Clingstone	Globose	Non-showy
(78) Sudanell 3	2213 AD	Local Spanish	Lérida, SP	233	Peach	Yellow	Non-melting	Round	Clingstone	Globose	Non-showy
(79) Sudanell Blanco	3099 AD	Local Spanish	Zaragoza, SP	231	Peach	White	Non-melting	Round	Clingstone	Globose	Non-showy
(80) Sudanell GF	2804 AD	Foreign	France	227	Peach	Yellow	Non-melting	Round	Clingstone	Globose	Non-showy
(81) Sudanell GF	2972 AD	Foreign	France	224	Peach	Yellow	Non-melting	Round	Clingstone	Globose	Non-showy
(82) Suncling	2805 AD	Foreign	USA	210	Peach	Yellow	Non-melting	Ovate	Clingstone	Reniform	Non-showy
(83) Super Crimson Gold	3657 AD	Foreign	USA	181	Nectarine	White	Melting	Round	Clingstone	Globose	Showy
(84) Tebana	3249 AD	Foreign	Italy	188	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Showy
(85) Tempranillo de Aytona	3138 AD	Local Spanish	Huesca, SP	186	Peach	Yellow	Non-melting	Round	Clingstone	Globose	Non-showy
(86) Tipo Campiel	2921 AD	Local Spanish	Zaragoza, SP	242	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(87) Venus	3660 AD	Foreign	Italy	221	Nectarine	Yellow	Melting	Round	Freestone	Reniform	Showy
(88) Vesuvio	2288 AD	Foreign	Italy	191	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Showy
(89) Vivian	2289 AD	Foreign	USA	249	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Showy
(90) Walgant	3247 AD	Foreign	South Africa	234	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Showy
(91) Wiser	3064 AD	Foreign	USA	243	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(92) Zaragozano	553 AD	Local Spanish	Zaragoza, SP	259	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Non-showy
(93) Zaragozano Amarillo	2857 AD	Local Spanish	Zaragoza, SP	253	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Showy
(94) Zaragozano Rojo	2858 AD	Local Spanish	Zaragoza, SP	246	Peach	Yellow	Non-melting	Round	Clingstone	Reniform	Showy

579 Table 2 Units, minimum, maximum and mean values for the traits evaluated, and ANOVA analysis of the effect of the 94 peach and nectarine accessions for the average of

580 all years of study

				-	Sou	rce of variat	tion ¹
Trait	Units/Description	Minimum	Maximum	Mean \pm SE	Cultivar (C)	Year (Y)	Y x C
Bloom beginning	Julian days	72	83	78 ± 0.19	ns	ns	ns
Full Bloom	Julian days	79	87	82 ± 0.15	ns	ns	ns
Harvest date	Julian days	185	275	224 ± 2.5	ns	ns	ns
TCSA	cm ²	44	280	92 ± 3.9	ns	ns	ns
Yield	Kg/tree	1.0	46.5	13.4 ± 1.9	ns	ns	ns
Yield efficiency	Kg/cm ²	0.11	1.31	0.30 ± 0.02	ns	ns	ns
Fruit weight (FW)	Grams	64	315	178 ± 2.8	ns	ns	ns
Soluble Solids Content (SSC)	°Brix	12	18	15 ± 0.13	***	ns	ns
Flesh firmness (FF)	Newtons	9	61	38 ± 0.9	ns	ns	ns
Titratable acidity (TA)	g malic acid/100 g FW	0.4	0.9	0.6 ± 0.01	***	ns	ns
Ripening index (RI)	SSC/TA	15	67	25 ± 0.43	***	ns	ns
L*	Lightness	10.6	76.8	61.9 ± 9.0	ns	ns	ns
a*	Greenness/redness	-1.18	60.8	22.4 ± 5.2	ns	ns	ns
b*	Blueness/yellowness	8.9	69.1	52.0 ± 11.5	ns	ns	ns
C*	Chroma	25.3	80.6	58.9 ± 9.1	ns	ns	ns
h*	Lightness's angle	16.9	91.4	62.7 ± 14.0	ns	ns	ns
Sucrose	g/kg FW	35	98	75 ± 0.9	ns	ns	ns
Glucose	g/kg FW	4	15	10 ± 0.19	*	ns	ns
Fructose	g/kg FW	2	14	11 ± 0.18	***	ns	ns
Sorbitol	g/kg FW	2	35	13 ± 0.76	***	ns	ns
Total sugars (TS)	g/kg FW	63	136	110 ± 1.35	ns	ns	ns
Vitamin C	mg AsA/100 g FW	3	28	13 ± 0.41	ns	ns	ns
Total phenolics	mg GAE/100 g FW	18	62	44 ± 0.65	ns	ns	ns
Flavonoids	mg CE/100 g FW	3	63	24 ± 1.49	ns	ns	ns
Anthocyanins	mg C3GE/kg FW	0.7	12	2.5 ± 0.21	ns	ns	ns
Relative Antioxidant Capacity (RAC)	μg TE/g FW	186	1184	840 ± 19.0	*	ns	ns

AsA ascorbic acid, GAE gallic acid equivalents, CE catechin equivalents, C3GE cyanidin-3-glucoside equivalents, TE trolox equivalents ¹Data were evaluated by two-way variance (ANOVA); *** $P \le 0.001$; * $P \le 0.01$; * $P \le 0.05$; ns, not significant

583 Table 3 Full bloom (BD), harvest date (HD), yield and annual yield efficiency (AYE), fruit weight (FW),

584 flesh firmness (FF), soluble solids content (SSC), titratable acidity (TA) and ripening index (RI) with

Trait	n	BD	HD	Yield	AYE	FW	FF	SSC	TA	RI
Local Spanish	43	81*	209*	17.3*	0.37*	185*	36	15*	0.62	25
Foreign	51	82*	229*	10.6*	0.23*	170*	40	16*	0.63	26
Peach	87	82	220*	14.6*	0.32*	178	39	15*	0.62*	25*
Nectarine	7	81	194*	9.1*	0.27*	158	31	14*	0.68*	22*
Yellow flesh	89	82	221	14.8	0.32	177*	40*	16	0.62*	26*
White flesh	4	82	201	10.7	0.26	148*	26*	14	0.73*	20*
Non-melting	84	82	220*	13.8	0.30	179	39*	16	0.61*	26*
Melting	10	81	202*	17.7	0.32	175	32*	15	0.70*	21*
Clingstone	89	82	218	13.6	0.29	179	38	15	0.62*	26*
Freestone	5	81	224	18.0	0.44	177	35	15	0.72*	21*

585 qualitative traits in peach and nectarine accessions

586 587 The number of observed accessions (n) is shown for each fruit type. Data are means over the three years

of study. In each trait column, means with * are significantly different according to t test ($P \le 0.05$)

588 Table 4 Chromatic parameters (L*= lightness; a*= redness and greenness; and b*= yellowness and

Trait	n	L*	a*	b*	C*	h*
Local Spanish	43	60*	25*	49*	58	60*
Foreign	51	64*	20*	51*	60	66*
Peach	87	63*	21*	53*	59*	64*
Nectarine	7	42*	35*	23*	43*	30*
Yellow flesh	89	63*	22	54*	60*	64
White flesh	4	56*	22	32*	46*	55

589 blueness; C*= chroma; H= lightness's angle) with qualitative traits in peach and nectarine accessions

590 591 The number of observed accessions (n) is shown for each fruit type. Data are means over the three years

of study. In each trait column, means with * are significantly different according to t test ($P \le 0.05$)

Table 5 SSC, sucrose, glucose, fructose, sorbitol, total sugars (TS), phenolics, flavonoids, anthocyanins, vitamin C and RAC (relative antioxidant capacity) with qualitative

593	traits in	neach and	nectarine	accessions
575	trants m	peach and	neetaine	accessions

Trait	n	SSC	Sucrose	Fructose	Sorbitol	TS	Vitamin C	Phenolics	Flavonoids	Anthocyanins	RAC
Local Spanish	43	15*	75	11	11*	107*	12*	42*	19*	2.2	771*
Foreign	51	16*	75	11	16*	113*	14*	47*	29*	2.8	926*
Peach	87	15*	75*	10	14*	110*	13*	45	24*	2.3*	861*
Nectarine	7	14*	65*	11	7*	94*	7*	35	8*	4.1*	606*
Yellow flesh	89	15	75*	11*	14	111*	12	45	25	2.3	864
White flesh	4	14	66*	8*	13	98*	10	42	21	3.6	821
Clingstone	89	15	76*	11	13	110*	13	46	24	2.5	848
Freestone	5	15	62*	10	13	95*	11	39	18	2.2	742

The number of observed accessions (*n*) is shown for each fruit type. Data are means over the three years of study. In each trait column, means with * are significantly different according to t test ($P \le 0.05$)

Trait	TCSA	YE	FW	SSC	FF	TA	RI	Sucrose	Glucose	Fructose	Sorbitol	TS	Vitamin C	Phenolics	Flavonoids	RAC
Yield	0.22*	0.82*	0.28**	ns	ns	-0.21*	ns	ns	ns	-0.29*	-0.27*	ns	ns	ns	ns	ns
HD	ns	ns	0.63**	0.63**	-0.52*	ns	ns	0.62**	ns	0.21*	0.78**	0.66**	ns	0.65**	0.79**	0.72**
YE		-	0.30**	ns	ns	ns	ns	ns	ns	-0.24**	ns	ns	ns	ns	ns	ns
FW			-	0.56**	ns	0.15*	ns	ns	0.36**	0.39*	ns	0.25*	ns	0.53**	0.21*	0.34*
SSC				-	0.49**	0.26**	ns	0.29**	0.27**	0.36*	0.77**	0.49**	ns	0.56**	0.60**	0.61**
FF					-	0.40**	-0.57*	-0.50**	-0.64**	-0.49**	-0.42*	-0.59*	ns	-0.52**	-0.26*	ns
TA						-	ns	ns	0.41**	ns	0.40**	ns	0.46**	ns	0.35**	ns
RI							-	0.42**	0.24*	0.35*	0.41**	0.27**	-0.21*	ns	ns	ns
Sucrose								-	0.57**	0.63**	0.48**	0.95**	ns	0.43**	0.47**	ns
Glucose									-	0.83**	0.44**	0.81**	ns	0.42**	0.44**	0.52**
Fructose										-	0.49**	0.83**	ns	ns	0.24**	ns
Sorbitol											-	0.56**	0.37**	0.52**	0.47**	0.64**
TS												-	0.42**	0.58**	0.61**	0.64**
Vitamin C													-	ns	ns	0.25*
Phenolics														-	0.68**	0.79**
Flavonoids															-	0.87**
RAC																-

Table 6 Pearson's correlation coefficients between pairs of traits studied

597 * $p \le 0.05$, ** $p \le 0.01$ represent significant values, *ns* not significant

598 Table 7 Eigenvectors of the two principal component (PC) axes of the agronomic, basic fruit quality traits, sugars and phytochemical compounds evaluated on 94 peach and

599 nectarine accessions

	Component loading	
	PC1 (35.2%)	PC2 (19.1%)
Harvest date	-0.738	0.408
Yield efficiency	0.138	-0.423
Fruit weight (FW)	-0.011	0.262
Soluble solid content (SSC)	-0.714	0.326
Flesh firmness (FF)	-0.551	0.375
Titratable acidity (TA)	-0.171	0.587
Sucrose	-0.319	-0.085
Glucose	-0.422	0.664
Fructose	-0.312	0.586
Sorbitol	-0.709	0.468
Total sugars	-0.700	0.371
Vitamin C	-0.660	0.230
Phenolics	-0.685	0.204
Flavonoids	-0.778	0.227
Anthocyanins	0.012	0.928
Relative Antioxidant Capacity (RAC)	-0.771	0.398
L*	-0.598	-0.731
a*	0.607	0.644
b*	-0.661	-0.667
C*	-0.662	-0.457
h*	-0.574	-0.707

603 Figure



